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Trends in land cover and in pollen concentration of *Quercus* genus in Alentejo, Portugal: Effects of climate change and health impacts☆

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ABSTRACT

Mediterranean forests dominated by *Quercus* species are of great ecological and economic value. The *Quercus* pollen season, peaking in April, varies in concentration due to geographical and climatic factors and has a remarkable allergenic potential. This study investigates *Quercus* trends in the Alentejo region of Portugal and examines the influence of meteorological parameters on DPC, PSD and SPIn, as well as the impact on allergic respiratory disease. The results show a progressive increase in *Quercus* Forest area from 1995 to 2018. Temperature and Precipitation are a key factor influencing pollen concentration, especially before peak of pollen season and prior to the pollen season. Particularly prior to the season, the precipitation of t-6 before influence, significantly, the pollen production. On the other hand, Global Srad and RH determine the beginning of the season. Using quartile-based categorization and multivariate statistical analysis, we identified years and scenarios within the IPCC projections where meteorological conditions influence may SPIn production. The study found a statistically significant correlation between high *Quercus* pollen concentrations in April and increased antihistamine sales. These findings are crucial for enhancing pollen forecast models and early warning systems.

1. Introduction

The *Quercus* genus ranges from trees to shrubs and is present mainly in the northern hemisphere, representing an important source of income in several countries (Deng et al., 2018; Denk et al., 2017; Manos et al., 1999). The genus *Quercus,* commonly known as oaks (cork oaks, holm oaks, executioners, etc.) is represented mainly by twenty taxa in the Iberian Peninsula, being well represented in the Atlantic and Mediterranean regions and dominate most mediterranean ecosystems (Rivas-Martínez et al., 2011). In Portugal, thirteen taxa of *Quercus* are represented, located from north to south, such *as Quercus robur* subsp. *broteroana* O. Schwartz, *Quercus pyrenaica* Willd., *Quercus canariensis* Willd., *Quercus faginea* Lam., *Quercus lusitanica* L., *Quercus suber* L., *Quercus rotundifolia* Lam., and *Quercus coccifera* L. and other subspecies and hybrids (Costa et al., 2012). The greatest diversity of *Quercus* is found in the transition areas from the mediterranean to the temperate macro-bioclimate, as is the case of Portugal (Rivas-Martínez et al., 2017). One of the species with the highest economic value is the cork oak (*Quercus suber* L.), associated with the extraction of its bark – cork (Sierra-Pérez et al., 2015). Due to its recognized thermal and acoustic properties (Poeiras et al., 2021; Silva et al., 2013), its applications are varied from corks for wine bottles, clothing, surfboards and even in the aerospace industry (Mateus et al., 2017; Peña-Neira et al., 2000; Reis & Silva, 2009). Due to the growing economic value of cork in recent decades, there is a tendency to increase the areas planted with cork oak, where irrigation and fertilization are even tested to increase their productivity and reduce the time between extractions, which is currently at least nine years (Camilo-Alves et al., 2022; Ribeiro et al., 2020).

Although the cork oak is native from the north to the south of mainland Portugal, it is in the Alentejo and Ribatejo regions where the largest continuous area of this species is found and where production is highest (Natividade, 1990). Normally, the cork oak is part of the land

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exploitation system known as montado/dehesa, formed by a domain of the vivacious herbaceous stratum and dispersed trees in different degrees of density, like a savannah (Pinto-Correia et al., 2013; Godinho et al., 2016). This type of exploitation is even recognized at European level as a habitat of the Natividade, 1990 Network (6310 – Montados de *Quercus* spp. with perennial leaves), through Annex I of Directive 92/43/EEC. Another common tree in the montados is the holm oak (*Quercus rotundifolia* Lam.), valued to produce sweet acorns, which fatten pigs, and for its high-quality wood (Tejerina et al., 2011; Quinto-Canas et al., 2021).

Quercus are anemophilous trees that produce large amounts of pollen (Gomez-Casero et al., 2004; Jato, Rodriguez-Rajo and Aira, 2007a,b; Molina et al., 1996). Their staminate inflorescences begin at the end of spring, one year before the next pollination and meiosis occurs next spring (Grewling, Jackowiak and Smith, 2014). The male flowers are evenly distributed in the long inflorescences, resemble catkins and their number varies between 19 and 26 flowers. For a given tree, the growth of the catkins is achieved 1–2 weeks after the opening of the buttons and pollination is completed in 2–4 days, however its rate of development will depend on external factors such as weather conditions (Rivas-Martínez et al., 2017). In central Europe, the flowering period of *Quercus* is limited to the month of March and April, however, in Portugal, the flowering period of *Quercus* species is wider, starting in early March to the end of May (Fernández-Rodríguez et al., 2016). *Quercus* forests produce a pollen mass ranging from 100 to 1000 kg/ha (Greenfield, 1996) Most pollen is deposited in lakes and forest soils, causing the ecosystem productivity to increase. Pollen is rapidly decomposing in aquatic and terrestrial ecosystems, releasing nutrient-rich matter to the environment. This material is available for bacteria's, fungi, protozoa, and other types of invertebrates, so pollen play an important role in forests through nutrient recycling (Filipiak, 2016). In northwestern Spain the presence of *Quercus* trees in the forest is an indicator of ecological maturity (Jato, Rodriguez-Rajo and Aira, 2007a,b; Recio et al., 2018), as they are responsible for maintaining the physical-chemical and microclimatic characteristics of the ecosystem (Recio et al., 2018), considering the use of *Quercus* pollen levels information is an important bioindicator to detect ecological variability induced by climate change (del Río et al., 2018; Cano-Ortiz et al., 2022).

In general, *Quercus* pollen is not considered an important aeroallergen, however, the high abundance of these species and areas of natural and seminatural vegetation increase the risk of allergy, mainly for agricultural and forestry people, since they are exposed to high levels of *Quercus* pollen (García-Mozo et al., 2008). In addition, it has already been verified that the relation between clinical and aerobiological data in the UK suggested that *Quercus* pollen may be an important cause of hay fever (Egger et al., 2008). This may be because there is a cross-reactivity between species of the Fagales family, such as Hazel, Almond and Betula (D'Amato et al., 2007; RODRÍGUEZ-RAJO et al., 2005). Cross-reactivity of *Quercus* pollen was observed with Bet v 1, Bet v 2 and Bet v 4 (Ickovic and Thibaudon, 1991). The aerobiological dynamics of *Quercus* pollen stations is being widely studied in the UK and Spain, however, there is still a scarcity of data about the influence of environmental factors on the production and release of *Quercus* pollen. The dynamics of the pollen concentrations and their relationship with the meteorological parameters have already been studied in some countries, giving particular importance to the long-term increase in temperature during winter and spring, a consequence of climate change that is causing early flowering in diverse pollen species from Europe. Changes in the intensity and duration of pollen seasons in plants that produce pollen considered allergenic have important consequences for allergy sufferers (Ziska et al., 2019).

The study aims to investigate the trends in *Quercus* Forest coverage in the Alentejo region and to assess the viability of these forests in terms of pollen production. The investigation focuses on understanding how meteorological conditions affect daily pollen concentrations, PSD and SPIn. Advanced statistical analysis is used to identify periods within the IPCC projection and scenarios where meteorological conditions may affect *Quercus* SPIn production. The allergenic effect of this pollen type is also considered. This in-depth analysis has important implications for the development and refinement of pollen forecasting models and early warning systems.

2. Methods

2.1. Study area

Fig. 1 shows the location map of Portugal and the 18 Portuguese continental administrative regions (districts), highlighting the Évora Station (red star) used in this work. The study area is characterized by a sub-humid thermos-Mediterranean bioclimatic class and at the geological level it is dominated by biotitic granite rocks, which gives the soil an acidic nature (Rivas-Martínez et al., 2017). The relief is flat to wavy, and the landscape dominates the traditional exploitation of cork oak forests (agro-silvo-pastoral system), resulting from the de-density of the evergreen *Quercus* forests, belonging to the *Asparago aphylli-Querco suberis* sigmetum series (Cancela d′ Abreu et al., 2004; Raposo et al., 2016). Although there is a domain of grazing with cattle, the legal protection of cork oak (*Quercus suber* L.) and holm oak (*Q. rotundifolia* Lam.), through Decree-Law no. 169/2001, of 25 May. D.R. No. 121, Series I-A, amended by Decree-Law No. 155/2004, of 30 June. D.R. 152, Series I-A, has contributed to its conservation. On the other hand, the economic value of cork has also encouraged landowners to plant new areas with *Q. suber*. The sampling point is located at the city of Évora, Fig. 1, and an area of greater pollen influence was defined, with a radius of about 20 km. To identify the trends of *Quercus* coverage over the years, all the official Land Use and Occupancy Charts were used, available through the Direção-Geral do Território for the years 1995, 2007, 2010, 2015 and 2018.

