



Floral biology variations in *Olea europaea* over 6 years in Southern Italy: implications for allergy

Alessandro Cinquantasei^{1,2*}, Stefano Palazzo^{1,2,3} , Nada Chaoul^{1,2,4}, Francesco Russano⁵, Maria Pia Rossi⁶, Lucia Anna Giliberti⁶, Marcello Albanesi^{1,2,7,8} 

¹Marcello Albanesi Allergy and Immunology Unit, 70126 Bari, Italy

²The Allergist Srls, 70122 Bari, Italy

³Department of Engineering and Science, Universitas Mercatorum, Rome, Italy

⁴Department of Interdisciplinary Medicine, University of Bari, 70124 Bari, Italy

⁵Farmacia Japigia, 70126 Bari, Italy

⁶Department DiMePRE-J, University of Bari, 70124 Bari, Italy

⁷Allergolys, 75009 Paris, France

⁸University of Bari Aldo Moro, Bari, Italy

***Correspondence:** Alessandro Cinquantasei, Marcello Albanesi Allergy and Immunology Unit, 70126 Bari, Italy. alessandro.cinquantasei@centroalbanesi.com

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Abstract

Aim: *Olea europaea*, an endemic plant of the Mediterranean basin, exhibits a flowering period from April to June, requiring high temperatures and sensitivity to low humidity, rainfall, and windiness. Allergy to *O. europaea* affects 13.85% of the Southern Italian population. This study investigated *O. europaea* pollen concentration, morphological and biochemical variations, and clinical symptoms over a 6-year period (2017–2022).

Methods: Pollen concentration in Southern Italy (Apulia, Bari) was analyzed alongside weather variables (temperature, precipitation, humidity, and windiness) using existing databases (Arpa Puglia; time and date). Optical and fluorescence microscopy techniques were employed to assess pollen morphology and biochemical characteristics. Additionally, the absolute number of prescriptions for various antihistamine drugs (cetirizine, ebastine, bilastine, desloratadine, rupatadine, levocetirizine, fexofenadine, loratadine) was calculated.

Results: The lowest pollen count occurred in the 2018 (91.1 pollen per m³/week), while the highest was recorded in the 2021 (2,545.3 pollen per m³/week). In 2019, the pollen peak was delayed by 2 weeks. Notably, 2018 exhibited more rainy days in May and June and higher humidity percentages (April 73%, May 70%, June 72%). In contrast, 2021 had lower humidity values (April 68%, May 61%, June 59%) and fewer rainy days (1 day in May and none in June). No changes in pollen size were observed, but modifications in *O. europaea* pollen fuchsin fluorescence were noted in 2018 and 2021. The number of drug prescriptions was highest in 2021.



Conclusions: This study highlights that the flowering period, morphology, and pollen production of *O. europaea* may influence patient symptomatology and the need for antihistamine medications.

Keywords

climate change, *Olea europaea*, pollen

Introduction

Climate change represents a significant contemporary challenge, originating with the second industrial revolution and escalating day by day primarily due to industrial activities and vehicular emissions [1]. Human population growth, coupled with the use of greenhouse gases, deforestation, and changes in land use, has disrupted climatic stability that evolved over millions of years within just two centuries. A critical issue associated with climate change is the rise in global temperatures, primarily driven by the concentration of greenhouse gases in the atmosphere, notably CO₂ (the predominant molecule in the atmosphere), CH₄ (resulting from material decomposition in landfills and biological activity of livestock), and NO₂ (with a Global Warming Potential 310 times higher than CO₂). Temperature increase induces alterations in weather and climate variables such as humidity, precipitation, and windiness.

Elevated concentrations of CO₂ can stimulate plant growth and pollen production, extending the flowering period [2]. Larger trees can produce more pollen compared to smaller ones [3, 4]. Industrial activities can also influence pollen biology, with observations indicating that pollen grown in industrialized areas tends to be smaller and possesses a higher concentration of allergenic proteins, alterations in the expression of proteins involved in abiotic stress, the difficulty for some plants to rapidly adapt their proteome to high or fluctuating temperatures, and the instability of certain functional proteins at elevated temperatures, with potential effects on photosynthesis, growth, and stress resistance [5, 6].

