

Airborne grass (Poaceae) pollen in southern Spain. Results of a 10-year study (1987–96)

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This work reports an exhaustive study of the aerobiology of the Gramineae in Seville, Spain, which is typical of coastal Mediterranean areas. Sampling was done with a Cour trap installed on the roof terrace of the School of Pharmacy, Seville, from 1987 to 1996, both inclusive. The climatic pattern of that period was characterized by two exceptionally wet years (1989 and 1996), between which were 5 consecutive years of drought (1990–5). This typically Mediterranean climate affects grass aerobiology. The annual amounts of total grass pollen are low, never exceeding 2500 grains/m³. The start, length, and intensity of the pollen season are significantly correlated with preseasonal meteorologic factors (precipitation and temperature), but intraseasonal meteorologic conditions have no effect on the three variables. The relationships are stated by three equations that, while further years of observations are anticipated, can be considered models to forecast the characteristics of the pollen season: the starting date depends on the mean temperatures of January and February, and the length and intensity of the season depend on the rainfall between the beginning of January and the starting date of the season. For the study period, the weekly concentrations (pollen curves) throughout the year showed no typical pattern of variation over the years, so that it was impossible to make mid- and long-term forecasts of the variation in weekly concentration. The most noteworthy aspects of grass pollen curves are a long pollen season, which starts in February or March and lasts until September or October; peaks of higher concentration (>100 grains/m³) in May and June, associated with increases in temperature and absence of precipitation; and other peaks in the summer months that may be as high as the spring peaks.

Key words: aerobiology; allergenic pollen; grass pollen; southern Spain.

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The great importance, from both the allergenic and epidemiologic points of view, of grass pollen in western European countries (1, 2) has been demonstrated in the last 25 years by numerous monographs dealing with various aerobiologic aspects of this plant family (3–8). The context includes Spain, where grass pollen is the most important cause of seasonal rhinitis and asthma (9). An interesting article by Subiza et al. (10) describes a model to forecast the intensity of the grass pollen season in the air of Madrid, according to climatic variables. The literature dealing specifically with grass aerobiology in the rest of the country is not particularly extensive, but of note are the studies carried out in Córdoba (11, 12) and Granada (13).

Now we present this work on various aspects of grass aerobiology observed in Seville (southern Spain), forecasting the starting date and the intensity of the pollen season from meteorologic varia-

bles, pollen counts, and the effect of drought on annual concentrations. The results of 10 years of observations extend the partial work already carried out in the city (14, 15) and attempt to extrapolate the grass aerobiology of the study area to southern Mediterranean regions in general. This would help allergists in similar geographic areas (southern Italy, southern Portugal, Greece, Israel, etc.) in treatment and prevention of allergies produced by grass pollen.

Material and methods

Study area: location, climate, and ecologic and allergenic importance of the Gramineae

The city of Seville is situated in the lower Guadalquivir valley, on the banks of the river of the same name, at 37°23'N, 5°58'W. Its population exceeds

1 000 000, making it one of the largest cities in Spain. Although it is not a coastal city, its closeness to the Atlantic Ocean (41.2 km) places it within the dry, coastal area, characterized by a temperate Mediterranean climate subject to marine influence (16). The average temperature and rainfall are 18.8°C and 600 mm (17). Rainfall is concentrated in autumn and early spring, with the minimum during the summer. The annual total rainfall is affected by periods of recurring drought, when the amount of rainfall is very much lower than the mean, one of the most specific features of the Mediterranean climate (18).

