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ORIGINAL PAPER

Trends in airborne grass pollen in Évora City (Portugal)

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Abstract Aerobiology could be used as complementary data or proxy for studying phenology, especially in species which usually are not long-distance transported, such as grasses. This 21-year aerobiological study took place in Évora (South Portugal), using a seven-day recording volumetric pollen trap with the aim of analysing the temporal trends of grass pollen seasons. To this end, data were statistically tested for correlation and regression to determine the features and temporal trends of pollen seasons. Main results show that Poaceae pollen has a high representation in pollen spectrum, 20% (min: 7%; max: 44%), with a long season starting in March and lasting till August/September or October. There was an overall, but not statistically significant trend, towards an increase in the annual pollen integral and peak values, and also an overall non-statistically significant trend

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towards an earlier start and later end of the pollen season. Main and high pollen seasons have been lasting longer, and the days with allergenic potential have been rising in line with the local temperature rising trend. It seems that grass pollen production is being aided by warmer temperatures and increased grassland areas in Alentejo region. This could promote the increase in annual pollen levels and in the number of high pollen days over the years. In clinical terms, it means that grass allergic patients are exposed to longer periods of airborne pollen and to higher counts over a longer time. The climate conditions projected for Alentejo region, including rising temperature, and the changes in local grassland areas may help to clarify the trends of grass pollen seasons in the next decades.

Keywords Airborne pollen · Poaceae · Pollen season · Pollen trends · Climate change · Portugal

1 Introduction

Grasses are perennial or annual herbaceous plants that grow almost everywhere (Chłopek, 13). They represent a plant community commonly known as grasslands, normally composed by a low-growing cover of plant species. It also has low abundance of woody plants and normally occurs in arid climates (Gibson, 33).

Grasses are members of the Poaceae family, within the Liliopsida class (the monocotyledons). In the Liliopsida, grasses are placed in the Poales order which includes 17 families. In Poaceae family, ± 12,074 species are recognised, divided among 711 genera into 12 subfamilies, 6 supertribes, 51 tribes and 80 subtribes (Soreng et al., 73). The subfamilies Pooideae, Panicoideae, Bambusoideae and Chloridoideae comprise the largest number of species (Hodkinson, 37). The largest genera correspond to *Panicum*, *Poa*, *Festuca*, *Eragrostis*, *Paspalum* and *Aristida*. Poaceae is economically, ecologically and evolutionarily one of the most successful groups in terms of species richness. Economically, grasses are an important plant group, providing cereals and bamboo for food, building and amenity materials. They also become important sources of raw material for the biomass and bioenergy industry and provide several species of horticultural/ornamental value (Hodkinson, 37).

Poaceae is classified as a monophyletic family, revealing inflorescence highly bracteate, perianth reduced or lacking (GPWG, 35). Most species are either morning or afternoon flowering (Norris-Hill, 59), albeit some species can be nocturnal (Takahashi et al., 76). Grass species are majority anemophilous, but entomophily may arise on few tropical and temperate grass species (Soderstrom & Calderon, 72; Adams et al., 1). They produce and release to the atmosphere large amounts of pollen. Grass pollens are morphologically similar, being identifiable until the family level, whereby referred as Poaceae pollen type. Grass pollen grains are monoporate, and their shape is spheroidal to sub-oblate, revealing an annulus. The pore is covered by the operculum composed by both layers of the wall (Juhász et al., 43). Pollen grains have a mean size of 35 × 40 µm and the largest belongs to cultivated species (García-Mozo, 30).

Over the human history, grasslands have been lived in and have been used by people. Therefore, native grasslands have changed and subject to threats, mainly due to agriculture practices, fragmentation of the landscape, and the occurrence of non-native species (Hoekstra et al., 39). There are evidences that several exotic plants have become invasive, were naturalised, and changed the native grasslands at a regional level (Gibson, 33). Furthermore, biological invasions among with land-use changes are altering the occurrence of plant species in a given region and modifying the spatio-temporal composition of airborne pollens (D'Amato et al., 15). This situation might pose a threat to the public health because it can increase the presence of grass allergens in the air (Bernard-Verdier et al., 5). According to D'Amato et al. (15), more than 80% of pollen allergy sufferers are allergic to grass pollen. A significant part of the world's population (10 to 40%) experiences allergic rhinitis and grass pollen is considered one of the major outdoor allergen triggers of this disease (Klimek et al., 47). Regarding this aspect, Poaceae is well represented in the Iberian Peninsula, particularly in Portugal, where it occurs throughout the country (Camacho, 9).

Poaceae pollen type prevails in the pollen spectrum of the country, occurring in the atmosphere throughout all the year, mostly between March till July or August (Todo-Bom et al., 77; Caeiro et al., 8). In Alentejo, Poaceae pollen is also considered a prevalent aeroallergen, reaching the highest concentrations mainly between May and early June (Caeiro et al., 8). In Portugal, the percentage of grass pollen sensitised patients in pollinic patients ranges from 44.9% in Cova da Beira (interior central region of Portugal) to 85.71/100% in Évora (Brandão & Lopes 6; Sánchez Mesa et al., 69; Loureiro et al., 50; Diamantino et al., 20; Newson et al., 58), According to Loureiro et al. (50) and Diamantino et al. (20), the most relevant genera are *Dactylis*, *Hordeum*, *Phleum*, *Poa*, *Avena*, *Festuca*, and *Lolium*, common species in our local grasslands.

