



Standardised index for measuring atmospheric grass-pollen emission



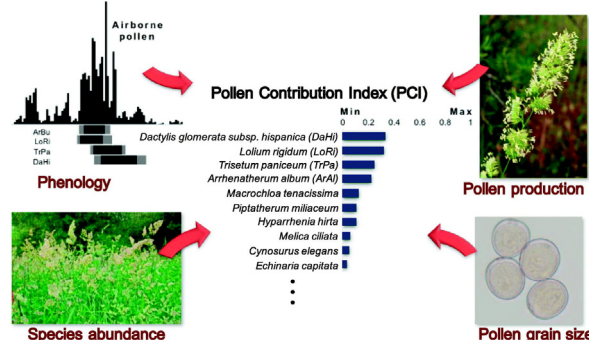
Jorge Romero-Morte, Jesús Rojo *, Rosario Rivero, Federico Fernández-González, Rosa Pérez-Badia

Institute of Environmental Sciences (Botany), University of Castilla-La Mancha, E-45071 Toledo, Spain

HIGHLIGHTS

- The Pollen Contribution Index evaluates the importance of the pollen emission of the species.
- The index considers phenology, pollen grain size, species abundance and pollen production.
- This standardised Index was applied to a case study of grass species.
- Late-flowering grass species bring the highest concentrations of airborne pollen.
- A small number of species were responsible for most airborne grass pollen.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 27 May 2017

Received in revised form 8 August 2017

Accepted 14 August 2017

Available online xxxx

Editor: D. Barcelo

Keywords:

Floral phenology

Pollen allergy

Poaceae

Pollen production

Airborne pollen

ABSTRACT

Grass pollen is the main cause of pollen allergy in Europe, and—given its marked allergenic potential and elevated airborne concentrations—constitutes a major public health risk. This study sought to identify the grass species triggering allergies during the highest-risk periods, and to measure the contribution of each species to airborne grass pollen concentrations. This type of research is particularly useful with a view to optimising the prevention and diagnosis of pollen allergies and developing the most effective immunological treatments. To that end, a total of 28 species potentially responsible for allergies were analysed. In order to assess the potential contribution of these species to overall airborne pollen concentrations, an index was designed (Pollen Contribution Index) based on the following parameters for each species: flowering phenology, pollen grain size (polar and equatorial axes), abundance of the species in the area and pollen production. The species contributing most to airborne pollen concentrations were, in order: *Dactylis glomerata* subsp. *hispanica*, *Lolium rigidum*, *Trisetum paniceum* and *Arrhenatherum album*. These species all shared certain features: small grain size (and thus greater buoyancy in air), high pollen production and considerable abundance. This Index was applied to a case study in a Mediterranean-climate area of the central Iberian Peninsula, but could equally be applied to other areas and other allergenic pollens. Findings showed that a small number of species were responsible for most airborne grass pollen.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Grass pollen poses a major health risk due to its elevated allergenic potential (Westritschnig et al., 2008; Scaparrota et al., 2013), and is

the main cause of pollen allergy in many parts of the world (D'Amato et al., 2007). In Europe, the prevalence of grass-pollen allergy is very high among allergy sufferers, who display marked sensitisation even when airborne pollen concentrations are low (Weger et al., 2013). In many countries, including the UK, Denmark and Switzerland, over 50% of allergy-sufferers are sensitive to grass pollen (Burbach et al., 2009; Heinzerling et al., 2009), while in some regions of Spain as many as

* Corresponding author.

E-mail address: jesus.rojo@uclm.es (J. Rojo).

80% of people displaying allergic reactions are sensitive to Poaceae (Pereira et al., 2006).

The problems posed by grass pollens for the allergy-sufferers are aggravated by a number of factors according to Thommen's postulates (Coca et al., 1931): these species are abundant and widely-distributed worldwide (Simon et al., 2017); they are also wind-pollinated and produce large amounts of pollen (Prieto-Baena et al., 2003), giving rise to elevated airborne concentrations (Skj  th et al., 2013). As a result, the degree of exposure is very high (Blanca-Lopez et al., 2016). It is estimated that up to 24% of the world's plant cover comprises communities in which grass species predominate (Judd et al., 2002). Moreover, grasses grow in all kinds of habitats, even in the most extreme environments, including those associated with saline substrates and high-mountain climates (Rivas-Mart  nez et al., 2002). In addition, around 70% of the world's arable land is given over to grasses grown as crops (Thomasson, 1988).

The Poaceae family comprises roughly 12,000 species grouped in 823 genera worldwide (Simon et al., 2017), and includes both perennial and annual species with a broad biological spectrum; this species diversity gives rise to marked phenological heterogeneity (Le  n-Ruiz et al., 2011). As a result of these characteristics, the pollen-season is very long—given the successive flowering of the different grass species in any given area (P  rez-Bad  a et al., 2010; Rojo et al., 2017).

Phenological data linked with airborne pollen records allows to identify the species whose flowering season coincides most closely with periods of maximum airborne pollen concentrations. For grasses, studies of this kind have been carried out by Le  n-Ruiz et al. (2011), Tormo et al. (2011), Cebrino et al. (2016) and Rojo et al. (2017) in central, western and southern Spain; similar research has been conducted in other Mediterranean countries, including Italy, by Frenguelli et al. (2010) and Ghitarrini et al. (2017), while Kmenta et al. (2016) have examined phenological data in conjunction with aerobiological records for grasses in Austria (central Europe). All these studies have sought to identify, by indirect means, those species contributing most to the Poaceae pollen curve. Other authors have addressed pollen production in grass species (Prieto-Baena et al., 2003; Aboulaich et al., 2009) or have attempted to establish correlations between certain pollen sources and airborne pollen concentrations (Skj  th et al., 2012). Yet there is clearly a need to investigate all these factors together, with a view to establishing not only which grass species are major sources of airborne pollen but also the contribution made by each of those species to airborne pollen concentrations.

Pollen grain size is another factor governing variations in the amount of grass pollen in the atmosphere (Friedman and Barrett, 2009), since it influences grain buoyancy and thus the length of time the grain remains in the air before being deposited (Oteros et al., 2015; Rojo et al., 2016). The pollen grains of different wild grass species vary considerably in size (20–80 µm), the smallest grains being the most buoyant (Subiza and Jer  z, 2002). Research into grass pollen grain size and morphology has hitherto focused on phylogenetic and ecological considerations (Sch  ler and Behling, 2011; Jan et al., 2015; Morgado et al., 2015), rather than on the crucial link between pollen grain size and dispersal of airborne pollen into the atmosphere.

As indicated earlier, awareness of the health risk involved has prompted a great deal of research into airborne Poaceae pollen (Brito et al., 2010), and there is widespread interest in those grass species with the greatest allergenic potential (Kleine-Tebbe, 2008; Johansen et al., 2009). But there is still a need to establish the contribution made by different species to the overall airborne allergen load, and to account for it in terms of species-specific characteristics. To that end, the present study sought to develop a standardised index (Pollen Contribution Index, PCI) based on the hypothesis that several characteristics of the grass species, included in Thommen's postulates, influence airborne pollen concentrations: flowering phenology in those species whose flowering season coincides most closely with periods of maximum airborne pollen concentrations; the abundance and distribution of those species; pollen production; and pollen grain size.

2. Material and methods

2.1. Aerobiological and phenological data

Airborne pollen amount was recorded at the Toledo City pollen station in central Spain (coordinates: 39° 51' 55"N, 4° 2' 31"W), over a 10-year period (2006–2015), using a Hirst-type volumetric sampler (Hirst, 1952). Sample collection and preparation, and pollen counts, were carried out using methods recommended by the Spanish Aerobiology Network (Gal  n et al., 2007).

Data on flowering phenology were collected between March and July in 2013 and 2015 (no data available for 2014), at 11 sampling sites in and around the city of Toledo, where the pollen station is located (Fig. 1; Table 1). Phenological observations were made for 25 individuals of each species growing at the sampling sites, which were selected as being representative of characteristic habitats for local grass species: forest vegetation (holm-oak forests with clearings mainly covered by xeric grasslands and shrublands); anthropic areas of ruderal grassland in urban and periurban areas; weed communities associated with the extensive croplands surrounding the urban area; and finally riparian vegetation containing Poaceae species. Flowering was studied in a total of 28 grass species (Table 2); the most representative species at each sampling point were selected following Le  n-Ruiz et al. (2011): 25 individuals/m² were selected for annuals and 25 individuals/10 m² for perennials. Therefore, the number of the studied species varied for each site according to the presence/absence of the species. For example, *Brachypodium phoenicoides* was only observed in the Peraleda park site and thus a total of 25 individuals were sampled for this species. Other species as *Trisetum paniceum* were found in 6 different sites, and thus the total observed individuals were 150 in this example (25 individuals × 6 sites).

Phenological observations were recorded weekly, following Barbieri et al., (1989) and using the phenophases defined in the international BBCH scale (Meier, 2001). The flowering season was deemed to have commenced when the first anthers were visible (BBCH code 61) and ended when all anthers were dehydrated and thus all pollen had been released (BBCH code 69). Linear interpolation was subsequently used to yield a continuous series of daily data on the phenological development of each species, as reported in previous phenological studies (Rojo and P  rez-Bad  a, 2014).

Phenological data were processed using Principal Factor Analysis to reduce dimensionality, thus yielding one result for each species (because most species were sampled at several sites). Given the similarity of phenological data for each species at different sites, the first principal component in the Factor Analysis accounted in all cases for over 90% of variance (Rojo et al., 2017). Phenological data were used to model the aerobiological data series and analyses the grass species contributing most to the pollen curve. In order to model the deterministic component of the series (seasonal + trend), the random component was eliminated using the Seasonal-Trend Decomposition procedure based on Loess, following Rojo et al. (2015a) and Rojo et al. (2017). The random component was attributed to very short-term sources of variability, such as daily variations in weather conditions (Petropavlovskikh et al., 2015; Rojo et al., 2017).