The olive tree (*Olea europaea* L.) originates from the Mediterranean region and stands as the most extensively cultivated oleiferous tree species globally due to its high economic value [7]. It thrives in semi-arid to sub-humid warm temperate regions characterized by wet winters and hot, dry summers. Presently, olives cover an area of 10 million hectares worldwide, with over 1,200 olive cultivars, more than 800 of which are dedicated to oil production. The olive tree demonstrates a high level of adaptability, invading regions in Australia and the Pacific Islands [8].

Olive pollination primarily occurs through wind, with some varieties being self-incompatible, necessitating cross-pollination (Biology of Plants, P. Raven). Olive pollen ranges in size from 10 to 25 µm, exhibiting radial symmetry and a tricolporate aperture. *O. europaea* undergoes flowering from April to June, peaking in mid-May. It is sensitive to lower temperatures, high humidity, precipitation, and elevated wind speeds. The tree thrives in dry weather with low rainfall. There are ten different allergens (Ole e 1–Ole e 10) in *O. europaea* pollen [9], with Ole e 1 being the main allergenic protein, causing IgE-mediated allergic responses in a significant proportion of individuals sensitized to olive pollen [10–12].

This study analyzes the impact of climate change on *O. europaea* allergy, focusing on pollen counts from 2017 to 2022 and comparing these values with meteorological parameters such as rainfall, wind, and temperature [13]. Additionally, we examined potential changes in pollen morphology and biochemistry during the specified years using optical and fluorescence microscopy methods. Finally, we correlate our findings with the absolute number of antihistamine prescriptions during the analyzed period [14].

Materials and methods

O. europaea pollen count

The *O. europaea* pollen data for the years 2017–2022 (except 2020) were extracted from the official database of the Apulian Regional Agency for the prevention and protection of the environment (ARPA-Puglia) (<https://www.arpa.puglia.it/>). The database provides day-to-day values, and the reported values represent the average concentration of pollen per month in the Bari area. These data were

aggregated to form a monthly average of pollen concentration. The 2020 analyses were provided by the sampler from the University of Bari, in central Bari (Figure 1A).

Morphological analysis utilized pollen samples collected using the Lanzoni VPPS 2000 pollen trap located on the rooftop at approximately 15 m above ground level, of the Agricultural Research Council (CREA) in central Bari. The sampler consists of a pump that sucks air at a flow rate of approximately 10 L/min. The air passes through a fissure and is deposited on a 2 cm wide acetate strip (Lanzoni) previously impregnated with silicone. Silicone is used to determine a surface with adhesive properties to be able to capture particles. The strip, for its part, is attached to a rotating drum. The drum is equipped with a clock mechanism that allows it to turn for seven days at a speed of 2 mm per hour. Each week, the strip was replaced and the drum was refilled again. Upon collection, the acetate strip was divided into seven segments representing the days of the week. Each segment was mounted on a slide, stained with basic fuchsin, and finally covered with a coverslip. The Nexiscope microscope (Model: NE620) with $\times 40$ optics was employed for the analysis. Images of the pollen were captured using Capture2.3 software, and subsequent analysis was conducted using ImageJ software, employing the region of interest (ROI) tool. An illustrative example of the analytical method is presented in Figure 2A.

Pollen morphology and biochemistry

Fluorescent images of fuchsin-stained pollen were acquired using a Nexiscope microscope (Model: NE620) coupled with a fluorescent lamp. For pollen staining with fuchsin, the preparation begins by placing a drop of glycerin jelly containing basic fuchsin on a microscope slide. This dye highlights the outer wall of the pollen grains. The sample is then covered with a cover slip. Pollen stained with fuchsin becomes clearly visible due to the contrast provided by the dye, which binds selectively to the sporopollenin present in the pollen wall, allowing for morphological analysis and species identification. Images were captured at different z-levels for subsequent three-dimensional (3D) fluorescence reconstruction. The reconstruction was carried out using Python algorithms, generating a 3D representation.

