

Aerobiological and phenological study of the main Poaceae species in Córdoba City (Spain) and the surrounding hills

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Abstract This study reports on the flowering phenology of 12 Poaceae species in different types of plant cover (scrub, riverbank and pasture), with a view to creating a phenological calendar for the major species contributing most to airborne pollen concentrations. The study has been carried out from March to June between 2000 and 2013. Seventeen sampling sites in the study area were selected by accessibility, and predominantly, grasses grow in different land covers for phenological monitoring. Airborne pollen was collected using a Hirst-type volumetric spore trap. Results showed that longer phenological ranges were recorded in the wettest year and shorter ranges in the driest year. Over the study period, certain species displayed changes in the timing of flowering phenology. In pastureland and scrubland, a slight phenological delay over the study period has been recorded. In pastureland, some species—i.e., *Bromus madritensis*, *B. diandrus* and *B. hordeaceus*, *Hordeum leporinum* and *Stipa capensis*—displayed a delay in the timing of phenophases over the study period, including *B. hordeaceus* and *B. madritensis* on riverbank. *Dactylis glomerata* and *Trisetaria panicea* in scrub have been the only species with a clear advance; *B. diandrus* and *B. madritensis* a clear flowering delay. *Lolium*

rigidum, *Dactylis glomerata*, *Aegilops geniculata*, in scrub, riverbank and pasture, and *Arrhenatherum album*, only in scrub, full flowering coincided with pollen peak in some populations during several years.

Keywords Poaceae · Grasses · Pollen · Phenology

1 Introduction

Phenology is the study of periodically occurring biological events as influenced by the environment, and especially by changes in temperature prompted by changing weather and climate patterns (Schwartz 2003). Phenological research, as a mainly descriptive science, requires precision, methodological fieldwork and direct observation of the annual life cycle of the species under study (Menzel 2002). In addition, phenology is an excellent tool for detecting and measuring the impact of climate on vegetation, since variations in climate give rise to changes or transformations in individuals over a particular period of time; these changes are most apparent over longer intervals, and for certain stages of the annual life cycle, known as phenophases (Roetzer et al. 2000). Aerobiological research studies the behavior, concentration and dispersal of airborne biological particles and charts their impact on the environment and on living organisms (Mandrioli and Ariatti 2001). As such, it is viewed as a valuable means of evaluating the

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behavior of airborne pollen from wind-pollinated species, such as Poaceae.

Poaceae comprises more than 700 genera containing around 10,000 species (Watson and Dallwitz 1992), being only overcome by Asteraceae, Fabaceae, Orchidaceae and Rubiaceae (Heywood et al. 1985). The importance of this family lies in terms not only of the number of species involved but also of the key ecological role played by these species in large herbaceous formations covering almost 25 % of the land surface (Dyer et al. 1982). Most grasses contain numerous flowers per inflorescence and thus release a large amount of pollen into the atmosphere; however, most perennial species produce 3.5 times higher pollen concentration *per* inflorescence than annual species (Prieto-Baena et al. 2003). Poaceae pollen grains are among the main causes of pollen allergy in Europe (D'Amato et al. 1998, 2007) and in Córdoba and are regarded as the second leading cause of allergy after olive pollen (Domínguez-Vilches et al. 1995).

Despite the ecological importance of this large family and the clear impact of its pollen on human health, only some papers have been published on field research into Poaceae phenology (i.e., Prieto-Baena et al. 2003; Sánchez-Mesa et al. 2003, 2005; Tormo et al. 2011; León-Ruiz et al. 2011, 2012).

Poaceae pollen grains are stenopalynous, and the present research sought to provide information about phenology on different species, as well as to ascertain which species contributed most to airborne pollen concentrations.

The specific aims were: (i) to monitor the phenological behavior of Poaceae species in different types of plant cover (scrub, riverbank and pasture) in Córdoba City and peri-urban area in spring; (ii) to create a phenological calendar of the main Poaceae species growing in Córdoba; and (iii) to analyze local Poaceae airborne pollen concentrations, indicating which species contribute most to the curve.

2 Materials and methods

2.1 Study area

This study was conducted in Córdoba City and the nearby Sierra Morena hills between 2000 and 2013; data were not collected in 2011 due to fieldwork problems. Córdoba City (30S UTM X: 341642; UTM

Y: 4192085), located in the southwestern Iberian Peninsula, has a population of 333,033 inhabitants (latest census, 2011); it lies at 123 meters above sea level and covers a surface area of 290.23 km². It has a Mediterranean climate with some continental features, characterized by cold temperate winters and hot dry summers. The mean annual temperature is 17.6 °C, and the mean annual rainfall is 535 mm (1971–2000) (AEMET, Spanish State Meteorological Agency 2001).

