

Clinical aspects of allergic disease

Allergenic pollen and pollinosis in Madrid

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Objective: A 15-year pollen count was performed in the atmosphere of Madrid, Spain, to determine the months in which the highest concentrations of allergenic pollens occur.

Methods: Pollen counts were done with a Burkard spore trap (Burkard Manufacturing, Rickmansworth, Herst., U.K.). The results were subsequently compared with results of skin tests in patients with pollinosis born and living in and around Madrid.

Results: The highest airborne presence (percent of total yearly pollen counts, mean of counts from 1979 to 1993) was for *Quercus* spp. (17%); followed by *Platanus* spp. (15%), *Poaceae* (15%), *Cupressaceae* (11%), *Olea* spp. (9%), *Pinus* spp. (7%), *Populus* spp. (4%), and *Plantago* spp. (4%). The most predominant pollens from January to April are tree pollens (*Cupressaceae*, *Alnus*, *Fraxinus*, *Ulmus*, *Populus*, *Platanus*, and *Morus*), although these are also abundant in May and June (*Quercus*, *Olea*, and *Pinus* spp.). The grass pollination period shows a double curve: the first peak occurs from February to April (8% of yearly grasses), and the second peak occurs from May to July (90% of yearly grasses). Among allergenically significant weeds, the most notable is *Plantago*; in contrast, *Rumex*, *Urticaceae*, *Cheno-Amaranthaceae*, and *Artemisia* spp. have very low concentrations ($\leq 2\%$ yearly total pollens). The most significant allergenic pollen is that of grasses, with a prevalence of positive prick test results of 94%, followed by *Olea europaea* (61%), *Plantago lagopus* (53%), *Platanus hybrida* (52%), and *Cupressus arizonica* (20%).

Conclusion: The population of Madrid is exposed to high concentrations of allergenic pollen from February to July, although the most intense period is from May to June. Grass pollens are the most important cause of pollinosis in this area. (*J ALLERGY CLIN IMMUNOL* 1995;96:15-23.)

Key words: Pollen calendar, airborne pollen, allergenic pollen, pollinosis

Persons who travel for work or leisure need to have reliable information about the likelihood of seasonal allergies when they visit another country. For this reason, knowledge of the atmospheric pollen concentration encountered in the different regions is of great interest to clinicians and patients with allergies to achieve better management of their hay fever symptoms. Every year Madrid receives millions of tourists from the United States and Europe; however, no pollen calendar has been published for this geographic area in English.

As result of the characteristic Mediterranean continental climatic conditions in this area, we find typical vegetation with production of allergenic

Abbreviation used

SPT: Skin prick test

pollens, such as that of *Trisetum paniceum* (a grass well adapted to the low-humidity soil found in the Madrid area) or *Olea* species, very different from that of central and northern Europe.¹⁻⁴

We present in this article the result of a 15-year aerobiologic study of airborne pollens in the atmosphere of Madrid and the results of a study on the frequency of positive skin test responses to aeroallergens among atopic patients living in this area.

METHODS

Pollen count

The pollen count was made according to a previously described technique^{5,6} with volumetric spore traps. A Hirst spore trap (C. F. Casella Co., London, U.K.) was used from 1979 to 1981, and a Burkard trap (Burkard

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Manufacturing Co., Rickmansworth, Herst., U.K.) was used from 1982 to 1993. The traps were installed on the roof of our center, which is 20 m high and is located in the east of the Madrid urban area (40° 26' N; 3° 40' E, 640 m above sea level), with no parks or gardens nearby. Ten liters of air was taken in every minute (14.4 m³/24 hours) through an opening (14 × 2 mm) permanently oriented in the direction of the wind thanks to its built-in vane. The airborne particles fall on a strip located very close to the opening. The strip is a transparent plastic film, which we cover with a thin, uniform layer of filamentous petroleum jelly and which is fixed around a drum, rotated by a 7-day clockwork at a speed of 2 mm/hr. Every week this strip is removed and cut into 48 mm long segments, each of which corresponds to 1 day of sampling. Each segment is mounted on a slide and covered with a 22 × 50 mm cover, which overlays the area of impact and is adhered to the strip by means of 2 to 3 drops of a solution of glycerol jelly and phenol at 40° C. To study the pollens that have fallen in a 24-hour period, a 48 mm sweep (2 mm × 24 hours = 48 mm) with the immersion lens is made (×10 ocular, ×100 objective and microscope field diameter of 0.18 mm). The counts made in this 48 mm sweep, once multiplied by a conversion factor of 5.4, give us the average number of grains of pollen per cubic meter of air in 24 hours.

