

Pollen production in anemophilous species of the Poaceae family in Tetouan (NW Morocco)

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Abstract Total pollen production per inflorescence and per square meter were studied in anemophilous species of the Poaceae family, to determine the relative contribution of each species to the total load of pollen released and to identify the species with the highest potential pollen emission. This was done by calculating the number of pollen grains per flower and per inflorescence and by estimating the density of inflorescences in an area of one square meter. Pollen production per inflorescence varied between 12,000 and 15 million grains, often being higher in the perennial species. Mountainous, grassland, riverside, and littoral areas contributed similarly to the amount of pollen grain production per square meter. Species such as *Gaudinia fragilis*, *Hordeum murinum*, *Lolium rigidum*, and *Trisetaria panicea*, with high pollen records, were found in all the studied areas. Aerobiological analysis will be undertaken to determine the main pollen season of Poaceae species. The results

obtained are of great importance for respiratory health management in this region.

Keywords Poaceae · Pollen production · Aerobiology · Allergy · Tetouan

1 Introduction

The large Poaceae family is distributed worldwide and represents almost 20% of world vegetation cover (Mabberley 1987). Most plants of this family are anemophilous with the exception of some cleistogamous and a smaller number of entomopollinated species (Adams et al. 1981). The Poaceae species grow in different habitats depending on the taxon (Fernandez-Gonzalez et al. 1999) and produce large amounts of pollen grains (20–40 µm diameter as average) during a short period ranging from a few hours to a few days, although some species develop new flowers continuously (Lewis et al. 1983; Knox et al. 1993). One concern is a family of different species with different allergenic properties, thus prolonging the period of time during which allergenic pollen can be found in the air. Given all the above-mentioned features, grass pollen is the major aeroallergen outdoors triggering hay fever and allergic asthma in spring (Knox 1993; Ong et al. 1995; D'Amato et al. 1998; Peternel et al. 2006). Poaceae pollen is one of the most important cause of allergic diseases in Morocco, because seasonal prevalence of

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its pollen-related asthma and rhinitis has risen to 10% of patients with clinical respiratory diseases (Yazidi Alaoui et al. 2001).

One palynological characteristic of Poaceae is the difficulty in distinguishing between pollen grains of the different genera and species, because few variations (stenopalynous) within the family are apparent from light microscopy (Driessen et al. 1989). It is, therefore, currently impossible to consider the partial contributions of the different species to the pollen spectrum in aerobiological studies.

In recent years, several papers have highlighted differences or similarities between pollen counts in the atmospheres of several specific locations in regions with different climates, and relationships between meteorological conditions and atmospheric pollen concentrations (Galán et al. 1995; Green et al. 2004; Stevenson et al. 2007).

Publications regarding flowering season, and pollen production related to the reproduction variability and aerobiology of Poaceae are extensive (Smart et al. 1979; Liem and Groot 1980; Knox and Singh 1987; Allisson 1990; Gutierrez and Medan 2006), but few studies report information about pollen production per anther or per inflorescence, and different findings have been obtained for the species studied (Subba Reddi and Reddi 1986; Prieto-Baena et al. 2003).

The objectives of this study were to estimate, for the most frequent species of Poaceae in the area of Tétouan, the density of inflorescences per square meter, to determine total pollen production per inflorescence instead of total production per plant, because of their morphological variability, and to provide information about the relative contribution of each species to total rate of the pollen release to the atmosphere, with the purpose of identifying the species with the highest potential pollen emission.

2 Material and methods

The study area was located in Tétouan city and its surroundings (35°33'–35°41'N and 05°19'–05°21'W) in the NW of Morocco, 10 m above sea level, in the mountainous area of Beni-Yeder (35°27' and 05°25'W, 147 m), 22 km southwest far from the city (Fig. 1). The climate conditions are predominantly Mediterranean warm subhumid (Benabid 1982), with

an annual rainfall average of 728 mm and an annual mean temperature average of 18.2°C. The plants were collected in five different areas comprising different habitats of grasses growing in the city (Fig. 1). The species were identified according the flora of Valdés et al. (2002).

The five selected areas were:

- Zone I: a mountainous zone of Beni-yeder located 22 km southwest of the town of Tétouan. The natural vegetation includes a forest of *Tetraclinis articulata* (Vahl) Masters with *Cistus monspeliensis* L., *Inula viscosa* Aiton, *Chamaerops humilis* L., *Calicotome villosa* Link, and *Pistacia lentiscus* L. as the principal species, together with a well developed herbaceous layer and fields of *Olea europea* L.
- Zone II: the vegetation of this metropolitan area, which contains the administration district and a new residential zone surrounded by ample unoccupied surfaces of semi natural meadow, is formed almost exclusively by grasses. This sector can be easily distinguished because of the total absence of agriculture.
- Zone III: crop land and pasture area. A rural zone with houses near fields of *Hordeum vulgare* L., *Triticum aestivum* L., *Zea mays* L., *Opuntia ficus-indica* Mill., and *Ficus carica* L. Other plants are used in the wake of the parcels, for example *Chamaerops humilis* L., wild *Olea europea* L., *Ceratonia siliqua* L., *Smilax aspera* L., *Rosa sempervirens* L., *Rosa canina* L., and *Pistachia lentiscus* L. This area is characterized by the presence of pasture rich in grasses.
- Zone IV: a riverside area located along the urbanized bank of the river M'hannech. The vegetation is made up of numerous riparian plants, including several grasses.
- Zone V: the littoral zone of M'Dik is a spa and tourist urban zone.