2.2. Aerobiological data, main pollen season (MPS) and pollen calendar

Pollen Data were collected for the European Aeroallergen Network (European Aeroallergen Network) (https://ean.polleninfo.eu/Ean/) and Pólen Alert database (https://lince.di.uevora.pt/polen/). Airborne *Quercus* pollen was collected with Hirst-type volumetric spore traps (Hirst, 1952) from 2002 to 2021 with a regional representation of >25 km radius (Oteros et al., 2019; Oteros et al., 2015). The sampler was placed at the Évora Atmospheric Sciences Observatory (EVASO), located on the roof-top of the Science and Technology School of the University of Évora, about 10 m above the ground. Briefly, by passing through a narrow intake orifice (2 × 14mm), the sampled air impacts onto a clock driven drum rotating at an angular velocity of 2 mm/h, taking 7 days to perform a complete turn. The drum is covered with a double-sided Mellinex tape, where airborne pollen is retained. After exposure, this tape is cut into seven 48 mm long segments, each representing one day of sampling, that are mounted on a microscope glass slide). Slides were analyzed daily under light microscope (400× magnification), and the results were expressed as pollen per cubic meter of air (Pollen/m^3). The Main Pollen Season (MPS) was determined by a logistic method developed for (Ribeiro et al., 2007) and modified by (Cunha et al., 2015) This method is based on fitting a non-linear logistic regression model to the daily accumulated curve for each pollen type (Cunha et al., 2015). Parameters such as, *start_date*, and *end_date* were determined based on asymptotes when pollen amounts are stabilized on the beginning and end of the accumulated curve. The Pollen Calendar, represented as a *Violinplot*, was used for representing the daily averages of pollen concentrations. This method is based on pollen intensity and adapted to the calendar published by (O'Rourke, 1990).

2.3. Meteorological parameters

The meteorological variables used were obtained from the

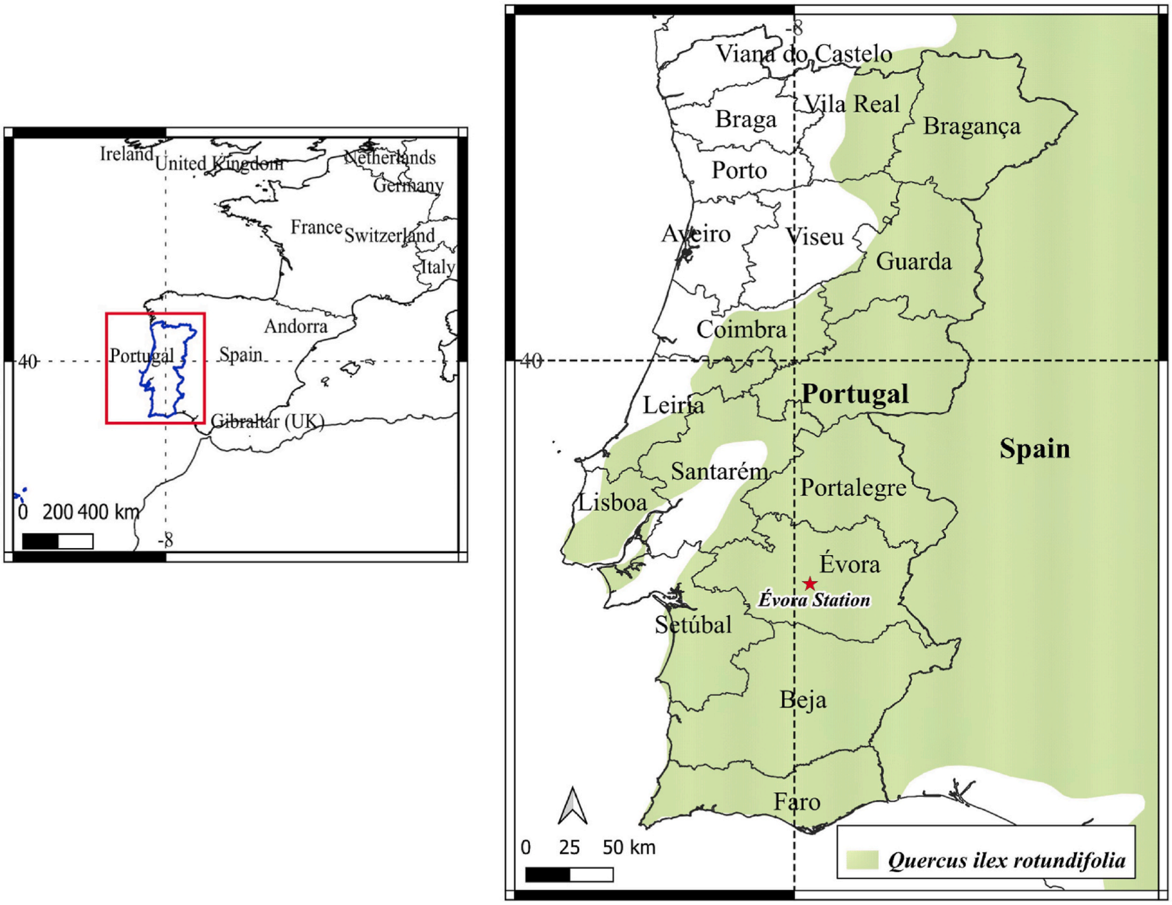

Fig. 1. Location map of Portugal and the location of the 18 Portuguese continental administrative regions (districts). The map also shows the locations of the Évora Station (red star) used in this work. The green color represents the *Quercus rotundifolia* Lam. chorological map distribution in Portugal, southwestern Europe. Chorological maps were based on the Caudullo et al. (2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

meteorological station installed at the Évora Atmospheric Sciences Observatory (EVASO) where the pollen sampler is also installed during the years 2002–2021, in the months prior to the beginning of pollination and during the *Quercus* genus pollen season. The measured variables, regularly checked, were the air temperature (°C), relative humidity (%), wind speed (m/s), wind direction (°), precipitation (mm) and Global solar radiation (kJ/m^2) at 10-s intervals, and then hourly and daily averaged (accumulated in the case of precipitation and solar radiation). The averaged wind direction values were obtained applying the arctangent to the ratio between the east-west and the north-south components of the wind. The averaged east-west and north-south components are calculated from the wind speed data, excluding zero values. This average is performed according to the procedure described in Campbell Scientific (https://s.campbellsci.com/documents/br/manuals/cr1000.pdf, last accessed June 21, 2024).

2.4. *Antihistamines sales data*

Data on antihistamines sales were obtained from the National Pharmacy Association (ANF), corresponding to monthly frequency from 2004 to 2021. Sales data correspond to antihistamines belonging to group 1 (G1 – histamines antagonists) during every year (histamine antagonists) and group 2 (G2) drugs sale in SOS, e.g., corticosteroids, β2-agonists and LTR antagonists.

2.5. *Statistical analysis*

The statistical analysis was used to study the influence of meteorological parameters on *Quercus* pollen concentrations. The data do not follow a normal distribution and therefore non-parametric tests were used. Statistical assessment of differences between meteorological parameters was performed using Kruskal-Wallis (significance level of 0.05) (Zar, 2007). The trend analysis of temperature (Fig. 4) was performed from the mean temperature in the months of the *Quercus* main pollen season in all years. Spearman's correlation analysis at significance levels of 5% and 1% was used to study the relationship between meteorological parameters and daily pollen concentrations, SPIn (Seasonal Pollen Index), DPC (Daily Pollen Concentrations) and PSD (Pollen Season Duration). For the study of the relationship between pollen counts/m^3/months and Antihistamines sales, the Spearman's correlation at a significance level of 5% was used.

