Allergol Immunopathol (Madr). 2020;48(2):194–201

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

Airborne pollen calendar of Portugal: a 15-year survey (2002–2017)

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Received 15 May 2019; accepted 25 June 2019
Available online 7 October 2019

KEYWORDS
Pollen;
Aeroallergens;
Pollen calendar;
Aerobiology;
Portugal

Abstract
Introduction: Data about the occurrence of airborne pollen enables the creation of pollen calendars with an approximation of flowering periods for the most common allergenic plant species in a specific area. The aim of this work is to provide pollen calendar for each of the seven monitoring regions of Portugal based on 15 years of airborne sampling, in order to chart the seasonal behaviour of the main allergenic pollen types.
Material and methods: Airborne pollen monitoring (2002–2017) was carried out by the Portuguese Aerobiology Network (RPA), using Hirst-type volumetric spore traps, following well-established guidelines.
Results: A total of 14 airborne pollen types were recorded at RPA monitoring stations, of which 64.2% belong to trees, 28.5% to herbs and 7.1% to weeds. The airborne pollen spectrum is dominated by important allergenic pollen types such as Poaceae, *Quercus* spp., Urticaceae and Cupressaceae. The average pollen index was 42.557 in mainland Portugal and 3.818 in the Islands. There was an increased trend in the airborne pollen levels over the years, namely in Coimbra, Évora and Porto, compared to the remaining regions.
Conclusion: This report provides accessible information about the main allergenic airborne pollen types occurring in the course of the year. The pollen calendars charted for each Portuguese region showed that the occurrence of most allergenic taxa was centred from March to July. Pollen peak concentrations were detected earlier in the Centre and Lisbon and Tagus Valley regions, and later in the remaining regions.

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https://doi.org/10.1016/j.aller.2019.06.012
0301-0546/

Introduction

Pollen allergens are bioaerosols considered an important risk to environmental health, being capable of inducing respiratory allergy symptoms in sensitised individuals.[1,2] It has been shown that the prevalence of respiratory allergies induced by pollens in Europe has been on the increase for the past decades.[2,3]

Despite the seasonal features of pollen allergens, they can occur in the atmosphere almost all year round in certain biogeographic regions.[4] In this respect, aerobiological research is essential in order to chart the behaviour of airborne allergens over the year.[5] Furthermore, aerobiological and clinical studies are generally conducted simultaneously in view to establish the prevalence and possible contribution of allergens in the aetiology of allergic disorders, particularly of allergic rhinitis and pollinosis.[6]

Since the first half of the twentieth century, pollen monitoring has been performed in Europe.[7] The data obtained from airborne pollen monitoring, commonly available as allergen or pollen calendars, are graphical representations of the time dynamics of major airborne pollen types from a specific geographic area. Such calendars provide readily accessible visual information on the various airborne pollen types occurring in the course of the year, informing about the pollen content in the air and pollen season characteristics.[5]

Pollen calendars can be used in allergy clinical facilities for symptoms correlation and subsequent selection of allergens panel for diagnosis and treatment.[6,8] It also corresponds to an important preventive tool for sensitised patients in the sense of diminishing exposure to aeroallergens and/or treatment adjustments when levels are above clinical thresholds.[9]

Previous studies performed in Portugal provided an overview about the distribution of aeroallergens, signalling regions and critical periods of exposure of the sensitised population.[10] Patients are mostly sensitised to grass pollen allergens (34.4%), to *Olea* (21.3%) and to *Parietaria* (17.5%).[11] Moreover, there is a high number of surveys carried out in Portugal on aeroallergen identification and several studies devoted to allergic sensitisation in the atopic population.[10,12,13] Despite all the aerobiological and clinical information gathered so far, previously published pollen calendars are based on short term monitoring.

The aim of the paper is to provide a pollen calendar for seven monitoring regions of Portugal based on 15 years of airborne sampling, in order to chart the seasonal behaviour of the main allergenic pollen types.

Material and methods

The study was carried out by the Portuguese Aerobiology Network (RPA) in five regions of mainland Portugal (North, Centre, Extremadura, Alentejo and Algarve regions), and in two insular sites: Azores and Madeira. Airborne pollen monitoring during the period 2002–2017 was conducted using a Hirst-type volumetric spore trap (Hirst 1952),[14] a Burkard seven-day recorder, placed in an open area, 10 m above ground level. Sampling and analysis followed the Minimum Recommendations proposed by the European Aerobiology Society Working Group on Quality Control.[15] All the samples were processed and analysed at the University of Évora, except the samples from Funchal and Ponta Delgada, which were analysed at Madeira University. Each sampler operated continuously aspirating a constant flow of 10 L per minute, where particles are trapped on a Melinex tape impregnated with silicone solution (Lanzoni). After sampling, the tapes were cut into 24 h fragments (48 mm in length), mounted on slides using glycerine jelly stained with glycerine gelatine solution with basic gel fuchsine (Lanzoni s.r.l., Italy).