The vegetation of the area is characterized by a very man-influenced environment in which there are no vestiges of the autochthonous forest (*Quercus*). This has been replaced by agricultural land on which grow numerous herbaceous plants (nitrophiles, ruderals, and weeds) having a short, rapid annual cycle: Gramineae, Urticaceae, Compositae, Euphorbiaceae, Chenopodiaceae, etc. Grasses colonize all ecologic environments and constitute the most abundant herbaceous stratum in the study area, with 83 genera and 224 species. The most important genera are *Poa*, *Avena*, *Vulpia*, *Hordeum*, *Brachypodium*, *Gaudinia*, *Bromus*, *Dactylis*, *Lolium*, *Panicum*, *Trisetaria*, *Piptatherum*, *Stipa*, *Aegilops*, *Phalaris*, *Melica*, *Agrostis*, *Holcus*, *Paspalum*, *Echinocloa*, *Cynodon*, and *Hyparrhenia* (19). Fig. 1 shows the flowering periods of these genera (19).

As in similar areas of southern Italy and Portugal (20, 21), grasses in Seville are the main cause of the pollen allergies reported, followed by olive (22, 23). Thus, the data of the Spanish Society of Clinical Allergy and Immunology reveal that 65% of the pollinosis reported in the city hospitals is caused by grass pollen (in patients also polysensitive to pollen of olive, plantain, mugwort, and fat hen), which also causes more than 90% of mono-sensitive cases (24).

Collection of airborne pollen

The counts of airborne grass pollen were made with a Cour trap. This was installed at a height of 15 m on a flat roof of the School of Pharmacy, Seville University. The trap monitored the city air uninterruptedly for 10 consecutive years (1987–96). The Cour trap uses 400 cm² of sterile gauze filters impregnated with silicone oil and fitted to a vane. The apparatus includes a totalizing anemometer, indicating the wind flow through the filter during the exposure period. Sample processing was done by the method proposed by Cour and designed at the Palynology Laboratory, Montpellier (France) (25). The filters undergo a series of chemical treat-

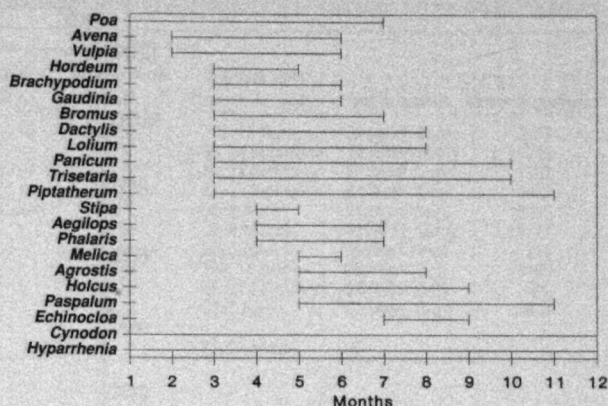


Fig. 1. Flowering periods of most abundant genera of Gramineae in study area (19).

ments in the laboratory to destroy the gauze and release the pollen, which is then acetolyzed and colored to help in its identification. (This treatment is very expensive and means 8 h of work per filter treated.) Lastly, a proportional part of the filter is analyzed. The period of sample exposure is 1 week; therefore, pollen concentrations are expressed in grains per week/m³ of air, and concentrations per hour or day cannot be calculated. In a 2-year study carried out by the authors, the weekly pollen concentrations obtained with the Cour method could be transformed into the weekly mean concentrations obtained by the Hirst method, by multiplying the former by 1.08. According to this result, the weekly concentrations of grass pollen obtained with the Cour and Hirst methods are equivalent (26).

The only way to obtain daily concentrations by the Cour method is to change the filter every day, a measure which was not taken by any of the various authors using this method (5, 27–30), given the high financial cost involved. Filters were changed only two to four times weekly, and only when making crop forecasts (31, 32).

Statistical and meteorologic data

Concepts such as pollen season (those weeks of the year when the pollen collected of a certain type represents between 5 and 95% of that collected by the end of the year) (33) and week of maximum pollen emission (that week of the year when the highest concentrations of a certain type of pollen are recorded) have been used.

The meteorologic data in this work (mean temperature and precipitation) were provided by the National Institute of Meteorology from its station in María Luisa Park, 1 km from the sampling point.