2.2 Aerobiological data

Airborne pollen was collected using a Hirst-type volumetric spore trap based on the impact principle (Hirst 1952). The trap is located 22 m above ground level, in the Campus of Rabanales, University of Córdoba.

Monitoring and data management were carried out in accordance with guidelines published in Spanish Aerobiology Network (REA) Management and Quality Manual (Galán et al. 2007) and with the minimum recommendations of the European Aeroallergen Network (EAN) (Galán et al. 2014). Data were expressed as average daily pollen grains/m³ of air.

The pollen season start was defined as the first day with ≥ 10 pollen grains/m³ of air and three subsequent days recording 10 or more pollen grains/m³. The pollen season end was defined as the last day with at least 10 pollen grains/m³ of air and with three subsequent days with lower values.

2.3 Meteorological data

Daily average temperature and rainfall data were provided by the Spanish State Meteorological Agency, located a few meters from the pollen-monitoring station in Córdoba. Southerly winds predominate. This airflow is coming from open areas, except for a few areas of olive cultivation. Cumulative average temperature and cumulative rainfall for March (M), February–March (FM) and January–February–March (JFM) for each study year are shown in Table 2.

2.4 Phenological data

Seventeen sampling sites within the study area were chosen for phenological monitoring in three different land covers: scrub with vegetation dominated by

woody perennials, pasture with grasses or other herbs and riverbank in which various grass species grow (Fig. 1). Sampling points were selected by accessibility, and grasses predominantly grow in different land covers. The scrub land cover occupies the greater extension, and it has been represented by 11 sampling points. Pasture and riverbank are less represented in the area with only four and two sampling points, respectively. Phenological monitoring was carried out weekly from March to June between 2000 and 2013. The most representative species at the various sampling sites were selected.

The following phenological phases were observed, based on the Barbieri system (Barbieri et al. 1989), and were translated into Biologische Bundesanstalt, BUNDessortenamt and Chemical industry (BBCH) phenological phases to facilitate international scientific communication: pre-flowering or phase 0 (<BBCH 60): the period from plant emergence to start of flowering; start of flowering or phase 1 (approx. from BBCH 60 to BBCH 63): until 25 % of florets open; full flowering or phase 2 (approx. from BBCH 63 to BBCH 67): from 25 to 75 % of florets open; end of flowering or phase 3 (approx. from BBCH 67 to BBCH 69): more than 75 % of florets open; and the fruiting or phase 4 (BBCH 69): during fruit ripening.

The phenological phase was defined as the mean phenological status of the population for each species, in a specific date and place, subsequently grouped by type of plant cover: scrub, riverbank and pasture. Phenophases were plotted from 1st of March of each year, at pre-flowering (phase 0).

This study was focused on 12 species displaying a plant cover of at least 30 inflorescences/m² per population. In summary, approx. 30 inflorescences of each species per sampling site were studied: *Bromus madritensis* L., *Bromus diandrus* Roth., *Bromus hordeaceus* L., *Hordeum leporinum* Link., *Avena barbata* Pott ex Link., *Vulpia geniculata* (L.) Link., *Stipa capensis* Thumb, *Arrhenatherum album* (Vahl.) W.D. Clayton, *Aegilops geniculata* Roth., *Lolium rigidum* Gaudin, *Trisetaria panicea* (Lam.) Paunero and *Dactylis glomerata* L. For this reason, those species with lower plant cover were omitted.

2.5 Statistical analysis

Analysis covered 12 species in the three studied plant covers over 13 years. The temporal trends for start of

flowering (phase 1) with a clear flowering advance or delay were analyzed by linear regression and significance values; p-values lower than 0.05 were considered significant. This analysis has been also applied to airborne pollen, both annual pollen index (API) and pollen peak day.

