



Meteorological factors driving airborne grass pollen concentration in central Iberian Peninsula

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Abstract Grasses are a taxonomic group of considerable environmental importance, playing a major ecological role as well as making a significant contribution to the world's economy, since they include valuable crop and pasture species. Grass pollen allergens are also among the main causes of respiratory allergies worldwide. The interpretation of airborne grass pollen concentrations is a particularly complex task, given the marked diversity of pollen-emitting species and the influence of weather-related variables. This paper examines the influence of meteorological variables on flowering in the species contributing most to airborne grass pollen concentrations. This study was carried out in the surroundings of the city of Toledo (Spain), a Mediterranean city located in the center of the Iberian Peninsula. Temperature was the variable most influencing flowering onset, which was also affected by relative humidity, rainfall and hours of sunlight. The flowering period of grass species that flower at the start of spring (*Bromus rubens* and *Hordeum leporinum*) began earlier in years with higher mean winter temperatures, while the

species that flower from mid to late spring (*Trisetaria panicea* and *Dactylis glomerata* subsp. *hispanica*), the flowering period began earlier in years with higher cumulative rainfall in winter and spring, which were also the years with the highest mean temperatures. Research into the influence of weather-related variables on grass phenology can shed important light on variations in airborne pollen concentrations, which determine the potential period of exposure for allergy sufferers.

Keywords Phenology · Grasses · Meteorological variables · Weather · Pollen exposure · Poaceae

1 Introduction

Grasses form a taxonomic group of species growing in abundance over a wide range of different habitats and dominate the grasslands which cover over one-fifth of the earth's surface (Simon et al. 2011; Soreng et al. 2015, 2017). This group, which includes both cereal crops and pasture species, is the most economically important of all the plant families (Wiersema and León 2016). Grasses are significant not only in ecological and socioeconomic terms, but also in terms of public health. Their pollen is a major cause of hay fever in Europe and elsewhere (García-Mozo 2017; Larsson et al. 2016; Zeb et al. 2018), due to the

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elevated allergenic potential of grass pollen proteins (Scaparrota et al. 2013; Westritschnig et al. 2008), the abundance and widespread distribution of grass species (Nyffeler 2003; Rivas-Martínez et al. 2002; Watson and Dallwitz 1992) and the high rates of pollen emission into the atmosphere (Aboulaich et al. 2009; Romero-Morte et al. 2018). In Europe, between 20 and 35% of the population suffer from grass pollen allergy, although allergy rates differ between countries (D'Amato et al. 2007; De Weger et al. 2013; García-Mozo 2017; Larsson et al. 2016). In some areas of Spain, for example, up to 80% of the population displaying allergic reactions are sensitized to grass pollen allergens (Pereira et al. 2006).

Aerobiological and phenological research has focused on identifying those species which contribute most to the airborne pollen load, and more particularly on those whose flowering period coincides with the highest airborne pollen concentrations (e.g., Cebriño et al. 2018; Ghitarrini et al. 2017; Kmenta et al. 2017; Rojo et al. 2017). The species most commonly responsible for allergic sensitization in Europe include members of the genera *Phleum*, *Poa*, *Dactylis*, *Trisetaria*, *Festuca*, *Holcus*, *Anthoxanthum* and *Lolium*, all belonging to the subfamily *Pooideae* (Hejl et al. 2009; Marcucci et al. 2010), which produce the airborne allergenic pollen grains prompting symptoms in allergy sufferers (Andersson and Lidholm 2003).

In Mediterranean areas, species like *Dactylis glomerata*, *Lolium* sp. pl., *Poa* sp. pl. and *Trisetaria panicea* are among those contributing most to airborne pollen concentrations (Prieto-Baena et al. 2003; Ghitarrini et al. 2017; León-Ruiz et al. 2011). However, a larger number of species make a moderate or small contribution in the course of the grass pollen season, among them *Bromus rubens*, *Avena sterilis* and *Hordeum murinum* subsp. *leporinum* (also members of the subfamily *Pooideae*) (Romero-Morte et al. 2018).

The Poaceae pollen season is the result of the flowering of a great diversity of species sharing this pollen type; the consecutive flowering of different grass species in any given territory leads to a long pollen season (Brennan et al. 2019; Rojo et al. 2017). As a result, airborne grass pollen concentrations depend to a great extent on the type of local vegetation and land use, which together govern the abundance and distribution of grass species (Bosch-Cano et al. 2011). At the same time, however, climate variables

strongly influence the phenological development of grass species (García-Mozo et al. 2010b) and thus also determine pollen emission patterns.

Temperature plays a key role in the regulation of flowering in herbaceous species such as temperate grasses, although other variables—including photoperiod and rainfall—are also involved (Heide 1994). Water availability may also delay or advance certain phenological phases in herbaceous plants, especially in Mediterranean climates, where water stress may lead to phenological dormancy, depending when it occurs (Clary et al. 2004). However, physiological and biological differences between species may give rise to different responses to given environmental stimuli (García-Mozo 2017).

Moreover, weather conditions during anther ripening have a crucial effect on pollen emission rates and thus strongly influence airborne pollen concentrations from wind-pollinated species such as grasses. High temperatures during anthesis, coupled with environmental xericity (absence of rainfall and low relative humidity), favor anther dehiscence and thus increase pollen release into the atmosphere (Aboulaich et al. 2013; Brighetti et al. 2014).

The study of the life cycle of grasses with respect to weather conditions will help to assess potential future variations resulting from climate change, which may prompt modifications in reproductive biology, in biomass production cycles and in species dominance patterns. Such modifications may be of particular ecological importance in grasses, given their wide range of ecological functions: grasses not only act as major carbon sinks but are also involved in regulating biogeochemical cycles and reducing soil erosion, as well as being key sources of biodiversity and offering many other ecosystem services (Bengtsson et al. 2019; Munson and Long 2017; Ziello et al. 2012). Moreover, any change in the grass reproductive cycle may have significant clinical consequences, since it may affect pollen emission patterns and thus cause changes in the pollen season and in aeroallergen exposure patterns (Beggs 2004).

In this context, a number of studies have examined correlations between grass pollen concentrations and the behavior of weather-related variables both during the pollen season and over the months prior to it (e.g., García-Mozo et al. 2010a; Recio et al. 2010). However, given the great complexity of the grass pollen season, little attention has been paid to the particular

influence of meteorological variables on each individual grass species as a function of its contribution to the airborne pollen curve. A better understanding of that influence would be of great value for a more accurate interpretation of airborne pollen levels. The present study sought to determine the influence of certain weather-related variables (temperature, hours of sunlight and rainfall), both during and prior to flowering, on the phenological development of several Mediterranean grass species of particular interest due to their considerable contribution to airborne pollen concentrations.