Modelling was performed using Partial Least Squares (PLS) regression, taking the phenological data for all the species studied as independent variables and airborne pollen concentrations as response variable. As part of this method, the relative influence of each species (phenology) on pollen concentrations was estimated by calculating the Variable Importance in Projection (VIP), thus identifying those grass species whose flowering period coincided with the highest peaks on the pollen curve. VIP index is the measure of the cumulative importance of the loading weights that each variable (phenology for each species) has for each component generated on the PLS regression (Mehmood et al., 2012). Here, the VIP variable ranged in value between 0 and 2 (minimum and maximum values for coincidence of flowering-period with highest pollen concentrations).

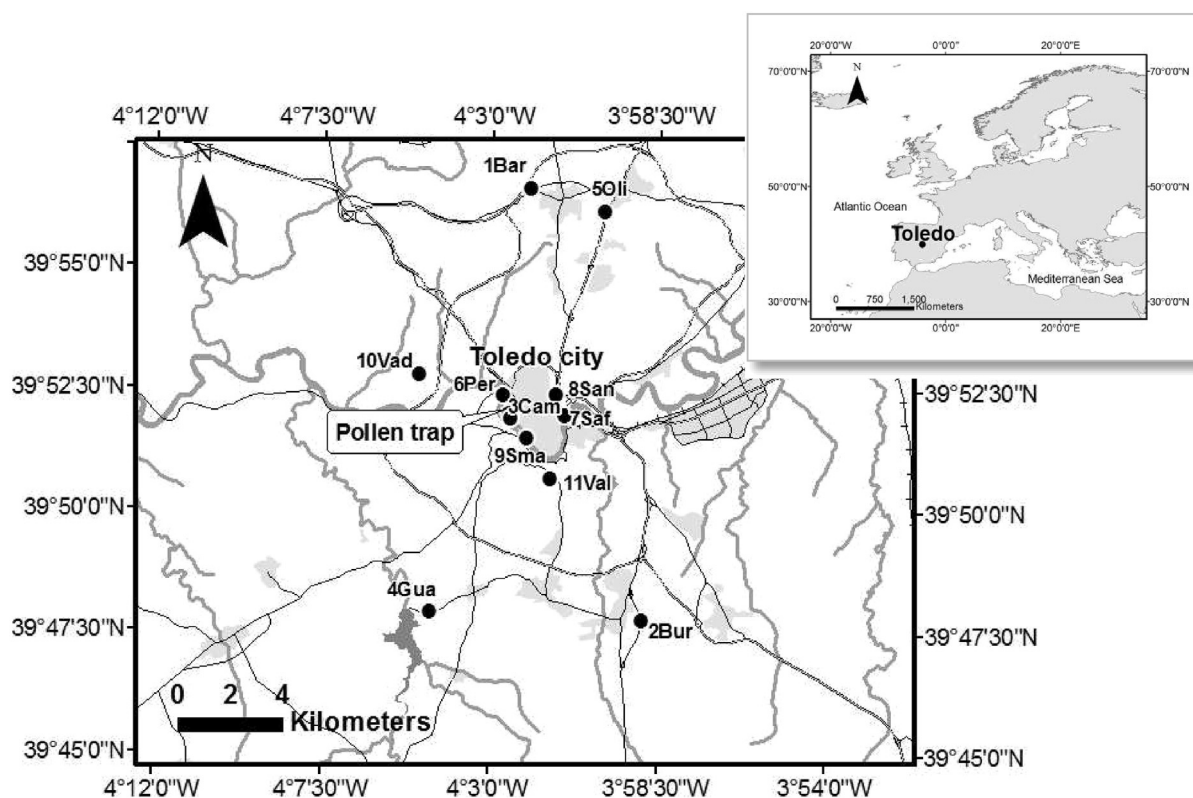


Fig. 1. Study area. Distribution of sampling sites. Phenological sites in the province of Toledo (central Spain): 1Bar, Bargas; 2Bur, Burguillos; 3Cam, Campus University; 4Gua, Guajaraz reservoir; 5Oli, Olías del Rey; 6Per, Peraleda park; 7Saf, Safont park; 8San, St Anton park; 9Sma, St Martin bridge; 10Vad, Valdelobos; 11Val, Tagus valley. The pollen trap is located in the city of Toledo.

Statistical analysis was carried out using the TANAGRA 1.4.50 software package (Rakotomalala, 2005).

2.2. Pollen grain size

Pollen grain size was measured for the 28 species included in the phenological study (Table 2); pollen grains were randomly chosen from 5 individuals per species at each sampling site. Two anthers were extracted per individual, i.e. 10 anthers per species. Following the adapted protocol published by Dafni (1992), pollen from these anthers was placed in Eppendorf tubes containing 200 µl of 70% ethanol solution and stained with fuchsin. Pollen grains were viewed at 400× magnification and the polar and equatorial axes were measured defining polar axis as the maximum diameter of the pollen grain and equatorial axis as the perpendicular plane to the polar axis, i.e. by geometrical criteria according to Faegri (1978). Diameters were measured using a light microscope equipped with a H101 ProScan II micropositioning stage and the Nikon NIS Elements software package (version 4.11).

A total of 100 randomly-selected pollen grains were measured for each species, this being deemed a sufficiently representative number (Mäkelä, 1996; Jan et al., 2015). Here an interval classification based on natural breaks according to Jenks' method was subsequently applied. This method is based on a data clustering using the average deviation between the classes (Jenks, 1967). As a result, four class intervals were used to calculate the value of the parameter pollen grain size in the Pollen Contribution Index (PCI). The classes were inverted and the highest category (class number 4) includes pollen with the smaller size and vice versa.

2.3. Estimation of species abundance

Species abundance was estimated using, as an indirect indicator, the surface area covered by the habitats of which the studied species was considered characteristic, in view of its ecological requirements and phytosociological traits. This ecological information about the type of habitat where the studied species can be found, was consulted in several

Table 1
Geographical characteristics of the phenological sites.

No.	Site	Code	Coordinates	Elevation (m a.s.l.)	Distance from the pollen trap (km)
1	Bargas	1Bar	39° 56' 45" N 4° 1' 32" W	581	8.9
2	Burguillos	2Bur	39° 47' 43" N 3° 58' 55" W	681	9.2
3	Campus University	3Cam	39° 51' 57" N 4° 2' 37" W	450	0.2
4	Guajaraz reservoir	4Gua	39° 47' 55" N 4° 04' 58" W	631	8.0
5	Olías del Rey	5Oli	39° 56' 9" N 4° 0' 2" W	623	8.6
6	Peraleda park	6Per	39° 52' 24" N 4° 02' 43" W	449	0.9
7	Safont park	7Saf	39° 51' 28" N 4° 1' 3" W	470	2.0
8	St Anton park	8San	39° 52' 25" N 4° 1' 16" W	494	2.0
9	St Martin bridge	9Sma	39° 51' 28" N 4° 2' 3" W	455	1.0
10	Valdelobos	10Vad	39° 52' 48" N 4° 4' 58" W	514	3.8
11	Tagus valley	11Val	39° 50' 41" N 4° 1' 12" W	600	2.7

Table 2

Characteristics of the grass species according to the phenological development (VIP), pollen production, pollen size and local distribution (potential habitat).

Grass species	Sampling sites ^a	VIP ^b	Pollen production ^c	Pollen size ^d	Potential habitat ^e
<i>Aegilops geniculata</i> Roth	5Oli, 8San, 11Val	1.41	51,212*	50.12	Ruderal vegetation
<i>Arrhenatherum album</i> (Vahl) Clayton	4Gua, 10Vad, 11Val	1.51	4,945,510 *	31.16	Forest vegetation (ca)
<i>Avena barbata</i> Link	2Bur, 4Gua, 7Saf, 8San	0.42	468,693*	51.19	Ruderal and weed vegetation
<i>Avena sterilis</i> L.	1Bar, 5Oli	0.93	347,702*	83.16	Ruderal vegetation
<i>Brachypodium phoenicoides</i> (L.) Roem. & Schult.	6Per	0.44	–	38.37	Riverbanks
<i>Bromus diandrus</i> Roth	3Cam, 6Per, 7Saf, 8San, 11Val	0.56	129,257*	56.76	Ruderal vegetation
<i>Bromus hordeaceus</i> L.	7Saf, 11Val	0.63	245,176*	39.56	Ruderal vegetation
<i>Bromus rubens</i> L.	2Bur, 5Oli, 8San, 9Sma, 11Val	0.52	193,035*	43.82	Ruderal vegetation
<i>Bromus tectorum</i> L.	1Bar, 2Bur, 5Oli	0.59	193,035* (<i>B. matritensis</i>)	38.76	Ruderal vegetation
<i>Celtica gigantea</i> (Link) F.M. Vázquez & Barkworth	4Gua	0.42	–	48.52	Forest vegetation (si)
<i>Cynosurus elegans</i> Desf.	8San	0.38	1,931,087* (<i>C. echinatus</i>)	30.73	Forest vegetation (ca)
<i>Dactylis glomerata</i> L. subsp. <i>hispanica</i> (Roth) Nyman	2Bur, 4Gua, 5Oli, 8San, 10Vad, 11Val	1.49	7,971,347*	29.99	Forest (ca/si) and ruderal vegetation
<i>Echinaria capitata</i> (L.) Desf.	10Vad, 11Val	0.52	–	32.86	Forest vegetation (ca)
<i>Elymus pungens</i> (Pers.) Melderis subsp. <i>campestris</i> (Godr. & Gren.) Melderis	3Cam, 6Per	0.70	1,635,984 * (<i>E. repens</i>)	36.42	Riverbanks
<i>Hordeum distichon</i>	1Bar, 2Bur	0.50	–	55.63	Herbaceous crops
<i>Hordeum murinum</i> L. subsp. <i>leporinum</i> (Link) Arcang.	2Bur, 3Cam, 6Per, 8San, 9Sma	0.43	245,607*	38.61	Ruderal vegetation
<i>Hyparrhenia hirta</i> (L.) Stapf	4Gua	1.46	2,820,582*	32.97	Rock vegetation
<i>Lamarckia aurea</i> (L.) Moench	9Sma	0.29	46,878*	28.92	Ruderal vegetation
<i>Lolium rigidum</i> Gaudin	1Bar, 2Bur, 8San	1.44	5,271,037*	35.89	Ruderal and weed vegetation
<i>Macrochloa tenacissima</i> (L.) Kunth	4Gua, 10Vad, 11Val	0.75	–	36.02	Forest vegetation (ca)
<i>Melica ciliata</i> L. <i>magnolia</i> (Gren. & Godr.) Husn.	2Bur, 4Gua, 10Vad	1.66	390,871**	32.83	Rock vegetation
<i>Piptatherum miliaceum</i> (L.) Coss.	3Cam, 7Saf, 8San, 9Sma	1.49	6,045,099*	29.21	Ruderal vegetation
<i>Poa annua</i> L.	6Per, 7Saf, 9Sma	0.94	115,511*	29.95	Ruderal vegetation
<i>Poa pratensis</i> L.	6Per	0.25	2,088,492** (<i>P. trivialis</i>)	27.88	Riverbanks
<i>Rostraria cristata</i> (L.) Tzvelev	2Bur, 5Oli, 6Per, 10Vad	0.76	157,658*	31.01	Ruderal vegetation
<i>Stipa capensis</i> Thunb.	9Sma, 11Val	0.47	172,790*	32.07	Ruderal vegetation
<i>Trisetum paniceum</i> (Lam.) Pers.	2Bur, 5Oli, 6Per, 7Saf, 9Sma, 11Val	1.65	21,278,803*	24.31	Ruderal vegetation
<i>Vulpia bromoides</i> (L.) Grey	4Gua, 11Val	0.54	14,458*	29.38	Forest vegetation (si)