The ornamental species of the Poaceae family which are used in the parks of the town of Tétouan were not included in this study. To estimate pollen production per inflorescence and total pollen production in the selected areas, several variables were studied for each species during 2007.

The number of pollen grains per anther was counted. A total of nine mature and undehiscent anthers were counted for three flowers from three



Fig. 1 Location of sampling sites near the city of Tetouan: *Zone I*, mountainous area; *Zone II*, area of grassland; *Zone III*, area of cropland; *Zone IV*, riverside area; *Zone V*, littoral area

different plants using the method proposed by Cruden (1977) also followed by Tormo Molina et al. (1996), Hidalgo et al. (1999), and Prieto-Baena et al. (2003). The pollen was obtained by crushing each anther in 100 μ l distilled water stained with 0.1% fuchsin. Total pollen was counted in three 10- μ l samples deposited in a chamber of a Mac-Master slide. To fill the slide cell, 140 μ l distilled water was added. A 4 $\times$ objective and a 10 $\times$ ocular were used for the counts. Finally, the number of pollen grains per flower was calculated by multiplying by the number of anthers per flower.

The number of flowers was estimated in 30 inflorescences from each species. In most species the number of spikelets per inflorescence (mean of 30 inflorescences) and the number of flowers per spikelet (mean of 30 spikelets) were counted. The number of flowers per inflorescence was obtained by multiplying the average number of flowers/spikelets by the average number of spikelets/inflorescence in accordance with Prieto-Baena et al. (2003). For species with compound inflorescences and a large number of flowers (*Cynodon dactylon*, *Piptatherum miliaceum*, and *Sorghum halepense*), second-order spikes or panicles of the inflorescence were counted (mean of 30 inflorescences), and multiplied by the number of spikelets of second order spike or panicle (mean of 30

spikes or panicles) and by the number of flowers per spikelet. Sterile flowers were not included in the counts.

Total pollen production per inflorescence was calculated by multiplying the average number of pollen grains per flower by the average number of flowers per inflorescence. Because of their morphological variability and the difficulty of counting the number of inflorescences on an individual plant, especially in perennial species, total pollen production was estimated per inflorescence. To calculate the number of inflorescences per square meter, a quadrant of 1 m² was deposited randomly, ten times, over the studied area as previously described by Prieto-Baena et al. (2003). The total number of inflorescences was counted in each quadrant. The species present in the area but always kept outside the quadrants were recorded as occasional.

Total pollen production per square meter was calculated by multiplying total pollen production per inflorescence by the average number of inflorescences per square meter. This value was not calculated for occasional species. For statistical analysis, ranges, averages, and coefficients of variation were computed by the program "Statistica" (Statistica software program 1984–2003). A comparison of means test was performed by means of

parametric analysis and applied to the data obtained in the different measurements for genera with more than one species.

3 Results

3.1 Number of pollen grains per anther and flower

The number of pollen grains per anther varied between 52 in *Polypogon monspeliensis* and 5,307 in *Sorghum halepense* (Table 1). Different results were observed for the different genera and species studied, including species of the same genera. However, no significant variation was observed for species of the genus *Avena* and between *Bromus hordeaceus* and *B. madritensis* (Table 3). Pollen production within species of the same genus varied by a factor of 1.5 (*Aegilops triuncialis* and *Aegilops geniculata*) to 2.75 (*Poa trivialis* and *P. annua*). In the genus *Bromus*, *B. maroccanus* produces 26 times more pollen than *B. diandrus* (Table 1). As said above, the number of pollen grains per flower was calculated by multiplying by the number of anthers per flower and the results can be seen in Table 2.

3.2 Number of flowers per inflorescence

The number of flowers per spikelet was generally constant in 50% of the species studied. In the others, the coefficient of variation varied from 4 to 23% (Table 1). Species of the same genus showed significant differences but no difference was observed for *B. diandrus* and *B. madritensis* (Table 3).

The number of spikelets per inflorescence varied between the different genera and species, ranging from five in *Elymus marginatus* to 2,767 in *Polypogon monspeliensis* (Table 1). Except for *Bromus hordeaceus* and *Bromus madritensis*, the number of spikelets showed significant differences between all species within the same genus (Table 3).