Pollen identification was based on comparison with reference slides and photographs, as described by Hyde and Adams.⁷ The sampling period included the months of January through December from 1979 to 1993.

Patients

We have evaluated the percentage of positive skin test responses to common aeroallergens in one group of 614 patients of both sexes, who had come spontaneously to our clinic consecutively in 1993.

Patients were selected on the basis of a history of seasonal or perennial rhinitis, asthma, or both. Each patient's evaluation was made by physician assessment that included a case history, clinical examination, skin prick tests (SPTs), and eventually, RAST. From this group we have selected the patients with SPT positivity to pollens and with seasonal clinical symptoms. There were 411 patients of both sexes and aged 4 to 77 years (average age, 29 years) born and still living in or around Madrid.

Skin tests

A battery of commercially available common inhalant allergens were used in prick tests. This battery included *Dermatophagoides pteronyssinus*, cat dander, and *Alternaria*, *Cladosporium*, *Aspergillus* species (Aristegui, Bilbao, Spain). Pollen extracts, prepared in our laboratory, were also used in prick tests. The pollens included in the battery were those that we considered to be the most representative of the atmosphere of our geographic area, according to pollen counts (pollen types that accounted for at least 3% of the yearly total) and studies

of the local vegetation Tables I and II. The pollen species were collected from plants and trees of our surrounding area in 1992 and were used to prepare extracts at 1:20 wt/vol.

Histamine chlorhydrate, 10 mg/ml, was used as a positive control, and 50% glycerol was used as a negative control. All skin test sites were read after 15 minutes. A positive reaction was defined as a wheal of at least 3 mm × 3 mm in the presence of a negative reaction to the 50% glycerol and a positive test result with histamine. Ten nonatopic subjects were also tested with all the above-mentioned extracts and served as a control group.

Collection of pollens

Selection and collection of mature male capitula were done in the morning. Collected inflorescences were immediately vacuum-dried at 35° C. Mechanical sieving was later undertaken with the dried product with 3 mm, 0.5 mm, 160 µm, 80 µm, and 40 µm mesh sieves. Microscopic control was then undertaken to ensure that the purity of the pollen was at least 98%. Finally, with the purified pollen, 24-hour vacuum-drying at 35° C was carried out.

Preparation of pollen extracts

Pollen extracts were prepared according to a previously described method.⁸ Two grams of each type of pollen was defatted with acetone, dried, and extracted in 40 ml of 0.01 mol/L phosphate and 0.15 mol/L NaCl, pH 7.4 (phosphate-buffered saline), and the mixture was stirred for 12 hours at 4° C. The suspension was filtered through filter paper (Whatman Ltd., Maidstone, U.K.), dialyzed against phosphate-buffered saline and sterilized by 0.22 µm filtration (Millipore, Molsheim, France), with a final concentration of 1:20 wt/vol.

RESULTS

Pollen counts

The months of maximum pollen concentration were March, April, May, and June. During this period, 80% of the annual total of pollen was recorded (Fig. 1). As can be seen in Table I, in the 15 years of research, 44 different pollen grains belonging to 32 families were identified and recorded.

Fig. 2 shows a pollen calendar for 19 pollen types for which the yearly concentrations were higher than 0.4% of the total pollen concentration. These account for 95% of all observations and are descriptive of Madrid anemophilous flora.