Statistical regressions relating meteorologic parameters to different aspects of grass pollen aerobiology (start, length, and intensity of the

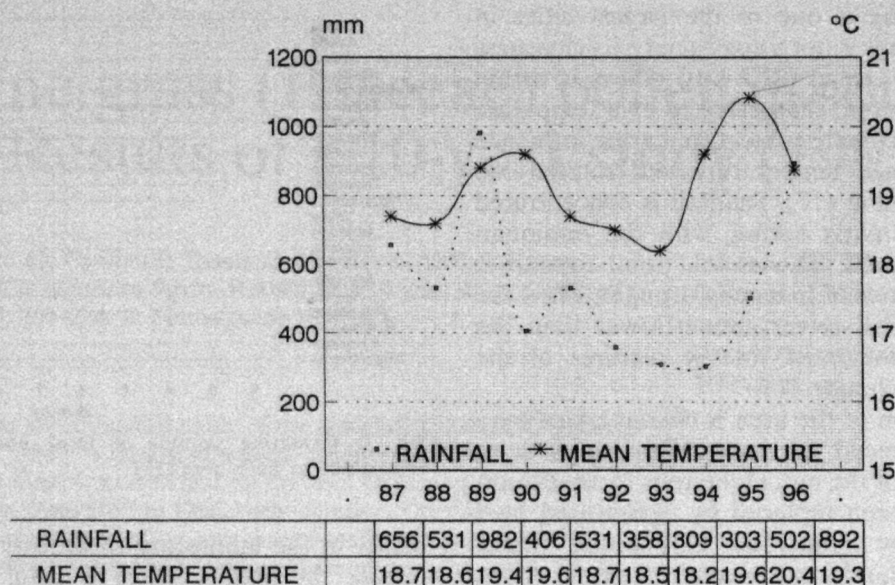


Fig. 2. Annual total precipitation and annual mean temperature (1987–96).

Table 1. Data of annual pollen of Gramineae: annual total concentrations, percentages of representation of these in total pollen and weed pollen, and number of weeks in year in which pollen of Gramineae appears in air

Year	Total annual (grains/m ³)	Representation in total pollen (%)	Representation in weed pollen (%)	Number of week of appearance
1987	2496.82	10.39	43.98	51
1988	1927.63	13.71	53.38	50
1989	1330.31	3.52	43.47	52
1990	1141.61	11.12	54.47	49
1991	2272.14	4.75	35.37	48
1992	1967.42	5.36	44.46	50
1993	822.42	7.35	56.27	51
1994	1616.21	11.64	46.83	50
1995	400.39	7.15	32.77	50
1996	2139.42	16.18	65.19	51
Mean		9.12	44.69	50.2

pollen season) were performed with the program SPSS for Windows. A cluster analysis was also performed of the variations in weekly concentration of grass pollen throughout the year (the annual pollen curves). A data matrix was constructed. The matrix included each study year considered in the analysis. Fifty-two variables were assigned to each year, coinciding with the values of the 52 weekly pollen concentrations (one for each week of the year). This analysis was performed by the program CSS. The Euclidean distances were calculated with Ward's method as rule of amalgamation (34).

Results

Climatology of the study years and annual grass pollen and its importance in the pollen spectrum of Seville

The study period included years when the total precipitation exceeded the mean of 600 mm (1987,

1989, and 1996), and others when this was below the mean (1988, 1990–5). The 3-year period 1993–5 was especially dry, with precipitation not exceeding 400 mm (Fig. 2). This interannual variation in precipitation became a drought of 6 consecutive years (1990–5) between two exceptionally wet years (1989 and 1996). The low precipitation in 1990, 1995, and 1996 was supplemented by annual mean temperatures above 18.8°C, that of 1995 being especially high (20.5°C) (Fig. 2).