2 Materials and methods

2.1 Phenological study

The reproductive phenology of grass species was studied at 10 sampling sites located around the city of Toledo (Supplementary material Fig. S1) over a 5-year period: 2013, 2015, 2016, 2017 and 2018 (Supplementary material Fig. S2). Flowering was studied in a total of 29 grass species (Fig. 2, Supplementary material Fig. S2), selecting at each sampling site the most abundant and most representative species, following the criteria recommended by León-Ruiz et al. (2011): 25 individuals/m² were selected for annuals and 25 individuals/10 m² for perennials.

Phenological observations were recorded weekly from March to July in 25 individuals for each species at each sampling site, following Barbieri (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-Badía 2014).

The phenological variable used for analysis was percentage of individuals in flower at each sampling time over the study period. The date of flowering onset was defined as the date on which the number of individuals in flower exceeded 25% (Rojo et al. 2017). The boxplot analyses showed phenological values per species and per site. However, for the analyses per

species (relationship between phenology of species and the meteorological variables) a single average phenological value was calculated for each species using a principal factor analysis to reduce dimensionality of the phenological data (Romero-Morte et al. 2018). This procedure allowed a weighted average value for each species to be computed over all sites.

2.2 Aerobiological study

Airborne pollen concentrations were recorded over a 14-year period (2005–2018) using a Hirst-type volumetric sampler (Hirst 1952) located in the city of Toledo, central Spain (coordinates: 39° 51' 55" N, 4° 2' 31" W). Sample collection and preparation and pollen concentration measurements were carried out using standard aerobiological methods (Galán et al. 2014). Pollen season start and end dates were calculated using a method which includes 90% of seasonal total pollen amounts, starting on the day by which 5% of total pollen has been recorded and ending on the day by which 95% of total pollen has been registered (Nilsson and Persson 1981).

2.3 Meteorological data

The city of Toledo has a Mediterranean climate, with low rainfall occurring mainly in spring and late autumn, and a marked period of summer drought (AEMET 2012). Winter temperatures are low, often falling below 0 °C, while summer temperatures are high, sometimes exceeding 40 °C (AEMET 2012).

The following weather-related parameters were monitored in a daily time resolution: maximum, minimum and average temperature (°C); rainfall (mm), relative humidity (%) and hours of sunlight. Meteorological data were provided by the Toledo city weather station (coordinates: 39° 53' 5" N, 4° 2' 43" W) belonging to the Spanish Meteorological Agency (AEMET) and located 2 km from the pollen trap.

2.4 Statistical analysis

The phenological status (start, end and length of the flowering period) of each grass species studied over the five-year period was plotted against the pollen curve. This pollen curve shows the mean daily concentrations over the period studied for field phenology (2013, 2015–2018).

The influence of weather-related variables on phenological status was studied in two early-flowering grass species –*Bromus rubens* and *Hordeum murinum* subsp. *leporinum*– and two late-flowering species –*Trisetaria panicea* and *Dactylis glomerata* subsp. *hispanica*. These species were selected due to their presence both at the largest number of sampling sites and over the largest number of sampling years (Supplementary material Fig. S2).

Correlations between the pollen season start date and weekly meteorological variables were compared and analyzed by statistical testing (Spearman's correlation test). The influence of accumulated values for weather-related variables (rainfall, maximum temperature and hours of sunlight) on the phenological status of selected species was analyzed, using values for accumulated maximum temperature between 1 January and flowering onset (expressed as Heat Units), following Jato et al. (2015).

All statistical analyses were performed using R software (R Core Team. 2018), and aerobiological databases were managed using the AeRobiology package (Rojo et al. 2019).

3 Results

The overall flowering period of the grass species studied here started in late March and ended in early July (Fig. 1). The flowering period of each species and the amounts recorded in successive pollen curve peaks indicate the phenological succession of all the grass species studied (Fig. 1). A first group comprised the earliest flowering species, in which flowering lasted from the third week of March (Day of Year DOY 75) to the end of April (DOY 120) and was associated with pollen peaks or concentrations which might be deemed low to moderate; this group included *Hordeum murinum* subsp. *leporinum*, *Vulpia ciliata* and a number of species of the genus *Bromus* (Fig. 1). A second group comprised species flowering between late May (DOY 121) and late June (DOY 180), a period coinciding with the highest airborne pollen concentrations or most intense peaks in the pollen curve; species included *Lolium rigidum*, *Arrhenatherum album*, *Trisetaria panicea*, *Dactylis glomerata* subsp. *hispanica*, *Melica ciliata* and *Piptatherum miliaceum*. However, there were some intermediate flowering species such as *Macrochloa tenacissima*,

Celtica gigantea, *Hyparrhenia sinaica* or *Rostraria cristata*, among other species, as the grass pollen season responds to a flowering succession of species rather than discrete groups of grasses. Also, perennial species such as *Hyparrhenia hirta* showed a very long flowering period (Fig. 1).

The pattern of the phenology curve—based on the number of individuals in flower—was also analyzed for the same species (*Bromus rubens*, *Hordeum murinum* subsp. *leporinum*, *Dactylis glomerata* subsp. *hispanica* and *Trisetaria panicea*) as a function of both mean temperature and cumulative rainfall over the months prior to flowering (winter: December–January–February DJF; spring: March–April–May MAM). In general, flowering began earlier in years with higher temperatures and lower rainfall in winter and spring (Fig. 2). Differences were noted, however, between early-flowering (*Bromus rubens* and *Hordeum murinum* subsp. *leporinum*) and late-flowering species (*Dactylis glomerata* subsp. *hispanica* and *Trisetaria panicea*). Lower rainfall and higher temperatures were recorded in 2015 and 2017. Flowering onset was brought forward in both early-flowering and late-flowering species, except in the case of *Bromus rubens* and *Hordeum leporinum* in 2015, when lower mean winter temperatures were recorded (Fig. 2).

The results thus highlight certain differences in behavior between early-flowering and late-flowering species, also apparent in Fig. 3. In general terms, similar phenological behavior was observed for *Bromus rubens* and *Hordeum leporinum*, on the one hand, and for *Dactylis hispanica* and *Trisetaria panicea* (*Trisetum paniceum*) on the other. In terms of flowering start date, the behavior of late-flowering species varied year-on-year, closely matching variations in weather conditions during the spring. By contrast, variations for early-flowering species more closely reflected variations in cumulative rainfall during the winter months. The duration of the flowering season in *Bromus rubens* and *Hordeum leporinum* varied in accordance with variations in weather patterns over the spring: years with greater cumulative rainfall and lower mean temperatures gave rise to the longest flowering periods. Late-flowering species (*Trisetaria panicea* and *Dactylis hispanica*) displayed more constant timing at each sampling site, as evident in the lower interquartile range values for the start and end of flowering (Fig. 3).