3 Results

3.1 Aerobiological data

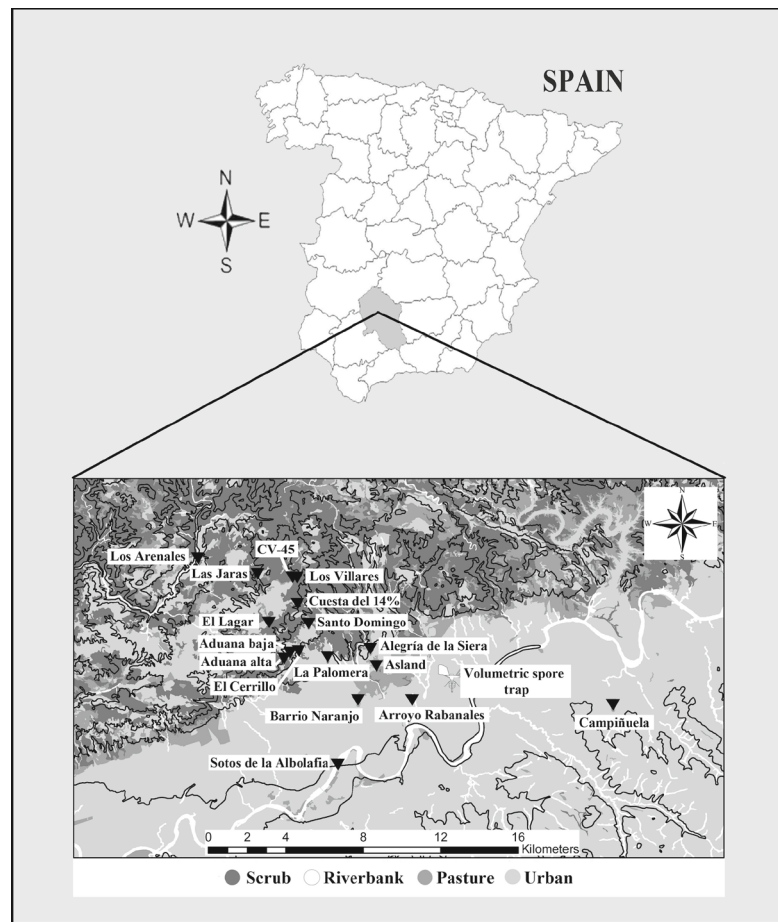
Characteristics of Poaceae pollen seasons for each study year (2000–2013) are shown in Table 1. Pollen season start occurred in the second half of April, except in 2000 and 2007 (early May), and in 2011 and 2012 (early April); the earliest pollen season start date was April 7, 2012, and the latest May 7, 2007. The pollen season ended roughly in the second half of June, except in 2005 and 2006 (early June); end dates ranged from 10th June (2006) to 30th June (2008, 2010 and 2011). The longest pollen season was recorded in 2011 (81 days) and the shortest in 2012 (43 days). The highest pollen peak was recorded in 2013 (1158 pollen grains/m³ of air), but the highest annual pollen index (API) was observed for 2007 (8767 pollen grains). A gradual and slight delay (not statistically significant) in the peak day has been shown during the study period. Regarding the annual pollen index (API), neither statistical significant trends have been obtained, but with a positive slight trend during the earlier years (2000–2007) and negative from 2008 until 2012. The highest values were recorded in 2013 (Table 1).

3.2 Meteorological data

Over the study period, the wettest period prior to the pollen season occurred in 2010 and 2013, while the driest years were 2000, 2005, 2007 and 2012 (Table 2).

3.3 Phenological data

Some study species flowered in early spring (*Bromus madritensis*, *Bromus diandrus*, *Bromus hordeaceus*, *Hordeum leporinum*, *Avena barbata* and *Vulpia geniculata*) and others in late spring (*Stipa capensis*, *Arrhenatherum album*, *Aegilops geniculata*, *Lolium*



Sample sites	UTM X	UTM Y
Aduana alta	340791	4199206
Aduana baja	340489	4198914
Alegría de la Sierra	344926	4199407
Arroyo Rabanales	347094	4196752
Asland	345252	4198511
Barrio Naranjo	344300	4196752
Campiñuela	357483	4196492
Cuesta del 14%	341181	4201771
CV-45	340908	4203137
El Cerrillo	341237	4199289
El Lagar	339695	4200782
La Palomera	342737	4198985
Las Jaras	339117	4203327
Los Arenales	336033	4204111
Los Villares	341149	4203129
Santo Domingo	341758	4200722
Sotos de la Albolafia	343274	4193395

Fig. 1 Sampling sites with UTM coordinates

Table 1 Characteristics and trends of Poaceae pollen seasons for each study year (2000–2013)

Years	Start	End	No. of days	Peak day	Pollen grains/m ³ of air Peak day	Annual pollen index
2000	125 (04-May)	171 (19-Jun)	46	152 (30-May)	404	3241
2001	115 (25-Apr)	171 (20-Jun)	56	145 (25-May)	775	7084
2002	115 (25-Apr)	171 (20-Jun)	56	141 (21-May)	807	7163
2003	118 (28-Apr)	168 (17-Jun)	50	140 (20-May)	856	6520
2004	121 (30-Apr)	177 (25-Jun)	56	152 (30-May)	475	3333
2005	112 (22-Apr)	163 (12-Jun)	51	125 (05-May)	130	1424
2006	110 (20-Apr)	161 (10-Jun)	51	139 (19-May)	356	4570
2007	127 (07-May)	177 (26-Jun)	50	134 (14-May)	863	8767
2008	113 (22-Apr)	182 (30-Jun)	69	140 (19-May)	757	6592
2009	117 (27-Apr)	179 (28-Jun)	62	135 (15-May)	273	3407
2010	114 (24-Apr)	181 (30-Jun)	67	149 (29-May)	510	7243
2011	100 (10-Apr)	181 (30-Jun)	81	135 (15-May)	277	5190
2012	128 (07-Apr)	171 (19-Jun)	43	151 (29-May)	202	2902
2013	108 (18-Apr)	176 (25-Jun)	68	132 (12-May)	1158	8154
			Slopes	R^2		p
Start			−0.5296	0.0836		0.3158
Peak day			−0.5054	0.0656		0.3764
Pollen grains/m ³ of air peak day			−5.0879	0.0047		0.8144
Annual Pollen Index			55.4769	0.0105		0.7272