Table 1 shows the total concentrations of grass pollen recorded each year. The data reveal three significant points:

- 1) In no year was 2500 grains/m³ exceeded.
- 2) There was a great interannual variation between the concentrations, with six times more pollen being collected in 1987 (maximum) than in 1995 (minimum).
- 3) Annual concentrations tended to decrease (Fig. 3).

Table 2. Data for pollen season of Gramineae: starting date, length, and intensity. Meteorologic factors (temperature and precipitation) before and during season

Year	Rainfall prior (mm) ^a	Rainfall prior (mm) ^b	Rainfall (mm) ^c	Mean temperature prior (°C) ^d	Mean temperature (°C) ^e	Starting date from 1 January	Length weeks	Intensity grains/m ³
1987	656.4	278.70	24.40	10.44	22.52	107, 17 April	22 (Apr–Sep)	2247
1988	530.4	152.70	177.50	11.77	23.07	86, 24 February	25 (Feb–Sep)	1735
1989	290.5	82.80	143.10	12.23	22.17	60, 27 February	27 (Feb–Sep)	1197
1990	833.1	75.30	140.40	12.66	23.17	47, 15 February	30 (Feb–Sep)	1027
1991	470.8	277.10	39	10.98	24.72	98, 8 April	24 (Apr–Sep)	2045
1992	268	77.50	220.80	11.99	19.93	67, 8 March	22 (Mar–Aug)	1771
1993	189.05	70.50	150.40	11.01	22.91	95, 5 April	26 (Apr–Oct)	740
1994	243.1	132.80	74.60	11.56	24.99	104, 14 April	25 (Apr–Oct)	1455
1995	171.05	67.70	46	12.48	23.39	58, 27 February	34 (Feb–Oct)	360
1996	724.7	352.80	131.20	12.11	24.32	91, 1 April	21 (Apr–Aug)	1925
Mean						81, 22 March	26	

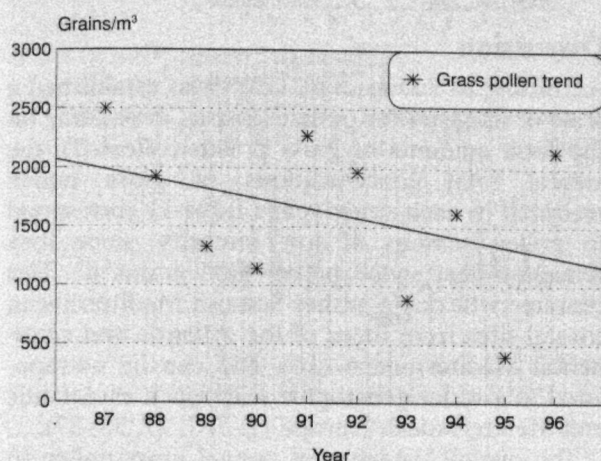
^a Rainfall between 1 September and starting date of season.^b Rainfall between 1 January and starting date of season.^c Rainfall recorded during season.^d Mean temperature of January and February.^e Mean temperature recorded during season.

Fig. 3. Trend of annual total concentrations of pollen of Gramineae (1987–96).

The absolute amounts in the pollen spectrum of Seville during these years were 9.12% of total pollen collected and 44.99% of weed pollen (means for 10 years). Grass pollen was present in the air for 50.2 weeks on average during the year (Table 1).

Relationship between the meteorologic parameters and the start, length, and intensity of the grass pollen season

The grass pollen season started on day 81 from 1 January (22 March) (mean of 10 years) (Table 2). This value is a guide, it fluctuated by 61 days when years were considered individually: the earliest date was 15 February (1990) and the latest was 17 April (1987) (Table 2). The differences could be related to the mean temperatures of January and February, given that the highest value was in 1990 (12.66°C) and the lowest in 1987 (10.44°C) (Table 2). The relationship is given by the following equation:

$$D = 382.899 - 25.50 \times T \quad (r = 0.807, r^2 = 0.651, P = 0.0048)$$

where D is the starting date of the pollen season, expressed as number of days from 1 January, and T is the mean of the mean temperatures of January and February.