The behavior of airborne grass pollen is shown in Fig. 2 and Table I. The start of the major grass pollen release period, defined as the day on which a concentration of 50 grains/m³ of air or greater occurs,⁹ varied between the ninth and twenty-ninth of May, although lower values were frequently

TABLE I. Annual relative frequency of atmospheric pollen types in Madrid (average 1979-93)

Family	Genus and species	Average	Family	Genus and species	Average
Fagaceae		18.81	Betulaceae		0.56
	<i>Quercus rotundifolia</i>	(17.08)		<i>Alnus</i> spp.	(0.43)
	<i>Quercus pyrenaica</i>	(1.10)		<i>Betula</i> spp.	(0.11)
	<i>Castanea</i> spp.	(0.63)		<i>Corylus</i> spp.	(0.02)
Platanaceae	<i>Platanus hybrida</i>	14.95	Ericaceae		0.29
Poaceae		14.85	Fabaceae		0.28
Cupressaceae		11.77	*Papilionoideae	<i>Sophora</i> spp.	(0.25)
Oleaceae		9.87	*Caesalpinioideae	<i>Gleditsia</i> spp.	
	<i>Olea europaea</i>	(8.72)	*Mimosoideae	<i>Mimosa</i> spp.	(0)
	<i>Fraxinus angustifolia</i>	(1.48)	Aceraceae	<i>Acer</i> spp.	0.25
	<i>Ligustrum vulgare</i>	(0)	Borraginaceae	<i>Echium plantagineum</i>	0.19
Pinaceae		7.25	Myrtaceae	<i>Eucalyptus</i> spp.	0.14
	<i>Pinus</i> spp.	(7.21)	Umbelliferae		0.14
	<i>Cedrus</i> spp.	(0.03)	Hippocastanaceae	<i>Aesculus</i> spp.	0.08
Salicaceae		4.48	Typhaceae	<i>Typha</i> spp.	0.06
	<i>Populus</i> spp.	(4.21)	Cistaceae		0.03
	<i>Salix</i> spp.	(0.27)	Juncaceae		0.02
Plantaginaceae	<i>Plantago</i> spp.	3.68	Buxaceae	<i>Buxus</i> spp.	0.02
Moraceae	<i>Morus</i> spp.	2.14	Tiliaceae	<i>Tilia</i> spp.	0.01
Polygonaceae	<i>Rumex</i> spp.	1.94	Labiatae		0.01
Ulmaceae	<i>Ulmus</i> spp.	1.66	Euphorbiaceae	<i>Mercurialis</i> spp.	0
Chenopo-Amaranthaceae		1.58	Juglandaceae	<i>Juglans</i> spp.	0
Compositae		1.13	Rosaceae		0
	<i>Artemisia</i> spp.	(0.45)	Unidentified		0.24
	<i>Taraxacum</i> spp.	(0.07)			
	Others	(0.59)			
Urticaceae		1.10			
Cyperaceae		0.80			
	<i>Carex</i> spp.	(0.80)			
	<i>Cyperus</i> spp.	0			

Pollen types are ranked according to the percentage contribution to the total pollen catch.

Pollen types >0 and <0.01 are represented by 0.

Numbers in parentheses indicate genus and species counted; numbers without parentheses indicate families counted.

*Subfamily.

recorded on some sunny days from the end of January onward. The end of the major grass pollen release period (last day on which ≥ 50 grains/m³ of air are recorded) varied between June 18 and July 25.

The number of consecutive days with over 50 grass pollen grains/m³ found in the air sampled in Madrid ranged from 11 (in 1992) to 39 (in 1985).

Likewise, there were wide year-to-year variations in total atmospheric grass pollen counts, expressed as the total sum of the mean daily concentrations from January 1 to December 31, ranging from 1674 in 1992 to 6624 in 1985 (Fig. 3).

The highest peak daily count (maximum day count of the season) was 546 grass pollen grains/m³ (May 29, 1988) and the lowest was 96 (May 14, 1992) (Fig. 3).