In addition to correlation analysis, this study adopts a comprehensive methodology that integrates correspondence analysis, Pearson's chi-square and Fisher's exact tests. This combined approach aims to explore the complex relationship between the *Quercus* SPIn and meteorological variables - wind speed (WS), mean temperature (T_{mean}), maximum temperature (T_{max}), minimum temperature (T_{min}), precipitation (Prep), relative humidity, and global solar radiation (GSRad). The importance of this methodology lies in its emphasis on categorizing variables, providing an alternative to correlation analysis for validating associations. To implement this methodology, the variables including SPIn and the meteorological variables were categorized using quartiles.

Quartiles, a statistical method of dividing data into four equal parts, effectively establish three ranges: Low (0–25%, values below the first quartile, Q1), Medium (25–75%, values in the interquartile range between Q3 and Q1) and High (75–100%, values above the third quartile, Q3). This classification not only facilitates the analysis of continuous variables, but also provides a clearer perspective for identifying patterns and trends. In addition, the quartile-based categorization facilitates the establishment of standards for each variable, reflecting variations in SPIn and meteorological components over time. By systematically organizing the data into quartiles, this approach enhances the interpretability of the results.

Following the quartile-based categorizations, Pearson's chi-squared test and Fisher's exact test (Fisher, 1922) was employed on the categorized data to evaluate the relationship between SPIn (as the dependent variable) and meteorological variables (considered as independent variables). The null hypothesis (H_0) states that there is no significant relationship between these variables, assuming independence, while the alternative hypothesis (H_1) states that there is a significant relationship,

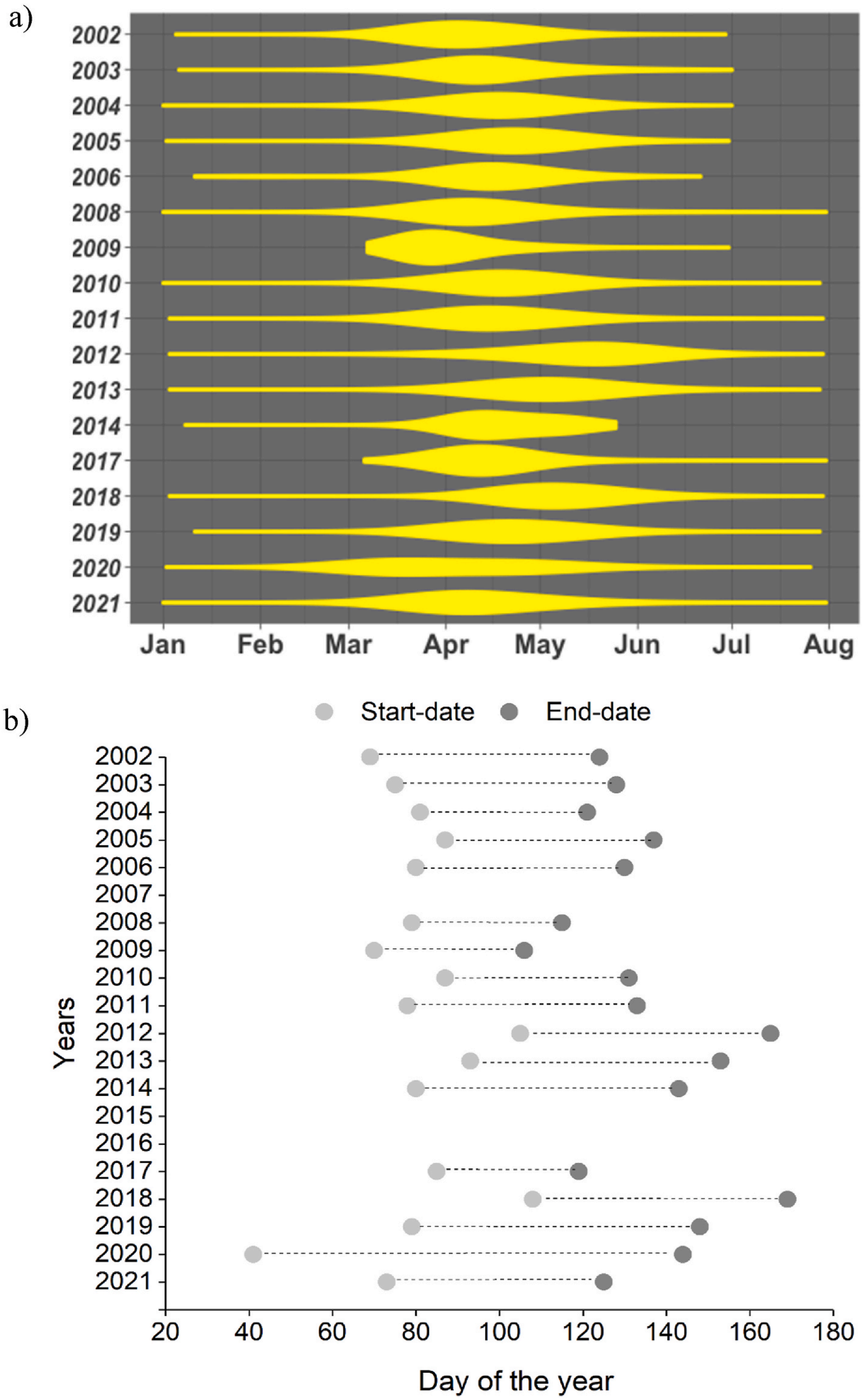

Fig. 2. a) *Quercus* pollen season between 2002 and 2006, 2008–2014 and 2017–2021. b) Start and end-date of *Quercus* pollen season between the years 2002–2021 in Evora region.

indicating dependence between the variables. Fisher's exact test, an extension of Pearson's chi-squared test, meticulously evaluates each degree of freedom to determine the true probability of observed variation. Unlike the chi-squared test, which may not be appropriate for small sample sizes, Fisher's exact test remains robust. By applying Fisher's exact test alongside chi-squared analysis, the study seeks to rigorously investigate the relationship between meteorological variables and SPIn, thereby providing valuable insights into their mutual interdependence. The resulting p-value derived from the tests plays a crucial role in assessing the statistical significance of the observed association. A p-value ≤ 0.05 indicates that the null hypothesis is highly improbable, leading to its rejection and indicating statistical evidence in favour of the observed association. Conversely, a p-value of >0.05 indicates that the null hypothesis is highly probable, leading to a declaration of "fails to reject", meaning that there is no statistical evidence to support a correlation between the two variables.

Finally, correspondence analysis (CA) (Greenacre, 2007), a multivariate statistical technique, was used to explore associations and patterns within the data. This method allows the visualisation of relationships within complex categorical data. CA works by visually representing the factor scores (coordinates) in a lower dimensional space. This visualisation ensures that the positions accurately reflect their associations. Each dimension in CA corresponds to the percentage of variance explained, with dimension 1 explaining the highest percentage of variance, followed by dimension 2, and so on. Effective dimension reduction is achieved when the original dimensions capture a substantial portion of the variability in the data.

3. Results

3.1. Characterization of genus Quercus land use between the years 1995–2018

The main *Quercus* identified in the study area were cork oak (*Q. suber* L.), holm oak (*Q. rotundifolia* Lam.) and kermes oak (*Q. coccifera* L.), however, the dominant species in the landscape is cork oak. The analysis of the area occupied by these species resulted in a progressive increase from 1995 to 2018. Thus, the increase in the area occupied by *Quercus* for twenty-three years was greater than one thousand hectares. This increase can have consequences on the quantity and quality of pollen in the atmosphere (Fig. S1).

3.2. Characterization of genus Quercus pollen season between the years 2002–2021

The characteristics of the *Quercus* pollen season in the periods of 2002–2006, 2008–2014 and 2017–2021 were analyzed and are shown in Fig. 2 (a)) and Table S1. Unfortunately, no data is available during the intermediate years missing from the plot in Fig. 2.

Quercus pollen was detected in large concentrations in the atmosphere of Évora. It was observed that the pollination of *Quercus* pollen occurs mainly in springtime, mostly between the months of March and May, Fig. 2. The pollen season is observed to start in March in all years except for the years 2018, 2012 and 2020, when the season began on April 18th (2018 and 2012) and February 10th (2020) (Table S1; Fig. 2 (a) and (b)). Regarding the end of the pollen season, it was observed that in most years the season ended in the month of May, however, 2008, 2009 and 2017 presented an earlier end of the season (April 25th and 16th, respectively), and the year 2018 presented a late season end, on June 18th (Table S1). The duration of pollen season varied mainly between 35 and 105 days (average: 55 ± 17 days), however it was observed that the year 2017 and 2020 presented an exceptional 35 and 105 days, respectively (Fig. 2(a) and (b)) and Table S1).