The coefficient of variation was relatively high and varied from 9 to 64%. *Bromus madritensis* and *Bromus hordeaceus* had approximately three times more spikelets than *B. diandrus*. In some genera, for example *Poa* and *Phalaris*, one of the two species studied had 3.5–3.8 times as many spikelets as the

other. *Melica magnolii* had seven times more spikelets than *Melica minuta*.

The number of flowers per inflorescence differed both between genera and within species of the same genus, except for *Bromus hordeaceus* and *Bromus maroccanus*. The genus *Aegilops* contained the lowest number of flowers per inflorescence (7–8) whereas *Trisetaria panicea* produced the highest number of flowers per inflorescence (3,784). Approximately 20% of the species produced a large number of flowers, exceeding 900 flowers per inflorescence (*Agrostis stolonifera*, *Ampelodesma mauritanica*, *Phalaris coerulescens*, *Piptatherum miliaceum*, *Polypogon monspeliensis*, *Rostraria cristata*, *Sorghum halepense*, and *Trisetaria panicea*). In the other species the number varied between 11 (*Taeniatherum caput-medusae*) and 634 (*Hyparrhenia sinaica*). In the species in which the number of spikelets per second-order spike or panicle was counted, the coefficient of variation ranged between 15 and 37% (Table 1).

3.3 Total pollen grain production

The number of pollen grains produced by an inflorescence was calculated on the basis of the number of pollen grains per flower and number of flowers per inflorescence. Pollen production varied between genera and between species within the same genus. The number ranged between 12,340 pollen grains for *Taeniatherum caput-medusae* and 15,765,000 for *Sorghum halepense* (Table 2). *Trisetaria panicea*, one of the most abundant species, produced 12,841,345 pollen grains per inflorescence. Figure 2 shows total pollen production per inflorescence. Pollen production potential was different for perennial and annual species, the perennial species often being much more productive than annual plants. In general, most annual plants produced less than 990,000 pollen grains per inflorescence whereas mean total pollen production by perennial species was, on average, 4.25 times higher than by annual species.

3.4 Number of inflorescence per m²

The number of inflorescences per m² of the different species studied varied depending on the area. In zone I, five species that had a significant presence: *Bromus madritensis*, with 73.1 inflorescences/m² (I/m²); *Vulpia geniculata*, with 52.5; *Gaudinia*

Table 1 Pollen production of the *Poaceae* species investigated

Species	No. of pollen grains/anther			No. of flowers/spikelet			No. of spikelets/inflorescence			No. of 2nd-order spikes versus panicles/inflorescence		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>Aegilops geniculata</i>	(620–1,420)	1,077.78	(23)	(5–11) ^a	8.07 ^a	(17) ^a						
<i>Aegilops triuncialis</i>	(1,220–2,630)	1,795.56	(29)	(6–8) ^a	7.07 ^a	(7) ^a						
<i>Agrostis stolonifera</i>	(100–280)	195.56	(28)	(1–1)	1	(0)	(927–2,000)	1,324.50	(22)			
<i>Ampelodesma mauritanica</i>	(2,400–7,560)	5,213.33	(33)	(2–2)	2	(0)	(74–797)	453.37	(40)			
<i>Anthoxanthum odoratum</i>	(2,000–4,800)	3,471.11	(29)	(1–1)	1	(0)	(20–106)	59.67	(42)			
<i>Avena barbata</i>	(1,040–4,250)	2,577.78	(44)	(2–2)	2	(0)	(15–112)	43.23	(53)			
<i>Avena sterilis</i>	(1,340–2,720)	1,993.33	(23)	(3–4)	3.73	(12)	(9–39)	19.80	(35)			
<i>Briza maxima</i>	(320–670)	491.11	(26)	(9–14)	11.73	(10)	(3–8)	5.53	(21)			
<i>Briza minor</i>	(42–110)	74.11	(31)	(5–9)	6.20	(14)	(50–228)	102.10	(40)			
<i>Bromus diandrus</i>	(80–270)	133.33	(42)	(5–7)	5.97	(9)	(6–27)	11.63	(50)			
<i>Bromus hordeaceus</i>	(310–660)	486.67	(23)	(6–11)	9.10	(13)	(15–51)	30.67	(27)			
<i>Bromus madritensis</i>	(180–530)	380.00	(34)	(4–7)	5.97	(14)	(15–45)	30.13	(26)			
<i>Bromus maroccanus</i>	(2,300–5,020)	3,505.56	(26)	(3–4)	3.70	(13)	(40–123)	77.80	(29)			
<i>Cynodon dactylon</i>	(620–1,500)	1,026.67	(31)	(1–1)	1.00	(0)	(27–75) ^b	47.63 ^b	(24) ^b	(4–7)	4.97	(15)
<i>Dactylis glomerata</i>	(1,330–4,000)	2,133.33	(41)	(3–4)	3.70	(13)	(70–193)	143.73	(23)			
<i>Desmazeria rigida</i>	(90–330)	233.33	(38)	(5–7)	6.20	(11)	(15–64)	36.10	(40)			
<i>Elymus marginatus</i>	(170–380)	273.33	(25)	(9–16)	12.27	(15)	(4–6)	5.03	(16)			
<i>Gaudinia fragilis</i>	(1,490–6,370)	3,528.89	(43)	(4–7)	4.97	(22)	(20–40)	29.27	(15)			
<i>Hordeum murinum</i>	(130–980)	531.11	(55)	(1–1)	1.00	(0)	(22–33)	29.50	(9)			
<i>Hyparrhenia sinaitica</i>	(440–1,240)	814.44	(30)	(15–17)	15.70	(4)	(20–92)	40.40	(42)			
<i>Lagurus ovatus</i>	(300–790)	612.22	(29)	(2–2)	2.00	(0)	(102–492)	215.67	(49)			
<i>Lamarckia aurea</i>	(80–180)	135.56	(22)	(1–1)	1.00	(0)	(95–220)	150.83	(15)			
<i>Lolium rigidum</i>	(2,770–4,860)	3,725.56	(19)	(7–14)	10.17	(17)	(15–32)	23.40	(18)			
<i>Melica magnolia</i>	(220–600)	412.22	(31)	(1–1)	1.00	(0)	(140–691)	316.07	(59)			
<i>Melica minuta</i>	(580–850)	726.67	(15)	(2–2)	2.00	(0)	(20–95)	45.63	(49)			
<i>Panicum repens</i>	(600–2,000)	1,125.56	(36)	(2–2)	2.0	(0)	(85–220)	157.60	(22)			
<i>Paspalidium obtusifolium</i>	(1,200–2,700)	1,933.33	(28)	(2–4)	3.07	(17)	(6–15)	10.83	(19)			
<i>Phalaris canariensis</i>	(1,000–1,640)	1,302.22	(14)	(1–1)	1.00	(0)	(160–480)	245.67	(32)			
<i>Phalaris coarulescens</i>	(1,370–3,190)	1,955.56	(28)	(1–1)	1.00	(0)	(605–1,730)	934.47	(33)			
<i>Piptatherum miliaceum</i>	(500–1,200)	862.22	(29)	(1–1)	1.00	(0)	(17–47) ^b	28.83 ^b	(24) ^b	(54–136)	83.30	(18)