Table 2 Cumulative rainfall and cumulative average temperature for March (M), February–March (FM) and January–February–March (JFM) for each study year (2000–2013)

Years	PrepJFM (mm)	PrepFM (mm)	PrepM (mm)	TmedJFM (°C)	TmedFM (°C)	TmedM (°C)
2000	59.3	35.2	35.0	1069.1	819.9	453.1
2001	362.8	227.3	197.1	1126.6	801.6	474.7
2002	175.2	134.7	130.6	1104.8	779.4	459.1
2003	231.1	155.6	53.9	1017.6	742.9	465.9
2004	190.6	173.0	85.6	1064.5	740.5	402.2
2005	75.4	75.4	17.5	894.6	665.8	435.1
2006	197.6	125.9	67.3	984.2	717.0	434.2
2007	107.1	93.8	30.5	1058.9	770.4	412.6
2008	128.5	58.5	6.5	1170.3	828.4	435.9
2009	217.9	153.8	70.7	1050.0	769.3	455.1
2010	456.0	310.6	104.0	1039.7	729.8	413.1
2012	19.5	5.6	3.9	829.7	605.0	405.3
2013	415.6	353.8	266.4	890.0	621.6	373.7

rigidum, *Trisetaria panicea* and *Dactylis glomerata*). Phenophases for the twelve species at different sampling sites (pasture, riverbank and scrub) throughout the study period are shown in Fig. 2.

A slight delay in flowering phases has generally been observed over the years when representing all species and populations (Fig. 3), only significant for phase 1 ($p < 0.1$); however, different behaviors have

been shown according to different species (Fig. 2): in pastureland, flowering took place progressively later in the three species of *Bromus* ($p < 0.05$), *Hordeum leporinum* ($p < 0.01$) and *Stipa capensis* ($p < 0.1$); in scrubland, *Trisetaria panicea* ($p < 0.05$) and *Dactylis glomerata* ($p < 0.001$) followed a flowering advance and *B. diandrus* ($p < 0.01$) and *B. madritensis* ($p < 0.05$) followed a flowering delay; and in riverbanks, no significant trend was observed in most species, with the exception of *B. hordeaceus* and *B. madritensis* that experienced a delay ($p < 0.05$) (Table 3).

Comparing trends in different plant covers for all species (Fig. 2; Table 3), a general delay in the timing of flowering phenophases has been observed in pastures and scrub ($p < 0.05$); on riverbanks, a slight delay in flowering was recorded in the course of the study period, but not statistically significant.

When comparing flowering phenophases vs. air-borne pollen, it has been observed that the full flowering period (phase 2) in most species occurred earlier than the pollen peak day. The species and population with full flowering during the pollen peak during several years are: *Lolium rigidum*, *Trisetaria panicea*, *Aegilops geniculata* and *Dactylis glomerata* in pastureland; *Lolium rigidum*, *Dactylis glomerata* and *Aegilops geniculata* in riverbanks; and *Dactylis glomerata*, *Arrhenatherum album*, *Lolium rigidum*, *Aegilops geniculata*, *Trisetaria panicea* and *Vulpia geniculata* in scrub (Fig. 2). *Lolium rigidum*, *Dactylis glomerata* and *Aegilops geniculata* full flowering coincided with the pollen peak in some populations during several years. *Arrhenatherum album* is only presented in scrub, but full flowering also coincided with the pollen curve in some years.

4 Discussion

Airborne pollen concentrations are regarded as a good biological indicator for studying reproductive phenology in anemophilous plants (Menzel 2002). The pollen season usually coincides with flowering, but in many cases, it also depends on a number of factors including weather conditions, atmospheric stability, soil status and plant age (Kasprzyk 2009). Herbaceous plants, including most Poaceae species, usually display a more immediate response to weather conditions than woody species (Alcázar et al. 2009; Dahl et al. 2013).