Regarding the SPIn, the highest SPIn was observed in 2014 with 25976 pollen/m^3 (average for the period 13050 ± 6330 pollen/m^3), on the contrary, 2020 was the year exhibiting the lower SPIn, with 4798 pollen/m^3 (Table S1). *Quercus* genus accounted for 19.1 ± 7.45% of the total annual pollen recorded in the atmosphere of Évora in the years under study. The highest percentage occurred in 2013 (31.25 %), while the lowest percentage was in 2010 (7.69 %) (Fig. S2).

3.3. Effects of meteorological parameters on Quercus daily pollen concentrations, PSD and SPIn

Figure S3 and **S3.1** shows the daily meteorological parameters during the pollen season for each year. Seasonal T_{mean} during pollen season of *Quercus* in all years varied between 7.0 °C and 27.8 °C with an average of 15.4 ± 3.7 °C. T_{max} ranged from 10.6 °C to 36.5 °C with an average of 22.0 ± 3.1 °C, while T_{min} ranged between −2.5 °C and 20.4 °C with an average of 9.8 ± 3.1 °C. Years 2003; 2009 exhibited the lowest T_{mean} (12.9 ± 1.9 °C and 13.5 ± 3.2 °C, respectively) while the year 2017 recorded a highest T_{mean} (17.4 ± 3.1 °C). Considering T_{min}, it was observed that the year 2005 and 2009 presented the lowest T_{min} (7.9 ± 4.2 °C and 7.8 ± 2.8 °C, respectively), while the highest T_{min} was observed in 2017 (11.4 ± 2.5 °C). Regarding T_{max}, the year 2003 and 2009 present the lowest T_{max} with 18.1 ± 3.0 °C and 20.1 ± 3.8 °C, respectively, while 2005 presents the highest T_{max} with 24.3 ± 4.0 °C. The significant differences observed between the previous factors are represented in Figure S3.1.

During the season, the precipitation ranged between 0.1 and 87.8 mm in all the years. For precipitation, no significant statistical differences between the years were found, however, the years when more precipitation occurred during the pollen season were 2002 with 236 mm (45% of occurrence: 25/56 days) and the year 2020 with 225 mm (39% of occurrence: 41/104 days) (Figure S3.1). A minimum of accumulated precipitation occurred in 2017 (26.7 mm).

The RH ranged from 16.74 % to 112.6 % with an average of 64.4 ± 14.8 % in all the years, however, was observed that 2002 presents the higher RH compared with other years (87.2 ± 14.1 %). Global Srad varied between 2287 kJ/m^2 and 49865 kJ/m^2, there was a great variability in all years except 2017 (Fig. 4(b)), however, there was a lower Global Srad in 2003 followed by 2020 with 17499 kJ/m^2 and 17452 kJ/m^2, respectively. The seasonal Global Srad was highest was 2014 at 34602 kJ/m^2 (Figure S3.1).

The WS ranged between 0.7 m/s and 8.6 m/s with an average of 2.4 ± 1.0 m/s and was observed that the year 2020 presents the lowest WS with, only, 1.8 m/s, contrary was observed in 2012, that the WS was higher compared with other years (4.1 ± 1.3 m/s). Regarding, WD the predominantly winds came from S-NE, however, in the year 2003 occurred winds predominantly of North and 2011 occurred winds of all directions, mainly NE and SE (Fig. S4).

To evaluate the influence of meteorological parameters on pollen concentrations, correlation analysis was performed.

The *Quercus* daily pollen concentration in Before peak and MPS showed the positive and significant correlation with Temperature, Global Srad and Wind, contrary was observed for the precipitation and RH, the same occurs for DPC After peak (Table 1). Considering, SPIn, was observed that the precipitation results showed that a negative correlation with Precipitation (R: −0.599*; p = 0.031), RH (R: −0.506*; p = 0.045) and WS (R: 0.689**; p = 0.002). Regarding PSD, there is no correlation with meteorological parameters (Table 1).

3.4. Effect of meteorological parameters in pollen production and start of Quercus season

The effect of the meteorological parameters during the 12-month period prior to the pollen season (Temperature, Precipitation, RH and Global Srad) were analyzed for the SPIn and for the Start-date (Fig. 3).

It was observed that different meteorological parameters prior to the season in different periods may affect the intensity of the *Quercus* pollen season. For Fig. 3 and Table S1, it is observed the negative and significant correlation with T_{mean} and T_{max} 3 months before pollen season (R:

Table 1

Spearman correlation between meteorological parameters and *Quercus* daily pollen concentrations (DPC) in Before peak, After peak and Main Pollen Season (MPS) period in the years 2002–2006; 2008–2010, 2013 and 2017–2021. Mean temperature (T_{mean}, °C), Maximum temperature (T_{max}, °C), Minimum temperature (T_{min}, °C), Precipitation (mm), Relative humidity (RH, %), Global Solar radiation (Global Srad, KJ/m^2), Wind Speed (WS, m/s) and Wind Direction (WD, °). *Correlation is significant at the 95% ($p<0.05$) and **Correlation is significant at the 99% range ($p < 0.01$).

	DPC Before peak	DPC After peak	DPC (MPS)	PSD	SPIn
T_{mean}	**0.554****	**0.312****	**0.381****	0.177	−0.103
$T_{\max}$	**0.544****	**0.339****	**0.402****	0.177	0
$T_{\min}$	**0.424****	**0.197****	**0.250****	0.352	−245
Precipitation	**−0.397****	**−0.378****	**−0.353****	0.399	**−0.549***
RH	**−0.485****	**−0.404****	**−0.412****	−0.064	**−0.506***
Global Srad	**0.548****	**0.232****	**0.402****	−0.046	0.380
WS	**0.186****	**0.108***	**0.128****	−0.028	**0.689****
WD	**0.152****	**0.122****	**0.139****	0.094	0.243

−0.495; p = 0.043 and R: −0.502; p = 0.040, respectively), T_{min} and T_{mean} 4 months before pollen season (R: −0.588; p = 0.013 and R: −0.527; p = 0.030), positive and negative correlation with precipitation 6 and 10 months before pollen season, respectively (R: −0.659; p = 0.004; R: −0.526; p = 0.030) with *Quercus* SPIn (Fig. 3 and Table S4).

Also, the effect between the meteorological parameters and start-date of *Quercus* pollen season was analyzed. Our results showed that at different periods the effect of temperature, Global Srad and RH was different. In the 10 and 11 months before the beginning of the pollen season, the effect of T_{max}, T_{mean} and Global Srad was positive and statistically significant, while RH and Precipitation showed a negative effect (Fig. 3 and Table S2). From t-8 months onwards, the influence of Global Srad is negative and statistically significant and remains so until t-5 months. In the t-5 months there is a turning point with the Temperature, starting to have a negative effect. From t-3 months onwards, Global Srad has a positive and significant effect up to t-1 and RH remains with the opposite effect (Fig. 3 and Table S2).

Table 2 shows the categorization of the variables based on quartiles for SPIn and meteorological variables during the February–March period preceding the *Quercus* pollen season. For each variable, the table defines three categories: Low, Medium and High, determined by quartiles (Q1 and Q3). This categorization helps to understand how different meteorological conditions preceding the *Quercus* pollen season can influence its intensity. In line with Table 2, Table S3 presents the results of Pearson's chi-squared, and Fisher's exact tests performed between SPIn and the meteorological variables during the February–March period preceding the *Quercus* pollen season for different time lags (t-1 to t-12). For each combination of variables, the table shows the chi-squared value, the degrees of freedom (DF), the chi-squared p-value and Fisher's exact p-value. Notably, significant associations were found at certain time points such as t-6 for SPInxT_{mean}, SPInxT_{max} and SPInxPrep and t-10 for SPInxRH. In addition, significant associations were found at t-1 and t-6 for SPInxGSRad, while significant associations were found at t-11 for SPInxT_{min}. These results show a statistically significant influence of previous meteorological conditions on pollen production levels and underline the importance of considering different factors for accurate allergy risk management and prediction.