The length of the grass pollen season ranged between 21 (1996) and 34 (1995) weeks, distributed between February and October depending on the year (Table 2). The length of the season could be related to the rainfall prior to the starting date: 1995 saw the lowest precipitation between 1 January and the starting date of the pollen season of all the years, and 1996 the highest (Table 2). The relationship is given by the following equation:

$$L = 29.40 - 0.00242 \times R \quad (r = 0.649, r^2 = 0.421, P = 0.0422)$$

where L is the length of the pollen season expressed in weeks, and R is the rainfall recorded between 1 January and the starting date of the season.

An attempt was made to relate the length of the pollen season to the rainfall recorded between 1 September of the preceding year (the beginning of the agricultural year in Spain) and the starting date of the season (Table 2). No statistically significant relationship was found ($r = 0.343$, $r^2 = 0.117$, $P = 0.331$).

Relationships were found between the intensity of the grass pollen season and meteorologic factors before or simultaneous with it. Table 2 shows that the most intense pollen seasons (1987, 1996) were those with the highest prior rainfall (recorded between 1 January and the starting date of the season), while the opposite occurred in 1993 and 1995 (low prior rainfall and low intensity of the pollen season). The relationship is given by the following equation:

$$I = 764.468 + 4.373 \times R \quad (r = 0.765, r^2 = 0.586, P = 0.0099)$$

where I is the amount of grass pollen, expressed in grains/m³, collected during the pollen season, and R is the prior rainfall recorded between 1 January and the starting date of the season.

Finally, the relationships between the intensity of the pollen season and 1) the prior temperatures (January and February), 2) temperatures recorded during the season, 3) prior rainfall (recorded from 1 September), and 4) rainfall recorded during the season were not significant ($r=0.276$, $r=0.009$, $r=0.325$, and $r=-0.072$, respectively).

Variation in the weekly grass pollen concentrations throughout the year (pollen curves)

Fig. 4 shows the variations in weekly grass pollen concentrations throughout the year (pollen curves) for each study year. The same figure shows the mean weekly temperatures and weekly total precipitation. For clarification of the figure, the most appropriate scale has been used for each coordinate, and the weekly precipitation has been divided by six. From analysis of the set of 10 years, various aspects stand out:

- Grass pollen appeared during practically the whole year (as already mentioned).
- The peaks of maximum concentration were between April and June, depending on the year; these peaks coincided with temperature increases of 15–25°C, and generally appeared in the absence of precipitation. During this period, the weekly mean concentration of 100 grains/m³ was often exceeded.
- Between July and October, there were numerous peaks of concentration, which often exceeded 50 grains/m³, and, in some years (1994 and 1995), were as large as or even larger than those of spring. There was a relationship between rainfall of late spring (June) and a marked presence of grass pollen during the summer (1988, 1992, 1994, and 1995).
- During the rest of the year, the weekly grass pollen concentrations remained below 50 grains/m³ (February–March) and 10 grains/m³ (November–January).

Fig. 5 shows the results of the cluster analysis, which associates the annual pollen curves by their similarity. In general, the resulting associations were weak, although there was a notable similarity between 1987 and 1991 (with a curve characterized by a large peak in May and a progressive decline in concentration in the months following), and between 1989 and 1992 (with a curve characterized by a significant presence of pollen, 50 grains/m³, from March, a large peak in May, and a significant presence of pollen, >50 grains/m³, again in June and July.

Finally, Table 3 shows different aspects of the week of maximum pollen production for the grasses. The most significant features of these data are the following:

- The dates of this week varied greatly over the years: the last days of April (1989 and 1992), May (1987, 1988, 1990, 1991, and 1994), the end of May and beginning of June (1993 and 1996), and October (1995).
- The range of temperature recorded for this week was between 18 and 25°C, except in 1996, when it was 27.90°C. There was no precipitation except in 1994.
- The amount of pollen collected ranged between 10 and 25% of the annual total, except in 1996, when it reached almost 45%.