To precisely quantify the fluorescence intensity of the pollen, ImageJ software was employed. A dedicated macro, known as Albanesi's grid, was programmed for this purpose. Initially, a picture was taken, and the image was cropped to 387×387 pixels for uniform measurement. The image was then converted into an 8-bit grayscale image. The Albanesi's grid macro was applied to divide the image into 387×387 squares. Subsequently, the grayscale intensity in each square was calculated for further analysis (Figure 2C–F).

Fluorescence-3D reconstruction and quantification

Comparisons of *O. europaea* pollen morphology through 3D reconstruction revealed no differences in fluorescence distribution and overall pollen shape (Figure 2B). Subsequently, fluorescence intensity was quantified, revealing a statistically significant difference between 2018 and 2021. The 3D reconstruction of pollen was carried out by implementing an algorithm in Python. The approach adopted relies on original images in TIFF format. The procedure involved the generation of 3D grids using the NumPy library, with 3D visualization performed through the Matplotlib library. The images used for the 3D reconstruction were acquired through fluorescence microscopy (Figure 2C–F). A comparison was made between the average pollen intensity values of 2018 and 2021 (Figure 2G). In addition, comparisons were carried out between four olive pollen samples collected during the three flowering months of 2018 and four samples from 2021 (Figure 2H).

Climatic variability analysis

O. europaea, being a heliophilic plant, exhibits drought tolerance but is sensitive to low temperatures, high precipitation, overhead windiness, and high humidity [15]. Climatic parameters, including medium, high, and low temperatures for each month in the specified years, humidity, precipitation, and windiness, were obtained from the site www.timeanddate.com (Figure 1B–E).

Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 9.2.0, employing an unpaired *t* test. *P*-values were interpreted as follows: ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, 95% CI.

Sources of prescriptions

Quantification of prescribed antihistamine medication: Data on prescribed antihistamine medications were sourced from Newline Ricerche di Mercato (Italy). The data used refer to absolute values, for example prescriptions by specialists or general practitioners.

Results

Variation in *O. europaea* flowering

We analyzed the variation in pollen concentration in the city of Bari from 2017 to 2022. With the exception of 2019, when a 20-day delay in the flowering period was observed, each flowering period occurred in the same month. Notably, 2021 exhibited the highest pollen count, while 2018 recorded the lowest count (Figure 1A). We saw a huge variation in annual pollen abundance in 2021.