^a Sampling sites: 1Bar, Bargas; 2Bur, Burguillos; 3Cam, Campus University; 4Gua, Guajazar reservoir; 5Oli, Olías del Rey; 6Per, Peraleda park; 7Saf, Safont park; 8San, St Anton park; 9Sma, St Martin bridge; 10Vad, Valdelobos; 11Val, Tagus valley.

^b VIP, Variable Importance Projection: Relationship between phenological development (flowering-period) and airborne pollen concentration. VIP values ranged between 0 and 2 (minimum and maximum values for coincidence of flowering-period with highest pollen concentrations).

^c Pollen production indicates number of pollen grains per inflorescence. Literature about pollen production: (*) Prieto-Baena et al. (2003); (**) Aboulaich et al. (2009).

^d Pollen size indicates the mean of the two diameters (polar and equatorial axes) of the pollen grains. Units: μm .

^e Potential habitat: forest vegetation areas have been divided according to the type of soil: ca, calcareous soils; si, siliceous soils.

reference manuals on flora and vegetation (Devesa Alcaraz et al., 1991; Rivas-Martínez et al., 2002). Habitats were mapped at a scale of 1:5,000, defining uniform tiles from the orthophoto provided by the Spanish Aerial Orthophotography Plan (PNOA). Then, a potential surface (abundance) was assigned for each species according to their habitat requirements. Finally, the abundance values of the species were grouped into four categories by applying the classification of intervals based on natural breaks according to Jenks' method (Jenks, 1967).

The surface areas of the habitats (riverbank vegetation, forest vegetation, rocky ground, ruderal vegetation, herbaceous crops and woody crops) were measured in a 20 km radius centered on the pollen station where the pollen trap was located. Since the main local atmospheric processes influencing pollen transport, such as convection, turbulence and storms (Orlanski, 1975) take place within this radius, most airborne pollen is likely to be originated in the mapped habitats. In this sense, although long distance transport can be responsible for some peaks, short-distance transport is responsible for the most important peaks of the pollen curve (Rojo and Pérez-Badía, 2015).

2.4. Estimation of pollen production

Pollen production for each species was estimated on the basis of data reported by Prieto-Baena et al. (2003) and Aboulaich et al. (2009). These works quantified the number of pollen grains from several parts of the

grasses (anthers, flowers, spikelets, inflorescences). We have used the variable “pollen grains/inflorescence” in order to value the pollen production.

The values of the parameter pollen production were grouped in four classes applying an interval classification based on natural breaks according to Jenks' method (Jenks, 1967) to the list of grass species included in the work of Prieto-Baena et al. (2003) (Fig. 2). This way, we obtain a wider classification for the parameter pollen production which can be applied for the species we study here as well as for other species that could be incorporated in other studies. Those species for which we do not have data on pollen production we have assigned the known value of pollen production of species that exhibit similar biological and morphological characteristics.

2.5. Pollen Contribution Index

The contribution of each species to overall airborne pollen concentrations was established by applying an index (PCI) comprising the following parameters: species phenology (VIP), pollen grain size with inverted classes (diameter, iD), pollen production (P) and surface area covered by the habitats studied (S), as a measure of the potential abundance of each species.

$$PCI = \frac{VIP \times iD^* \times P \times S}{128^{**}}$$
 where VIP = Variable Importance Projection of the species (mean for the two study years), iD = pollen diameter (mean of

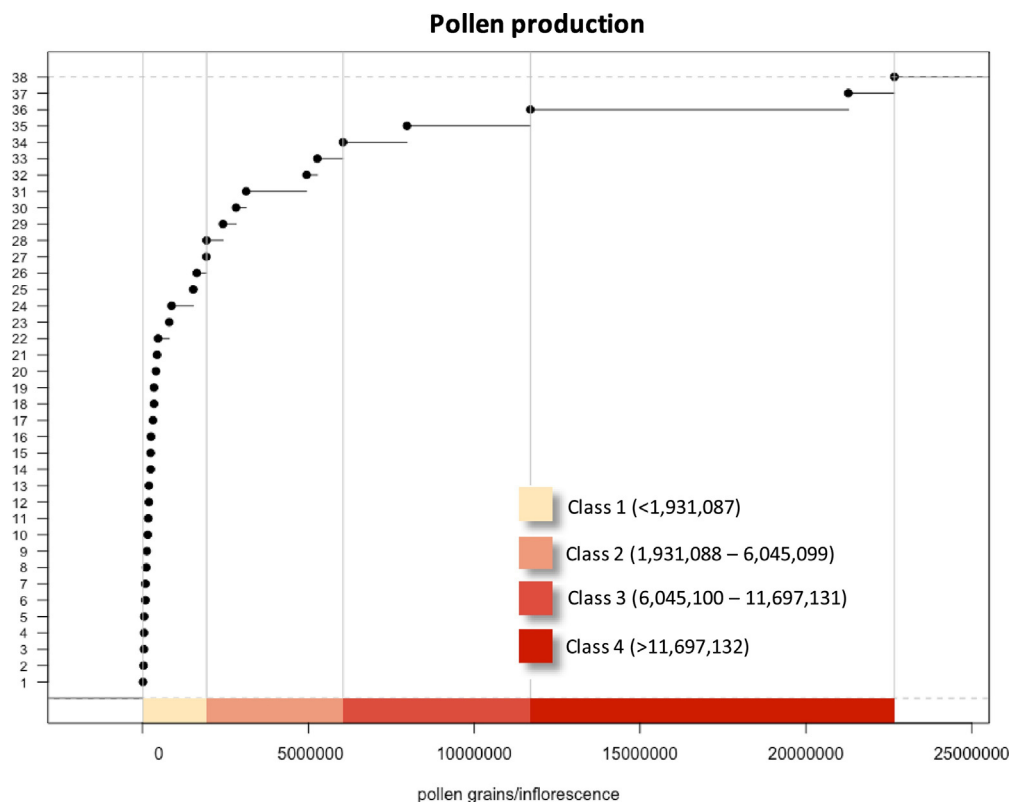


Fig. 2. Natural breaks (Jenks) method for classifying pollen production of bibliographic data from Prieto-Baena et al. (2003). These class intervals were used in order to calculate the Pollen Contribution Index (PCI). Species considered in Prieto-Baena et al. (2003): 1 *Vulpia myuros*, 2 *Hordeum marinum*, 3 *Brachypodium distachyon*, 4 *Lamarckia aurea*, 5 *Aegilops geniculata*, 6 *Aegilops triuncialis*, 7 *Desmazeria rigida*, 8 *Poa annua*, 9 *Bromus diandrus*, 10 *Rostraria cristata*, 11 *Stipa capensis*, 12 *Bromus lanceolatus*, 13 *Bromus matritensis*, 14 *Bromus hordeaceus*, 15 *Hordeum leporinum*, 16 *Phalaris paradoxa*, 17 *Briza maxima*, 18 *Paspalum paspalodes*, 19 *Avena sterilis*, 20 *Polypogon monspeliensis*, 21 *Panicum repens*, 22 *Avena barbata*, 23 *Phalaris minor*, 24 *Holcus lanatus*, 25 *Cynodon dactylon*, 26 *Elymus repens*, 27 *Holcus setiglumis*, 28 *Cynosurus echinatus*, 29 *Agrostis stolonifera*, 30 *Hyparrhenia hirta*, 31 *Vulpia geniculata*, 32 *Arrhenatherum album*, 33 *Lolium rigidum*, 34 *Piptatherum miliaceum*, 35 *Dactylis glomerata*, 36 *Festuca arundinacea*, 37 *Trisetaria panicea*, 38 *Sorghum halepense*.

polar and equatorial axes), P = pollen production, S = surface area covered by the habitats studied.

(*) Pollen grain size is inversely related to PCI. Thus the classes of this parameter (iD) have been inverted i.e. the highest category (class 4) includes pollen with the smaller size, and vice versa.

(**) This figure represents the maximum values attained by parameters $VIP \times iD \times P \times S$ ($2 \times 4 \times 4 \times 4$).

In this formula, VIP displayed values ranging between 0 and 2; in order to simplify calculations, values for pollen production, pollen grain size and surface area of habitat were transformed and reduced to four classes (values 1, 2, 3 and 4), using natural break method classification (Jenks' method) implemented in the R package "classInt" (Bivand, 2017), thus enabling the index to be standardised. Values for the index therefore ranged from 0 (i.e. no contribution by the species to airborne pollen concentrations) and 1 (i.e. maximum contribution). Finally, Principal Component Analysis (PCA) was carried out using R software (R Core Team, 2016) to examine the relationship between the parameters studied (i.e. phenology, pollen production, pollen grain size and species abundance) and to group species as a function of their contribution to the pollen concentrations shown by the pollen curve. Two principal components were used to relate the data.