Table 1 continued

Species	No. of pollen grains/anther			No. of flowers/spikelet			No. of spikelets/inflorescence			No. of 2nd-order spikes versus panicles/inflorescence		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>Poa annua</i>	(260–550)	405.56	(26)	(2–2)	2.00	(0)	(42–92)	58.73	(20)			
<i>Poa trivialis</i>	(600–1,690)	1,128.89	(27)	(3–3)	3.00	(0)	(108–350)	205.56	(31)			
<i>Polypogon monspeliensis</i>	(30–80)	52.22	(33)	(1–1)	1.00	(0)	(800–4,500)	2,767.13	(41)			
<i>Rostraria cristata</i>	(70–170)	108.89	(33)	(4–5)	4.77	(9)	(49–600)	212.17	(64)			
<i>Sorghum halepense</i>	(2,400–9,000)	5,307.78	(41)	(1–1)	1.00	(0)	(3–13) ^b	7.60 ^b	(25) ^b	(49–248)	130.27	(37)
<i>Taenatherum caput-medusae</i>	(250–450)	356.67	(19)	(1–1)	1.00	(0)	(9–21)	11.53	(25)			
<i>Trisetaria panicea</i>	(600–1,800)	1,131.11	(34)	(3–5)	4.07	(11)	(200–1,850)	929.80	(48)			
<i>Vulpia geniculata</i>	(210–520)	352.22	(27)	(2–4)	3.27	(23)	(36–117)	79.37	(28)			

1, Range; 2, mean; 3, coefficient of variation (%)

^a Number of flowers per inflorescence in *Aegilops*^b Number of spikelets per spike or panicle

fragilis, with 51.5; *Aegilops geniculata*, with 43.5, and *E. marginatus*, with 30.4. In the meadow area (zone II), the most dominant species were *Gaudinia fragilis*, with 111.6 I/m²; *Trisetaria panicea*, with 105.9; *Cynodon dactylon*, with 52.0; *Lolium rigidum*, with 49.9, and *Bromus hordeaceus*, with 24.8. In the crop land and pasture area (zone III), the most frequent species were *Vulpia geniculata*, *Bromus hordeaceus*, *Gaudinia fragilis*, *Elymus marginatus*, and *Hordeum murinum* with 104.5, 29.5, 16.4, 15.6 and 15.3 I/m², respectively. In the riverside area (zone IV), the most abundant species were *Trisetaria panicea* with 96.4 I/m², *Bromus madritensis* with 65.5, *Hordeum murinum* with 59.6, *Elymus marginatus* with 27.7, and *Poa annua* with 20. Finally, in the coastal area (zone V), the most representative species were *Gaudinia fragilis* with 180.5 I/m², *Cynodon dactylon* with 93.1, *Polypogon monspeliensis* with 71.1, *Hordeum murinum* with 52.0, and *Bromus hordeaceus* with 28.9. The area with the highest inflorescence density was the coastal zone, followed by the meadow area, the riverside area, the mountain area and the crop land and pasture zone.