To complement the results of Pearson's chi-squared and Fisher's exact tests, Table S3, correspondence analysis (CA) was used to explore the relationships between categorized levels of Quercus SPIn and meteorological variables. Two-dimensional space biplot analysis was used in this research, as dimensions 1 and 2 account for most of the variance explained. Illustrated in Fig. 4(a)–(f), the symmetrical biplot shows the results of the correspondence analysis for SPIn and meteorological variables. In particular, the closeness between high SPIn (H_SPIn) and low mean temperature (L_TEMP) at t-6, Fig. 4(a), indicates their statistically significant association, Table S2, while high mean temperature (H_TEMP) is associated with low SPIn (L_SPIn). Similarly, the association between H_SPIn and L_TMAX at t-6, Fig. 4(b), is evident, while H_TMAX is associated with L_SPIn. These results suggest that the average and maximum temperature over the last six months (t-6) may influence SPIn production levels. Statistically significant chi-squared and Fisher's exact tests suggest that the observed relationship is less likely to be due to chance. Conversely, Fig. 4(c) shows associations between high SPIn (H_SPIn) and low minimum temperatures (L_TMIN), as well as between low SPIn (L_SPIn) and high minimum temperatures (H_TMIN) for the last t-6, but these association were not statistically significant for t-6, Table S3. Similarly, Fig. 4(d) shows an association between high relative humidity (H_RH) and high SPIn as well as L_RH and L-SPIn at t-6 period prior to pollen season, but the associations are not statistically significant, Table S3.

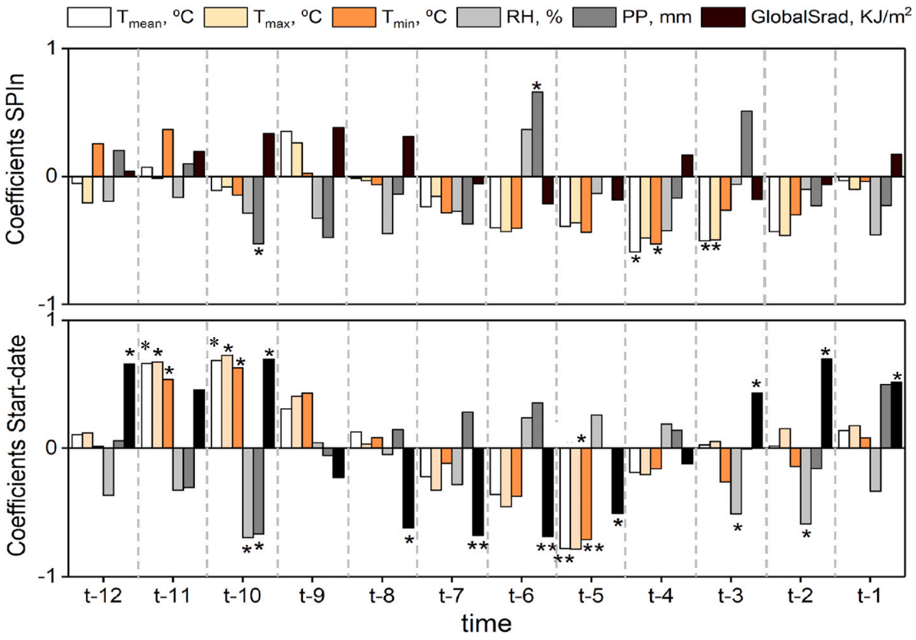

Fig. 3. Spearman's correlation between the time-lags of meteorological parameters and *Quercus* SPIn.

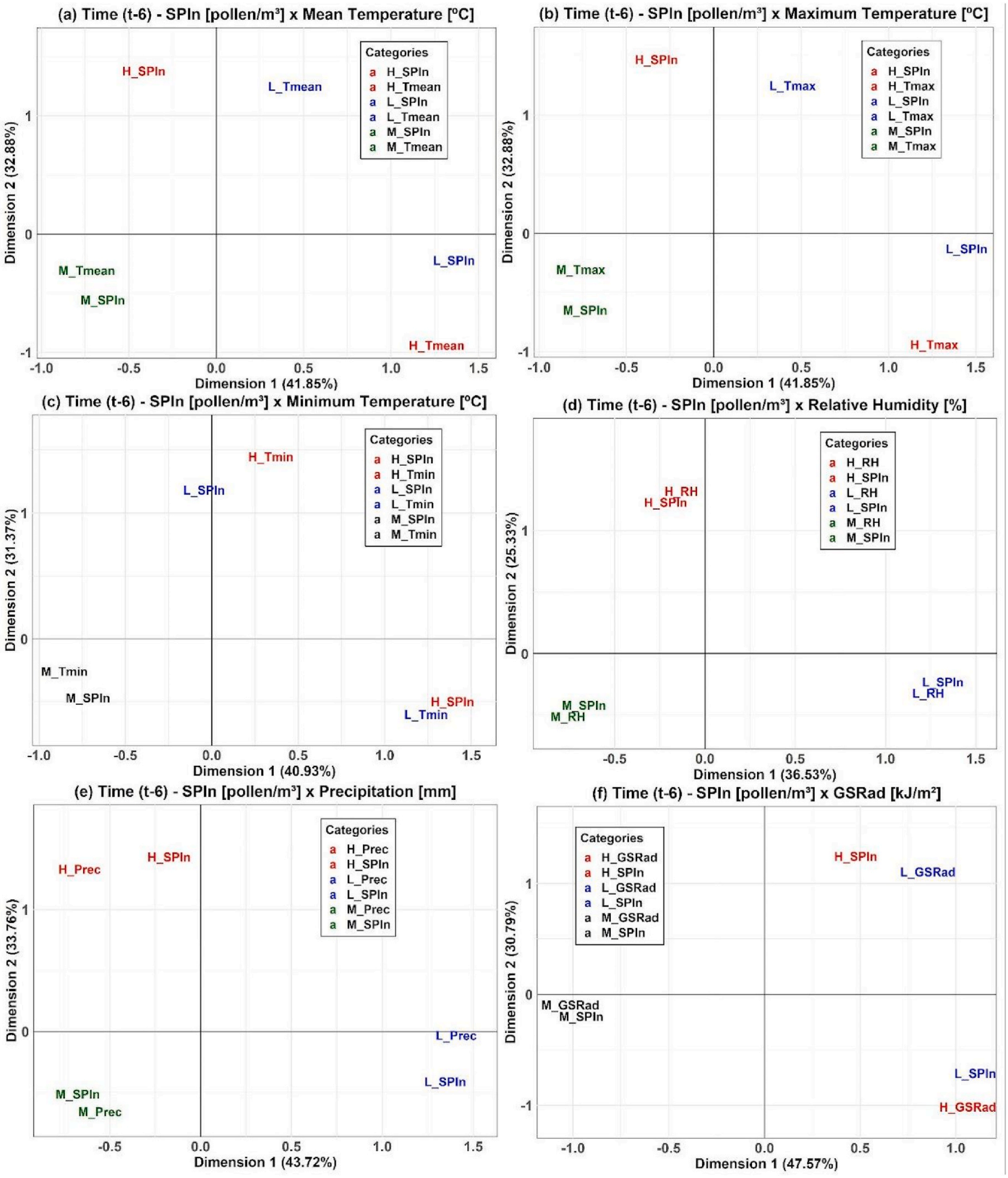

Fig. 4. Correspondence analysis biplot for SPIn and meteorological parameters during the Feb–March period prior to the pollen season of *Quercus* based on quartile categories: (a) SPIn x mean temperature (Tmean); (b) SPIn x maximum temperature (Tmax); (c) SPIn x minimum temperature (Tmin); (d) SPIn x relative humidity; (e) SPIn x precipitation (Prec); (f) SPIn x global solar radiation (GSRad).

On the contrary, Fig. 4(e) shows an association between high precipitation levels (H_Prec) from the prior t-6 period and high SPIn levels (H_SPIn), as well as between low precipitation levels (L_Prec) and low SPIn levels (L_SPIn). Table S3 confirms that these relationships are statistically significant, indicating the importance of t-6 precipitation levels and their impact on SPIn production levels. These observed associations are not random occurrences as confirmed by Pearson's chi-square test and Fisher's exact test, but rather suggest an important relationship between prior t-6 precipitation and SPIn production. Similarly, Fig. 4(f) shows an association between low global solar radiation (L_GSRad) at t-6 and high SPIn levels (H_SPIn), as well as between high global solar radiation (H_GSRad) and low SPIn levels (L_SPIn). Table S3 confirms these associations as statistically significant, indicating that the relationship between global solar radiation levels six months prior and SPIn production levels is not random. These results emphasize that t-6 global solar radiation can affect SPIn production levels.