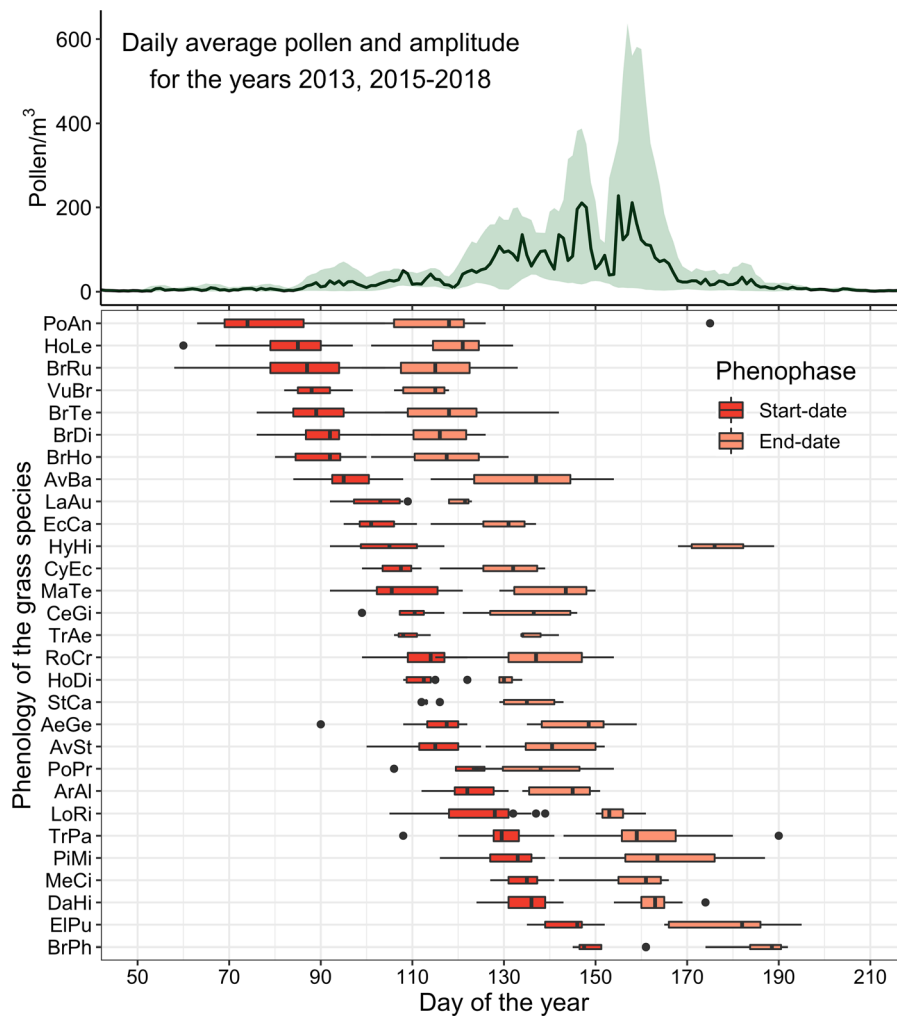


Fig. 1 Variations in daily grass pollen concentrations and species pollen distribution over the period 2003–2018 versus flowering season start and end dates for the grass species sampled. The mean flowering period is given for each species over the five study years (2013, 2015, 2016, 2017 and 2018). Days are expressed in DOY. Sampled Species abbreviations: AeGe *Aegilops geniculata*; ArAl *Arrhenaterum album*; AvBa *Avena barbata*; AvSt *Avena sterilis*; BrDi *Bromus diandrus*; BrHo *Bromus hordeaceus*; BrPh *Brachypodium phoenicoides*; BrRu *Bromus rubens*; BrTe *Bromus tectorum*; CeGi *Celtica*

gigantea; CyEc *Cynosurus echinatus*; DaHi *Dactylis glomerata* subsp. *hispanica*; EcCa *Echinaria capitata*; ElCa *Elymus pungens* subsp. *campestris*; HoDi *Hordeum distichon*; HoLe *Hordeum murinum* subsp. *leporinum*; HyHi *Hyparrhenia sinaica*; LaAu *Lamarckia aurea*; LoRi *Lolium rigidum*; MaTe *Machrochloa tenacissima*; MeCi *Melica ciliata*; PiMi *Piptatherum miliaceum*; PoAn *Poa annua*; RoCr *Rostraria cristata*; StCa *Stipa capensis*; TrAe *Triticum aestivum*; TrPa *Trisetaria panicea*; VuBr *Vulpia bromoides*

The influence of weather-related variables on flowering onset in the grass species studied can also be analyzed in terms of the effect of cumulative values over a given period prior to flowering. Flowering began when cumulative daily maximum temperatures reached a certain number of Heat Units (Fig. 4). A narrower heat unit (HU) range was observed for late-flowering than for early-flowering species: *Dactylis*

hispanica, for example, flowered between 9 and 20 May (DOY 129–140) over the 5 study years, once a threshold level of 2241 ± 90 HU had been reached. *Trisetaria panicea* flowered between 4 and 16 May (DOY 124–136), at a cumulative temperature level of 2128 ± 100 HU. The heat accumulation recorded immediately prior to flowering in *Bromus rubens* and *Hordeum leporinum* showed greater variation:

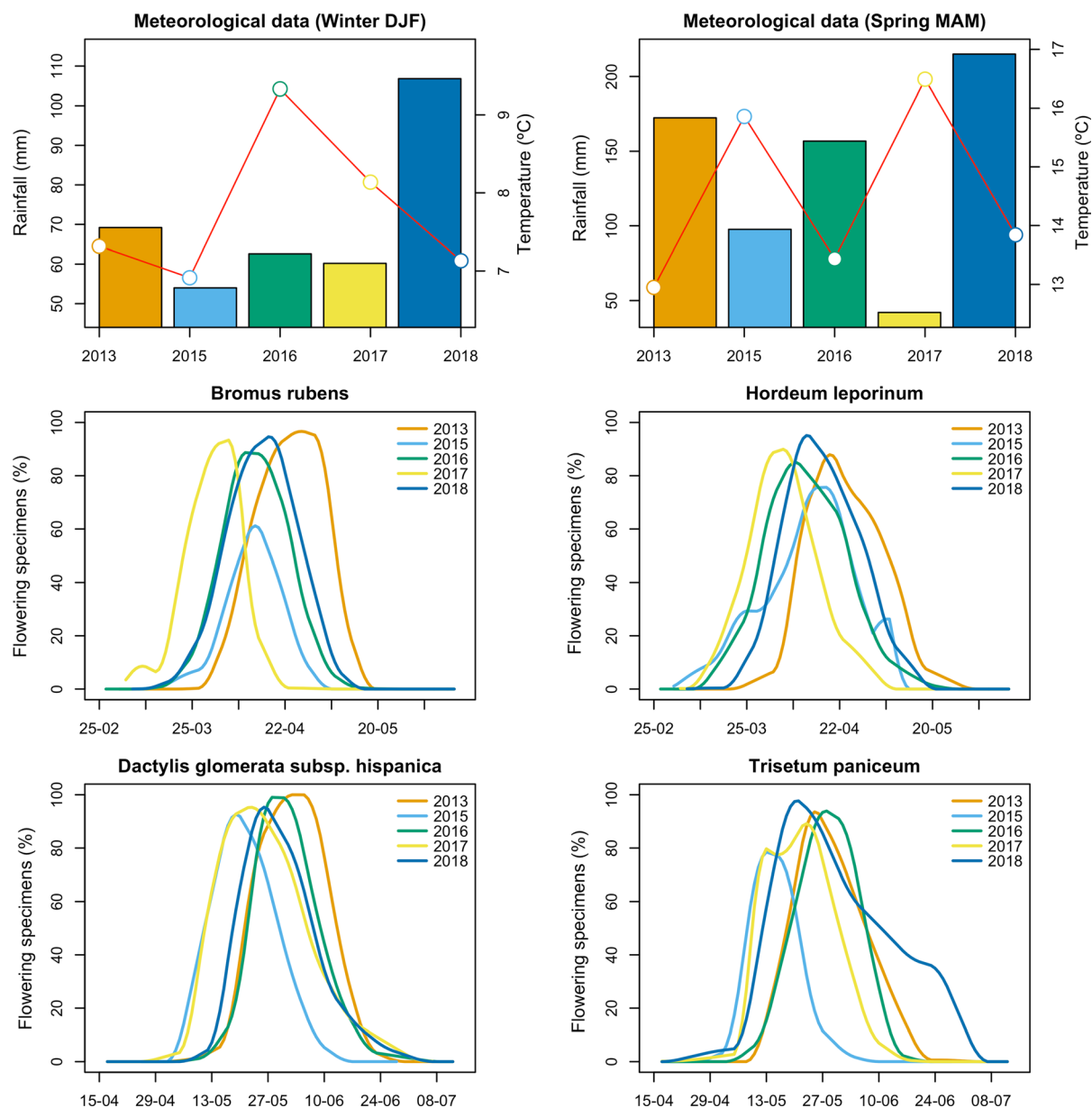


Fig. 2 Percentage of individuals in flower (*Trisetaria panicea* (*Trisetum paniceum*), *Dactylis glomerata* subsp. *hispanica*, *Bromus rubens* and *Hordeum murinum* subsp. *leporinum*) over

the study period (2013, 2015, 2016, 2017 and 2018) versus influence of temperature (points and lines) and rainfall (bars) in winter and spring

1203 ± 112 HU and 1176 ± 134 HU, respectively. Similar results were obtained for cumulative hours of sunlight: flowering began in *Dactylis hispanica* when cumulative sunlight hours reached 983 ± 46 , while for *Trisetaria panicea* the threshold was 933 ± 47 . Cumulative daily rainfall varied considerably between study years and had an observable influence on

flowering onset, which was delayed in years with higher cumulative rainfall (Fig. 4).

Another approach when analyzing the impact of weather-related variables on grass floral phenology is to chart airborne pollen concentrations. A positive correlation was observed between pollen season start date and temperatures (minimum and mean) in late December, while a negative correlation was found

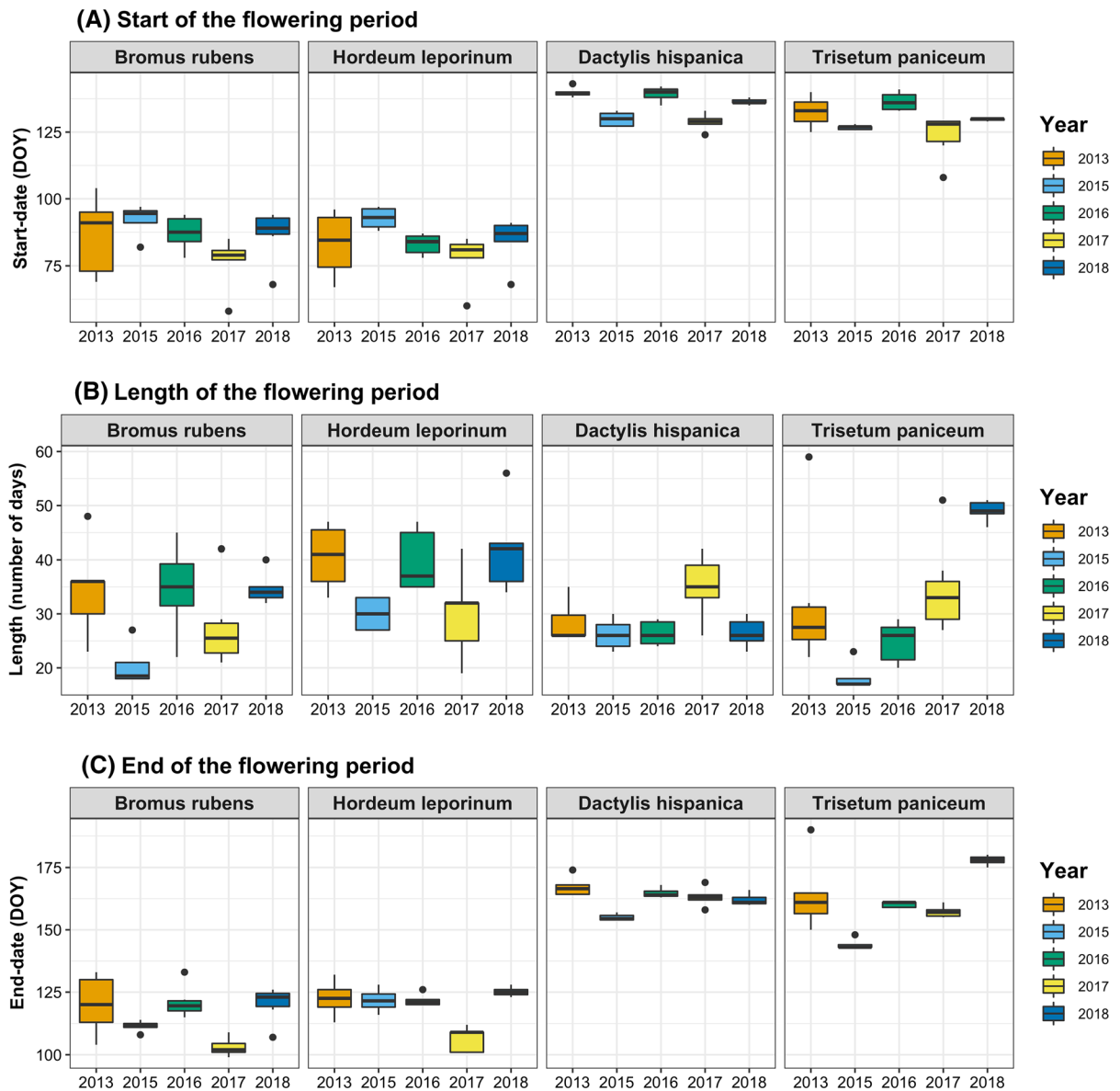


Fig. 3 Start date, duration and end date of flowering for species *Trisetaria panicea* (*Trisetum paniceum*), *Dactylis glomerata* subsp. *hispanica*, *Bromus rubens* and *Hordeum murinum* subsp. *leporinum* over the 5-year study period (2013, 2015, 2016, 2017 and 2018)