3. Results

The flowering season of the grass species in the study area generally started in late March and ended in early July. Analysis of the flowering period for each species and its coincidence with pollen-curve peaks revealed a phenological succession (Fig. 3). The earliest-flowering grass species included *Hordeum marinum* subsp. *leporinum*, species of the genera *Vulpia*, *Bromus* and *Avena*, as well as *Cynosurus elegans* and

Echinaria capitata (Fig. 3). All these species flowered between the third week in March and the second week in May, coinciding with low to moderate peak airborne pollen concentrations (all peaks below 200 pollen grains/m³). From early May to late June, late-flowering species coincided with maximum airborne pollen concentrations, i.e. the most intense peaks several days exceed the threshold of 200 pollen grain/m³: these included *Lolium rigidum*, *Arrhenatherum album*, *Trisetum paniceum*, *Dactylis glomerata* subsp. *hispanica*, *Melica ciliata* and *Piptatherum miliaceum* (Fig. 3).

Modelling using PLS regression showed that flowering phenology accounted for 87% of variance in contribution to airborne pollen concentrations ($R^2 = 0.87$) in 2013 and for 89% ($R^2 = 0.89$) in 2015. Observed values correlated well with predicted values from the model according to the Kendal rank correlation (Fig. 4). Mean VIP values which indicate the importance for each grass species on the model of the airborne pollen concentrations are shown in Table 2. Therefore, the greatest VIP values are found for the species whose flowering-period coincided with the highest pollen peaks as indicated above (*Melica ciliata*, *Trisetum paniceum*, *Arrhenatherum album*...).

The most abundant habitat was annual herbaceous cropland, which accounted for 59.4% of the study area (Fig. 5). The main agricultural crop in this habitat was two-rowed barley (*Hordeum distichon*), while slender wild oats (*Avena barbata*) and ryegrass (*Lolium rigidum*) were the most abundant weed species in this area (Table 2). Woody cropland, accounting for 11.4%, also included a number of weed species. Ruderal communities, growing on abandoned arable land, in peri-urban areas and at roadsides, covered 4.4% of the study area and contained a considerable variety of species belonging to the genera *Hordeum*, *Bromus*, *Avena*, and *Trisetum*, among others. Forest vegetation comprised mostly sclerophyllous shrubland and scrub as well as xerophyllous pastureland,

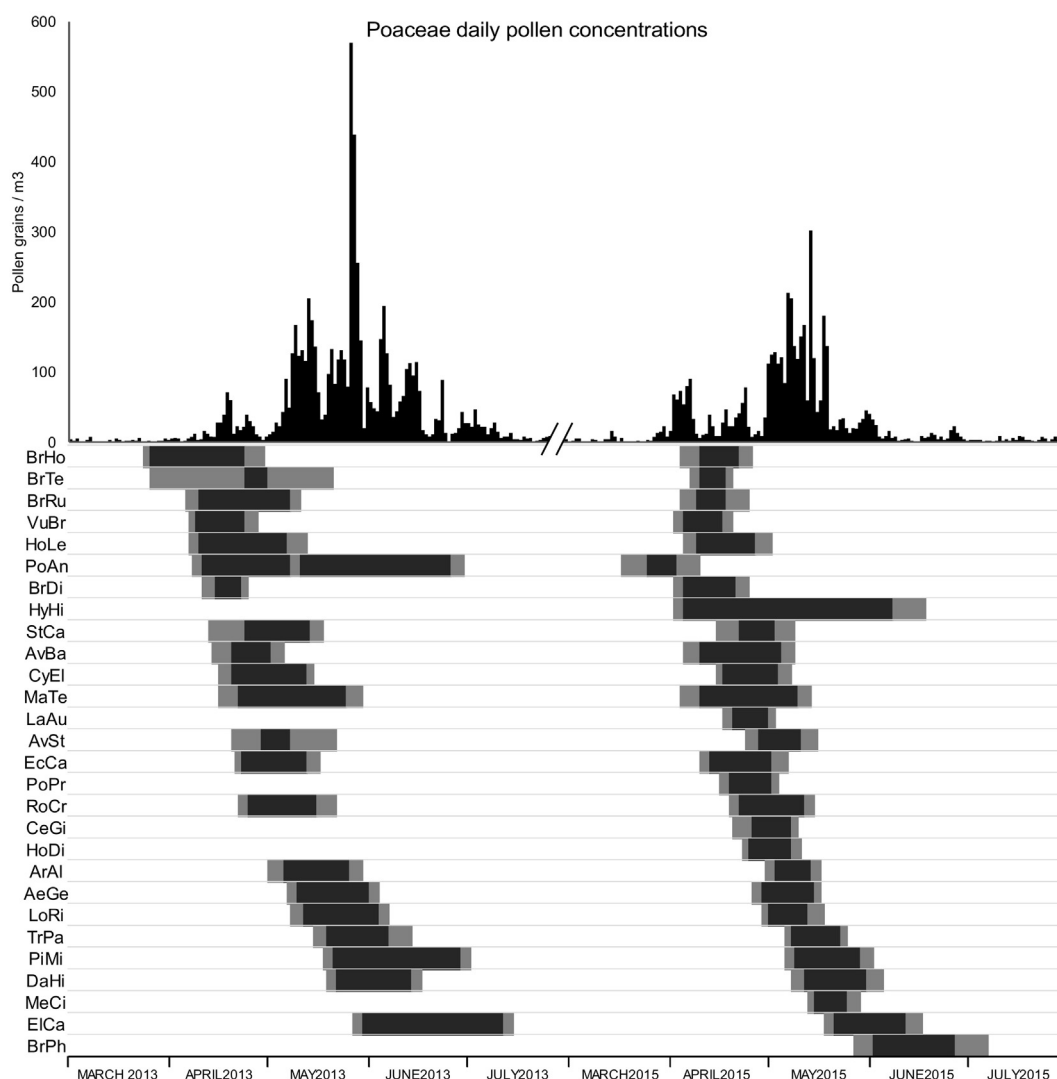


Fig. 3. Variations in daily grass-pollen concentrations (grains/m³) and floral phenology of grass species in 2013 and 2015. Floral phenology: light grey bar indicates 25–50% of individuals in flower; dark grey bar indicates >50% of individuals in flower. Abbreviations of sampled species: AeGe *Aegilops geniculata*, ArAl *Arrhenatherum album*, AvBa *Avena barbata*, AvSt *Avena sterilis*, BrPh *Brachypodium phoenicoides*, BrDi *Bromus diandrus*, BrHo *Bromus hordeaceus*, BrRu *Bromus rubens*, BrTe *Bromus tectorum*, CeGi *Celtica gigantea*, CyEl *Cynosurus elegans*, DaHi *Dactylis glomerata* subsp. *hispanica*, EcCa *Echinaria capitata*, ElCa *Elymus pungens* subsp. *campestris*, HoDi *Hordeum distichon*, HoLe *Hordeum murinum* subsp. *leporinum*, HyHi *Hyparrhenia hirta*, LaAu *Lamarckia aurea*, LoRi *Lolium rigidum*, MaTe *Macrochloa tenacissima*, MeCi *Melica ciliata* subsp. *magnolii*, PiMi *Piptatherum miliaceum*, PoAn *Poa annua*, PoPr *Poa pratensis*, RoCr *Rostraria cristata*, StCa *Stipa capensis*, TrPa *Trisetum paniceum*, VuBr *Vulpia bromoides*.

and accounted for 18.9% of the surface area studied (calcareous soils 14.8%; siliceous soils 4.1%). Grass species growing in these habitats (woodlands, scrubs and grasslands of the forest vegetation) included perennials such as *Macrochloa tenacissima* and *Celtica gigantea*, and annuals like *Echinaria capitata* and *Vulpia bromoides* (Table 2).

Riverbank vegetation was among the least common habitats, representing 1.5% of the surface area studied, while rocky ground accounted for 0.1% (Fig. 5). Given the surface area of the various types of habitat, the most abundant species in terms of potential habitat were weeds commonly found in cropland, and species characteristic of forest vegetation; surface areas in all cases exceeded 5473 ha (>5% of the total study area). (Fig. 6A).

Analysis of pollen grain size (Table 2) yielded a first group (class 1, inverted classes) of species with a pollen grain size >56.9 μm , a second group comprising species with pollen grains ranging between 44.0 and 56.8 μm , a third group with grain sizes of between 33.1 and 43.9 μm , and a fourth group of less 33.0 μm (Fig. 6B).

Revision of pollen production data (Table 2) showed that the species producing the largest amount of pollen was *Trisetum paniceum*, with 21,278,803 pollen grains per inflorescence, although

high pollen production was also recorded for other species, including *Arrhenatherum album*, *Dactylis glomerata* subsp. *hispanica*, *Hyparrhenia hirta*, *Lolium rigidum* and *Piptatherum miliaceum* (Prieto-Baena et al., 2003). The lowest pollen-production values were observed for studied species as *Vulpia bromoides*, *Lamarckia aurea*, *Aegilops geniculata*, *Poa annua*, *Bromus diandrus* or *Rostraria cristata* (Table 2). Interval classification for all grass species from Prieto-Baena et al. (2003) is shown in the Fig. 2.

The application of the Pollen Contribution Index (PCI) revealed that *Dactylis glomerata* subsp. *hispanica*, with a score of 0.34, makes the greatest contribution to the pollen curve (Fig. 7). Other species with a great contribution (PCI values over 0.20) were *Lolium rigidum*, *Trisetum paniceum* and *Arrhenatherum album*, followed by perennial species as *Macrochloa tenacissima*, *Piptatherum miliaceum* or *Hyparrhenia hirta*. The remaining species had low scores of <0.10 (Fig. 7).

The PCI, establishing the contribution of each study species to air-borne pollen concentrations (the pollen curve), enables species to be classified in terms of the four parameters used to calculate the index (phenology, abundance, pollen size and pollen production) (Fig. 8). It also enables the relationship between the parameters to be analysed.