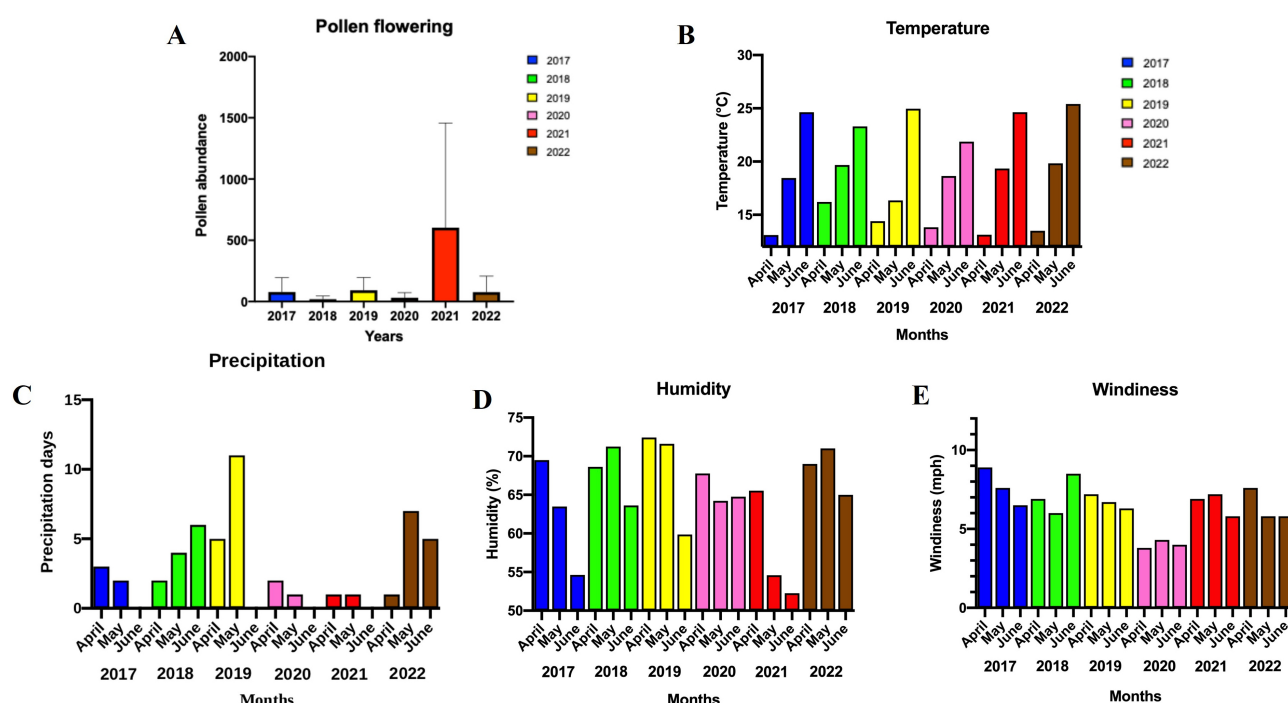


Figure 1. *O. europaea* pollen concentration per year (pollen per m³) during the flowering period, April–June, with the sum of monthly pollen concentrations in the city of Bari. (A). We analyze the weather variables in this area and in the flowering months of *Olea* for each year (2017–2022), with average monthly temperature in °C (B), precipitation, which is expressed in rainy days (C), humidity in percentage (D), and windiness expressed in mph (E).

Climatic variables

To elucidate the flowering period variation, we analyzed climatic variables, including temperatures, precipitation, humidity, and windiness during April, May, and June for the specified years.

Temperatures

In 2019, May experienced the lowest temperature among the analyzed years (16°C; Figure 1B), while no significant changes were observed in the other years (2017, 2018, 2020, 2021, and 2022).

Precipitations

In 2018, May recorded the second-highest number of rainy days (4 days), with June having the highest (6 days, Figure 1C). In 2019, May witnessed the highest number of rainy days (11 days, Figure 1C),

contrasting with 2017, 2020, 2021, and 2022, which had low precipitation days in May. Remarkably, 2021 experienced minimal rainy days in May (1 precipitation day).

Humidity

In 2018 and 2019, humidity values were high during April and May, consistently exceeding 65%. Conversely, 2021 had the lowest humidity rates in May and June, dropping below 55% (Figure 1D).

Wind

No discernible changes in windiness were observed during the six years analyzed (Figure 1E).

Pollen morphology

Pollen size

To compare pollen size across different years, we used Capture2.3 to photograph *O. europaea* pollen with a microscope at $\times 40$. Pollen area (approximated as a circle) was measured using a calibrated instrument. Individual pollens were chosen for analysis in both 2018 and 2021, representing the lowest and highest pollen concentration data, respectively. Results indicated no significant modification in pollen size between these years (Figure 2A–B). On the other hand, fluorescence analysis revealed a significant change ($P < 0.05$) in fuchsin staining between 2018 and 2021 (Figure 2C–H).