Long-term phenological databases provide an opportunity to chart phenological trends and monitor the impact of climate change on plants. Most research has focused on Northern and Central Europe (Menzel et al. 2006, Cook et al. 2012), while few papers have addressed the Mediterranean region (Peñuelas et al. 2002; Gordo and Sanz 2005). A number of studies have looked at the behavior of populations in a range of environmental conditions, taking into account the degree of sun exposure, soil type and different types of plant cover. The present study examined flowering phenology in different plant covers following prior studies (Frenguelli et al. 2010; León-Ruiz et al. 2011; Tormo et al. 2011).

According to León-Ruiz et al. (2011), longer phenophases over the last decade, and so a longer pollen season, are observed during wetter years in Córdoba. Conversely, drier years are associated with shorter phenophases and in general a shorter pollen season. These findings were confirmed here over a longer study interval with recent years, with a clear statistically significant advance or delay in start of flowering (phase 1) in some studied species. Shorter pollen seasons with lower annual pollen concentrations were recorded during the driest years (2000, 2005, 2007 and 2012). The pollen season started around the second fortnight in April and ended around the second fortnight in June. The longest pollen season was observed in the wet year 2011 (81 days) and the shortest in the driest year of the study period, 2012 (43 days). The highest pollen peak (1158 pollen grains/m³ of air) was recorded during the rainiest year (2013). The peak pollen day usually occurred in the second half of May, as observed in earlier studies (Fernández-González et al. 1999; García-Mozo et al. 2009). Rainfall exerts a major influence during the pollen season, washing out the atmosphere and/or blocking pollen emission; this is evident in the variations in pollen season severity reported in earlier papers (Silva-Palacios et al. 2000; Barnes et al. 2001; Sánchez-Mesa et al. 2005; Smith and Emberlin 2006; Pérez et al. 2009; Recio et al. 2010).

When comparing flowering phenology of different species and airborne pollen, this study has shown that most species bloom before detecting the pollen peak in the curve and similar results have been observed in Badajoz, Spain (Tormo et al. 2011). The authors attributed this to grass flowering phenology not being clear probably because many species do not open their