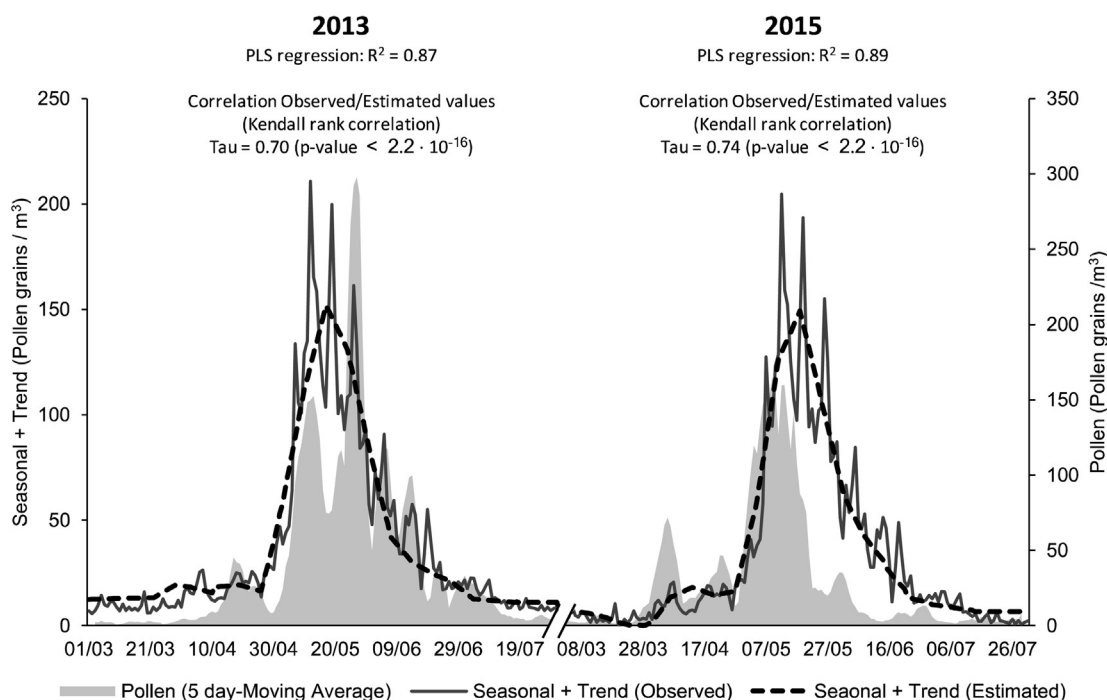


Fig. 4. Pollen data curve (seasonality + trend) with values estimated using a PLS regression model (R^2 coefficient of determination); Kendal rank correlation shows the relationship between observed values and estimated values for 2013 and 2015.

Phenology, which determined the coincidence of grass flowering with the highest airborne pollen concentrations, displayed a direct correlation with species pollen production but an inverse correlation with pollen grain size (Fig. 8).

4. Discussion

The grass pollen season is long and complex, often lasting 4 or 5 months (García-Mozo et al., 2009; Gücel et al., 2013), and sometimes as long as 7 months (Pérez-Badía et al., 2010). Moreover, as the present

study showed, plotting of airborne grass-pollen concentrations reveals numerous oscillations, though maximum concentrations in central Spain tend to be recorded in May and June (Pérez-Badía et al., 2011; Sabariego et al., 2011; Rojo et al., 2017). This pollen-curve behaviour makes it difficult to determine the main sources of pollen emission (Skjøth et al., 2013), since for any given study area there are contributions from numerous species with highly-varied phenological patterns (Rojo et al., 2017).

Phenology enabled airborne pollen concentrations to be modelled as a function of flowering intensity (measured here as the percentage of

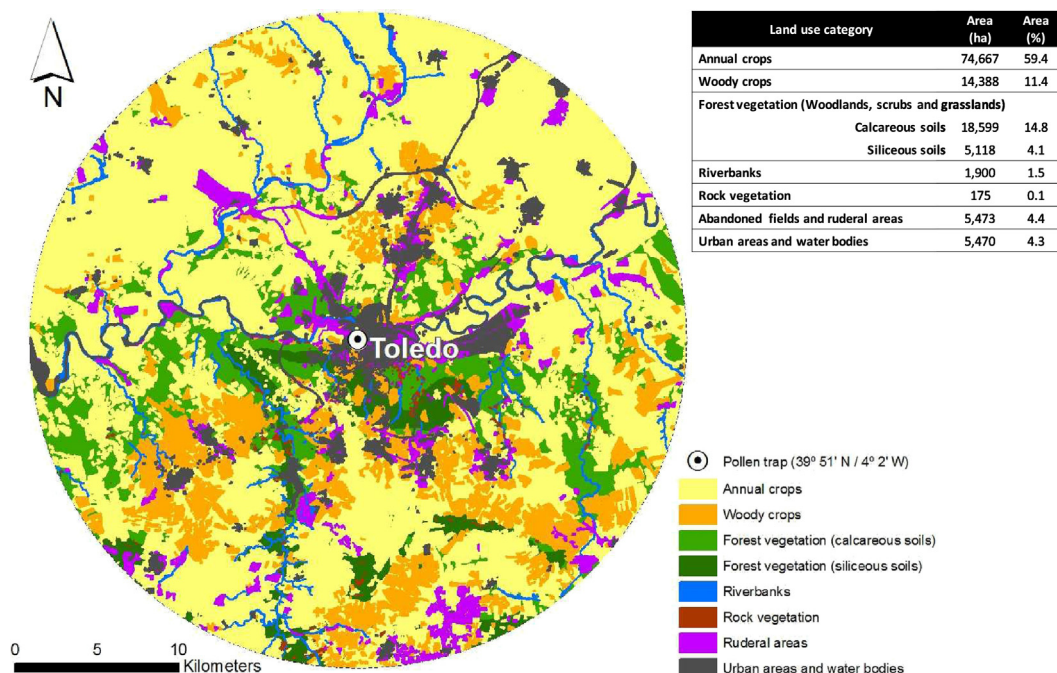


Fig. 5. Map of habitat types in the study area. Land use category according to the study area (125,790 ha).

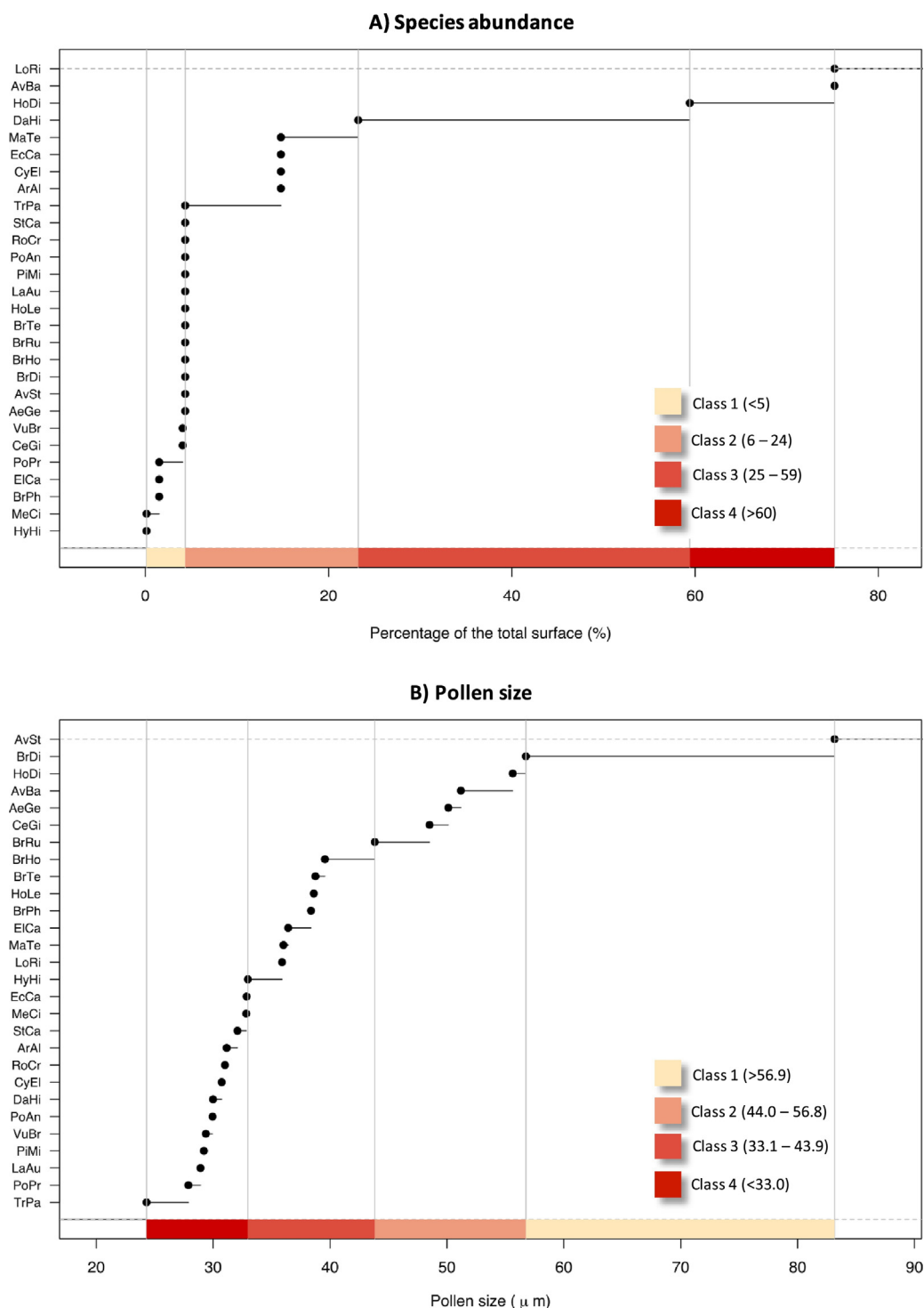


Fig. 6. Natural breaks (Jenks) method for classifying species abundance (A) and pollen size (B). The classes of the pollen size (B) have been inverted i.e. the highest category (class 4) includes pollen with the smaller size, and vice versa Abbreviations of species names are defined in Fig. 3.

individuals in flower) of the grass species most representative of the study area. Findings confirmed the close relationship between phenology and aerobiology, phenological data yielding a pollen curve (eliminating the random component) with a coefficient of determination approaching 0.9.