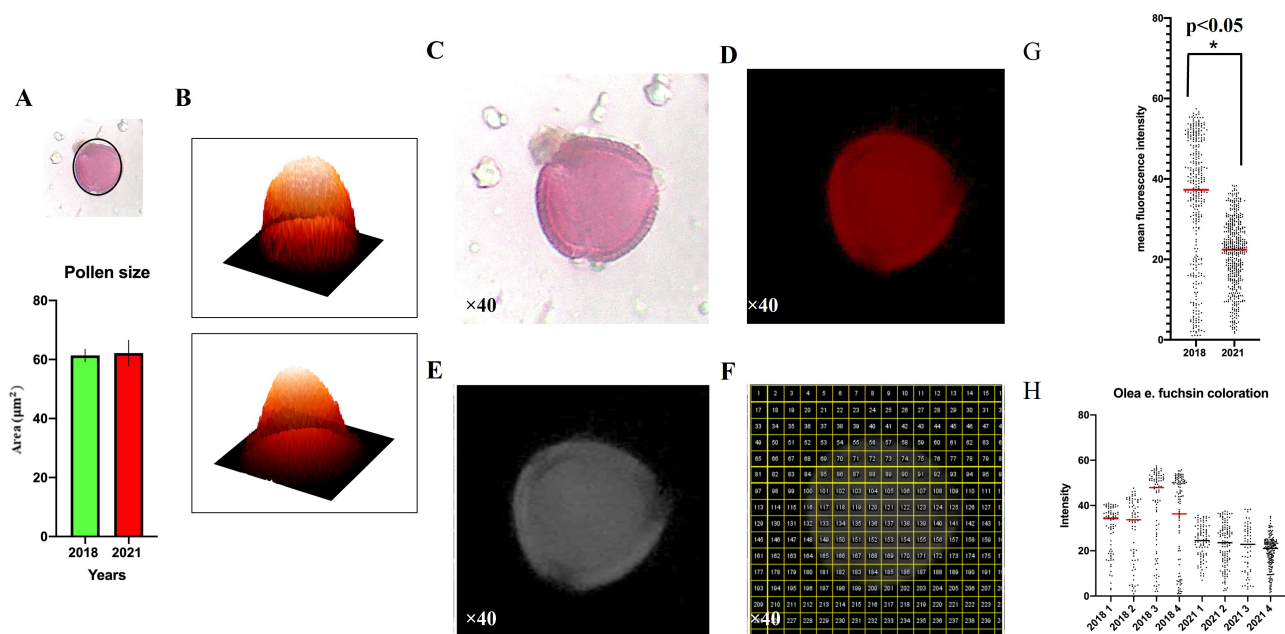


Figure 2. *O. europaea* pollen stained with basic fuchsin, $\times 40$ magnification. The black circle indicates the region of interest (ROI) used for quantification (A, upper panel); quantification of pollen size in years 2018 and 2021 (A, bottom panel); fluorescence distribution and 3D reconstruction of *O. europaea* pollen (B, 2018, upper panel; 2021, bottom panel); *O. europaea* pollen stained with basic fuchsin, $\times 40$ magnification (C); fluorescence microscopy of *O. europaea* pollen, $\times 40$ magnification (D); 8 bit image generated with ImageJ software (E); application of the Albanesi's grid macro (F); quantification of mean fluorescence intensity in *O. europaea* pollen in 2018 and 2021 (G) and the comparison of 4 pollen of the 2018 and 4 from 2021 (H). 3D: three-dimensional. $*P < 0.05$.

Antihistamine prescriptions

Variations in antihistamine prescriptions were analyzed over the six-year period, focusing on April, May, and June (Figure 3A). Data on different antihistamine molecules were obtained for the city of Bari, including cetirizine (Figure 3B), ebastine (Figure 3C), bilastine (Figure 3D), desloratadine (Figure 3E), rupatadine (Figure 3F), levocetirizine (Figure 3G), fexofenadine (Figure 3H), and loratadine (Figure 3I). Results indicated that 2021 had the highest total number of drug prescriptions (Figure 3A), with elevated values for all antihistamines analyzed. Additionally, 2018 recorded the lowest number for all the considered drugs except desloratadine, rupatadine, levocetirizine, and loratadine.