3.5 Total pollen production per m²

In the different areas studied, pollen grain production per m² was highest in the meadow area, with 1.86×10^9 , followed by the riverside zone with 1.44×10^9 , the coastal area with 0.76×10^9 , and the mountain area with 0.43×10^9 . The value was lowest for crop land and pasture area, with 0.23×10^9 pollen grains/m².

The number of species identified per area was between 15 and 22, depending on the area investigated. The greatest number of species harvested was observed in zone III (22), followed by zone II and zone IV (21). The smaller number (15) was in the coastal area.

Gaudinia fragilis, *Hordeum murinum*, *Lolium rigidum*, and *Trisetaria panicea* were found in all the areas studied, with substantial inflorescence incidence and high pollen production, exceeding 3,000 pollen grains per flower, in some areas.

4 Discussion

With the exception of some cleistogamous species, anemophily is the predominant method of pollination

Table 2 Number of pollen grains per inflorescence

Species	1		2		3		4		5				
	I	II	I	II	III	IV	V	I	II	III	IV	V	
<i>Aegilops geniculata</i>	A	3,233.34	8.07	26,093	43.50	4.50	12.60	–	–	1,135,046	117,419	328,772	–
<i>Aegilops triuncialis</i>	A	5,386.68	7.07	38,084	6.30	–	*	–	–	239,929	–	+	–
<i>Agrostis stolonifera</i>	P	586.68	1,324.50	777,058	*	*	*	1.00	–	+	+	777,058	–
<i>Ampelodesma mauritanica</i>	P	15,639.99	986.74	15,432,604	*	–	–	–	*	+	–	–	+
<i>Anthoxanthum odoratum</i>	P	10,413.33	59.67	621,363	–	3.00	*	*	–	–	1,864,089	+	–
<i>Avena barbata</i>	A	7,733.34	86.46	668,625	8.00	6.80	1.70	13.80	1.00	5,349,000	4,546,650	1,136,663	668,625
<i>Avena sterilis</i>	A	5,979.99	73.85	441,622	15.10	*	5.40	14.00	*	6,668,492	+	2,384,759	6182,708
<i>Briza maxima</i>	A	1,473.33	64.87	95,575	7.20	19.00	1.40	*	–	688,142	1,815,925	133,805	+
<i>Briza minor</i>	A	222.33	633.02	140,739	–	4.70	–	–	–	–	661,473	–	–
<i>Bromus diandrus</i>	A	266.66	69.43	18,514	–	*	*	5.00	3.00	–	+	92,570	55,542
<i>Bromus hordeaceus</i>	A	1,460.01	279.10	407,489	8.40	24.80	29.50	9.80	28.90	3,422,908	10,105,727	12,020,926	3,993,392
<i>Bromus madritensis</i>	A	1,140.00	179.88	205,063	73.10	20.50	10.70	65.60	6.00	14,990,105	4,203,792	2,194,174	13,452,133
<i>Bromus maroccanus</i>	P	10,516.68	287.86	3,027,332	–	10.50	–	–	–	–	31,786,986	–	–
<i>Cynodon dactylon</i>	P	3,080.01	236.72	729,100	*	52.00	1.00	20.40	93.10	+	37,913,200	729,100	14,873,640
<i>Dactylis glomerata</i>	P	6,429.99	531.80	3,419,469	–	–	1.50	–	*	–	–	5,129,204	+
<i>Desmazeria rigida</i>	A	699.99	223.82	156,672	–	*	1.60	3.00	*	–	+	250,675	470,016
<i>Elymus marginatus</i>	P	819.99	61.72	50,610	30.40	15.90	15.60	27.70	–	1,538,544	804,699	789,516	1,401,897
<i>Gaudinia fragilis</i>	A	10,586.67	145.47	1,540,043	51.50	111.60	16.40	11.20	180.50	79,312,215	171,868,799	25,256,705	17,248,482
<i>Hordeum murinum</i>	A	5,096.67	29.50	150,352	20.20	23.40	15.30	59.60	52.00	3,037,110	3,518,237	2,300,386	8,960,979
<i>Hyparrhenia sinaica</i>	P	2,443.32	634.28	1,549,749	9.00	–	3.00	–	–	13,947,741	–	4,649,247	–
<i>Lagurus ovatus</i>	P	1,836.66	431.34	792,225	–	–	–	*	2.50	–	–	–	1,980,563
<i>Lamarckia aurea</i>	A	406.68	150.83	61,340	–	*	*	*	–	–	+	+	–
<i>Lolium rigidum</i>	A	12,983.34	237.98	3,089,775	27.90	49.90	10.30	7.60	27.40	86,204,723	154,179,773	31,824,683	23,482,290
<i>Melica magnolia</i>	A	1,236.66	316.07	390,871	–	–	2.50	–	–	–	–	977,178	–
<i>Melica minuta</i>	A	2,180.01	91.26	198,948	–	–	1.50	–	–	–	–	298,422	–
<i>Panicum repens</i>	P	3,376.68	315.20	1,064,330	–	9.00	–	9.00	12.00	–	9,578,970	–	12,771,960
<i>Paspalidium obtusifolium</i>	P	5,799.99	33.25	192,850	–	5.50	–	8.20	*	–	1,060,675	–	1,581,370
<i>Phalaris canariensis</i>	A	3,906.66	245.67	959,749	*	3.00	–	15.30	2.70	+	2,879,247	–	14,684,160
<i>Phalaris coerulea</i>	P	5,866.68	934.47	5,482,236	13.80	3.30	1.90	*	–	75,654,857	18,091,379	10,416,248	2,591,322
<i>Piptatherum miliaceum</i>	P	2,586.66	2,401.54	6,211,967	*	*	1.00	5.30	–	+	+	6,211,967	32,923,425