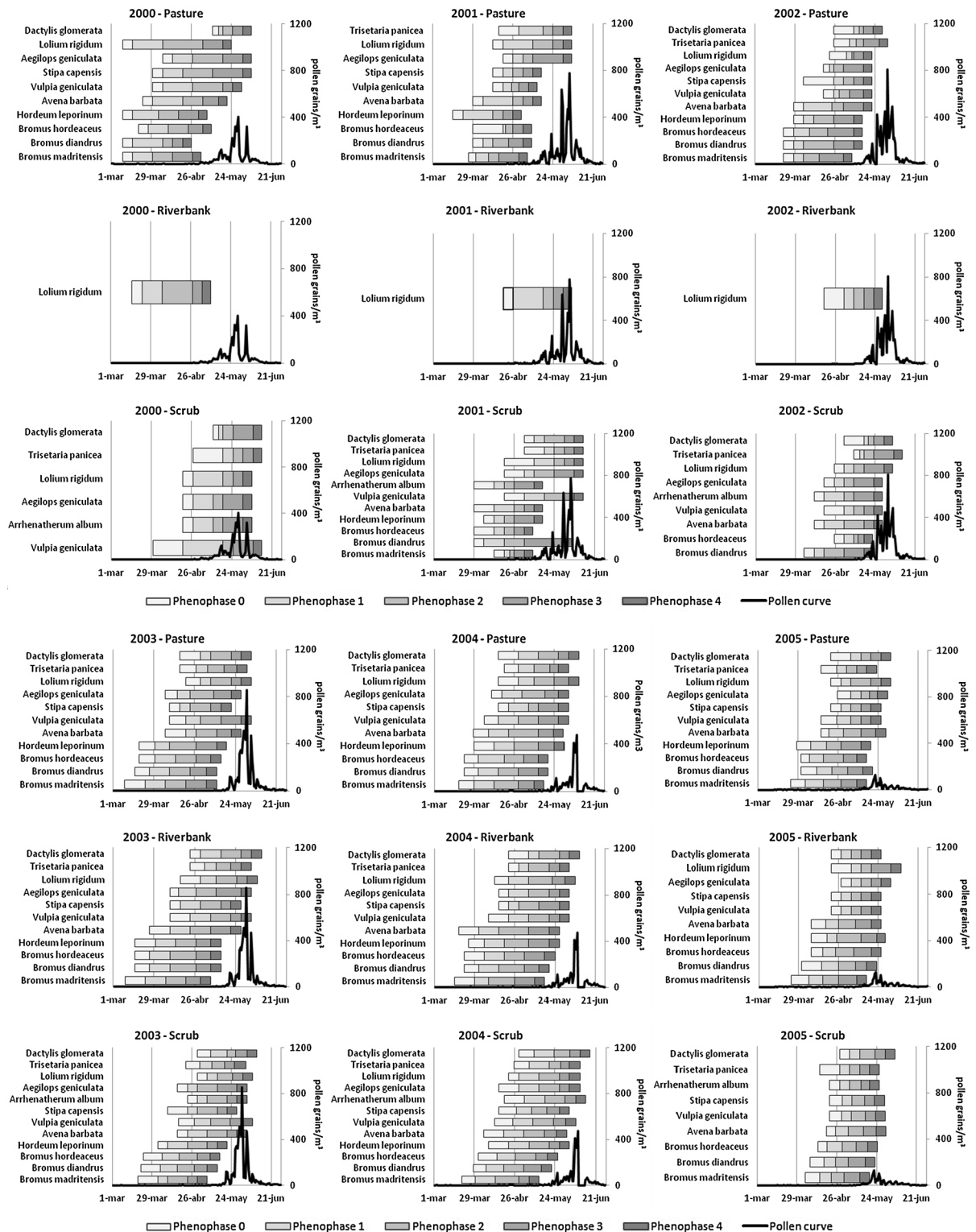


Fig. 2 Pollen curve and phenological behavior of different Poaceae species in different land covers for each year (2000–2013)

