



# Standardised index for measuring atmospheric grass-pollen emission



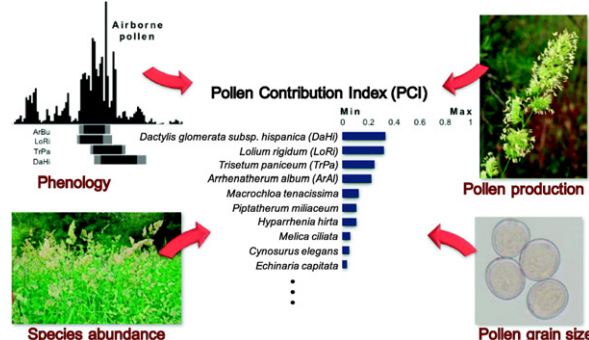
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## HIGHLIGHTS

- 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 1 September 2017

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.

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## 1. Introduction

Grass pollen poses a major health risk due to its elevated allergenic potential (Westritschnig et al., 2008; Scaparrotta 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

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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 (Skjøth 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-Martínez 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 (León-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 (Pérez-Badia 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 León-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 (Skjøth 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 Jeréz, 2002). Research into grass pollen grain size and morphology has hitherto focused on phylogenetic and ecological considerations (Schüler 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 (Galán 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 León-Ruiz et al. (2011): 25 individuals/m<sup>2</sup> were selected for annuals and 25 individuals/10 m<sup>2</sup> 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 Pérez-Badia, 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).

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