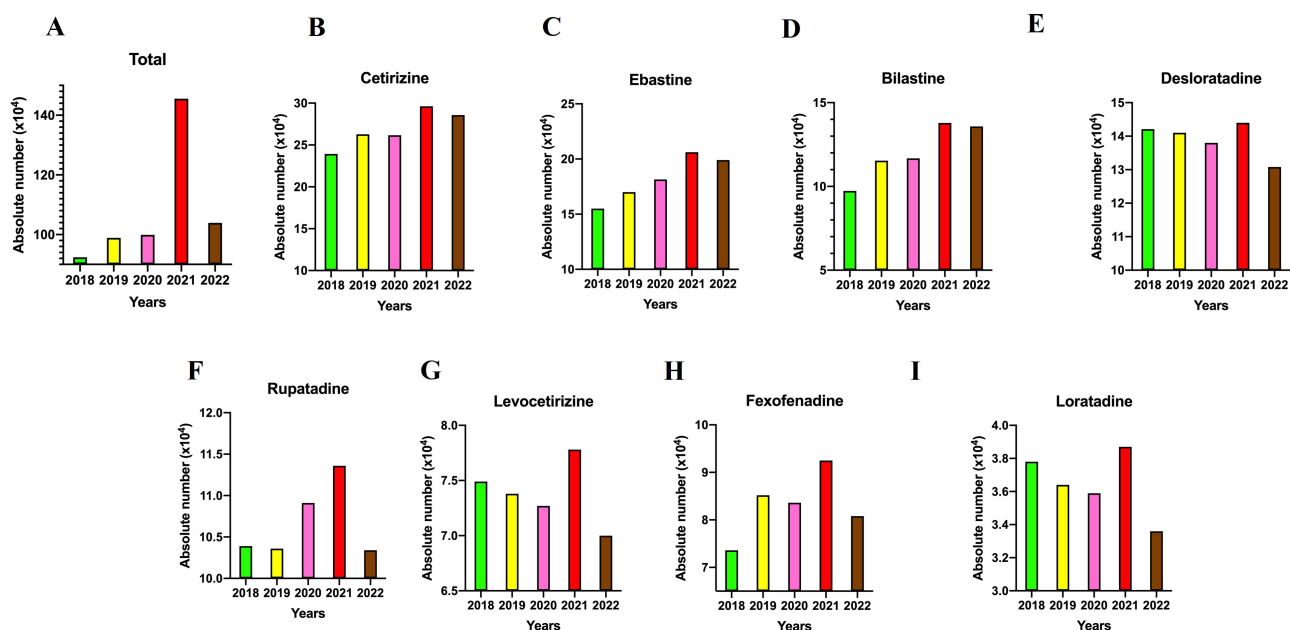


Figure 3. Total number of antihistamine prescriptions in 2018, 2019, 2020, 2021, and 2022. In the metropolitan area of Bari, the total number (A); the number of prescriptions for cetirizine (B); ebastine (C); bilastine (D); desloratadine (E); rupatadine (F); levocetirizine (G); fexofenadine (H); and loratadine (I).

Discussion

Climate change is a major contemporary issue, and one of its main consequences is the progressive increase in temperature. This phenomenon has a significant impact on plants, particularly on floral biology. Anemophilic plants are of major importance in the field of allergy [4]. We used *O. europaea* as a model, due to its short flowering period, making it simpler to analyze the minimum time variations over the years studied, and for its high allergenicity of its pollen related to human health in the Mediterranean region [16].

Moreover, few studies exist on *O. europaea* and climate change, and this is the first study focusing on the Apulian region.

The data presented in this manuscript suggest that, concerning *O. europaea*, climate change might influence pollen concentration and, consequently, the number of drug prescriptions. Temperature shifts and a higher number of precipitation events can be more dangerous in terms of pollen abundance. In our study, we observed that a mere 2-degree increase can lead to a significant rise in pollen concentration. The same trend is shared with the number of precipitation days; in fact, it is inversely proportional to pollen abundance. Temperatures in Bari are increasing every year, leading to a progressive decrease in rainy days and humidity. This induces a lengthening of the flowering period and pollen concentration, potentially resulting in a 2 to 3-week longer pollen peak and an earlier starting period, even in March [2, 17]. The observed climate trends are similar to other Mediterranean cities suggest that these changes may also occur in the analyzed cities (Figures S1–4).