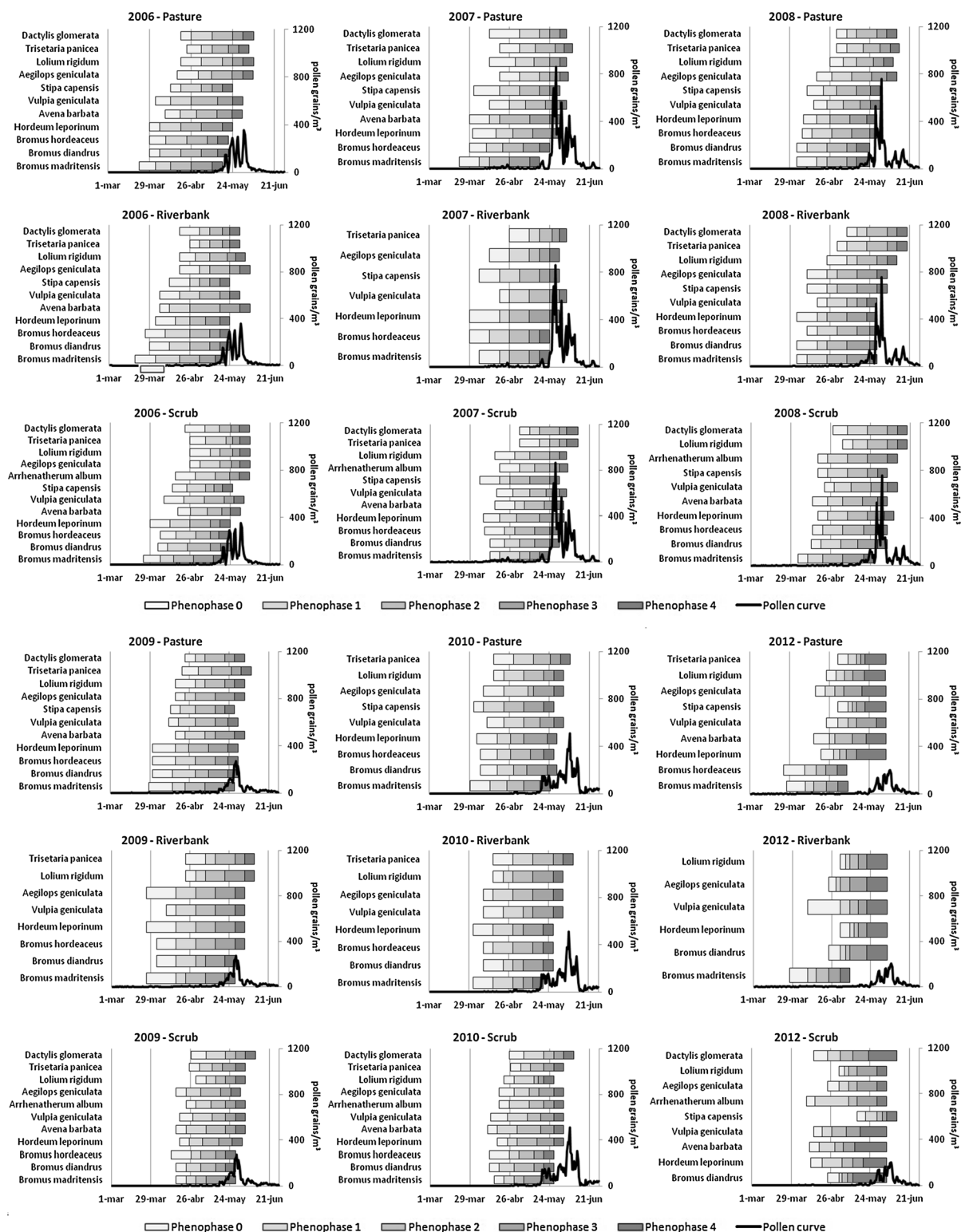


Fig. 2 continued

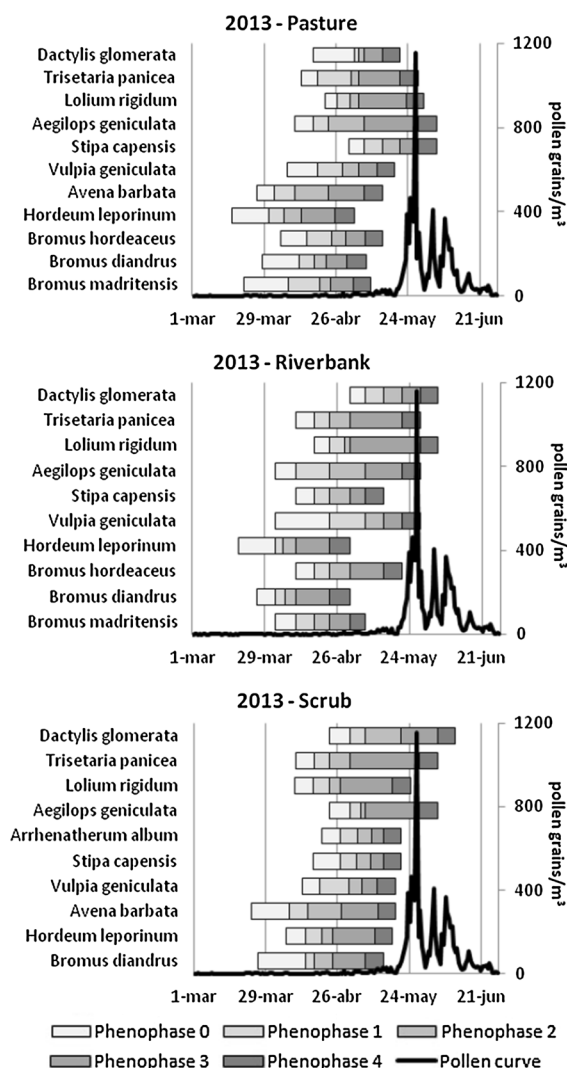


Fig. 2 continued

spikelets for pollen emission. On the other hand, there are also important differences in pollen production among the different species (Prieto-Baena et al. 2003; Aboulaich et al. 2009), and it can also contribute to the species being better represented in the pollen curve. In Italy, Frenguelli et al. (2010) have also observed grass species blooming before and after the maximum pollen concentrations in the air. Due to the fact that airborne grass pollen is the main cause of pollinosis in Europe, and grasses represent high number of species with different presence and behavior, these researchers recommend phenological observations when planning clinical trials with medications for grass pollen allergy.

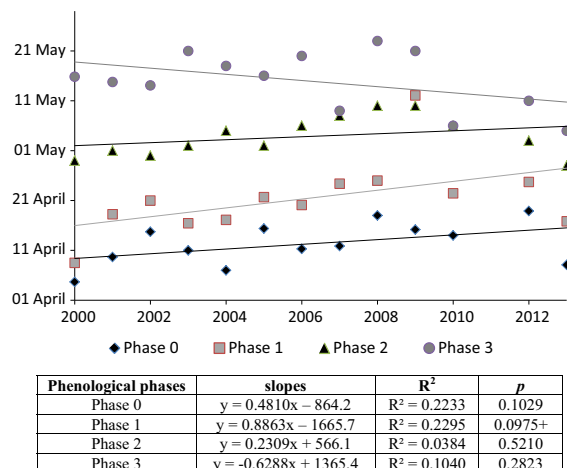


Fig. 3 Mean dates of phenological phases vs. time (year) with linear regression line showing trends during the study period. +if trend at $\alpha = 0.1$ level of significance

Previous research in Córdoba has shown that the greatest contribution to the pollen curve is from natural areas including mountains and riverbanks (León-Ruiz et al. 2011). Grass species flower later at higher altitudes, but the pollen released from mountainous populations can be transported to the city (León-Ruiz et al. 2011; Jochner et al. 2012); our data support these results, scrub areas and—to a lesser extent—riverbanks were probably the greatest contributors to the pollen curve. This study has focused on spring flowering species that display a plant cover of at least 30 inflorescences/m², and it is likely that less represented species have contributed to the pollen curves.