These results represent a further contribution to the study of the main grass species contributing to the pollen curve, complementing the findings reported by other authors (Frenguelli et al., 2010; León-Ruiz et al., 2011; Cebrino et al., 2016). The present study sought not only to analyse the relationship between grass phenology and

aerobiology, but also to investigate the characteristics of key species in terms of pollen emission rates and capacity for airborne pollen dispersal: pollen production and abundance of each species in the geographical area as a measure of potential pollen release into the atmosphere (Rojo et al., 2015b; Maya Manzano et al., 2017), and pollen grain size as an indicator of dispersibility (Oteros et al., 2017).

The resulting Pollen Contribution Index, applied here to grass species in a limited geographical area, offers a standardised tool for assessing the contribution of individual species to airborne pollen levels, and thus provides a valuable means of comparing the contribution of

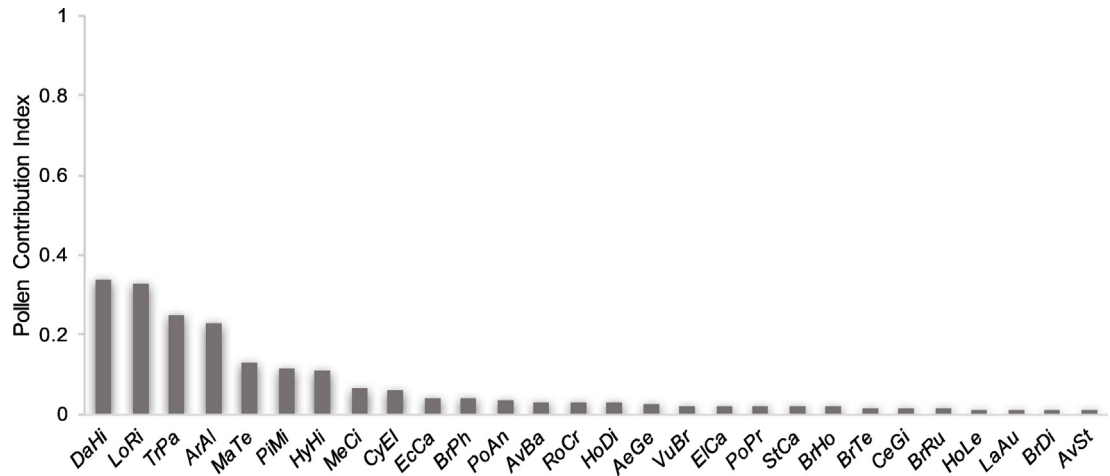


Fig. 7. Chart showing Pollen Contribution Index for the grass species studied. Abbreviations of species names are defined in Fig. 3.

each species to the pollen curve in periods when the flowering periods of several species coincide.

The highest airborne pollen concentrations coincided with the flowering-period of later-flowering species (between May and June), including *Aegilops geniculata*, *Arrhenatherum album*, *Avena sterilis*, *Dactylis glomerata* subsp. *hispanica*, *Hyparrhenia hirta*, *Lolium rigidum*,

Melica ciliata subsp. *magnolii*, *Poa annua* and *Trisetum paniceum*. Although the presence of any given grass species obviously depends on the biogeographical features of each study area, the findings broadly agree with those reported in southern Spain (León-Ruiz et al., 2011), Italy (Ghitarrini et al., 2017) and Austria (Kmenta et al., 2016), where the strongest correlation with pollen concentrations was observed for

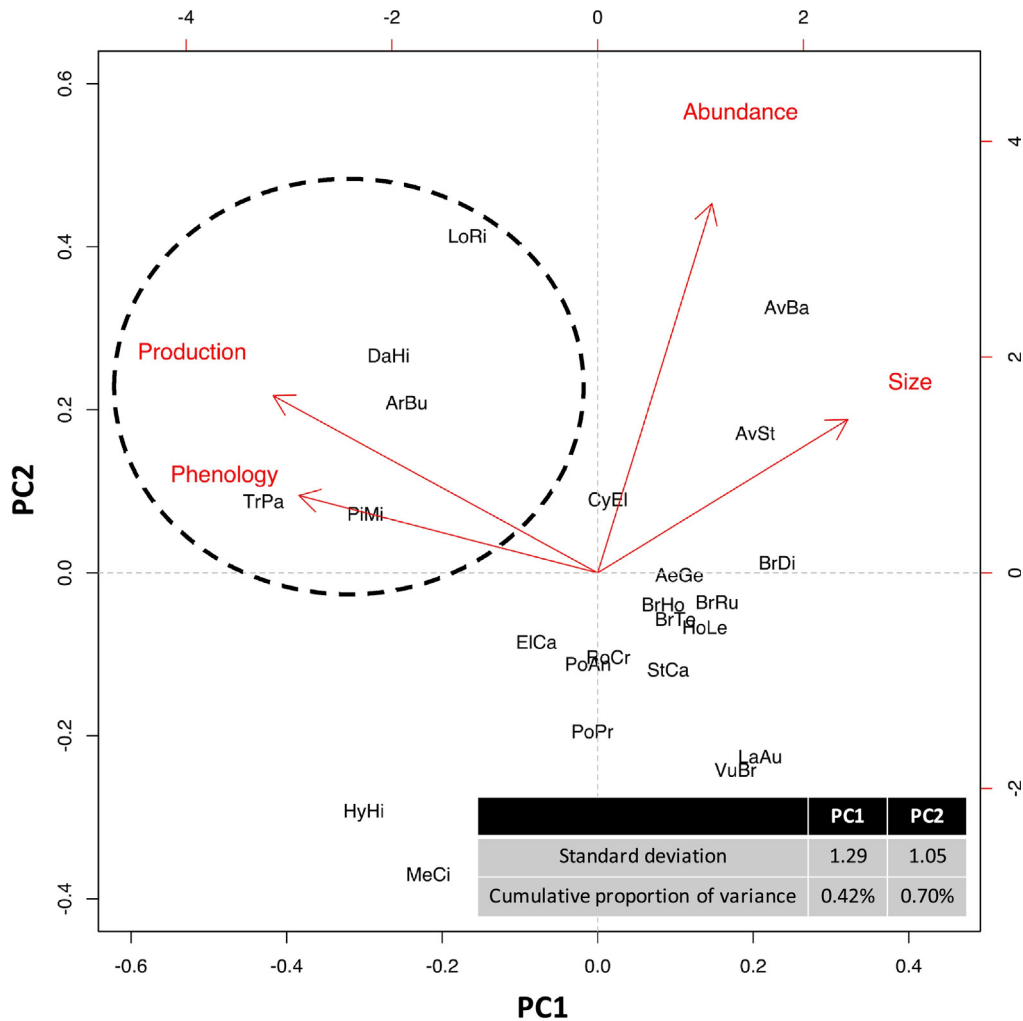


Fig. 8. Results of Principal Component Analysis (PC1 vs. PC2) taking into account the classification of the grass species with respect to phenology, species abundance, pollen grain size and pollen production. Abbreviations of species names are defined in Fig. 3.

the genera *Arrhenatherum*, *Dactylis*, *Lolium*, *Poa* and *Trisetum*, all species of the subfamily *Pooideae* (Subiza et al., 2003). However, application of the Pollen Contribution Index, incorporating data on abundance, pollen production and pollen grain size for each species, revealed that only a few species as *Dactylis glomerata* subsp. *hispanica*, *Lolium rigidum*, *Trisetum paniceum* or *Arrhenatherum album* was found to make a considerable contribution to the pollen curve.

The species releasing most pollen into the atmosphere share certain characteristics: high pollen production per individual, small pollen-grain size, and marked abundance in the study area (Cruden, 2000; Aboulaich et al., 2009). *Dactylis glomerata* subsp. *hispanica* is notable for its elevated pollen output (7,971,347 pollen grains per inflorescence, class 3; Prieto-Baena et al., 2003), its very small pollen-grain size (29.99 μm , class 4), its flowering period also coincides with the highest pollen-intensity peaks (VIP 1.49) although this species shows a moderate abundance (class 2). By contrast, *Avena sterilis* and *Aegilops geniculata* displayed a very low PCI score and are thus of negligible importance, although a phenological approach alone would indicate otherwise (Rojo et al., 2017).

The Pollen Contribution Index described here also enables a reduction of the bias generated in phenological sampling, or in the estimation of other parameters such as the indirect measurement of species abundance on the basis of habitat surface area, since these species are assigned interval or category values, which absorb errors of quantification.

A further contribution made by this study is the generation of a database on pollen-grain size and morphology for the grass species most representative of habitats in central Spain. This complements the information hitherto available for members of the Poaceae family (Jan et al., 2015; Morgado et al., 2015), and seems to confirm that in the Mediterranean region the shape and size of pollen grains is closely linked to their buoyancy (Rojo et al., 2016; Oteros et al., 2017).

The findings serve to confirm certain aspects regarding the grass-dominated habitat types constituting major sources of pollen emission and thus making the greatest contribution to airborne pollen concentrations. Aboulaich et al. (2009) argued that species growing in natural and semi-natural habitats produce more pollen than those growing in cropland and ruderal areas; this view is generally supported by the present findings, although with certain exceptions such as *Trisetum paniceum* and *Lolium rigidum*, which were found to produce large amounts of pollen. Pollen-grain size in species characteristic of urban, ruderal or cropland habitats, belonging to the genera *Aegilops*, *Avena*, *Bromus* and *Hordeum*, was classed as large (over 35 μm), thus hindering their airborne dispersal with respect to other species (Hernández-Ceballos et al., 2014). Bosch-Cano et al. (2011) and Rojo et al. (2015a) reported that pollen levels originating in rural areas were higher than those associated with urban areas. The present study included the crop species *Hordeum distichon*, growing widely in the study area. Though highly abundant, this species scored very low on the Pollen Contribution Index, having a large pollen grain (class 1) and low pollen production (class 2). As a result, the degree of coincidence with maximum pollen concentrations was also low (VIP 0.50). The application of PCI index to urban scale can be used to identify allergenic grass species of grasslands and lawns in the cities. Knowing these species, it would be possible to recommend the authorities and gardening and environmental services, not to use seeds of these species in the composition of the seed mix used in the grasslands and lawns in urban green spaces.