The Coupled Model Intercomparison Project (CMIP) of the World Climate Research Programme (WCRP) analyzes global climate models (GCMs) to understand past, present and future climate systems (Tran-Anh et al., 2023; Calvin et al., 2023; Copernicus Climate Change Service, 2021). CMIP6 data support the IPCC Sixth Assessment Report (AR6) with scenarios including SSP1 (Sustainability), SSP2 (Middle of the road), SSP3 (Regional Rivalry), SSP4 (Inequality) and SSP5 (Fossil-fuelled Development). The CMIP6 experiments, categorized as Tier 1

Table 2
Categorization of variables based on quartiles for SPIn and meteorological parameters during the Feb–March period prior to the pollen season of *Quercus* for mean temperature (Tmean) [°C], maximum temperature (Tmax) [°C], minimum temperature (Tmin) [°C], relative humidity (RH) [%], precipitation [mm], global solar radiation [kJ/m^2]. Low values are below Q1, Medium values are the interquartile range between Q3 and Q1, High values are above Q3.

Variable	Low	Medium	High
SPIn	<8201	8201 ≥ SPIn ≤ 15364	>15364
Tmean (t-6)	<17.47	17.47 ≥ Tmean ≤ 19.60	>19.60
Tmax (t-6)	<24.13	24.13 ≥ Tmax ≤ 26.62	>26.62
Tmin (t-6)	<12.72	12.72 ≥ Tmin ≤ 14.53	>14.53
RH (t-6)	<56.25	56.25 ≥ RH ≤ 68.88	>68.88
Prep (t-6)	<23.08	23.08 ≥ Prep ≤ 81.20	>81.20
GSRad (t-6)	<13148.91	13148.91 ≥ GSRad ≤ 15893.50	>15893.50

(SSPs 1–2.6, 2–4.5, 3–7.0 and 5–8.5) and Tier 2 (SSPs 1–1.9, 4–3.4, 4–6.0 and 5–3.4), outline different societal trajectories in the absence of climate policy (Tran-Anh et al., 2023). Fig. 5 illustrates the results of the CMIP6 future scenarios for daily average temperature (Fig. 5(a)) and total daily precipitation (Fig. 5(b) at t-6. SSP1-1.9 aims for sustainable economic development with even less dependence on fossil fuels, while SSP1-2.6 prioritises sustainable economic growth with a focus on reducing dependence on fossil fuels. SSP2-4.5 is moderate compared to other SSPs. SSP5-8.5 examines economic expansion driven by fossil fuels. Fig. 5 shows projections of mean surface temperature and total daily precipitation for the reference period (2015–2100) under CMIP6. The coloured lines represent the ensemble means of the models, while the shaded areas indicate the ranges of uncertainty (1 standard deviation) for each scenario. Specifically, the coloured lines correspond to the average ensemble mean of two available CMIP6 models for SSP1-1.9, SSP1-2.6, SSP2-4.5 and SSP5-8.5 (ensemble mean of IPSL-CM6A-LR and CNRM-ESM2-1). In addition, the low and high thresholds based on the categorized variables in Table 1 for mean temperature (Tmean) [°C] and precipitation (Prec) [mm] were applied to the CMIP6 future climate scenarios to determine whether conditions at t-6 could be considered favorable or unfavorable for high or low SPIn levels in the coming *Quercus* pollen seasons.

The statistically significant association between high SPIn production levels and low mean temperature at t-6 (T_{mean}_SSP1-1.9 and T_{mean}_SSP1-2.6, Fig. 5(a)) suggests that cooler temperatures prior to the *Quercus* pollen season may contribute to higher pollen production. Cooler temperatures at t-6 could influence the growth and release of *Quercus* pollen, leading to increased SPIn values during the subsequent pollen season. This association shows the sensitivity of *Quercus* pollen production to temperature variations. Conversely, the association between low mean temperature at t-6 (T_{mean}_SSP1-1.9 and Tmean_SSP1-2.5) and high SPIn levels suggests that lower mean temperatures at t-6 prior to the pollen season may be associated with increased pollen production. Warmer temperatures could inhibit or delay the growth and release of *Quercus* pollen, resulting in lower SPIn levels during the pollen season. Considering the projected T_{mean} values under different CMIP6 scenarios, scenarios with higher mean temperatures may lead to contrasting effects on SPIn sensitivity. Under scenarios associated with

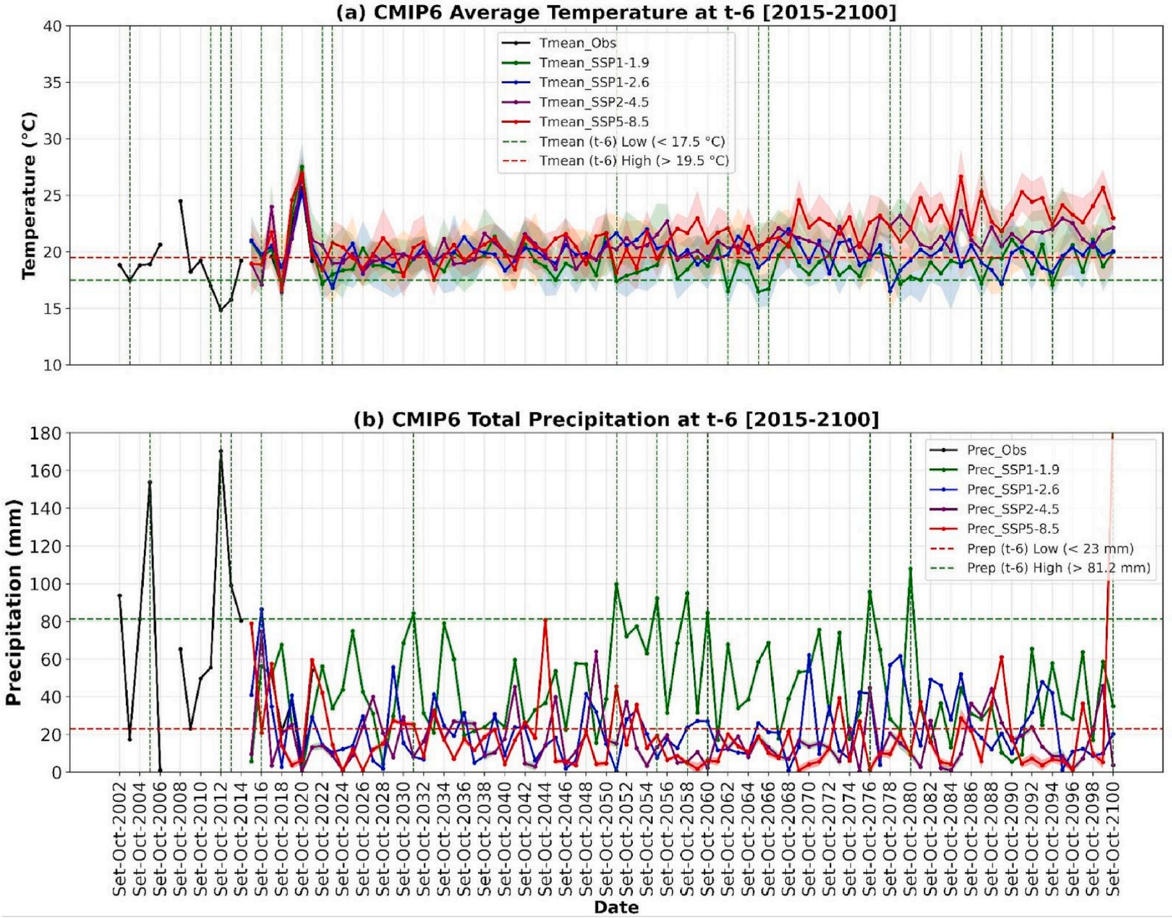

Fig. 5. Projections for t-6 (a) average surface temperature [°C] and (b) total precipitation for the reference period (2015–2100) under CMIP6. The coloured lines show the ensemble means of the models, while the coloured shaded areas show the ranges of uncertainty (1 standard deviation) for each scenario. The coloured lines correspond to the average ensemble mean of two available CMIP6 models for the scenarios SSP1-1.9, SSP1-2.6, SSP2-4.5 and SSP5-8.5 (ensemble mean of IPSL-CM6A-LR (France) and CNRM-ESM2-1 (France)).

higher emissions and higher temperatures (SSP5-8.5), the association between high temperature and low SPIn levels could indicate a potential decrease in pollen production, thereby reducing allergy risks for sensitive individuals. Conversely, scenarios with lower emissions and lower temperatures (SSP1-1.9) may have the opposite effect, potentially leading to increased or stable pollen production and higher SPIn levels.