Table 2 continued

Species	1	2	3	4		5					IV	V		
				I	II	III	IV	V	I	II			III	
<i>Poa annua</i>	A	1,216.68	117.46	142,911	–	*	*	20.00	*	–	+	+	2,858,220	+
<i>Poa trivialis</i>	A	3,386.67	616.68	2,088,492	17.20	15.20	–	3.00	28.10	35,922,062	31,745,078	–	6,265,476	58,686,625
<i>Polypogon monspeliensis</i>	A	156.66	2,767.16	433,499	*	11.40	1.00	3.00	71.10	+	4,941,889	433,499	1,300,497	30,821,779
<i>Rostraria cristata</i>	A	326.67	1,012.05	330,606	–	–	12.20	*	–	–	–	4,033,393	+	–
<i>Sorghum halepense</i>	P	15,923.34	990.05	15,764,903	*	–	*	2.00	–	+	–	+	31,529,806	–
<i>Teniattherum caput-medusae</i>	A	1,070.01	11.53	12,337	*	–	–	–	–	+	–	–	–	–
<i>Trisetaria panicea</i>	A	3,393.33	3,784.29	12,841,345	7.20	105.90	6.80	96.40	15.70	92,457,684	1,359,898,436	87,321,146	1,237,905,658	201,609,117
<i>Vulpia geniculata</i>	A	1,056.66	259.54	274,246	52.50	16.70	104.50	*	8.60	14,397,915	4,579,908	28,658,707	+	2,358,516
Total pollen production					391.3	516.6	257.40	400.90	533.60	434,966,471	1,856,162,351	227,479,175	1,438,789,772	762,885,970

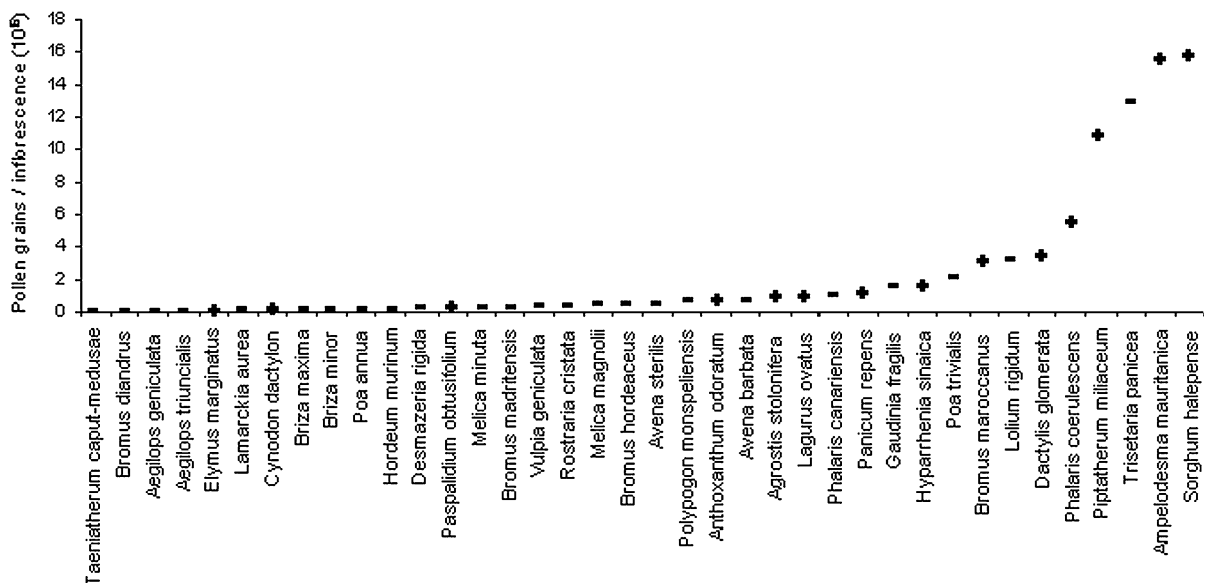
I, Number of pollen grains per flower; 2, number of flowers per inflorescence; 3, number of pollen grains per inflorescence; 4, number of inflorescences per square meter; 5, number of pollen grains per square meter; I, mountain area; II, grassland area; III, abandoned crop land; IV, riverside area; V, littoral area; (–) no presence; (*) occasional; (+) insignificant; A, annual; P, perennial