from mid-February to late May, also with maximum temperature. A positive correlation was recorded between rainfall and pollen season start date, though it attained statistical significance only between December and February (Table 1). As shown in greater detail in Fig. 5, winter rainfall influenced pollen season start dates; the years in which the pollen season started later (2008, 2010, 2013 and 2016) were those in which high levels of winter or spring rainfall were recorded. They were also those in which the

shortest pollen seasons were observed, statistically significant correlations being recorded between pollen season length and both winter rainfall (-0.59 , $p < 0.05$) and spring rainfall (-0.65 , $p < 0.05$). However, 2007 and 2018 were an exception, they registered large amount of rain in spring, but part of the rainfall occurred in the later spring and was not reflected in the start date of the pollen season (Fig. 5).

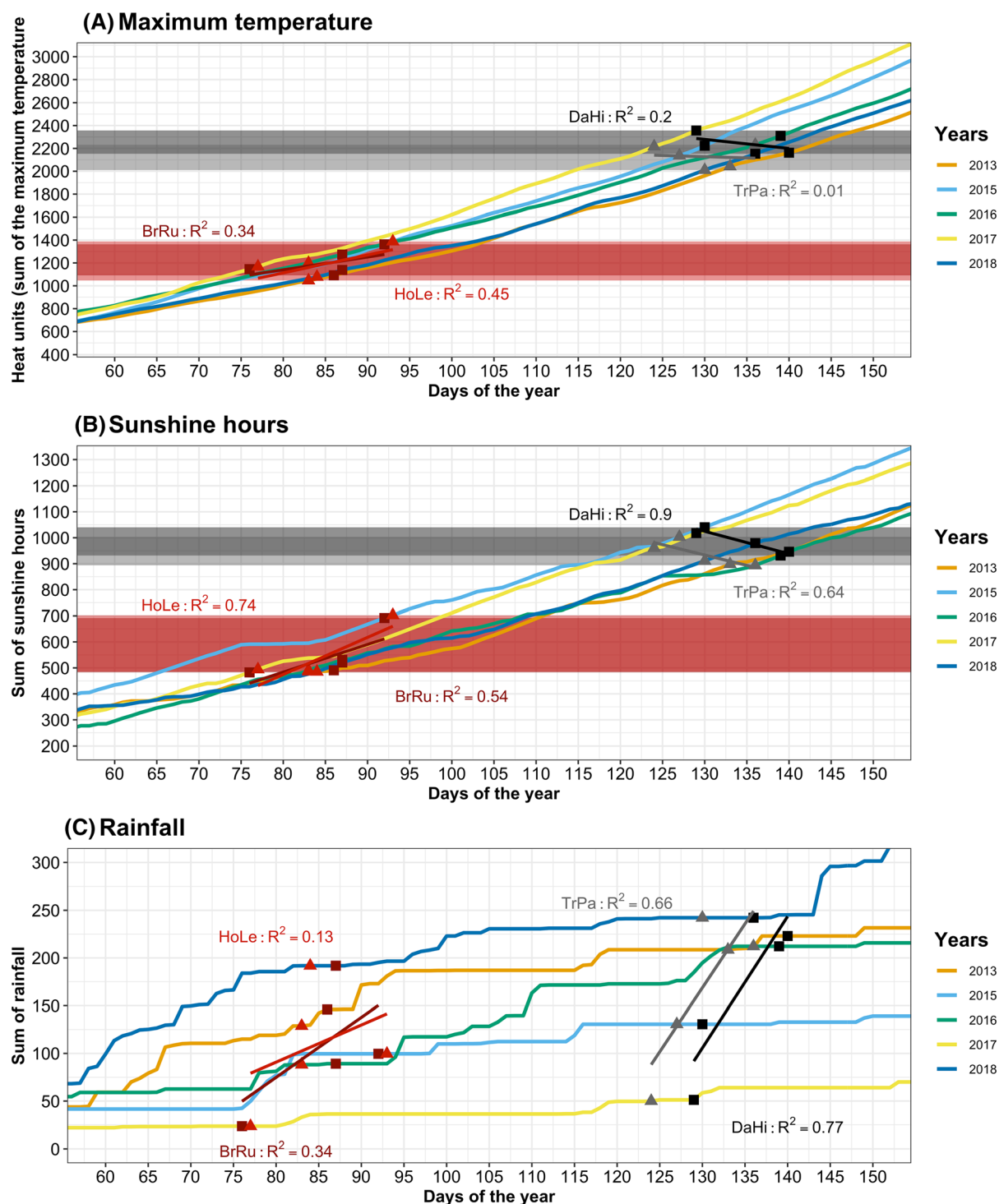
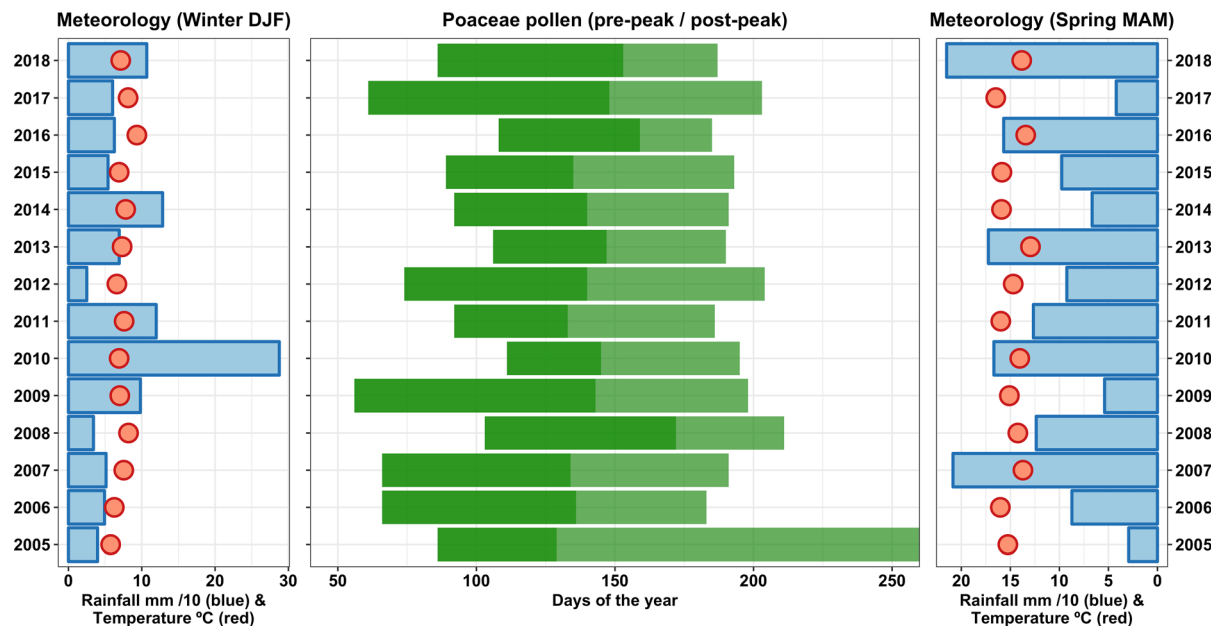