One fact that attracted our attention was the abundance of small-grain grass pollen in the atmo-

sphere of Madrid. The percentage of sampled grass pollen with a diameter of less than 30 μ m ranged from 50% to 76%, with an average of 63% (Fig. 3). This information is even more interesting because field studies have shown that the only grass that grows in abundance around Madrid and is able to produce such small pollen is *Trisetum paniceum* (Fig. 4), a grass that, before the studies, had not been described as a cause of pollinosis.¹⁰

SPT results

The percent frequency of positive skin reactions for all studied pollen allergens in the group of 411 patients with pollinosis of both sexes is summarized in Table II. The percent values have been calculated on the total number of patients with positive pollen skin test results, considering also the polysensitizations.

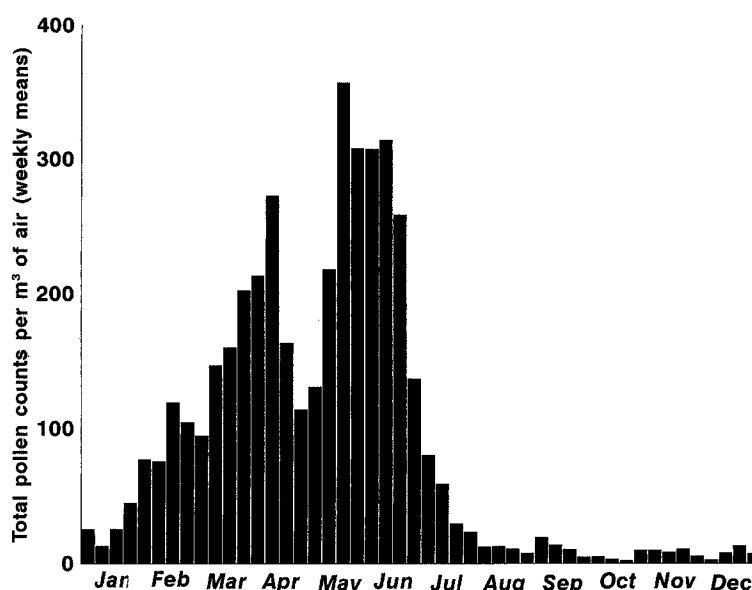


FIG. 1. Weekly mean values of the total pollen counts in the Madrid atmosphere from 1979 to 1993.

TABLE II. Presence of airborne pollen and skin test positivity

Airborne presence		Skin test positivity		
Family or genus	(%)	Species	Common name	(%)
<i>Quercus</i> spp.	17	<i>Quercus rotundifolia</i>	Live oak	14
<i>Platanus</i> spp.	14.9	<i>Platanus hybrida</i>	London plane tree	52
Poaceae	14.8	<i>Trisetum paniceum</i>	Lesser oat grass	85
		<i>Dactylis glomerata</i>	Orchard grass	87
		<i>Trisetum</i> and/or <i>Dactylis</i>		94
Cupressaceae	11.7	<i>Cupressus arizonica</i>	Arizona cypress	20
		<i>Cupressus sempervirens</i>	Italian cypress	16
		<i>C. arizonica</i> and/or <i>C. sempervirens</i>		23
<i>Olea</i> spp.	8.9	<i>Olea europaea</i>	Common olive	61
<i>Pinus</i> spp.	7.2	<i>Pinus sylvestris</i>	Scotch pine	7
		<i>Pinus halepensis</i>	Aleppo pine	4
		<i>Pinus pinea</i>	Italian stone pine	4
		<i>Pinus pinaster</i>	Maritime pine	2
<i>Populus</i> spp.	4.2	<i>Populus alba</i>	White poplar	29
<i>Plantago</i> spp.	3.6	<i>Plantago lagopus</i>	Hare's foot plantain	53
		<i>Plantago lanceolata</i>	English plantain	32
		<i>P. lagopus</i> and/or <i>P. lanceolata</i>		55

Airborne presence (percent of total yearly pollen count, mean of 1979 to 1993) and skin test positivity (percent positive reactions in patients with pollinosis) in Madrid.