in the Poaceae family. The different species flower successively (Lewis et al. 1983; Knox et al. 1993), increasing the period of time in which their allergenic pollen can be found in the air. This behaviour of successive flowering is especially remarked in the Mediterranean basin, because the orography of the territory means the same species has different phenology periods, depending on altitude. On the other hand, Poaceae pollen is one of the main cause of pollinosis everywhere, with a well-known phenomenon of cross-reactivity among the species, probably because of their close phylogenetic relationships (Andersson and Lidholm 2003).

The mean variation of pollen grains per anther, ranging from 14 to 55%, is less than 31% in 60.5% of the species. In the remaining species (39.5%), it is equal or higher than 31%. This character thus has low mean variation in the former group and is probably genetically controlled. In the latter group, it may be affected by environmental factors. The results for *Avena barbata* with 2,558 pollen grains per anther, *Bromus hordeaceus* with 486, *Lamarckia aurea* with 135, *Poa annua* with 405, and *Rostraria cristata* with 109 are similar to those for the same species in the study carried out by Prieto-Baena et al. (2003). In contrast, discrepancies were found regarding the pollen content of other species. In fact, values for *Briza maxima* with 491 pollen grains per anther, *Cynodon dactylon* with 1,027, *Dactylis glomerata* with 2,133, *Hordeum murinum* with 531, *Lolium rigidum* with 3,725, *Piptatherum miliaceum* with 862, *Polypogon monspeliensis* with 52, *Trisetaria panicea* with 1,131 and *Vulpia geniculata* with 352 are different from those recorded by Prieto-Baena et al. (2003) (pollen grains per anther for the same species being, respectively: 1,572, 2,500, 3,475, 371, 10,453, 1,052, 91, 1,392, and 3,544). Different pollen production values were also observed for species of the same genera. Prieto-Baena et al. (2003) estimated 1,000 pollen grains per anther for *Phalaris minor* and *Phalaris paradoxa*, 2,224 for *Hyparrhenia hirta*, and 5,410 for *Elymus repens*, values different from the 1,955 pollen grains for *Phalaris coerulescens*, 814 for *Hyparrhenia sinaica*, and 273 for *Elymus marginatus* observed in this study. As documented by Stanley and Linskens (1974), pollen production may vary from year to year because of physiological and environmental factors. This might explain the different results obtained in our study in comparison to

Table 3 Means comparison test performed by means of parametric analysis (paired samples student *t* test) from data obtained in the measurements (Table 1)

Genera	Paired samples <i>t</i> -test	Pollen grains per anther	Flowers per spikelet	Spikelets per inflorescence
<i>Aegilops</i>	<i>A. geniculata</i> – <i>A. triuncialis</i>	0.011	0.001 ^a	–
<i>Avena</i>	<i>A. barbata</i> – <i>A. sterilis</i>	0.240	0.000	0.000
<i>Briza</i>	<i>B. maxima</i> – <i>B. minor</i>	0.000	0.000	0.000
<i>Bromus</i>	<i>B. diandrus</i> – <i>B. hordeaceus</i>	0.000	0.000	0.000
	<i>B. diandrus</i> – <i>B. madritensis</i>	0.002	1.000	0.000
	<i>B. diandrus</i> – <i>B. maroccanus</i>	0.000	0.000	0.000
	<i>B. hordeaceus</i> – <i>B. madritensis</i>	0.068	0.000	0.808
	<i>B. hordeaceus</i> – <i>B. maroccanus</i>	0.000	0.000	0.000
	<i>B. madritensis</i> – <i>B. maroccanus</i>	0.000	0.000	0.000
<i>Melica</i>	<i>M. magnolii</i> – <i>M. minuta</i>	0.000	–	0.000
<i>Phalaris</i>	<i>P. canariensis</i> – <i>P. coerulea</i>	0.007	–	0.000
<i>Poa</i>	<i>P. annua</i> – <i>P. trivialis</i>	0.000	–	0.000

^a Number of flowers per inflorescence**Fig. 2** Total pollen production per inflorescence for the 38 species studied. Dashes, annual species; plus symbols, perennial species

those of other authors (Subba Reddi and Reddi 1986; Prieto-Baena et al. 2003). Variation of pollen production can also be attributed to local variations in climatic factors and to differences in the taxa recorded in the different regions.