Previous works have documented a straight relationship between weather conditions and airborne grass pollen counts in regions with distinct climatic features (Emberlin, 21; González Minero & Candau, 34; Sánchez Mesa et al., 70). Abiotic factors such as temperature, photoperiod and water availability influence the growth and development of plant species, specially before the flowering stage. During flowering, other secondary weather-related factors, such as rainfall and relative humidity, influence pollen release (Laaidi, 48). Besides the pre- and in-season weather, other factors can affect the magnitude of grass pollen seasons, such as latitude, pasture greenness and regional biogeography (Davies et al., 19). On this respect, two of the world's macro bioclimates are represented in Portugal: Temperate and Mediterranean, with most locations belonging to the Mediterranean macro bioclimate. In sequence, there are 4 bioclimates in Portugal segregated in different thermotypes and ombrotypes which align with several phytogeographical typologies and floristic diversity (Rivas-Martínez et al., 66; Capelo & Aguiar, 11). In such a complex biogeography, pollen allergens originated from different Poaceae subfamilies such those of temperate Pooideae and subtropical Panicoideae may arise. Both subfamilies are well widespread on different biogeographical regions, in all terrestrial biomes, and their distribution usually tends to overlap (Gallaher et al., 29), a condition that can turn grass aerobiology more complex, including in the Portuguese territory. Due to the clinical importance of Poaceae pollen in the country and its ubiquitous presence, it is important to monitor this pollen type from an aerobiological perspective which helps protect the population more exposed and sensitised to such aeroallergens.

This study focused on the analysis of long temporal trends of grass pollen data in the atmosphere of Évora (Alentejo region, southern Portugal), over a 21-year period. The potential influence of meteorological factors in the trends of grass pollen and its season-related features were also evaluated.

2 Materials and methods

2.1 Study area

The study took place in Évora City (N 38.57143° W 7.9135°) located in Alentejo in the south region of Portugal mainland in the Iberian Peninsula (Fig. 1). Évora has 53.568 inhabitants (Statistics Portugal, 75), being for that reason the fifth largest municipality in the country, with an area of 31,551.2 km^2 (Suwanu Europe. Deliverable 1.1-State of play analyses for Alentejo, Portugal, 2020).

The municipality of Évora is in the middle of Alentejo plain with a mean altitude of 240 m above sea level. The climate of Alentejo is Mediterranean type, characterised by mild winters and hot dry ad prolonged summers, with high thermal amplitudes. The wettest months are from September to May, with most precipitation in the form of torrential downpours. It is characterised by enormous variability, alternating between dry and rainy periods in a random way, however, with a predominance of dry periods. The dry or rainy periods can either correspond to isolated years or to groups, which can reach six years (Ventura, 78). Average annual precipitation in Évora is 609.4 mm and average annual temperature is 15.9 °C (Climate normals, 1971–2000 IPMA), in summer the maximum temperatures can regularly exceed 40 °C.

In terms of bioclimate, Évora presents a Mediterranean Pluviseasonal—Oceanic Macrobioclimate—and is part of the Lower Subhumid Lower Mesomediterranean bioclimatic floor (Pinto-Gomes, 63; Rivas-Martinez, 65; Rivas-Martínez et al., 67), which determine the distribution of potential and current vegetation in the Alentejo. In most of the Alentejo territory, the natural vegetation corresponds to forest communities, except for smaller proportions of dune vegetation, sea cliffs, salt marshes, riparian vegetation and wetland vegetation or rocky outcrops. The landscape is made up of a mosaic of types of vegetation that includes thickets, bushes, pastures as well as cork oak and holm oak forests, which correspond