Discussion

According to Subiza et al. (10) (who established a scale of intensity for pollen seasons, depending on the total amounts of grass pollen collected), the annual total concentrations of grass pollen recorded in each study year (Table 1) correspond to pollen seasons of low intensity, since they remained very much below 4000 grains/m³. This characteristic distinguishes Spanish Mediterranean coastal sites from those of the Atlantic and continental Mediterranean (16), and can be extrapolated to a wider geographic scale, such as Atlantic and Mediterranean Europe (2, 5, 7, 27, 35–37).

The overall tendency of annual grass pollen to decrease during the 10 years of the study (Fig. 2) is interesting. The explanation undoubtedly lies in the six consecutive years of drought (1990–5) (Fig. 1). Drought has a negative effect on the biologic cycle of these plants: it impedes the germination of many of their seeds, and leads to a lack of vigor in growth and a lack of intensity in flowering (18). Such a notion is reinforced by the example of the 400 grains/m³ collected as annual total in 1995, the last year of drought (Fig. 2), and indirectly by the low pollen production of olive (a plant resistant to the rigors of the Mediterranean climate) already reported in the area for those years (23).

The amount of grass pollen in the pollen spectrum of Seville was 9.12% and 44.69% of total pollen and weed pollen, respectively (mean of the 10 years) (Table 1). The first value is very much lower than other pollen types identified in the city air, such as *Olea europaea*, *Quercus*, *Platanus hispanica*, and Cupressaceae (38). This percentage of representation in the total pollen is small, especially when we consider that grasses are strongly anemophilous (39) and that they are widespread