Ninety-four percent of the patients showed a clear positive reaction to grass pollens, although 95% of them showed positive SPT responses to other pollen as well.

The scarcity of monosensitizations in the rest of the pollen types tested attracted our attention: 1.2% for *Cupressus* spp, less than 1% for *Olea* and *Platanus*, and 0% for the other pollens used. None

of the control subjects showed positive skin reactions to the allergen battery.

DISCUSSION

The majority of patients living in Madrid experience their pollinosis symptoms mainly during May and June. During this period the two most frequent pollen types collected in our spore trap

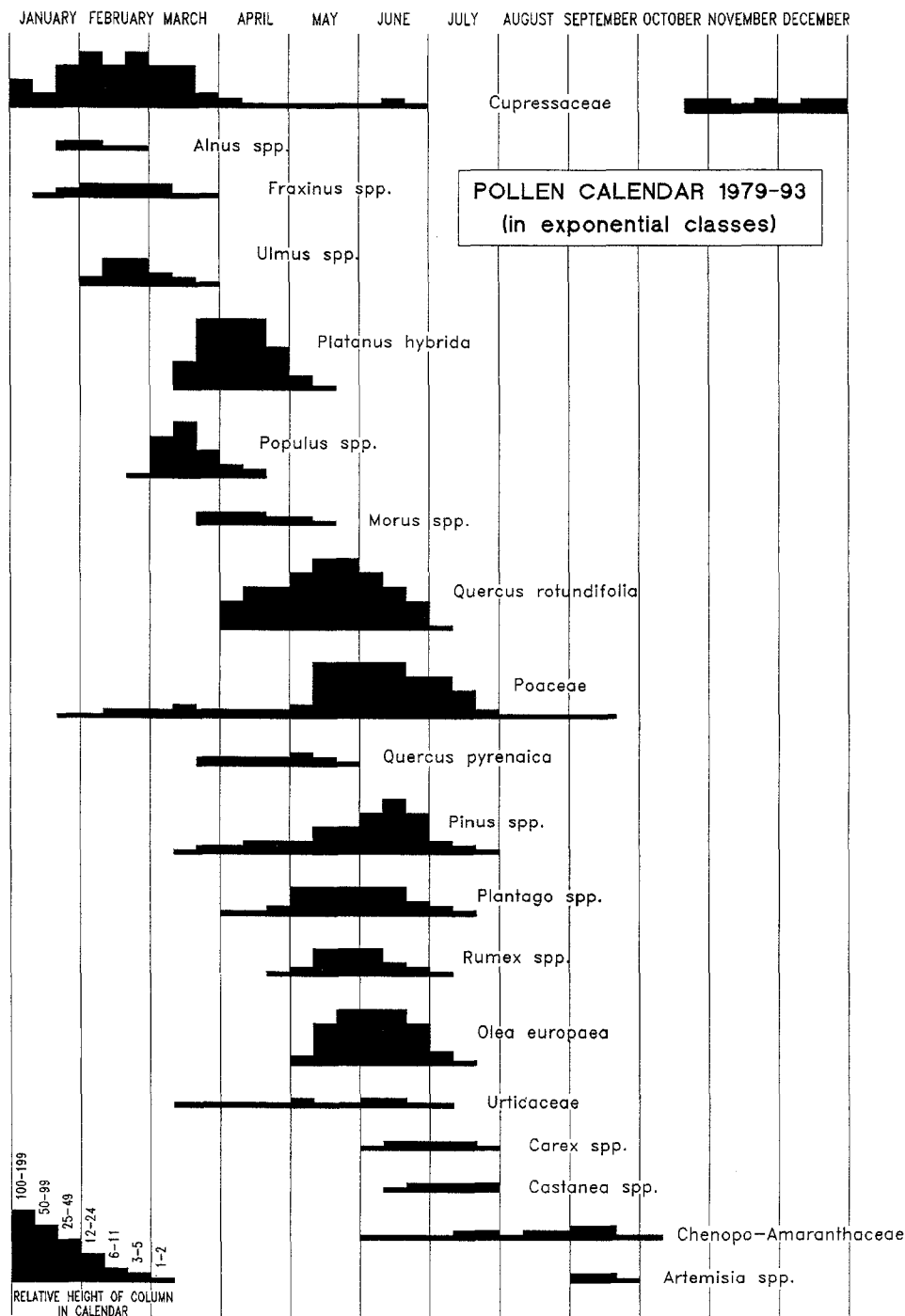


FIG. 2. Madrid pollen calendar. The figure shows, in exponential classes, the logarithmic means of 10 days of pollen counting (totals of 10 mean daily concentrations) for 15 years measured in Madrid with a volumetric pollen trap. An arbitrary limit has been set: included are those species (or groups of species; taxa), which accounted for at least 0.4% of the year's total.