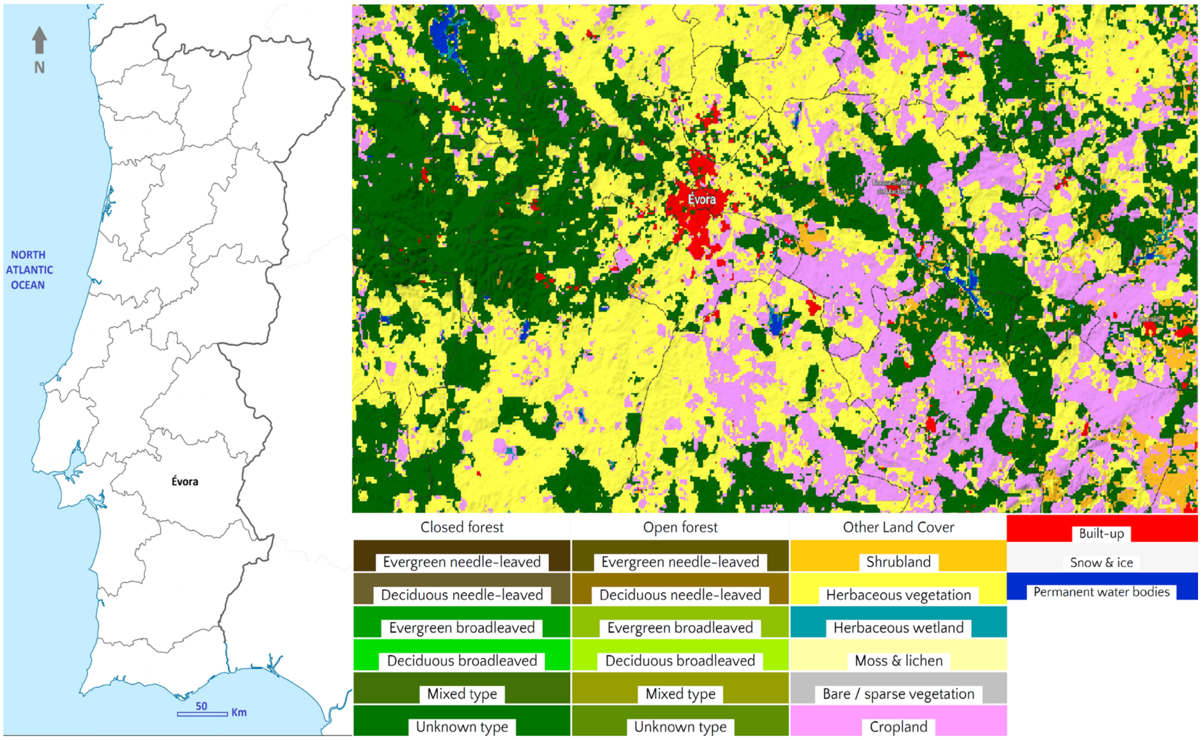

Fig. 1 Location of Évora city (southern Portugal) and land cover inventory (Buchhorn et al. 2020; © Copernicus Service Information 2019)

to a semi-natural structure originating from an agro-silvopastoral system exploited at various levels: trees, shrubs and herbaceous. The arboreal level may consist of oaks such as the cork oak (*Quercus suber* L.), the holm oak (*Q. rotundifolia* Lam.) and more rarely the Pyrenean oak (*Q. pyrenaica* Willd) and the "cerquinho" oak (*Q. faginea* Lam.), in pure or mixed stands with a variable density. The sub-cover is composed by pastures used by livestock or is cultivated with dryland arable crops in a rotation system. Natural pastures can be occupied by bushes, to a greater or lesser extent (Capelo & Vila-Viçosa, 10; Montado in Alentejo Natura 55 sites -Portugal, 2021).

2.2 Aerobiological data

Airborne pollen monitoring in Alentejo was performed daily from 2001 to 2021 by the Portuguese Aerobiology Network (https://www.rpaerobiologia.com/) with a Burkard Seven Day Volumetric Spore-trap® (Burkard Manufacturing Co. Ltd., UK) and a Hirst type methodology associated. The pollen trap is located at Alentejo Regional Directorate for Culture (N 38.57142° W 7.90882°), on the top of a flat roof, placed 10 m above the ground level.

Sampling and analysis were performed in accordance with the European Norm EN 16868:2019 and the Minimum Recommendations proposed by the European Aerobiology Society Working Group on Quality Control (Galán et al., 28). The Burkard trap has a vacuum pump able to suck 10 L of air per minute, which reflects the average human ventilation capacity. The airborne particles are projected into a clockwork-driven drum which rotates 2 mm/h, where holds a melinex tape coated with silicone solution (Lanzoni). After one week sampling, the melinex tape is cut in seven daily portions of 48 mm each and mounted on glass slides. Each sample is stained with glycerine gelatin melted with basic fuchsine. The identification and counting of pollen grains were done using a light microscope at a magnification of 400 × with a filed view of 0.45 mm following a 4 longitudinal transects along each slide, which corresponds to 13% of the daily sample.

Poaceae pollen concentrations were calculated and expressed as number of pollen grains per cubic meter of air (pollen/m^3). The annual sum of daily grass pollen concentrations was expressed as the Annual Pollen Integral (APIn). In order to determine the airborne permanence of Poaceae pollen in the atmosphere, the Main Pollen Season (MPS) was calculated according to Pfaar et al. (62), which defines de "beginning" of the pollen season as the 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥ 3 pollen/m^3 and with a sum of these 5 days of ≥ 30 pollen/m^3. The "end" of the season corresponds to the last day of a series of 5 days (out of 7 consecutive days) with ≥ 3 pollen/m^3 and with a sum of these 5 days of ≥ 30 pollen/m^3. It was also determined the High Pollen Season (HPS), calculated according to Pfaar et al. (61), that defines the "beginning" of the peak pollen period as the 1st day out of 3 consecutive days, each with at least ≥ 50 pollen/m^3. The "end" of the peak pollen period consists to the last day of at least 3 consecutive days, each with ≥ 50 pollen/m^3.

The pollen peak date was obtained as the day on which the highest daily concentration of grass pollen of the year was recorded. The intensity of the Poaceae season was established as the number of days where average daily concentrations exceeded 50 pollen grains/m^3 (Pfaar et al., 61). It is a specific parameter where can be inferred if most sensitised people are at risk to develop allergic symptoms. According to Galán et al. (27), pollen-allergy sufferers start to show serious symptoms when the average grass pollen counts exceed 25 grains/m^3. For that reason, additional categories for risk allergenicity were considered to analyse the intensity to grass pollen exposition over the years: No days > 25 pollen grains/m^3; No days > 30 ≤ 50 pollen grains/m^3; No days > 50 ≤ 100 pollen grains/m^3 and No days > 100 pollen grains/m^3. In sequence, it was calculated the number of weeks of the year for each risk level (level 0: no risk, 0 grass pollen grains/m^3/week; level 1: low risk, 0.1–4.9 grass pollen grains/m^3/week; level 2: medium risk, from 5–19.9 grass pollen grains/m^3/week; level 3: high risk, from 20–29.9 grass pollen grains/m^3/week; and level 4: very high risk > 30 grass pollen grains/m^3/week), described by Belmonte et al. (4). Such categorisation was applied on stations with similar geographic and phytogeographic characteristics of Évora.