Fig. 4 Cumulative values for meteorological variables (maximum temperature, hours of sunlight and rainfall) vs. phenological status for the species *Trisetaria panicea*, *Dactylis glomerata* subsp. *hispanica*, *Bromus rubens* and *Hordeum murinum* subsp. *leporinum*, using the cumulative value for

maximum temperature from 1 January to flowering onset over the study period (2013, 2015, 2016, 2017 and 2018). The linear regression (R^2) for each species by meteorological variable is also plotted

Table 1 Spearman's correlation results for start date of the pollen season versus weekly maximum temperature, weekly mean temperature, weekly minimum temperature and weekly accumulated rainfall. Only significant results are shown

Time period	$T_{\max}$	$T_{\min}$	T_{mean}	Prec
December week 4 (year 1)		0.64*	0.6*	0.68***
January week 2				0.55*
February week 3	− 0.64*			0.69***
February week 4				0.54*
May week 3	− 0.55*	− 0.53*	− 0.53*	

Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ **Fig. 5** Correlation of mean temperature and rainfall in winter and spring with pollen season pre-peak and post-peak for the period 2005–2018

4 Discussion

In the center of the Iberian Peninsula, the grass pollen season begins with the flowering of species belonging to the genera *Bromus*, *Hordeum*, *Vulpia* and *Avena*, all of which are annuals. Flowering in this group coincides with the first low-to-moderate peaks in the pollen curve, i.e., from early March to late April. By contrast, late-flowering grasses—i.e., those flowering in May and June—coincide with the highest airborne pollen concentrations; species include annuals such as *Aegilops geniculata*, *Lolium rigidum* and *Trisetaria panicea*, and perennials like *Arrhenatherum album*, *Dactylis glomerata* subsp. *hispanica*, *Hyparrhenia*

sinaica and *Melica ciliata* subsp. *magnolii*. Although the presence of these and other grass species is governed by the particular biogeographical features of each territory, these findings agree in large measure with those reported for southern Spain (Cebrino et al. 2016), Italy (Ghitarrini et al. 2017), and Great Britain (Brennan et al. 2019), and even for temperate areas of northern and central Europe (Kmenta et al. 2017), where species contributing to maximum pollen concentrations include members of the genera *Dactylis*, *Festuca*, *Lolium*, *Poa* and *Trisetaria*.

Interpretation of the grass pollen curve, in terms of identifying the main species responsible for pollen emission and their location, is hindered by the

complexity of the grass pollen season, which involves numerous species growing in highly varied habitats (Brennan et al. 2019; Rojo et al. 2017). Moreover, their phenological behavior differs as a function of variations in weather patterns; this also gives rise to changes in the pattern of airborne pollen concentration (Fernández-Martínez et al. 2012).

Temperature exerts a marked influence on reproductive phenology of plants (García-Mozo et al. 2010a). While phenology of woody species is mostly dependent on temperature (Chuine et al. 1999, 2010), photoperiod and rainfall play a more important role in herbaceous species, including grasses (Bennie et al. 2018; Quinn and Wetherington 2002; Swanton et al. 2000). The present study confirmed the findings of earlier reports: while temperature exerted the greatest influence on phenological development, it was by no means the only influential variable; others included relative humidity, rainfall and hours of sunlight, the latter also displaying year-on-year variations.

Cumulative rainfall over the period prior to flowering is known to influence the timing of flowering onset (García-Mozo et al. 2010a). Earlier research has shown that rainfall over the period prior to the pollen season not only has an impact on grass phenology but also prompts an increase in plant biomass (Miranda et al. 2011), as well as a rise in flower production and thus increased airborne pollen concentrations, as reported for other plant species (Llorens and Peñuelas 2005). Variations in relative humidity, a variable closely linked to temperature and rainfall, also affect plant physiology and thus phenology (Oduber et al. 2019).

Differences in phenological response have been observed between annual and perennial grass species. In general terms, annuals respond more quickly than perennials to changes in environmental conditions (Alcázar et al. 2009; Dahl et al. 2013), although the response depends on phenophase status (Recio et al. 2010). Garnier (1992) reported a higher relative leaf production rate in several annual grass species than in perennials, which might facilitate a prompt response of annuals following certain episodes of environmental stress. The findings of the present study partly confirmed the existence of greater phenological plasticity in annuals like *Trisetaria panicea*; at high mean temperatures, phenological development was quickly activated.

Grass phenology is also affected by habitat and the optimal ecological characteristics of each species. Grass species growing in scrublands and pasturelands are more sensitive to changes in temperature and water availability (Cebrino et al. 2018). Water availability has a more marked influence on the development of grass species in Mediterranean biogeographical areas than elsewhere (Clary et al. 2004) and is a key factor in plant growth. This was evident in the early-flowering species studied here (*Hordeum murinum* subsp. *leporinum* and *Bromus rubens*), in which the pollen season started in years with higher winter rainfall (2008, 2010, 2013 and 2016) and also lasted longer than in years with lower rainfall. It should also be noted that environment type governs pollen emission in each habitat as a function of the characteristic species growing there. Perennial grass species growing in natural and seminatural habitats (e.g., *Dactylis hispanica* in the present study) are generally associated with higher pollen production (Aboulaich et al. 2009), as well as with smaller and thus more buoyant pollen grains, and the timing of their flowering season more closely matches the timing of maximum pollen curve peaks (Romero-Morte et al. 2018), compared with species growing on cropland or in ruderal environments (e.g., *Hordeum murinum* subsp. *leporinum* and *Bromus rubens*), with the exception of certain species such as *Trisetaria panicea* and *Lolium rigidum*, which also make a considerable contribution to airborne pollen concentrations (León-Ruiz et al. 2011).