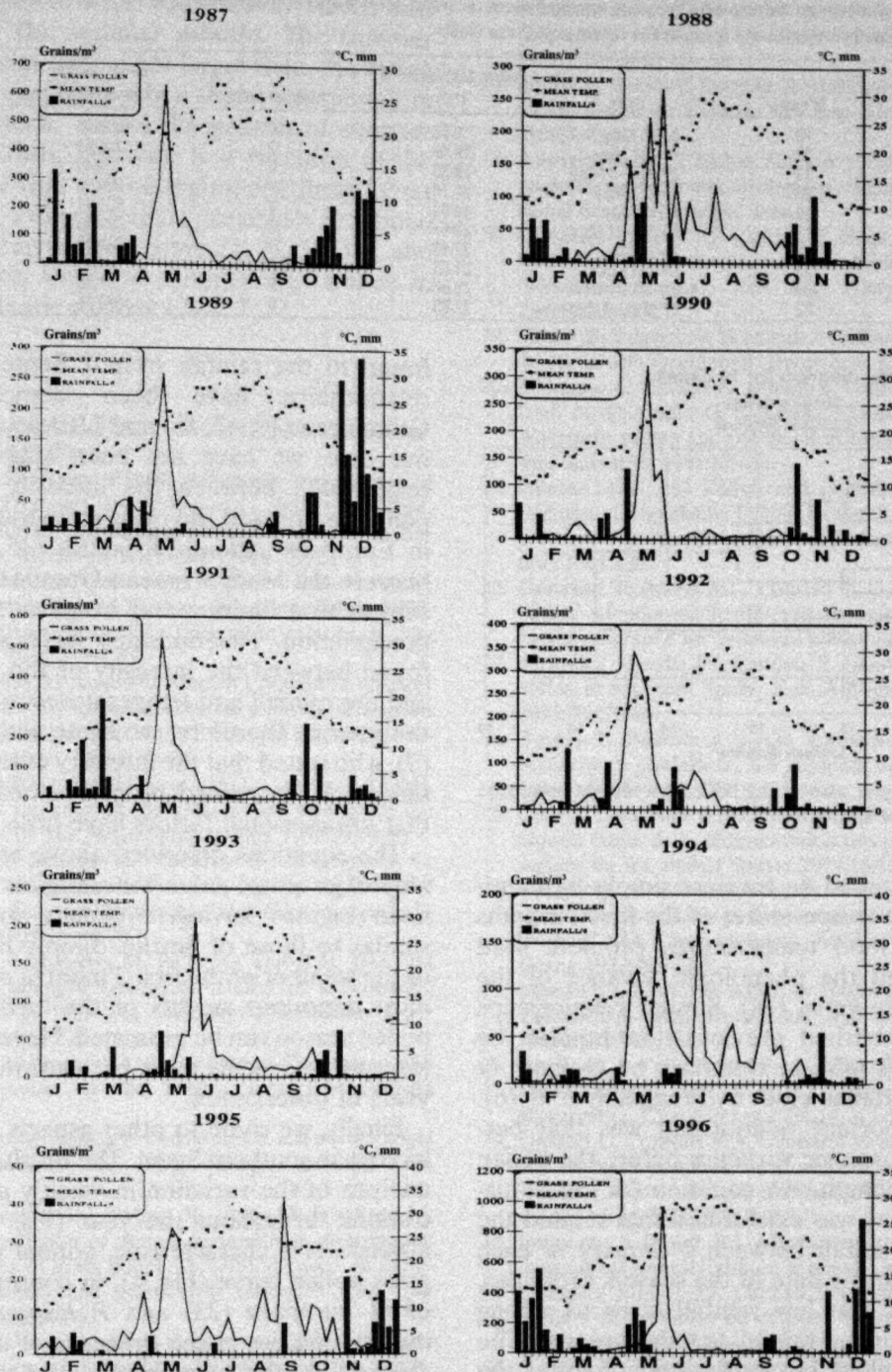


Fig. 4. Annual pollen curves, or variation through year, of weekly pollen concentrations of Gramineae (grains/m³), weekly mean temperatures (°C), and weekly total precipitation (mm). Precipitation is divided by six.

on the plant scene (19). This discordance, also observed in other Mediterranean areas (6, 36, 40), can be explained by the low dispersion of grass pollen (41), with more than 90% remaining close to the plant (42), and thus reaching the height of the trap only with difficulty (43). The second value (44.69%) further confirms the predominance of the grasses in the herbaceous stratum of the vegetation.

The central argument of our work concerns the data of Table 2 and the equations relating them, with different degrees of statistical significance.

First of all, the relationship between the starting date of the pollen season and the mean of the mean temperatures of January and February is interesting. It is an inverse relationship, with a good level of significance, which enables the starting date of

Table 3. Data for week of maximum pollen production: date, concentrations recorded, percentages of representation of those concentrations in annual total for Gramineae, and values of temperature and precipitation recorded in those weeks

Year	Week	Date	Mean temperature (°C)	Rainfall (mm)	Grass pollen (grains/m ³)	Representation in annual total (%)
1987	20	8–15 May	19.56	0	629.05	25.19
1988	22	14–21 May	24.16	0	266.64	13.8
1989	19	29 Apr–6 May	20.35	0	258.45	19.42
1990	20	13–20 May	21.78	0	320.90	28.1
1991	19	6–13 May	18.90	0	528.71	23.26
1992	18	26 Apr–3 May	20.57	0	340.40	17.30
1993	23	2–9 June	22.48	0	211.60	23.98
1994	22	23–30 May	21.05	13.50	172.50	10.67
1995	36	23–30 October	23.60	0	41.96	10.48
1996	22	27 May–3 June	27.90	0	960.16	44.88