Temporal trends of MPS features, APIn, and intensity to grass pollen exposition were analysed by linear regression, and significance values, where *p* values lower than 0.05, were considered statistically significant. To check statistical differences between the APIn, a Kruskal–Wallis test was applied. Differences were reported for *p* values ≤ 0.05. As the analysed variables did not show a normal distribution, the Spearman's rank correlation was applied to explore the relationships between the daily grass pollen counts and meteorological variables. Statistical analysis and plots generation were performed by using SPSS (Statistical Package for the Social Sciences) 22.0 programme.

2.3 Meteorological data

Meteorological data such daily temperature (maximum, mean and minimum; °C), relative humidity (%), rainfall (mm), sunshine (*h*), total global radiation (KJ/m^2) wind speed (m/s) and wind direction were supplied by the Portuguese Institute of Ocean and Atmosphere (IPMA). The selection of the weather parameters was based on previous research that has demonstrated the important influence of such variables on the grasses pollen season features (Puc & Puc, 64; Sánchez Mesa et al., 69). Due to a technical failure, it was not possible to obtain data of relative humidity between 2013 and 2016 and rainfall values in 2016.

3 Results and Discussion

3.1 Trends on timing and length of grass pollen seasons

One of the aims of the present study was to analyse the long temporal trends of airborne grass pollen data in Évora. The annual variation of grass pollen in the atmosphere of Évora shows multiple pronounced pollen peaks, restricted to a short period of time (on a weekly basis) (Fig. 2), a pattern that has persisted over the years.

This annual pollen distribution and the length of the MPS may suggest the contribution of different grass pollen species with overlapping flowering times or repeated flowering of the same grass species (Fernández-González et al., 26; Mercuri et al., 53; Addison-Smith et al., 3). The high variability in the number of peaks and dates of their occurrence was

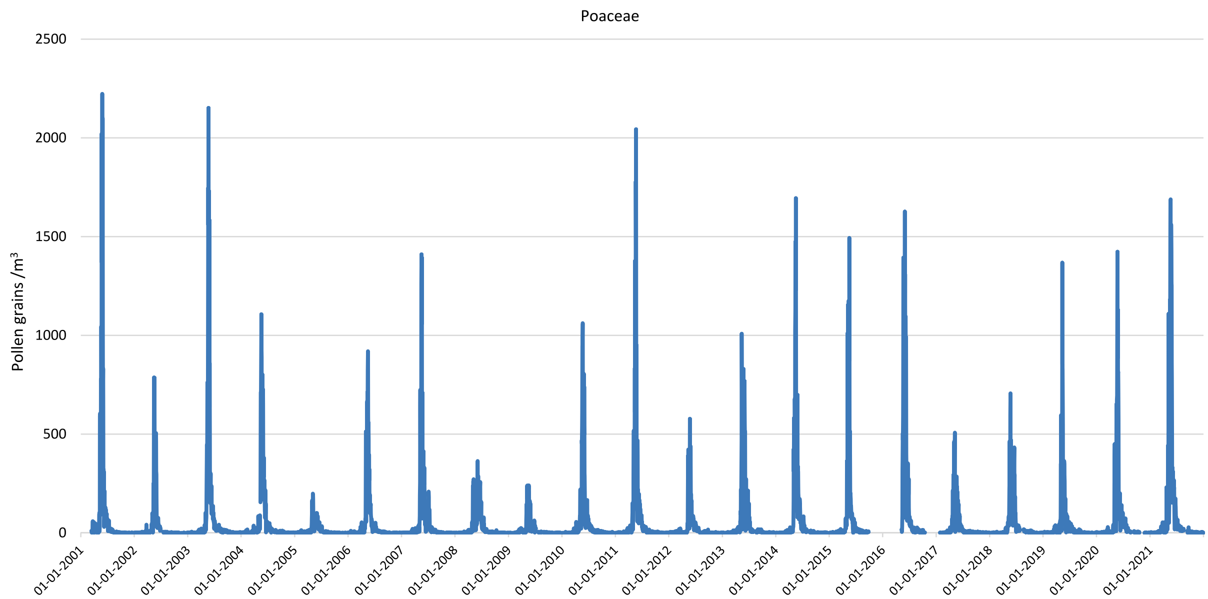

Fig. 2 Annual Poaceae pollen records in in Évora during the study period

also previously reported (Spieksma et al. 74; Sánchez Mesa et al., 69).

During the study period, grass pollen was observed in the atmosphere of the city since early March to August/September or until the first week of October. Grasses pollen season of Évora is long, likewise in other European cities (Chłopek, 13; Malkiewicza & Klaczak, 52; Mercuri et al., 53), which in the case of Alentejo region matches with low precipitation and average maximum temperature varying between 32–34 °C in the summer (Miranda et al., 54). It was shown that patients with allergic rhinitis and also sensitive to grass pollen usually suffer allergic symptoms mainly between May to July (Myszkowska, 56).