Year-on-year variations were observed in the start date, duration and end date of the flowering season due mainly to changing weather patterns; this is attributable to the phenotypic plasticity displayed by species which adjust rates of growth and reproductive development to the prevailing environmental conditions (Munson and Long 2017). However, the plasticity is limited, and for this reason, the succession of the flowering periods in grass species showed a similar sequence from year to year, as confirmed by our results. Reflecting higher rainfall and lower spring temperatures, the flowering period in the late-flowering species (*Dactylis hispanica* and *Trisetaria panicea*) occurred considerably earlier in 2015 and 2017. By contrast, in 2013, 2016 and 2018 a delay in flowering was observed, as a response to adverse temperature and rainfall patterns. Similar findings have been noted by other authors, including Cebrino

et al. (2016) and León-Ruiz et al. (2011), who reported a delay in flowering in years with higher spring rainfall. By contrast, higher temperatures and lower rainfall accelerate anther dehiscence and favor earlier flowering (Dahl et al. 2013; Emberlin et al. 1999). Bloor et al. (2010) experimentally demonstrated a marked advance in flowering as a response to increased temperature in grass species growing in a natural grassland ecosystem.

However, species' phenological behavior varied from year to year over the study period, and inter-species differences were observed in terms of the response to weather-related variables, as reported for other taxonomic groups in the Mediterranean area (Peñuelas et al. 2004). Unsurprisingly, early-flowering species such as *Bromus rubens* and *Hordeum leporinum* generally appeared to be more influenced by winter weather conditions, while late-flowering species were more influenced by conditions in spring, these being the seasons immediately prior to floral development. Greater variation in phenological parameters (onset and duration of flowering season) is observed among early-flowering species, whereas in species whose flowering season ends in May and June, the higher temperatures favor synchronous flowering of populations across a whole geographical area (Schauber et al. 2002). Over the period 2013–2018, flowering onset occurred at a given threshold level of maximum temperatures and cumulative hours of sunlight; threshold values lay within a narrow range, particularly in the case of late-flowering species. A significant delay in flowering also tends to be recorded for these species as annual cumulative rainfall increases (Ghitarrini et al. 2017). These findings have major implications when modeling the timing of flowering in grass species on the basis of their need to meet certain threshold requirements in terms of cumulative heat and hours of sunlight (McMaster and Wilhelm 1997).

Improved knowledge of the heat requirements to be met in order for grass species to complete their reproductive cycle is of value in evaluating the potential impact of climate change on grasses and on the phenological parameters of the grass pollen season (Ghitarrini et al. 2017).

5 Conclusions

This work highlighted the great effect of the temperature on the phenological development of grasses; however, other variables influencing phenological timing included relative humidity, rainfall and hours of sunlight, all of them displaying year-on-year variations. In the same way, year-on-year phenological variations were observed due mainly to changing weather patterns which were studied using a short-term approach. One important point of the findings of this work was that inter-species differences were observed in terms of the response to weather-related variables. A better understanding of that influence would be of great value for a more accurate interpretation of airborne pollen levels in important wind-pollinated taxa like as grasses. This type of research is interesting to be developed in other taxa that include species sharing the same pollen type such as Cupressaceae or *Quercus* pollen types whose study would have interest from clinical or ecological point of view. According to our results, the future projected changes in the Mediterranean region will cause phenological changes in the flowering period of allergenic species. These changes will influence on pollen exposure for the allergic population in the coming years as a result of climate change.

6 Supplementary data

Supplementary data consist of Figure S1: map of the study area; Figure S2: species of grasses sampled by sampling point; and year and Figure S3: flowering season start and end dates for the grass species sampled for each study year.