These findings point clearly to an inverse correlation between pollen production per individual and pollen-grain size, reported earlier by Cruden (2000) and attributed to a simple size/number trade-off (Charnov, 1982). This is of particular importance in terms of airborne pollen production, given the limited capacity of species with large pollen grains to produce abundant sufficiently-buoyant pollen (Subiza and Jeréz, 2002). Moreover, a number of species producing large pollen grains—among them *Hordeum*

distichon—reproduce by cleistogamy, a floral selfing mechanism through which pollen is transferred from anther to stigma within the closed flower, thus preventing the entry of external pollen (Campbell et al., 1983). This pollination mechanism also hinders pollen release and dispersal.

Another key factor is species morphology. As reported by Tormo et al. (2011), the species contributing most to airborne Poaceae pollen concentrations include numerous perennials. Pollen production per inflorescence is greater in perennials than in annuals (Prieto-Baena et al., 2003), although certain annual species such as *Trisetum paniceum* are known for their high inflorescence density (Aboulaich et al., 2009). Since pollen production is also directly linked to anther size, the most productive species are those with large anthers (Tormo et al., 1996), including the majority of perennials. High pollen production may ensure cross-fertilisation in perennial species, most of which display marked self-incompatibility (Prieto-Baena et al., 2003).

Identification of the grass species releasing the largest amount of pollen into the atmosphere, and measurement of their abundance in any given area, is crucial to establishing which periods constitute a real public health risk (Héguy et al., 2008) and to the planning of more effective campaigns for the prevention and treatment of allergies. At present, grass-pollen vaccines used in immunotherapy generally involve a mixture of pollens of various species. The most widely-used mixture is *Dactylis glomerata*, *Phleum pratense*, *Anthoxanthum odoratum*, *Lolium perenne* and *Poa pratensis*, these being the most common species; they are widespread throughout Europe, and are known for their allergen content (Jutel et al., 2005). Although a number of studies report that tests using any species belonging to the subfamily *Pooideae* are effective both for diagnosis and for immunotherapy (Johansen et al., 2009), different species-specific allergens prompt a differing degree of sensitisation in patients (Moingeon et al., 2008); symptoms may even be linked to the phenology of each species (Kmenta et al., 2016). In this sense, the results of the application of the Pollen Contribution Index (PCI) can be used to encourage new works to determine allergens from wild species little known or studied (such as *Lolium rigidum*, *Macrochloa tenacissima* or *Piptatherum miliaceum*) and to initiate specific immunotherapy treatments with the allergens of wild grass species that obtain the highest PCI values.

5. Conclusions

The Pollen Contribution Index for grass species presented here is a valuable tool for identifying the species releasing the largest amounts of pollen into the atmosphere, coinciding with periods of greatest allergy risk. The fact that a small number of species account for most of the airborne pollen concentrations recorded has important implications when drawing up recommendations for grass-allergy sufferers, in that it enables the main sources of pollen emission to be identified, and exposure to them avoided. Since calculation of the index is standardised, it allows the contributions of different grass species to be compared, and can readily be extended to other geographical areas of the Mediterranean region where the vegetation (natural or anthropic areas) is dominated by the same grass species or taxonomically close. The Pollen Contribution Index could also be usefully applied to other pollen types, especially those involving species whose flowering-periods overlap, such as *Plantago* and *Amaranthaceae*, which also comprise numerous and highly-diverse species.

Acknowledgements

The authors are grateful to all the members of the Castilla-La Mancha Aerobiology Network (AEROCAM) for their help in carrying out this study.