3.1.1 The dynamics of the annual pollen integral (APIn)

The APIn was highly variable, being the obtained average for grasses of 16,749 ± 7414 pollen/m^3. The irregular distribution of annual Poaceae totals was confirmed by the statistical test of Kruskal–Wallis, whose H_0–the annual distribution of APIn is the same over the years, was rejected. The highest total amount of grass pollen was observed in 2021 (30,554 pollen/m^3) and in 2005 (4302 pollen/m^3) was detected the lowest (Table 1).

Grass pollen represents on average 20% of the annual total pollen recorded in the atmosphere of Évora city, ranging between 7% (2005 and 2009) and 44% (2001). The higher percentage rates were detected in the first decade of the study (23% on average), whilst in last decade, the average percentage was 18%. Even though it is a common airborne pollen type worldwide, grasses pollen contribution to the global pollinic spectrum of a region (20% in Évora) is seldom greater than 25% (Juhász et al., 43).

The variability on the APIn observed in Évora has been also reported in other cities, considering that grass pollen seasons differ from region to region owing to factors that condition the release to the atmosphere and abundance of pollen grains (Sánchez Mesa et al., 70). According to the results herein, a positive but not significant trend for Poaceae APIn was noticed. This apparent trend of increase in APIn is also shown in Fig. 3a.

Different trends in the APIn have been previously found in different ways in similar bioclimatic regions, either revealing negative trends (Jato et al., 41; Mercuri et al., 53; Lind et al., 49; Hoebeke et al., 38), or positive trends (García-Mozo et al., 31; Cebrino et al., 12). Such contrasting patterns stem from biotic and abiotic factors (biology, ecology, climatology, latitude) (Emberlin et al., 22) that rule the reproduction

Table 1 Characteristics and trends of Poaceae pollen seasons in Évora (2001–2021)

Year	APIn (pollen × day/m^3)	Main Pollen Season					Pollen peak		Representation in the pollen spectrum (%)	Pollen categorisation (No. of weeks)				
		Start Date	Day of the Year	End Date	Day of the Year	No. of days	Date of the Year	Poaceae pollen/m^3		Nil 0	Low 0,1–4,9	Medium 5–19,9	High 20–29,9	Very high > 30
2001	28,519	20/Mar	79	11/Aug	223	145	27/May	2222	44	1	10	13	2	11
2002	9523	19/Mar	79	29/Jun	180	103	26/May	786	18	4	15	18	1	12
2003	28,450	11/Mar	71	29/Sep	272	203	23/May	2151	36	0	10	10	3	30
2004	18,416	14/Mar	75	30/Sep	274	201	18/May	1106	32	0	7	16	1	29
2005	4302	4/Apr	95	14/Jul	195	102	5/May	199	7	3	10	16	7	17
2006	15,118	25/Mar	85	11/Aug	223	140	16/May	920	20	0	17	9	6	21
2007	19,462	09/Mar	69	24/Aug	236	169	17/May	1410	27	0	17	6	5	24
2008	10,100	28/Mar	89	14/Aug	227	140	04/Jun	363	13	1	13	12	8	19
2009	5559	12/Mar	72	07/Jul	189	118	19/May	240	7	3	18	10	3	18
2010	18,107	3/Apr	94	22/Sep	265	173	22/May	1062	22	4	12	9	2	26
2011	23,522	18/Mar	78	1/Aug	213	137	23/May	2043	26	1	12	10	8	22
2012	8982	06/Mar	67	4/Sep	248	183	24/May	577	10	2	11	14	5	18
2013	16,474	04/Apr	95	27/Sep	270	177	13/May	1008	19	1	10	14	2	23
2014	22,743	08/Mar	68	2/Sep	245	179	23/May	1695	19	4	14	6	3	21
2015	17,851	04/Mar	64	23/Aug	235	173	18/May	1493	12	0	5	5	4	25
2016	23,849	21/Apr	111	7/Oct	281	170	31/May	1627	22	0	1	1	2	20
2017	9355	14/Mar	74	21/Aug	233	161	8/May	507	10	2	10	13	5	20
2018	11,606	16/Apr	107	6/Oct	279	174	23/May	706	18	7	11	8	2	22
2019	13,618	18/Mar	78	13/Sep	256	180	12/May	1368	14	4	12	9	5	23
2020	20,395	11/Mar	71	13/Sep	257	187	23/May	1423	30	3	8	14	1	21
2021	30,554	14/Mar	73	3/Oct	276	204	21/May	1689	20	1	8	12	3	26
Average	16,976	19/Mar	81	2/Sep	242	163	20/May	1171	20	2	11	11	4	21
maximum	28,519		111		281	204		2151	44	7	18	18	8	30
minimum	4302		64		180	102		199	7	0	1	1	1	11