The prior analysis identified a significant association between high precipitation levels (H_Prec) from the prior t-6 period and high SPIn levels (H_SPIn), as well as between low precipitation levels (L_Prec) and low SPIn levels (L_SPIn). The variability in precipitation levels under different scenarios, as observed in the analysis of Prec_SSP1-1.9, Prec_SSP1-2.6, Prec_SSP2-4.5 and Prec_SSP5-8.5, Fig. 5(b), highlights the importance of considering different socio-economic and emission pathways on future precipitation patterns. Given the relationship between prior t-6 precipitation and SPIn *Quercus* production levels, these differences in precipitation scenarios could potentially affect SPIn production differently. The observed fluctuations in prior t-6 precipitation levels across scenarios in Fig. 5(b) complement the previous analysis by illustrating how changes in precipitation, influenced by socioeconomic and emissions factors, may contribute to variations in SPIn production levels. Scenarios with lower emissions (SSP1-1.9) may exhibit higher precipitation at t-6, which could potentially lead to correspondingly higher SPIn *Quercus* production levels. Scenarios with higher emissions and lower precipitation levels, Prec_SSP5-8.5, might correspond to periods of lower SPIn production if the association between low precipitation and low SPIn *Quercus* production levels continued to be observed.

3.5. Impact of genus Quercus pollen on allergic disease

To evaluate the relationship between pollen counts/months and total sales/months correlation analysis was performed (Fig. 6).

Fig. 6 shows that there is a statistically significant correlation between the sale of antihistamines and pollen concentration in the month of April, mainly for antihistamines of G1 ($R = 0.0656$; $p = 0.028$) and the G1+G2 set (R: 0.642; $p = 0.033$). For antihistamines of G2, although there was a moderate to strong correlation with *Quercus* pollen concentrations, the statistical significance was 0.057, which is just above the significance threshold and should be interpreted with caution. This suggests a possible correlation between G2 and *Quercus*, albeit not conclusively (Fig. 6).

4. Discussion

4.1. Evolution of land cover of Quercus

Portugal is considered the world's largest producer and exporter of cork (49.6%) of global annual production). Our results showed the growing presence of plants of the genus *Quercus* (Fig. 1), particularly, between the years 1995–2018 (Fig. S1), there was an increase of 1132 kha in a 20 km radius around the sampling site (data collected as described in Methods section), contrarily to the 16 kha decrease of the *Quercus* Forest global area in Alentejo region between the years 2005–2015 observed by Instituto da Conservação da Natureza e das Florestas (ICNF). The reason for the increased *Quercus* forests can be mainly the installation of new areas by the project-funded cork production (PRODER). The same has already been observed for other countries in the Mediterranean area, such as Spain, particularly in the Andalucía region (Fernández-González et al., 2020; Recio et al., 2018). The continuous increase in the *Quercus* areas, as well as the change in land use, can have direct consequences on the quantity of pollen in the atmosphere. Our results showed maximum values > 200 pollen/m^3 within the pollen season, which coincides with the classification level recommended by REA (REA, 2024). In the Évora region the presence of *Quercus* species is around the city (Raposo et al., 2016), and the pollen found in the pollen season is possibly originating from local sources, however, pollen can be deposited in regions other than those that gave rise to it due to its morphological and aerodynamic characteristics (Sofiev et al., 2013). Long-distance transport has already been documented by several authors and in this study can be considered as a possible contribution to the annual total of *Quercus*, as can be seen for other pollen types, such as Cupressaceae (Galveias et al., 2021). Long-distance transport may also be responsible for the appearance of several concentration peaks within MPS's present in this study (data not shown here) (Hernández-Ceballos et al., 2015; Rojo et al., 2015).

4.2. Effects of meteorological parameters on Quercus daily pollen concentrations, PSD and SPIn during the pollen season

Meteorological parameters influence the release, dispersal, transport and deposition of pollen. (Makra et al., 2004; Veriankaitė et al., 2010). Our results indicate that the meteorological parameters T_{mean}, T_{min}, and T_{max} positively affect *Quercus* pollen concentrations during the before-peak, after-peak, and Main Pollen Season (MPS) periods. However, the correlation coefficients are lower in the after-peak period, likely due to the plant's reduced pollination capacity post-peak. Additionally, the before-peak period shows higher correlation values compared to the MPS period, which includes both before-peak and after-peak periods, suggesting that the after-peak period has a diminishing influence. These results agree with other authors who considered the temperature to be a factor that has a great influence on pollen concentrations (Galán et al., 2001; Jato, Rodriguez-Rajo et al., 2007a,b; Recio et al., 2018), due do the drying of the anthers which gives rise,

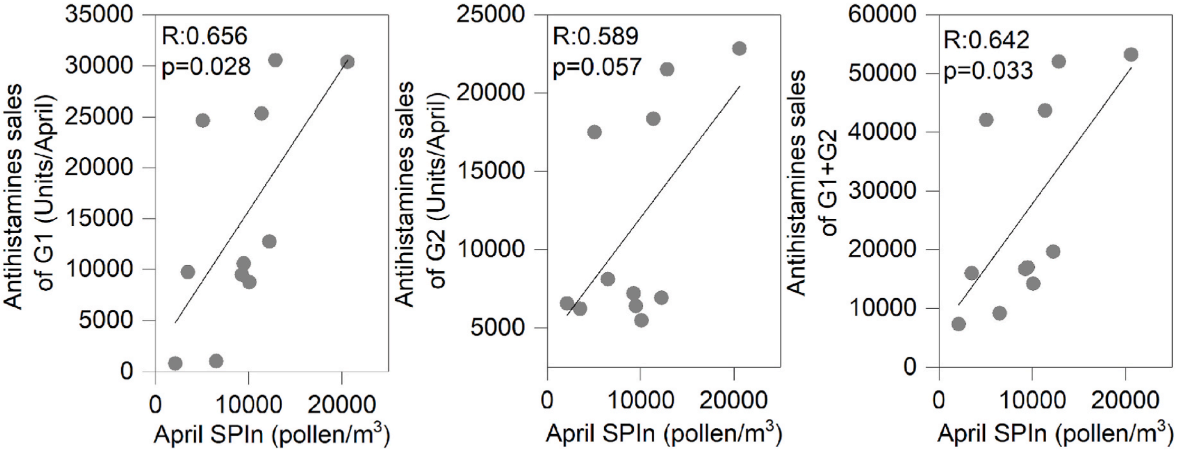

Fig. 6. Relationship between antihistamines sales and April SPIn values.

together with other meteorological factors, to the release of pollen for atmosphere (Rojo et al., 2015). On the contrary, it is observed that precipitation and relative humidity present a negative and significant correlation with concentrations and pollen in the air. These observations have already been reported by several authors, demonstrating that, mainly, precipitation has a leaching effect in the suspended particles, whether organic or inorganic, which are deposited (Docampo et al., 2007; Garcia-Mozo et al., 2015; Gomez-Casero et al., 2004). Wind speed was another parameter that showed a positive influence on pollen concentrations in the air, demonstrating that it may be an important factor due to the transport over short and long distances and the possibility of external contribution from other regions where there is a high predominance of the genus *Quercus* (Sofiev et al., 2013; Recio et al., 2018). Pollen season parameters, such as, SPIn and PSD, can also influenced by meteorological parameters (Recio et al., 2018; Ziska et al., 2019; Schramm et al., 2021). Although our results do not demonstrate a significant effect of the influence of temperature on pollen intensity, there is evidence that this factor has a direct impact on plant phenology, more specifically, on the anther maturation and flowering process, contributing significantly to the increase in pollen concentrations during the period of the main pollination season (Recio et al., 2018; Garcia-Mozo et al., 2015; Hajkova et al., 2015; Menzel et al., 2006; Walther et al., 2002). Precipitation and RH, being covariates, also affect the intensity of the season and its duration. Although our results do not show a significant effect of these two factors on the length of the season, the same is not observed for SPIn, there are reports that the occurrence of precipitation during the main pollination season contributes to the decrease of pollen concentrations in the air, since it promotes the leaching process by depositing suspended particles. Regarding RH, this factor has a direct effect on plant anthers. By Linkens and Cresti (2000) the low relative humidity of the air favors the anthesis process and consequently the dehiscence of the anthers, controlling the flowering process and pollen emission (Cresti and Linskens, 2000). In fact, the anthesis process, which consists of opening the anthers, is controlled by temperature, humidity, and intensity of illumination (Cresti and Linskens, 2000). Considering Wind Speed, acting as a dispersal factor in anemophilous plants, contributes significantly to the increase of pollen concentrations in the atmosphere, as it enhances its release into the atmosphere.