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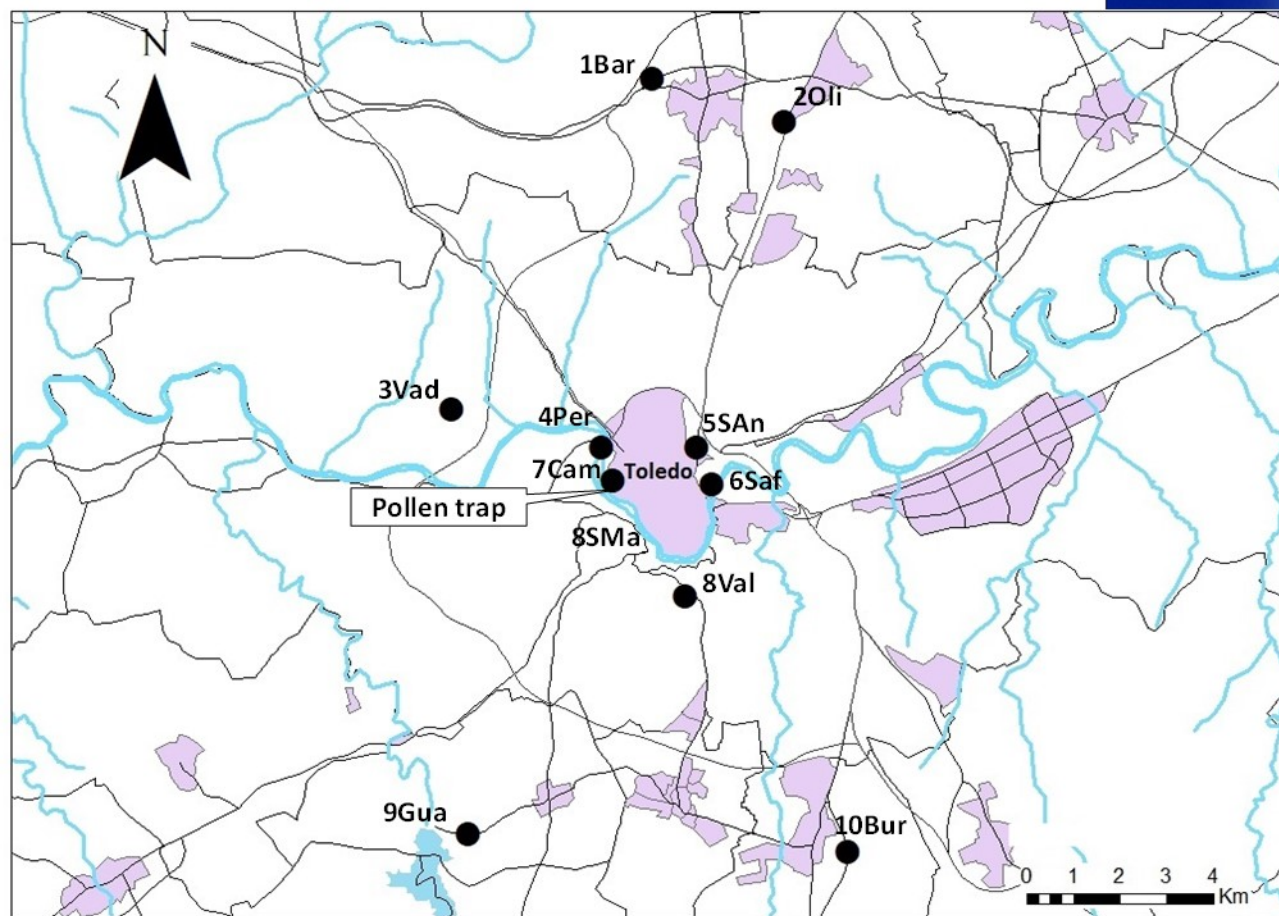
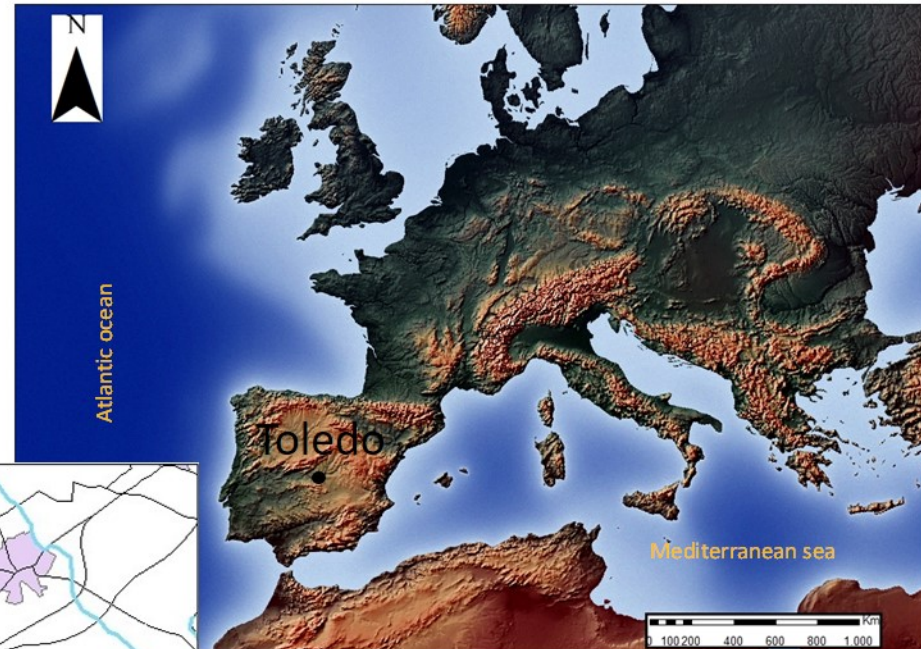
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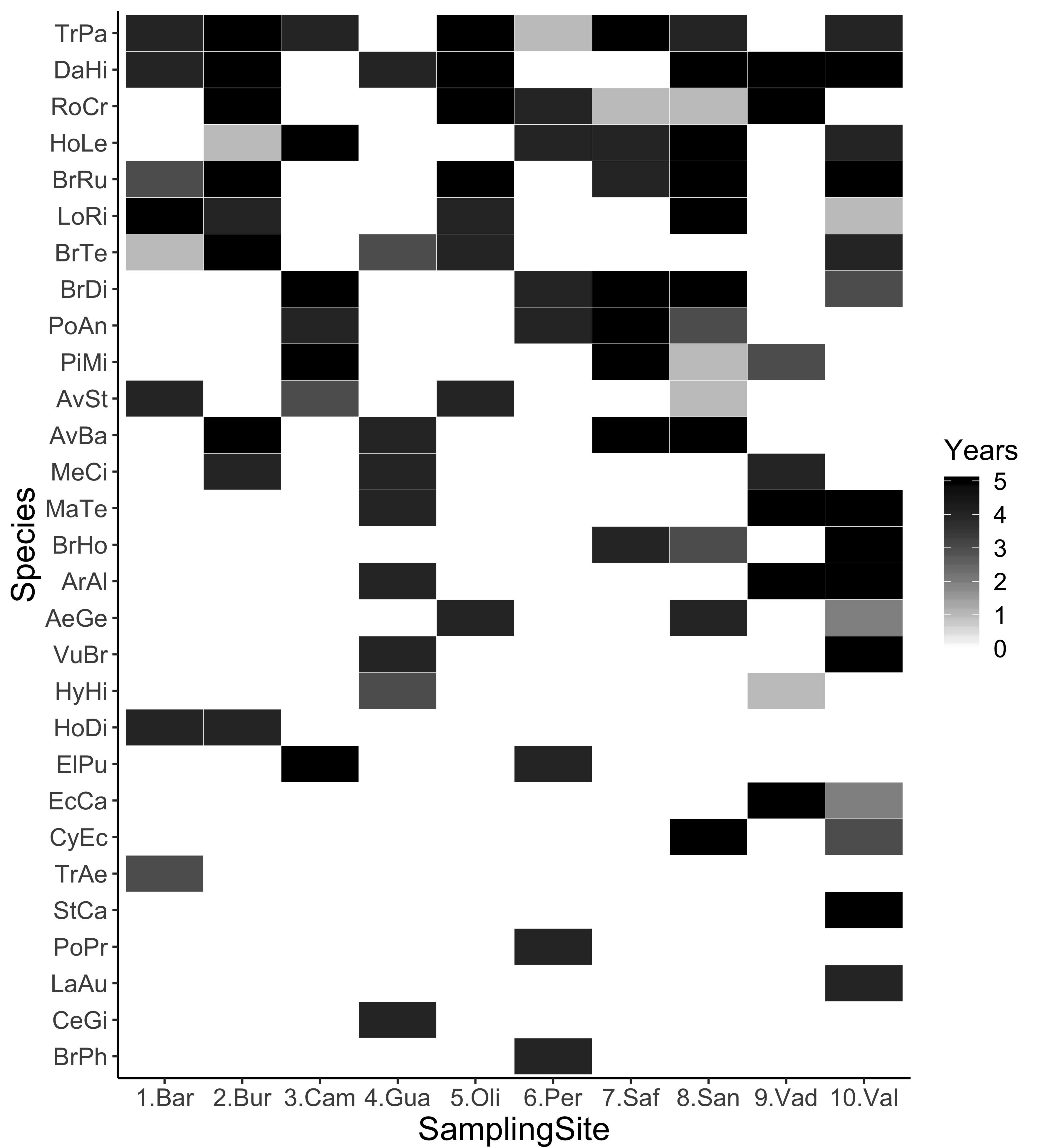
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Phenology of the grass species

2013

2017

2015

2018

2016

PoAn
HoLe
BrRu
VuBr
BrTe
BrDi
BrHo
AvBa
LaAu
EcCa
HyHi
CyEc
MaTe
CeGi
TrAe
RoCr
HoDi
StCa
AeGe
AvSt
PoPr
ArAl
LoRi
TrPa
PiMi
MeCi
DaHi
EIPu
BrPh

PoAn
HoLe
BrRu
VuBr
BrTe
BrDi
BrHo
AvBa
LaAu
EcCa
HyHi
CyEc
MaTe
CeGi
TrAe
RoCr
HoDi
StCa
AeGe
AvSt
PoPr
ArAl
LoRi
TrPa
PiMi
MeCi
DaHi
EIPu
BrPh

50 70 90 110 130 150 170 190 210

50 70 90 110 130 150 170 190 210

Day of the year

Phenophase

Start-date
End-date