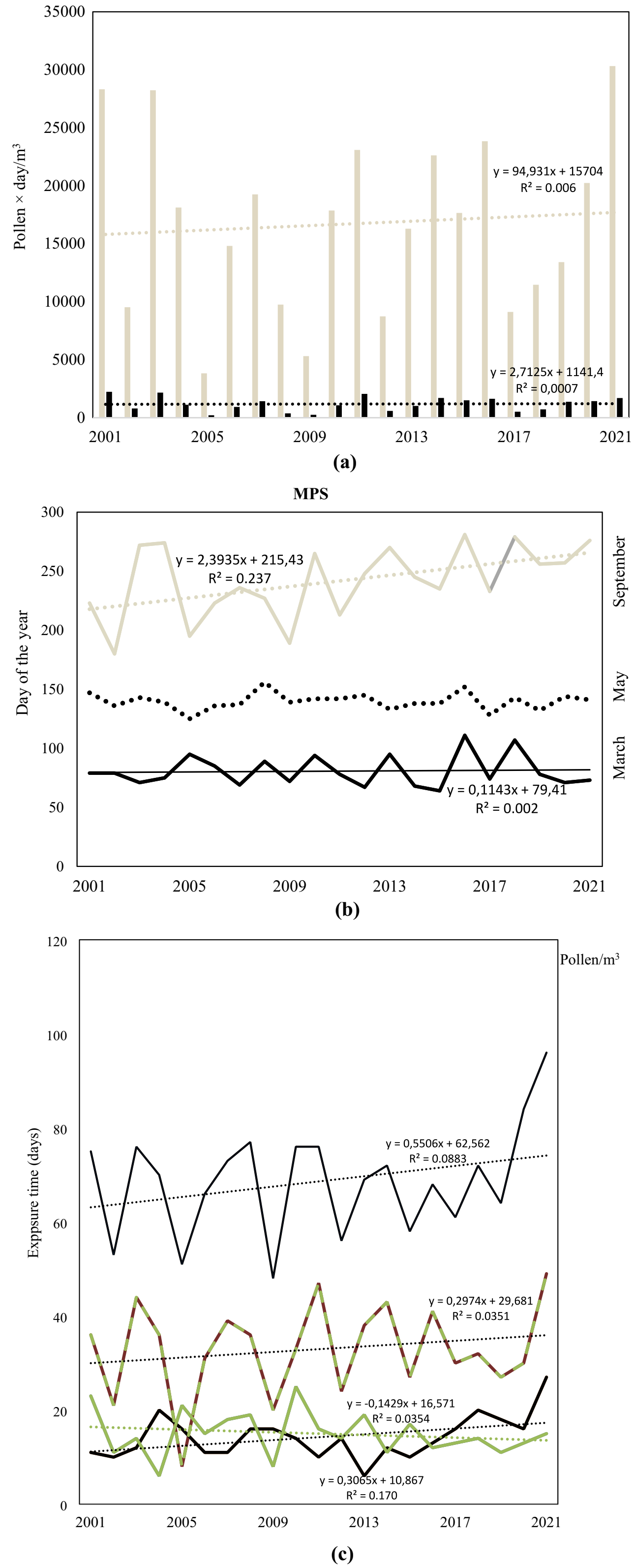

Fig. 3 Representation of the main features of Poaceae pollen seasons in Évora over the 20-year study period. **a** Annual Pollen Integral (grey histograms) for Poaceae pollen from 2001 to 2021 and Peak value (maximum daily pollen concentration) (black curve). Linear trends are represented by regression lines in their respective colour. **b** Pollen seasons start dates (black curve), end dates (grey curve) and peak dates (dashed black dots) for Poaceae pollen from 2001 to 2021. Linear trends of the start and end dates are represented by regression lines in their respective colour. **c** Number of days for which pollen concentration exceeded 25 grains/m^3; varied between 30 and 50 grains/m^3; between 50 and 100 grains/m^3 and surpassed 100 grains/m^3. Linear trends are represented by regression lines in their respective colour

pattern of grasses and its capacity to adapt to an environment.

The apparent increase in annual pollen counts agrees with the tendency of increase in grassland areas in Portugal (Eurostat, 25), in Alentejo region in particularly, where the permanent grassland varied from 17 to 48% (Jones et al., 42). Beyond the anemophily capacity, the land conditions of Alentejo and the low altitude can favour grass species proliferation (Kazakis et al., 45).

3.1.2 Main pollen season (MPS) features

The MPS for airborne grass pollen in Évora generally starts in the second week of March and finishes in August or in September, eventually in the beginning of October (Supplementary Fig. 1, Table 1). The onset of the MPS in March in other Southern European regions was also reported by Puc & Puc (64).

The average length of the MPS is 165 ± 27 days, being the maximum grass pollen daily concentration attained in May. The shortest grass pollen seasons occurred in 2005 which lasted 102 days, whilst the longest one was detected in 2021 with 204 days. The earliest onset of Poaceae pollen season was observed on 4 March 2015, and the latest on 16 April 2018. The earliest end date was detected on 7 July 2009, and the most delayed was on 7 October 2016. It was detected that some years with the earliest pollen onsets (i.e., 2004, 2009, 2015, 2017 and 2019) had higher annual maximum temperatures, whilst years with late starts (i.e., 2005, 2008 and 2016) presented lower annual minimum temperatures. Regarding the High Pollen Season (HPS), it normally occurs from May to June and lasts 52 ± 12 days on average. The average number of high pollen days tended to increase during the study period. The peak values occurred mainly in May, except at 2008 which was on 4 June, corresponding to the latest peak, whereas the earliest grass pollen peak was observed on 5 May, in 2005. The highest peak value was detected on 23 May of 2003 with 2151 pollen/m^3 and the lowest on 5 May of 2005, with 199 pollen/m^3.

As the summer in Alentejo is characterised by high temperatures and low rainfall levels, that conditions the annual life cycle of grasses, consequently the end of the main grass pollen season. The intensity and temporal trends of grass MPS and HPS features for the entire period of study were calculated by means of simple linear regressions, as shown in Table 2. Despite considered non-statistically significant, the onset of MPS and HPS showed an advance of 0.22 and 1.09 days per year, respectively, whilst the end of both seasons tended to occur later, with a delay of 1.82 and 0.40 days per year, respectively.