4.3. Effect of meteorological parameters prior to the Quercus pollen season in pollen intensity and start-date

Anemophilous plants release large amounts of pollen into the atmosphere. This high abundance is due to the damage of the reproductive process, and its high production is directly related to the production of flowers and inflorescences, as well as to the conditions in which the plants develop. Adverse environmental conditions, such as temperature, solar radiation and humidity, affect all phases of pollen development (Mesihovic et al., 2016). Since pollen development occurs within the anthers and flowers of the plant, the negative effect on the sporophyte will influence the pollen (Hinojosa et al., 2019), having effects on its production and viability. In the last 50 years, the influence of meteorological parameters on plant phenology has been described (Schwartz, 2003), mainly in Mediterranean plants, even more so in a context of climate change where the temperature change in winter and spring (Peñuelas et al., 2002; Bertin, 2008; Wolkovich et al., 2013). The results demonstrated in this study suggest a complex relationship between meteorological parameters and the *Quercus* pollen season, particularly in intensity and start of season. Regarding pollen intensity, the temperature 3 and 4 months before the main pollen season was shown to be an important factor for pollen production. It was shown that higher temperatures 3 months and 4 months before the pollination season may lead to a decrease in SPIn. These observations are corroborated by other authors for the Mediterranean area (Fernandez-González et al., 2020). In the case of temperature, it has been reported that temperatures above the optimal range (Houston et al., 2016; Mihajlovíc et al., 2014) whether transient or constant, have a negative impact on plant yield, since such conditions bring changes in the morphology, physiology, and biochemistry of plants, significantly affecting their growth and development (Begcy and Dresselhaus, 2018; Hatfield and Prueger, 2015). In the case of precipitation, it proved to be a preponderant factor for pollen production in the 6 months prior to the pollen season. The same is true of precipitation, and water conditions in the early stages of pollen development are the crucial factor for high production (Cresti and Linskens, 2000). These observations have already been reported for the other species, included *Cupressaceae* and *Olea europeae* but also, Poaceae and Urticaceae families, noting a significant impact of accumulated rainfall on the total pollen load. Such observations may suggest that the effect of climate change on pollen production is based not only on the flowering period, but also on the period leading up to it (Oteros et al., 2014; Mousavi et al., 2024). The same was observed in a study carried out in Belgium, in which the temperature and rainfall of the summer months were important factors in the pollen production observed (Verstraeten et al., 2023). Understanding how important meteorological parameters are to explain the high pollen production in different years gives us information about the vitality of *Quercus forests*. According to the IPCC, in the year 2050 the forecast is that there will be high precipitation and low temperature, with increase in pollen production expected. As consequence of increased exposure to *Quercus* pollen, the occurrence of allergic reaction will increase, causing the quality of life of the allergic population to decrease.

Considering the start of pollen season, between the years, differences are also observed, possibly related to the climatic conditions felt daily, and conducive to their release and dispersion and not to the physiological development of the pollen, since at this time the pollen grains are already formed (Pacini and Dolferus, 2019). In fact, temperature and precipitation are factors that influence plant phenology, conditioning of all adjacent mechanisms (Armstrong-Herniman & Greenwood, 2021). Our results showed at 2 and 3 months before, Global Srad and RH were shown to be important factors, probably annual differences in these two parameters explain the differences in the periods between the years under study. In fact, most anemophilous species exhibit diurnal cycles of pollen release (Dowding, 1987; Cresti and Linskens, 2000) and depending on the diurnal climatic conditions, the anther anthesis and dehiscence process are influenced differently, i.e., meteorological parameters influence the anthesis process inducing the release of pollen by driving the dehydration of the anther walls, giving rise to dehiscence. However, in stressful conditions, particularly conditions of extreme cold, humidity the dehiscence process can be interrupted or even reversed (Greene, 2005; Greene et al., 2008).

Thus, our results can contribute to the observations that several authors have already found, in which temperature and precipitation are probably the most important factors for the pollen production which becomes even more relevant in a scenario of climate change, since there is evidence that the Earth's average temperature is increasing (IPCC, 2023). The statistically significant association between high SPIn production levels and low mean temperatures prior to the *Quercus* pollen season suggests that cooler temperatures may contribute to higher pollen production. Conversely, warmer temperatures preceding the pollen season may inhibit or delay pollen growth and release, resulting in lower pollen production levels. Projected temperature trends under different emission scenarios indicate potential contrasting effects on pollen production, with scenarios associated with higher emissions and temperatures possibly leading to decreased pollen production which could lead to lower allergy risks. Conversely, scenarios with lower emissions and temperatures may result in increased or stable pollen production and higher SPIn levels.

Similarly, the association between precipitation levels prior to the pollen season and pollen production underscores the influence of socioeconomic and emission factors on future precipitation patterns. Variations in precipitation levels across scenarios presents potential differences in pollen production. Scenarios with higher emissions and reduced precipitation levels may correspond to lower pollen production, while scenarios with lower emissions and increased precipitation levels could lead to higher pollen production. These findings emphasize the complexity of factors influencing pollen production and allergy risks, necessitating consideration of both meteorological parameters and socio-economic factors in future pollen forecasting and mitigation strategies (D'Amato et al., 2015).

4.4. *Impact of genus Quercus pollen on allergic disease*

Increased concentrations of *Quercus* pollen in the atmosphere may have a negative impact, because of the development of allergic sensitization in the population. Some studies have emerged, considering that this type of pollen is responsible for the main cases of allergy in an area where its abundance is high, mainly in Europe, due since pollen production in many species pollinated in similar period (RODRÍGUEZ-RAJO et al.). Our results showed the same. When comparing the sale of antihistamines with *Quercus* pollen concentrations in the month where this species is predominant (April), it is observed that there is a correlation, mainly with antihistamines of G1 and the junction of G1+G2. When we refer to the SOS antihistamines sales or corticosteroids (G2), the trend is positive and the correlation is close to significance, which indicates, probably, that this pollen type can cause severe symptoms in susceptible individuals, however, more analyses should be necessary for validate these observations (D'Amato et al., 2007).

5. Conclusion

Large concentrations of *Quercus* pollen in the atmosphere of Évora in the years 2002–2021. Meteorological parameters have been shown to be important factors that can influence pollen dynamics and concentrations of *Quercus* pollen, and consequently the risk of exposure. Temperature and precipitation were shown to be factors with significant effect on daily pollen concentrations and pollen production. Regarding the beginning of the pollen season, other parameters are important, such as, Global Srad and RH.

This research has important implications for understanding and predicting *Quercus* pollen seasons, which is crucial for allergy management and public health planning. By considering these meteorological parameters and the different effect of each one, predictive models for pollen seasons could be defined, and mitigation strategies proposed for individuals susceptible to pollen allergies. In addition, all these findings may also be important for understanding the changing dynamics of ecosystems in the context of climate change.

CRediT authorship contribution statement

Ana Galveias: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Ediclê de Souza Fernandes Duarte:** Writing – original draft, Visualization, Formal analysis, Data curation. **Mauro Raposo:** Writing – original draft, Visualization, Formal analysis, Data curation. **Maria João Costa:** Writing – original draft, Visualization, Resources, Formal analysis, Data curation. **Ana Rodrigues Costa:** Writing – review & editing, Visualization. **Célia M. Antunes:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124996.

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