were *Quercus rotundifolia* (30% of the total pollen collected in May and June) and grasses (27%). Ninety-four percent of patients with pollinosis from this geographic area showed clearly positive SPT responses to grass pollen, whereas only 14%

showed a positive reaction to *Quercus* spp. These facts seem to suggest that grasses are the main cause of pollinosis in Madrid. Furthermore, in the 15-year period of study, we observed that the number of consecutive days with concentrations

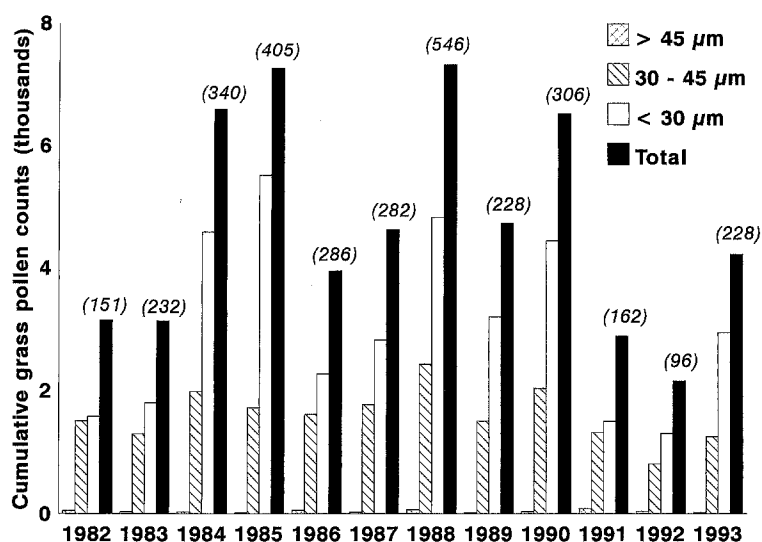


FIG. 3. Totals of daily averages of airborne grass pollen concentrations (grains per cubic meter) from 1979 to 1993 in Madrid. The cumulative grass pollen counts are also expressed according to the size of the pollen grain. The maximum daily count of the season is indicated in parentheses.

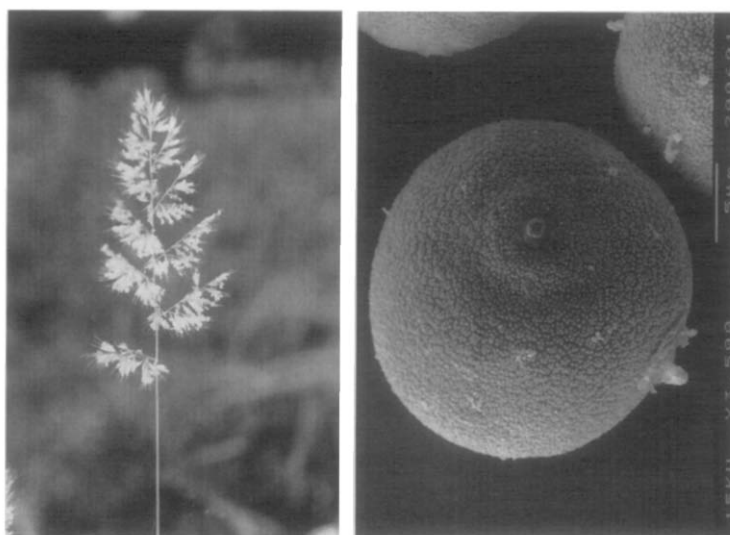


FIG. 4. *Trisetum paniceum*.