Pasture ecosystems are among the plant cover types most sensitive both to water availability (Dickinson and Dodd 1976) and to temperature changes (French and Sauer 1974). A phenological delay has been recorded, i.e., *Bromus* sp., over the study period in pastureland; in scrubland, *Dactylis glomerata* and *Trisetaria panicea* have been the only species that have shown a clear flowered advance. The same conditions can influence phenology in different grass species differently due to responses to global warming (Chuine et al. 2000; Grzegorz 2011). However, no significant trend was observed in riverbanks, as water availability or other environmental factors probably influence the growth of flowering species. The development of grasses is positively influenced by water availability, especially in Mediterranean areas (Clary

Table 3 Temporal trends for start of flowering (phase 1) of 12 species in three plant cover over the study period (2000–2013)

Poaceae species	Plant cover	Slopes	R^2	p
<i>Aegilops geniculata</i>	Pasture	0.2221	0.0174	0.6671
	Riverbank	−0.8041	0.1020	0.3683
	Scrub	0.2000	0.0594	0.4971
<i>Arrhenatherum album</i>	Pasture	–	–	–
	Riverbank	–	–	–
	Scrub	−0.4147	0.0958	0.3033
<i>Avena barbata</i>	Pasture	0.9066	0.1747	0.2007
	Riverbank	2.5000	0.2319	0.5184
	Scrub	−0.2972	0.0340	0.5656
<i>Bromus diandrus</i>	Pasture	1.8851	0.6468	0.0028**
	Riverbank	1.7845	0.3008	0.1262
	Scrub	1.5705	0.5192	0.0081**
<i>Bromus hordeaceus</i>	Pasture	1.1504	0.3411	0.0360*
	Riverbank	1.7541	0.5752	0.0178*
	Scrub	1.1078	0.1791	0.2229
<i>Bromus madritensis</i>	Pasture	1.7336	0.6324	0.0011**
	Riverbank	1.6463	0.4223	0.0419*
	Scrub	3.1388	0.6850	0.0112*
<i>Dactylis glomerata</i>	Pasture	−0.6057	0.1645	0.2447
	Riverbank	0.8320	0.2480	0.3147
	Scrub	−1.1131	0.6657	0.0006***
<i>Hordeum leporinum</i>	Pasture	2.0199	0.4778	0.0088**
	Riverbank	1.3566	0.1629	0.2472
	Scrub	0.6316	0.1684	0.2387
<i>Lolium rigidum</i>	Pasture	1.3587	0.1867	0.1402
	Riverbank	1.1012	0.1548	0.2056
	Scrub	−0.4241	0.0650	0.4235
<i>Stipa capensis</i>	Pasture	1.2245	0.2864	0.0594
	Riverbank	−0.3173	0.0527	0.6202
	Scrub	1.3900	0.2640	0.1926
<i>Trisetaria panicea</i>	Pasture	−0.0964	0.0043	0.8386
	Riverbank	−1.0675	0.2279	0.2315
	Scrub	−1.6848	0.5156	0.0128*
<i>Vulpia geniculata</i>	Pasture	0.7402	0.2082	0.1170
	Riverbank	0.2247	0.0280	0.6439
	Scrub	−0.5335	0.2239	0.1023
All species	Pasture	0.6809	0.3542	0.0318*
	Riverbank	0.6478	0.0810	0.3458
	Scrub	0.6536	0.3418	0.0358*

*** if trend at $\alpha = 0.001$
level of significance

** if trend at $\alpha = 0.01$ level
of significance

* if trend at $\alpha = 0.05$ level
of significance

et al. 2004). On the other hand, these results notice that *Lolium rigidum*, *Dactylis glomerata*, *Aegilops geniculata* and *Arrhenatherum album* full flowering coincide with the pollen peak in some populations during several years.

The present study found that *Dactylis glomerata*, *Lolium rigidum*, *Trisetaria panicea* and *Vulpia geniculata* are possible contributors to the pollen curve, which supports the work in Spain by León-Ruiz et al. (2011). Although *Aegilops geniculata* full flowering

coincided with pollen peak in the three plant covers, this species is not a main grass pollen contributor (Prieto-Baena et al. 2003; Aboulaich et al. 2009). In addition, other populations that were not studied are also likely contributors to the pollen curve. Future studies should be focused on different grass population densities in the Córdoba City and the surrounding hills.

5 Conclusion

Although most Poaceae species displayed similar flowering phenology profiles under different weather conditions and in different types of plant cover, some species exhibited differences in the timing of flowering over the study period in different environments. In pastureland and scrubland, a trend toward a slight delay trend has been recorded over the study period; *Dactylis glomerata* and *Trisetaria panicea* have been the only species that have experienced a clear trend in earlier flowering in scrubland. For *Lolium rigidum*, *Dactylis glomerata*, *Aegilops geniculata* and *Arrhenatherum album* full flowering coincided with the pollen peak in some populations during several years. These studies are important for better understanding the airborne pollen curve; however, it is important to support these results with future studies on different grass population densities in the Córdoba City and the surrounding hills.

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