The results obtained in this work demonstrated that the number of spikelets per inflorescence is the most variable characteristic. Prieto-Baena et al. (2003) also suggested that this characteristic depends

on environmental factors. The number of second-order spikes or panicles per inflorescence showed a range of variation between 15 and 37% and the number of flowers per spikelet remained constant for most species.

According to Faegri and Van der Pijl (1979), anemophily is accompanied by production of large amounts of pollen to increase the probability of wind-pollination. Weak production of pollen per anther can

be compensated by a large number of flowers per inflorescence. This is proved for *Polypogon monspeliensis*, *Rostraria cristata*, and *Agrostis stolonifera*. Reduction of the number of flowers is normally balanced by high production of pollen per anther, as is observed for *Anthoxanthum odoratum*, *Bromus maroccanus*, *Gaudinia fragilis*, and *Lolium rigidum*. This important variability in the production of pollen is also related to the method of pollination, i.e. if the species are autogames or allogames.

Total mean pollen production per anther of the species studied was approximately 1,400 grains. If we take into account only the common species included in our study and that of Prieto-Baena et al. (2003), a value of 1,148.5 pollen grains per anther was obtained in Tetouan, which differs from the mean of 1,972.5 recorded in the same species studied in Cordoba by Prieto-Baena et al. (2003). This demonstrates that the values genuinely differ between the two studies and, although for most of the species were similar, in our case the anthers often contained less pollen. Furthermore, the high pollen-content species in the work of Prieto-Baena et al. (2003), for example *Arrhenatherum album*, *Cynosurus echinatus*, *Elymus repens*, and *Festuca arundinacea*, were not collected in our investigation.

Regarding pollen production per square meter, the values were between 0.44 and 1.85×10^9 pollen grains/m² in the different areas studied. A total of $1.24\text{--}1.36 \times 10^9$ pollen grains/m² were estimated for *Trisetaria panicea* in the meadow area and the riverside zone. This was twice as high than the amount detected in the study of Prieto-Baena et al. (2003) and approximately similar to the 1.25×10^9 grains of *Phalaris tuberosa* and the 1.89×10^9 grains of *Vulpia geniculata* (Smart et al. 1979). Smart et al. (1979) measured production of 2.11×10^9 pollen grains per square meter for *Lolium perenne*. *Trisetaria panicea*, by itself, accounted for 74% and 86% of total pollen production in the grassland and riverside zones, respectively.

Regarding maximum pollen production per square meter, with the exception of the crop land with 0.52×10^9 pollen grains, areas I, II, IV, and V with 1.55×10^9 , 1.53×10^9 , 1.52×10^9 , and 2.25×10^9 contributed similarly to total pollen production per square meter. Production was much higher on natural surfaces which still preserve semi-natural vegetation than in the crop land. In this area, despite of the high

variety of species (22), lower pollen production per square meter was observed. These results are because of the low pollen content of the most frequent species and the low grass species density, related mainly to the pasture but also to the occurrence of other species.

The amount of pollen in the atmosphere depends not only on pollen production but also on the duration of the flowering season and on environmental factors (Rogers 1993). It cannot, therefore, be deduced solely from pollen production by the inflorescence. According to Galán et al. (1995), the most important meteorological factors are moisture, minimum temperature, and sunshine hours, and Peternel et al. (2006) found that low pollen concentrations correlated with high levels of rainfall and humidity. In contrast, Green et al. (2004) found that the major factors affecting airborne pollen counts were maximum and minimum temperatures. To understand the temporal distribution of the pollen emission by the Poaceae, phenological studies and aerobiological analysis are necessary. Subba Reddi et al. (1988) demonstrated that flowering season periods can differ by several months between genera, and some Poaceae species have a circadian mechanism of pollen shedding, each species having their own temporal features of pollen release, with duration that may vary between 2 and 13 h.

There is a need to assess further phenological studies to determine the anthesis periods of the different species of grass and the response to meteorological conditions, together with aerobiological sampling, to analyze the eventual annual variation in the atmospheric pollen content of the region. This will enable monitoring of air quality, and determine the extent to which grass pollen can cause respiratory allergies.

5 Conclusions

This study indicates that pollen production by grasses is higher in natural areas that still preserve semi-natural vegetation than in crop lands in which the occurrence of such vegetation is reduced, with a few species being responsible for most pollen production. To ensure their wind-pollination, the species of Poaceae studied produce enough pollen grains per anther or, if not, develop an inflorescence with several flowers when the number of pollen grains per

anther is low, or both at the same time; this means they release sufficient quantities of pollen to cause allergenic disorders. The perennial species produce much more pollen grains per anther than the annual species. In contrast, an important inflorescence density was recorded for the annual species. This work provides many data that must be taken into account in the phenological studies and aerobiological analysis that are increasing because of their importance for epidemiology and immunotherapy, and for health improvement and management of respiratory allergy diseases in the atopic population within our region.

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