greater than 50 grass pollen grains/ m^3 of air (considered high in general for patients clinically sensitive to grasses)⁹ ranged from 11 to 39 days during May and June every year. It is surprising that in a climate as dry as that of Madrid such high concentrations of grass pollen can be reached. These concentrations are similar to those obtained in other Northern European cities (e.g., London) where there is more rainfall and therefore grasses are more abundant.^{9, 11-15}

This may be explained by the fact that Madrid is located in the center of the Iberian peninsula,

more than 300 km from the nearest coast. Oceanic humidity cannot penetrate the peninsula because of the numerous mountain ranges, which practically wall off most of the coastal areas. (Spain is the most mountainous country in Europe after Switzerland.) This endows Madrid (and the majority of the other geographic areas in the interior of the peninsula) with the low relative humidity, which characterizes its Mediterranean extreme continental climate.¹⁶ The lack of humidity in the continental climate is largely responsible for significant daily temperature variations and above all for very

brusque changes in temperature with the change of seasons. Very cold winter temperatures turn into very hot and dry summers in scarcely 4 weeks (mid-May to mid-June) and finally provoke an almost simultaneous anthesis of the majority of grass species in the area.¹⁷ These 2 months account for 79% of the year's grass pollen loads, and if the figures for April and July are added, this increases to 92% of the year's total grass pollen (Fig. 2).

It is interesting to note that there appears to be a predominance of small-grain grass pollen (with a diameter of less than 30 μm) in the atmosphere of Madrid, as demonstrated by the pollen counts taken over the last 12 years (mean of 63% of small grasses over total grasses) (Fig. 3). This is hardly surprising in a climate as stable as that of Madrid, which rarely has strong winds in May and June and which seems to favor small-grain pollens.

In previous studies we found that *Trisetum panicum* was the most common of the grasses that produce small-grain pollens in the Madrid area.¹⁰ This plant tends to grow in calcium-rich plateaus, at times forming huge meadows, above all to the south and east of the capital.¹⁸ This is an annual plant and therefore depends on the soil moisture in autumn for its germination. The high interannual variation of autumn rainfall in Madrid appears to play an important role in the large interannual total atmospheric grass pollen counts.⁶ Therefore it is not surprising that these large interannual variations in the total grass pollen counts are mainly due to the number of small grass pollens (Fig. 3).

Considering allergenic pollen as a whole in Spain, grasses are the most important cause of pollinosis. Allergic sensitization to grasses is prevalent in central and northern Spain, with the exception of the Mediterranean coast because of its climate, where in contrast *Parietaria judaica* pollen is the primary cause of pollinosis.^{4, 17, 19} The grass pollination season in Spain varies from one region to another. In Seville (southern Spain) it lasts from late April to late June, in contrast in Coruña (northern Spain) where it lasts from late May to late July. The highest atmospheric grass pollen concentration varies from 40 to 75 grains/ m^3 of air, which is the most frequent level in the Spanish Mediterranean coastal area, and from 200 to 400 grains/ m^3 in some areas such as Badajoz or Salamanca (central Spain), which like Madrid are interior areas with an extreme continental climate.^{17, 20, 21}

In May and June the third type of pollen most frequently collected in our air sampler was *Olea* spp. (15% of the total pollen sampled in this

period). The highest weekly averages were around 100 grains/ m^3 of air, with peak days around 200 grains/ m^3 of air. It is probable that these concentrations are higher in the area south of Madrid, because the nearest olive groves (22,400 hectares) are located 14 km from our sampler.