References

- Aboulaich, N., Bouziane, H., Kadiri, M., Del Mar Trigo, M., Riadi, H., Kazzaz, M., Merzouki, A., 2009. Pollen production in anemophilous species of the Poaceae family in Tetouan (NW Morocco). *Aerobiologia* 25, 27–38.
- Barbieri, R., Botarelli, L., Salsi, A., Zinoni, F., 1989. Guida Alle Rilevazioni Agrofologiche Ed Alla Compilazione Delle Schede di Rilevamento per le Colture Erbacee Ed Arboree.
- Bivand, R., 2017. classInt: choose univariate class intervals. R package version 0.1–24. <https://CRAN.R-project.org/package=classInt>. Accessed date: 28 July 2017.
- Blanca-Lopez, N., Campo, P., Salas, M., García, R.C., Palomares, F., Blanca, M., Canto, G., Feo, B.F., Rondon, C., 2016. Seasonal local allergic rhinitis in areas with high concentrations of grass pollen. *J. Investig. Allergol. Clin. Immunol.* 26, 83–91.
- Bosch-Cano, F., Bernard, N., Sudre, B., Gillet, F., Thibaudon, M., Richard, H., Badot, P.M., Ruffaldi, P., 2011. Human exposure to allergenic pollens: a comparison between urban and rural areas. *Environ. Res.* 111, 619–625.
- Brito, F.F., Gimeno, P.M., Carnés, J., Fernández-Caldas, E., Lara, P., Alonso, A.M., García, R., Guerra, F., 2010. Grass pollen, aeroallergens, and clinical symptoms in Ciudad Real, Spain. *J. Investig. Allergol. Clin. Immunol.* 20, 295–302.
- Burbach, G.J., Heinzerling, L.M., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., Bousquet, J., Bousquet-Rouanet, L., Bousquet, P.J., Bresciani, M., 2009. GA(2)LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. *Allergy* 64, 1507–1515.
- Campbell, C.S., Quinn, J.A., Cheplick, G.P., Bell, T.J., 1983. Cleistogamy in grasses. *Annu. Rev. Ecol. Syst.* 14, 411–441.
- Cebriño, J., Galán, C., Domínguez-Vilches, E., 2016. Aerobiological and phenological study of the main Poaceae species in Córdoba City (Spain) and the surrounding hills. *Aerobiologia* 32, 595–606.
- Charnov, E.L., 1982. The theory of sex allocation. *Monogr. Popul. Biol.* 18, 1–355.
- Coca, A.F., Thommen, A.A., Walzer, M., 1931. Asthma and hay fever in theory and practice. Thomas, Springfield.
- Cruden, R.W., 2000. Pollen grains: why so many? *Plant Syst. Evol.* 222 (1), 143–165.
- Dafni, A., 1992. *Pollination Ecology: A Practical Approach*. Oxford University Press, Oxford, New York (250 pp.).
- D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., Van Cauwenberge, P., 2007. Allergenic pollen and pollen allergy in Europe. *Allergy* 62 (9), 976–990.
- Devesa Alcaraz, J.A., Ruiz Tellez, T., Viera Benítez, M.d.C., Ortega Olivencia, A., Tormo Molina, R., Carrasco Claver, J.P., 1991. Las gramíneas de Extremadura. Universidad de Extremadura-Servicio de Publicaciones.
- Faegri, K., 1978. What is the polar axis? *Grana* 17 (1), 15–16.
- Frenguelli, G., Passalacqua, G., Bonini, S., Fiocchi, A., Incorvaia, C., Maruccci, F., Tedeschini, E., Canonica, G.W., Frati, F., 2010. Bridging allergologic and botanical knowledge in seasonal allergy: a role for phenology. *Ann. Allergy Asthma Immunol.* 105, 223–227.
- Friedman, J., Barrett, S.C.H., 2009. Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Ann. Bot.* 103, 1515–1527.
- Galán, C., Cariñanos, P., Alcázar, P., Domínguez, E., 2007. Spanish Aerobiology Network (REA): Management and Quality Manual.
- García-Mozo, H., Galán, C., Belmonte, J., Bermejo, D., Candau, P., Díaz de la Guardia, C., Elvira, B., Gutiérrez, M., Jato, V., Silva, I., Trigo, M.M., Valencia, R., Chuine, I., 2009. Predicting the start and peak dates of the Poaceae pollen season in Spain using process-based models. *Agric. For. Meteorol.* 149, 256–262.
- Ghitarrini, S., Galán, C., Frenguelli, G., Tedeschini, E., 2017. Phenological analysis of grasses (Poaceae) as a support for the dissection of their pollen season in Perugia (Central Italy). *Aerobiologia* <http://dx.doi.org/10.1007/s10453-017-9473-7>.
- Gucel, S., Guvensen, A., Ozturk, M., Celik, A., 2013. Analysis of airborne pollen fall in Nicosia (Cyprus). *Environ. Monit. Assess.* 185, 157–169.
- Héguyl, L., Garneau, M., Goldberg, M.S., Raphoz, M., Guay, F., Valois, M.F., 2008. Associations between grass and weed pollen and emergency department visits for asthma among children in Montreal. *Environ. Res.* 106, 203–211.
- Heinzerling, L.M., Burbach, G.J., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., et al., 2009. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 64, 1498–1506.
- Hernández-Ceballos, M.A., Skjøth, C.A., García-Mozo, H., Bolívar, J.P., Galán, C., 2014. Improvement in the accuracy of back trajectories using WRF to identify pollen sources in southern Iberian Peninsula. *Int. J. Biometeorol.* 58, 2031–2043.
- Hirst, J.M., 1952. An automatic volumetric spore trap. *Ann. Appl. Biol.* 39, 257–265.
- Jan, F., Schüler, L., Behling, H., 2015. Trends of pollen grain size variation in C3 and C4 Poaceae species using pollen morphology for future assessment of grassland ecosystem dynamics. *Grana* 54, 129–145.
- Jenks, G.F., 1967. The data model concept in statistical mapping. *Int. Yearb. Cartography* 7, 186–190.
- Johansen, N., Weber, R.W., Ipsen, H., Barber, D., Broge, L., Hejl, C., 2009. Extensive ige cross-reactivity towards the Pooideae grasses substantiated for a large number of grass-pollen-sensitized subjects. *Int. Arch. Allergy Immunol.* 150, 325–334.
- Judd, W.S., Campbell, C.S., Kellogg, E.A., Teven, P.F., 2002. *Plant Systematics: A Phylogenetic Approach*. 2nd edition. Sinauer (464 pp.).
- Jutel, M., Jaeger, L., Suck, R., Meyer, H., Fiebig, H., Cromwell, O., 2005. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J. Investig. Allergol. Clin. Immunol.* 116, 608–613.
- Kleine-Tebbe, J., 2008. One grass or many? Does allergy distinguish among species? *Drugs Today* 44, 65–67.
- Kmenta, M., Bastl, K., Kramer, M.F., Hewings, S.J., Mwange, J., Zetter, R., Berger, U., 2016. The grass pollen season 2014 in Vienna: a pilot study combining phenology, aerobiology and symptom data. *Sci. Total Environ.* 566–567, 1614–1620.
- León-Ruiz, E., Alcázar, P., Domínguez-Vilches, E., Galán, C., 2011. Study of Poaceae phenology in a Mediterranean climate. Which species contribute most to airborne pollen counts? *Aerobiologia* 27, 37–50.
- Mäkelä, E.M., 1996. Size distinctions between Betula pollen types - a review. *Grana* 35, 248–256.
- Maya Manzano, J.M., Tormo Molina, R., Fernández Rodríguez, S., Silva Palacios, I., Gonzalo Garijo, Á., 2017. Distribution of ornamental urban trees and their influence on airborne pollen in the SW of Iberian Peninsula. *Landsc. Urban Plan.* 157, 434–446.
- Mehmood, T., Liland, K.H., Snipen, L., Sæbø, S., 2012. A review of variable selection methods in Partial Least Squares Regression. *Chemom. Intell. Lab. Syst.* 118, 62–69.
- Meier, U., 2001. Growth Stages of Mono- and Dicotyledonous Plants. BBCH Monograph. 2nd ed. Federal Biological Research Centre for Agriculture and Forestry.
- Moingeon, P., Hrabina, M., Bergmann, K.C., Jaeger, S., Frati, F., Bordas, V., Peltre, G., 2008. Specific immunotherapy for common grass pollen allergies: pertinence of a five grass pollen vaccine. *Int. Arch. Allergy Immunol.* 146, 338–342.
- Morgado, L.N., Gonçalves-Esteves, V., Resendes, R., Ventura, M.A.M., 2015. Pollen morphology of Poaceae (Poales) in the Azores, Portugal. *Grana* 54, 282–293.
- Orlanski, I., 1975. A rational subdivision of scales for atmospheric processes. *Bull. Am. Meteorol. Soc.* 56, 527–530.
- Oteros, J., García-Mozo, H., Alcázar, P., Belmonte, J., Bermejo, D., Boi, M., Cariñanos, P., Díaz de la Guardia, C., Fernández-González, D., González-Minero, F., Gutiérrez-Bustillo, A.M., Moreno-Grau, S., Pérez-Badía, R., Rodríguez-Rajo, F.J., Ruiz-Valenzuela, L., Suárez-Pérez, J., Trigo, M.M., Domínguez-Vilches, E., Galán, C., 2015. A new method for determining the sources of airborne particles. *J. Environ. Manag.* 155, 212–218.
- Oteros, J., Valencia, R.M., del Río, S., Vega, A.M., García-Mozo, H., Galán, C., Gutiérrez, P., Mandrioli, P., Fernández-González, D., 2017. Concentric Ring Method for generating pollen maps. Quercus as case study. *Sci. Total Environ.* 576, 637–645.
- Pereira, C., Valero, A., Loureiro, C., Dávila, I., Martínez-Cócera, C., Murio, C., Rico, P., Palomino, R., 2006. Iberian study of aeroallergens sensitisation in allergic rhinitis. *Eur. Ann. Allergy Clin. Immunol.* 38, 186–194.
- Pérez-Badía, R., Rapp, A., Morales, C., Sardinero, S., Galán, C., García-Mozo, H., 2010. Pollen spectrum and risk of pollen allergy in central Spain. *Ann. Agric. Environ. Med.* 17, 139–151.
- Pérez-Badía, R., Rapp, A., Vaquero, C., Fernández-González, F., 2011. Aerobiological study in east-central Iberian Peninsula: pollen diversity and dynamics for major taxa. *Ann. Agric. Environ. Med.* 18, 99–111.
- Petrovavlovskikh, I., Evans, R., McConville, G., Manney, G.L., Rieder, H.E., 2015. The influence of the North Atlantic Oscillation and El Niño-Southern Oscillation on mean and extreme values of column ozone over the United States. *Atmos. Chem. Phys.* 15, 1585–1598.
- Prieto-Baena, J.C., Hidalgo, P.J., Domínguez, E., Galán, C., 2003. Pollen production in the Poaceae family. *Grana* 42, 153–160.
- R Core Team, 2016. A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria <http://www.R-project.org/>. Accessed date: 11 May 2016.
- Rakotomalala, R., 2005. TANAGRA: une plate-forme d'expérimentation pour la fouille de données. 32. Revue MODULAD, pp. 70–85.
- Rivas-Martínez, S., Díaz, T., Fernández-González, F., Izco, J., Loidi, J., Lousa, M., Penas, A., 2002. Vascular plant communities of Spain and Portugal. Addenda to the syntaxonomical checklist of 2001. *Itiner. Geobot.* 15 (1–2), 5–922.
- Rojo, J., Pérez-Badía, R., 2014. Effects of topography and crown-exposure on olive tree phenology. *Trees Struct. Funct.* 28, 449–459.
- Rojo, J., Pérez-Badía, R., 2015. Spatiotemporal analysis of olive flowering using geostatistical techniques. *Sci. Total Environ.* 505, 860–869.
- Rojo, J., Rapp, A., Lara, B., Fernández-González, F., Pérez-Badía, R., 2015a. Effect of land uses and wind direction on the contribution of local sources to airborne pollen. *Sci. Total Environ.* 538, 672–682.
- Rojo, J., Salido, P., Pérez-Badía, R., 2015b. Flower and pollen production in the 'Cornicabra' olive (*Olea europaea* L.) cultivar and the influence of environmental factors. *Trees Struct. Funct.* 29, 1235–1245.
- Rojo, J., Orlandi, F., Pérez-Badía, R., Aguilera, F., Ben Dhiab, A., Bouziane, H., Díaz de la Guardia, C., Galán, C., Gutiérrez-Bustillo, A.M., Moreno-Grau, S., Msallem, M., Trigo, M.M., Fornaciari, M., 2016. Modeling olive pollen intensity in the Mediterranean region through analysis of emission sources. *Sci. Total Environ.* 551–552, 73–82.
- Rojo, J., Rivero, R., Romero-Morte, J., Fernández-González, F., Pérez-Badía, R., 2017. Modeling pollen time series using seasonal-trend decomposition procedure based on LOESS smoothing. *Int. J. Biometeorol.* 61, 335–348.
- Sabariego, S., Pérez-Badía, R., Bouso, V., Gutiérrez, M., 2011. Poaceae pollen in the atmosphere of Aranjuez, Madrid and Toledo (central Spain). *Aerobiologia* 27, 221–228.
- Scaparrota, A., Verini, M., Consilvio, N.P., Cingolani, A., Rapino, D., Attanasi, M., Cerasa, M., Di Pillo, S., Chiarelli, F., 2013. Sensitization to timothy grass pollen allergenic molecules in children. *Multidiscip. Respir. Med.* 8 (1), 17.
- Schüler, L., Behling, H., 2011. Characteristics of Poaceae pollen grains as a tool to assess palaeoecological grassland dynamics in South America. *Veg. Hist. Archaeobotany* 20, 97–108.
- Simon, B.K., Clayton, W.D., Harman, K.T., Vorontsova, M.S., Brake, I., Healy, D., Alfonso, Y., 2017. Grassworld. <http://grassworld.myspecies.info>. Accessed date: 22 February 2017.
- Skjøth, C.A., Ørby, P.V., Becker, T., Geels, C., Schlünssen, V., Sigsgaard, T., Bønløkke, J.H., Sommer, J., Søgaard, P., Hertel, O., 2012. Identifying urban sources as cause of elevated grass pollen concentrations using GIS and remote sensing. *Biogeosciences* 10, 541–554.

- Skjøth, C.A., Šikoparija, B., Jäger, S., Ean, N., 2013. Pollen sources. In: Sofiev, M., Bergmann, K. (Eds.), *Allergenic Pollen: A Review of the Production, Release, Distribution and Health Impacts*. Springer, pp. 9–27.
- Subiza, J., Jeréz, M., 2002. Gramíneas. In: Valero, A.L., Cadahia, A. (Eds.), *Polinosis. Polen y alergia*. SEIAC, pp. 25–40.
- Subiza, F.J., Pola, J., Feo, F., Moral, A.J., 2003. Pólenes de interés en alergología en nuestro medio. In: Peláez, A., Dávila, I.J. (Eds.), *Tratado de Alergología*. Ergon, pp. 425–446.
- Thomasson, J. (Ed.), 1988. *International Symposium on Grass Systematics and Evolution*, Washington, DC (USA), 27–31 Jul 1986. Smithsonian Institution Press.
- Tormo, R., Muñoz, A., Silva, I., Gallardo, F., 1996. Pollen production in anemophilous trees. *Grana* 35, 38–46.
- Tormo, R., Silva, I., Gonzalo, Á., Moreno, A., Pérez, R., Fernández, S., 2011. Phenological records as a complement to aerobiological data. *Int. J. Biometeorol.* 55, 51–65.
- Weger, L.A., Bergmann, K.C., Rantio-Lehtimäki, A., Dahl, A., Buters, J., Déchamp, C., Belmonte, J., Thibaudon, M., Cecchi, L., Besancenot, J.P., Galán, C., Waisel, Y., 2013. Impact of pollen. In: Sofiev, M., Bergmann, K. (Eds.), *Allergenic Pollen: A Review of the Production, Release, Distribution and Health Impacts*. Springer, pp. 161–215.
- Westritschnig, K., Horak, F., Swoboda, I., Balic, N., Spitzauer, S., Kundi, M., Fiebig, H., Suck, R., Cromwell, O., Valenta, R., 2008. Different allergenic activity of grass pollen allergens revealed by skin testing. *Eur. J. Clin. Investig.* 38, 260–267.