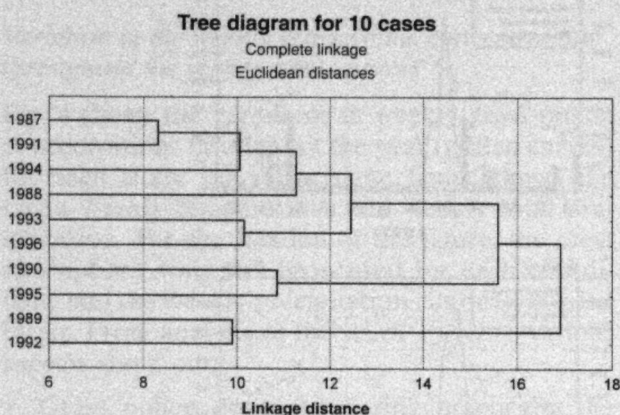


Fig. 5. Cluster analysis, associating pollen curves of Gramineae with their degree of similarity.

the pollen season to be forecast simply by monitoring the mean temperatures of the first 2 months of the year. Good temperatures promote seed germination and the phenologic advance of the grasses, and vice versa (44). Similar relationships have been established previously in London by Davies & Smith (45), in Denmark by Golberg et al. (46), and in Italian cities by Frenguelli et al. (6).

Another important relationship was that between the meteorologic variables before the pollen season and its length. An equation (of low statistical significance) was established that related the length to the rainfall between 1 January of each year and the starting date of the season. From this, it was deduced that low rainfall leads to a long season, and abundant rainfall to a short season. The biologic explanation lies in the phenology of the Mediterranean Gramineae, which exhibit a flowering that is irregular and spread throughout the year in periods of drought, while their growth is slower and more concentrated in time when the soil has sufficient water reserves (44).

The third important aspect that can be forecast with an acceptable degree of reliability is the intensity of the pollen season. The severity of the pollen season increases with increasing rainfall between 1 January and the starting date of the season, and vice versa. Such a relationship was not

found to the rainfall from 1 September. Similar relationships have been demonstrated for Gramineae (10, 47, 48) and Urticaceae (49, 50). In our case, we have not been able to show the relationship between the intensity of the grass pollen season and the prior temperatures reported in European Atlantic localities (45, 46), possibly because the Mediterranean Gramineae are closely subject to a limiting and irregular factor such as precipitation. Relationships have also not been found between the intensity of the pollen season and the rainfall and temperatures recorded during the season. Therefore, we agree with Subiza et al. (9), who stated that the intensity of the grass pollen season is determined by preseasonal factors, and that intraseasonal factors have little effect.

The equations described above are unquestionably of practical value for allergists in Mediterranean regions having a climate and vegetation similar to those of Seville. Simply by observation of the weather of the first 2 months of the year, the most important aspects of the forthcoming grass pollen season can be estimated. Nevertheless, these forecasting models must be confirmed with more years of observation.

Finally, we come to other aspects of grass aerobiology in southern Spain. The results of the cluster analysis of the variation in weekly pollen concentrations throughout the year (Fig. 5) reveal the absence of a characteristic, annual pattern in the grass pollen curve (Fig. 4), in contrast to the case of *O. europaea* (23) and *P. hispanica* (51). The explanation lies in the existence of numerous species within the Gramineae (19) with flowering spread throughout the year (Fig. 1), and each with a particular phenology. This leads us to reaffirm our agreement with Spijkma et al. (4) on the impossibility of long- or mid-term forecasts of week-by-week variation in pollen concentrations. The annual pollen curves of Gramineae are characterized by the appearance of the highest concentrations in May and June, associated with increases in temperature of 15–25°C and absence of precipitation, as reported previously for the nearby cities of Córdoba (11, 12) and Granada (13). Depending

on the year, peaks were recorded in March and April, and in the summer months. The summer peaks could be as large as, or larger than, the spring ones (Fig. 4), especially when there was rainfall in late spring, which favors the growth of summer grasses (44). Thus, our case is a variation in the concentrations that can be highly distributed over many months, a characteristic previously described in other Mediterranean regions (2, 27, 35, 36), and which contrasts with the summer appearance in European Atlantic regions (2–4, 7, 8).

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