Olea pollen has been described as an important cause of pollinosis in the Mediterranean area,²²⁻³⁰ especially in regions with intense cultivation of olive trees, such as Granada, Cordoba, Jaén, (southern Spain),³⁰⁻³³ or Bari (southern Italy)³⁴ where the peak concentrations expressed as daily means are frequently in these two last cities higher than 2000 to 3000 pollen grains/ m^3 of air. In the latter regions *Olea* is the most relevant allergenic pollen, and about 14% to 20% of patients with pollinosis have been found to be monosensitized to it.^{4, 32-34}

In our study 61% of patients with pollinosis showed a positive skin test response to this pollen, although in nearly all cases a positive reaction to grasses was also observed. For this reason, it is difficult to estimate its true clinical relevance in the Madrid area.

Pinus spp. were also an abundant airborne pollen in May and June (13% of the total pollen sampled in this period), although with low allergenicity, as previously described.^{1-3, 35} Among our patients only 2% to 7% showed a positive skin test reaction to *Pinus* pollen (Table II).

In contrast to *Pinus* pollen, *Plantago* pollen showed a low airborne presence (5% of the total pollen sampled in May and June) but with a much higher frequency of positive skin reactions (Table II). The species that probably contributes the most in the *Plantago* airborne pollens sampled in Madrid is *Plantago lagopus*. The greater exposure of the Madrid residents to this species may explain the greater prevalence of positive skin test reactions to *Plantago lagopus* (53%) as compared with *Plantago lanceolata* (32%). This fact is also interesting because *Plantago lagopus* had not been previously reported as a possible cause of hay fever. It is probable that both pollens are allergenically very similar because most patients who reacted to *Plantago lanceolata* also reacted to *Plantago lagopus* (Table II).

No monosensitizations were found among the patients with positive *Plantago* skin test reactions and pollinosis.

Platanus pollen mainly appeared in March and April and was one of the most frequently found pollens in the atmosphere of Madrid (Table I), and at the time of the study in this geographic area a high percentage of patients with pollinosis were

sensitive to this pollen (Table II). This is surprising because plane tree pollen is usually described in literature as being only slightly allergenic, in spite of the high concentrations captured in the air samplers of a large part of Europe and the United States.^{1, 2, 4, 36-40} This information is consistent with the low prevalence of positive skin test reactions (only 2%) for plane-tree pollen obtained in a study undertaken in 1979 among our patients with pollinosis in Madrid.³⁸ However, it strongly contrasts with the present high prevalence of skin sensitivity (52%) found among the 411 patients with pollinosis studied. Furthermore, in a recent study we reported that at least in some of these patients *Platanus* pollen is capable of inducing allergic rhinitis and bronchial asthma in natural conditions.⁴¹ Although further research is necessary to determine its true clinical relevance, we believe that at present its importance in contributing to pollinosis symptoms should not be underestimated, at least in the Madrid area.

Remarkable amounts of airborne Cupressaceae pollens were recorded in our sampler during the period of January to March (Fig. 2). Although evident fluctuations from year to year were observed, there seems to be an increasing tendency, probably caused by the high number of Cupressaceae trees, mainly *Cupressus arizonica*, planted extensively in the North Madrid area during the last few years (data not shown).

Airborne Cupressaceae pollen has been recognized as a cause of pollinosis in Mediterranean and extra-Mediterranean areas.^{2, 3, 28, 42-47} Positive SPT responses to *Cupressus arizonica* and/or *Cupressus sempervirens* were observed in 23% of our patients with pollinosis, although such responses were frequently associated with a response to grasses. Some but not all of these *Cupressus*-sensitized patients have pollinosis symptoms during sunny days from mid-January to mid-March. However, airborne grasses (probably *Poa annua*) frequently appear together with Cupressaceae pollens in the atmosphere of Madrid during these sunny days and may also contribute to their winter pollinosis symptoms (Fig. 2).

In conclusion, in the Madrid area grasses are one of the major pollinators and they are also the major cause of pollinosis. *Olea*, *Plantago*, *Platanus*, and *Cupressus* spp. are important, but their sensitivity alone is rare.

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