Fuchsin is a colorant used to stain pollen, creating a link with exine (the external part of pollen surface, which consists of a lipid-protein matrix that protects from desiccation) [18]. An analysis of fuchsin absorption spectra suggests possible differences in the degree of fuchsin connection with pollen, indicating potential changes in the biochemistry of this pollen part. We reconstructed the 3D form of fuchsin abundance in pollen with the objective of identifying differences in adsorbent spectra. While no modifications in pollen fluorescence distribution were observed, we detected a change in the fuchsin IR spectra, which in 2021 had lower values. This leads us to hypothesize that climate change influences pollen abundance and its morphology. We also developed an innovative technique to demonstrate the abundance of fuchsin pollen link (*Albanesi's grid*), where the difference in grey-black coloration corresponds to higher levels of fuchsin. Thus, this innovative technique serves as an indirect method to demonstrate a modification in pollen exine biochemistry.

Interestingly, pollen concentration influences the drug prescriptions recorded (Figure 3A–I). An increase in pollen is responsible for a rise in sensitive subjects to this plant and, consequently, an increase in the number of prescriptions. Although climate change plays an indirect role in antihistamine prescriptions, it has a significant impact on healthcare costs. Therefore, studies on artificial intelligence for projecting antihistamine consumption and immunotherapy changes could be useful.

We also monitored the abundance of *Parietaria* and *Graminaceae* in the same *O. europaea* flowering period for each year analyzed; these are used as controls for our study. No significant increase was observed in 2021; in fact, their absolute number was below 250 mg/m³ per month (Figure S5).

To summarize, our results have indicated a potential point to link between climate change, pollen concentration, and the number of antihistamine prescriptions, although 6 years of analysis is a small data to study. Through the matrices, we can observe that certain parameters may be interconnected, such as wind and humidity, which appear to influence both the dispersion and abundance of olive pollen. For instance, higher wind speeds can enhance pollen spread over larger areas, while humidity levels may affect pollen viability and airborne concentration. These relationships suggest that specific climatic conditions can significantly impact pollen behavior and seasonal patterns (Figure S6). According to our preliminary findings, the increase in temperature and decrease in humidity percentage and the number of rainy days induce a higher pollen concentration compared to lower temperatures and higher humidity. However, further research is needed for a better understanding of *O. europaea* pollen in relation to climate change; in fact, it can also modify other environments, so this phenomenon could be related globally. Indeed, there are high possibilities that climate change can also induce an increase in the spread of *O. europaea* in regions which nowadays absent [19].

Finally, for a more in-depth study, several years of analysis are needed to better understand the connection between the points we have examined and relate them to climate change. Indeed, an abstract poster of this study was presented at the Conference “Adapting to change, Emerging infectious diseases in a shifting climate (Paris, 2024)”.

Abbreviations

3D: three-dimensional

O. europaea: *Olea europaea*

Supplementary materials

The supplementary materials for this article are available at: https://www.explorationpub.com/uploads/Article/file/100996_sup_1.pdf.

Declarations

Author contributions

AC: Conceptualization, Data curation, Formal analysis, Supervision, Writing—original draft. SP: Data curation, Formal analysis. NC: Data curation, Supervision, Visualization, Project administration, Writing—review & editing. FR: Data curation. MPR: Data curation. LAG: Data curation. MA: Conceptualization, Data curation, Formal analysis, Supervision, Validation, Project administration, Writing—original draft. All authors read and approved the submitted version

Conflicts of interest

The authors declare no conflicts of interest with regard to this project.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The datasets that support the findings of this study are available from the corresponding author upon reasonable request.

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