Also, the peak day tended to occur earlier, with a tendency to increase its concentration along the years, as did the MPS and HPS lengths. However, the increase in pollen peak was shown not to be significant, neither the corresponding linear trend represented in Fig. 3a revealed a clear pattern. The grass pollen seasons are considered long, as revealed by the large interval between start and end dates (Fig. 3b) which is corroborated by the positive slope of the MPS and HPS lengths (Table 2). Given the fact that the annual pollen totals are apparently increasing over time and the HPS counts are decreasing over time, this means that the pollen peak is getting reduced and extended over the years.

Flowering advance has been observed in several European regions (Adam-Groom et al., 2), largely due to the capacity of herbaceous plants such grasses respond more immediate to weather conditions than

Table 2 Trends of the MPS and HPS for Poaceae pollen during the whole data period. The shown parameters of the linear regression analysis were slopes of the regression, reported in unit/year; *p* value < 0.05, indicates a significant fit to linearity; n represents the number of observations; *R*-squared or coefficient of determination indicates how close the data are to the fitted regression line

	n	Linear trend regression		
		Slope	*p*	R^2
Annual totals	21	0.325	0.749	0.006
Start of MPS	21	− 0.222	0.827	0.003
End of MPS	21	1.817	0.087	0.163
Length of MPS	21	1.556	0.137	0.119
Peak value	21	0.120	0.906	0.001
Date of maximum value	21	− 0.044	0.966	0.0001
Sart of HPS	21	− 1.093	0.288	0.059
End of HPS	21	0.402	0,692	0.008
Length of HPS	21	0.981	0.339	0.048
Average of HPS	21	− 0.139	0.891	0.001
No days > 25 grains/m^3	21	1.357	0.191	0.088
No days > 30 ≤ 50 grains/m^3	21	1.977	0.063	0.171
No days > 50 ≤ 100 grains/m^3	21	− 0.835	0.414	0.035
No days > 100 grains/m^3	21	0.832	0.416	0.035

tree species (Dahl et al., 18). In our study, a tendency towards an advance in the onset and peak of grasses pollen seasons has been detected. In addition, the pollen season tends to end later and last longer, a behaviour observed in central Europe (Makra et al., 51). As the overall grass pollen seasons has been tending to advance over time, it poses a higher risk to patients develop allergic symptoms. Further, the abovementioned shifting in timing and magnitude of grasses pollen seasons in Évora along with the mentioned changes in land use in Alentejo might explain the increasing trend in the length and severity of the grass pollen seasons in this region as a whole. According to Schleussner et al. (71), the evidence on observed and future impacts of climate change in Portugal reveals that the country is already experiencing increasing climate change, with direct impact on humans and ecosystems. The mean and extreme temperatures have increased in the past decades and are projected to continue to do so. Annual precipitation has decreased, and it will decrease about 30% in the southern part, and about 15% in the northern regions (Schleussner et al., 71). Moreover, in Alentejo, water stress can occur with varying degrees of severity throughout the growing season of grasses economically important such wheat (Rocha et al., 68) and pastures (for animal food). The rising water deficits projected for southern Portugal, in March/April–June for the incoming years can be a limiting factor for the ecological success of grasses species. Water availability is essential on Poaceae development, pollen production and anthesis (Sánchez Mesa et al., 69), especially in the pre pollen season where drought may influence the decline of annual grass pollen counts (Jato et al., 41). In addition to water stress and rising temperature scenario in Portugal, another important disturbing factor is occurring more frequently and on a greater scale than expected. Centro and the Alentejo are among the Portuguese regions on which there is an extreme risk of wildfire over 2071–2100 (Schleussner et al., 71). After an ecological disturbance such a wildfire, grasses are common elements of early or intermediate stages of primary succession (Gibson, 33), a fact that ultimately should condition the severity of Poaceae pollen seasons in Portugal, particularly in Alentejo.

The results obtained in this study based on the linearity model lacks statistical significance despite the fitting of the data set to the regression line, as given by the coefficient of determination. The long-term evolution of Poaceae pollen season could be statistically proven using a large period of analysis, a similar drawback described by other aerobiological sites that also operate less than 3 decades (Hobeke et al., 38; Adams-Groom et al., 2). This shall lessen the high variability effect of pollen data underlying the variable annual pollen production cycles.

3.2 Intensity of grass pollen allergens exposure

The number of days on which the concentration of Poaceae pollen exceeded the allergenic risk threshold of 25 pollen/m^3 is 69 ± 11 days on average, being 33 ± 10 days for > 100 pollen/m^3 category. The highest number of days for both categories was recorded in 2021, with 96 and 49 days, respectively. It was noticed that 11 weeks per year reveal low or medium allergenic risk of Poaceae pollen in the atmosphere of Évora, and 21 weeks per year on average revealed higher critical levels of grass allergens (Table 1). On regards to the allergenic risk threshold**,** a tendency of increase along the years on all pollen categories was noticed, except on the No days $> 50 \leq 100$ grains/m^3 (Fig. 3c, Table 2). In fact, such general positive trend for the annual number of days above the exposure thresholds is in line with the positive values calculated for the long-term trend in APIn (Table 2). The No days > 100 grains/m^3 increased in Évora in comparison to the last survey (Caeiro et al. 8), a trend that was in line with the long-term increased in APIn which represents an issue for grass allergic suffers. There are different grass pollen thresholds indicated in the literature as triggering allergy symptoms. According to Negrini et al. (57), grass pollen counts of over 50 grains/m^3 can cause clinical symptoms in allergy sufferers, but levels over 35 grains/m^3 are considered sufficient to display such effects. In addition, a concentration higher than 120 grains/m^3 may cause dyspnoea in allergic people (Malkiewicz & Klaczak, 52).

In the next decades, temperature and precipitation patterns are projected to change heterogeneously across Portugal (Schleussner et al., 71), thus, it is expected that both variables could directly affect future local pollen emissions. The extreme drought and water stress might play negative effects on grass communities and consequently on pollen records (Jato et al., 41). Nonetheless, it is well accepted that higher temperatures above the average values can enhance pollen production and dispersal, which usually result in extended seasons of pollination on several species of grasses (Ianovici, 40; Mercuri et al., 53). Hence, the monitoring and analysis of the main Poaceae pollen seasons features can help researchers track the effective response of plants in a changing climate and infer their severity from a clinical perspective. The abovementioned climatic conditions in Alentejo, such as rising temperature, low precipitation, and the impact of several abiotic disturbances (water stress, increased grassland areas) that are currently taking place, shall determine the trends of grasses pollen seasons in decades to come.

3.3 Meteorological influences on airborne grass pollen

In an attempt to identify the possible causes that can influence the trends of aerobiological data in Évora, correlation analyses between these and meteorological variables were tested. Significant positive correlations between aerobiological data with maximum temperature (0.642; $p<0.01$), average temperature (0.599; $p<0.01$), minimum temperature (0.462; $p<0.01$), wind speed (0.106; $p<0.01$), wind direction (0.163; $p<0.01$), sunshine (0.602; $p<0.01$) and total global radiation (0.773; $p<0.01$) were observed. Relative humidity (−0.581; $p<0.01$) and rainfall (−0.397; $p<0.01$) correlated significantly and negatively with Poaceae pollen counts (Table 3).

Table 3 Results of correlation analysis between daily average of Poaceae pollen concentrations and meteorological parameters (2001–2021)

Meteorological variables	Correlation Coefficient Spearman
Mean temperature (°C)	0.599**
Maximum temperature (°C)	0.642**
Minimum temperature (°C)	0.462**
Relative humidity (%)	−0.581**
Wind direction	0.163**
Wind speed (m/s)	0.106**
Rainfall (mm)	−0.397**
Total Global Radiation (KJ/m^2)	0.773**
Sunshine (*h*)	0.602**

**a statistically significant correlation coefficient ($p<0.01$)

Flowering and pollen emission are process closely associated with environmental drivers (Ziello et al., 79). As reported previously (Palacios et al., 60; Dąbrowska, 17), our findings indicate that warm temperatures and stronger wind contribute to greater grass pollen concentrations and dispersion in Évora over the years.

As previously reported by Caeiro et al. (8), we also observed a positive but not statistically significant trends on Poaceae main pollen season, length, and intensity, which are in line with local temperature increasing trend in Alentejo. Nevertheless, the inverse effect was observed with respect to days with relative humidity and rainfall, which seem to be a limiting factor for pollen occurrence and dispersion. These weather parameters have been considered important factors in controlling pollen production in spring and their influence prevails during the pollen season (Emberlin et al., 22; García-Mozo et al., 32; Kasprzyk & Walanus, 44). In reality, each grasses species responds differently to atmospheric conditions, but in general, temperature and light intensity determine the opening of anthers and pollen release (Laaidi, 48), whilst rainfall and high air humidity inhibit anthesis (Green et al., 36).

Ultimately, it seems that temperature and light factors had a positive effect on daily Poaceae pollen records, and that was reflected on the increasing trends on APIn and in the MPS/HPS features. More, with a general rising trend of the annual number of high pollen days in Alentejo, it is expected a worsening of allergic symptoms in patients sensitized to grasses in the coming years. Longer and more intense grass pollen seasons was detected in our survey, a phenomenon that has being described for other pollen types, with consequently increase on respiratory allergy burden on sufferers, healthcare providers and society in general (D'Amato et al., 16; Kishikawa & Koto, 46).

4 Conclusions

This study shows that important changes are occurring in Poaceae pollen seasons in Évora. The long pollen seasons (March to beginning of October) may reflect the pollination of multiple flowering species that normally bloom between May and June. It seems that grass pollen production is being aided by warmer temperatures and increased grassland areas in Alentejo. This could promote the rising annual pollen totals and the number of high pollen days over the years.

The temperature and light factors were considered important climate drivers of grasses pollen seasons. Poaceae species seem to response to temperature increase whereby this pollen type can be a bio-indicator to monitor local climate change. Poaceae pollen seasons are starting earlier, lasting longer, and ending later. The peak day also tended to occur earlier, with higher levels over the years. Both MPS and HPS lengths tend to augment, as well as the number of days for which pollen concentration exceeds clinical thresholds. In consequence, grasses seasons become more severe over the years and greater exposure times to grasses allergens may occur in the coming years. The aforementioned ecological disturbances that are already affecting Alentejo need to be taken into consideration on future aerobiological studies to explain more accurately yearly changes and trends in grasses pollen counts.

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Declarations

Conflict of interest The authors declare no competing interests.

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