The identification and counting of pollen grains were performed with the aid of a light microscopy (400×) along four longitudinal transects. The pollen concentrations were expressed as the number of pollen grains per cubic meter of air (p/m^3). The annual sum of daily average airborne pollen concentrations for all taxa combined was expressed as the Annual Pollen Index (API).

Statistical analysis was carried out using the SPSS 22.0 programme (IBM Corp. Armonk, NY, USA). Correlation analysis was applied in order to assess the variation of API by region, and over the period of study.

The pollen calendar was constructed for each Portuguese region, where the different taxa follow the order in which the maximum peaks appear, and only those taxa that showed a minimum 10-day mean equal to or greater than one pollen grain/ m^3 of air were included.

Portugal has a warm temperate climate, mostly Mediterranean, characterised by hot, dry summers and cool, wet winters.[16] Mainland Portugal includes both temperate and Mediterranean territories.[17] The north western half of Portugal reveals Meso-temperate and Meso-Mediterranean thermoclimates, while the central eastern region of Portugal which includes Alentejo, also exhibits Meso-Mediterranean features. The central and southwestern part of Portugal, which includes Extremadura (Lisbon and Tagus Valley) and Algarve region shows a Termo-Mediterranean thermoclimate. The climate in the islands ranges from Temperate/Hyper-oceanic-sub-Mediterranean in Madeira, to Mediterranean pluviseasonal oceanic/Temperate hyperoceanic sub-Mediterranean in the Azores.[18]

On regards to vegetation cover, 36.2% of mainland Portugal is covered by forest, 31% by bushes and pasture and 23% by agricultural area. In the forest prevails species of eucaliptus, cork oak and pines. *Pinus pinaster* (maritime pine) stands prevail in the north and centre coast of the country, whereas *Eucalyptus globulus* (blue gum) are abundant along the western half of Portugal and interior parts of central and southern regions. Evergreen oak woodlands predominate in the southern half of Portugal, while *Quercus suber* (cork oak) woodlands are the main forest land cover type in south-western Portugal, and *Quercus rotundifolia* (holm oak) predominates in the southeast.[16,19,20]

In the Azorean islands the land has been cleared for agriculture and settlement, but the native vegetation persists in several isolated areas, dominated by *Myrica faia* (faya tree), *Myrsine africana* (Cape myrtle) and *Erica azorica* (Azores heather).[21]

In Madeira, about two-thirds of the island is a natural park dominated by a humid laurel forest. The urban green spaces comprise exotic plants, besides several tree species, such as *Tipuana tipu*, *Jacaranda mimosifolia* and *Casuarina equisetifolia*, and species belonging to Arecaceae, Cupressaceae

and Cyatheaceae. In the outskirts of the city exotic forest from the genera *Acacia*, *Eucalyptus* and *Pinus* prevails.[22,23]

Results

During the studied period, a total of 14 different pollen types with allergenic importance were recorded in the atmosphere of Portugal. Among them, 64.2% belong to trees, 28.5% to herbs and 7.1% were weeds. The average pollen index was 42.557 in mainland Portugal and 3.818 in the Islands. The highest total pollen level was attained in the Alentejo region and the lowest in Madeira. Overall, there was a high variability with regards to daily pollen concentrations (Table 1).

Pollen calendars for the studied regions are shown in Fig. 1, with the pollen types ordered by the timing of their appearance in the atmosphere. The greatest diversity of pollen types was detected between March and June including Poaceae which in the main pollen season predominates until the summer. Pollen peak concentrations were detected earlier in the Centre and Lisbon regions, and later in the remaining regions. Airborne pollen concentrations remained at lower levels during late summer and particularly during the autumn.

In January and February *Cupressus* pollen were recorded in all Portuguese regions, along with *Alnus*, except in the islands. Cupressaceae pollen continued into March-April, the period when *Pinus* and *Rumex* increased their pollen counts which persisted into May. By March, other pollen types started to appear, including *Platanus* and *Quercus*, whilst *Parietaria* pollen prevailed.

In April, *Betula pendula* started its main pollination period that last two months, besides *Plantago* and *Olea* the pollen of which prevailed in the atmosphere until June and September, respectively. During April-May pollen from Amaranthaceae species that occurred in the atmosphere were recorded until the end of the autumn. *Castanea* pollen was prevalent during June and August, as well as *Eucalyptus* which revealed an extended pollination period during the year, especially in Lisbon, Algarve and Islands. In August, the main pollination period of *Artemisia* pollen started, which lasted until October-November in the Lisbon and Alentejo regions.

Over the years a positive increase in airborne pollen levels was observed in Coimbra ($R^2 = 0.83$), Évora ($R^2 = 0.66$) and Porto ($R^2 = 0.56$), whereas in the remaining regions there were no significant changes in the amount of pollen in the air.

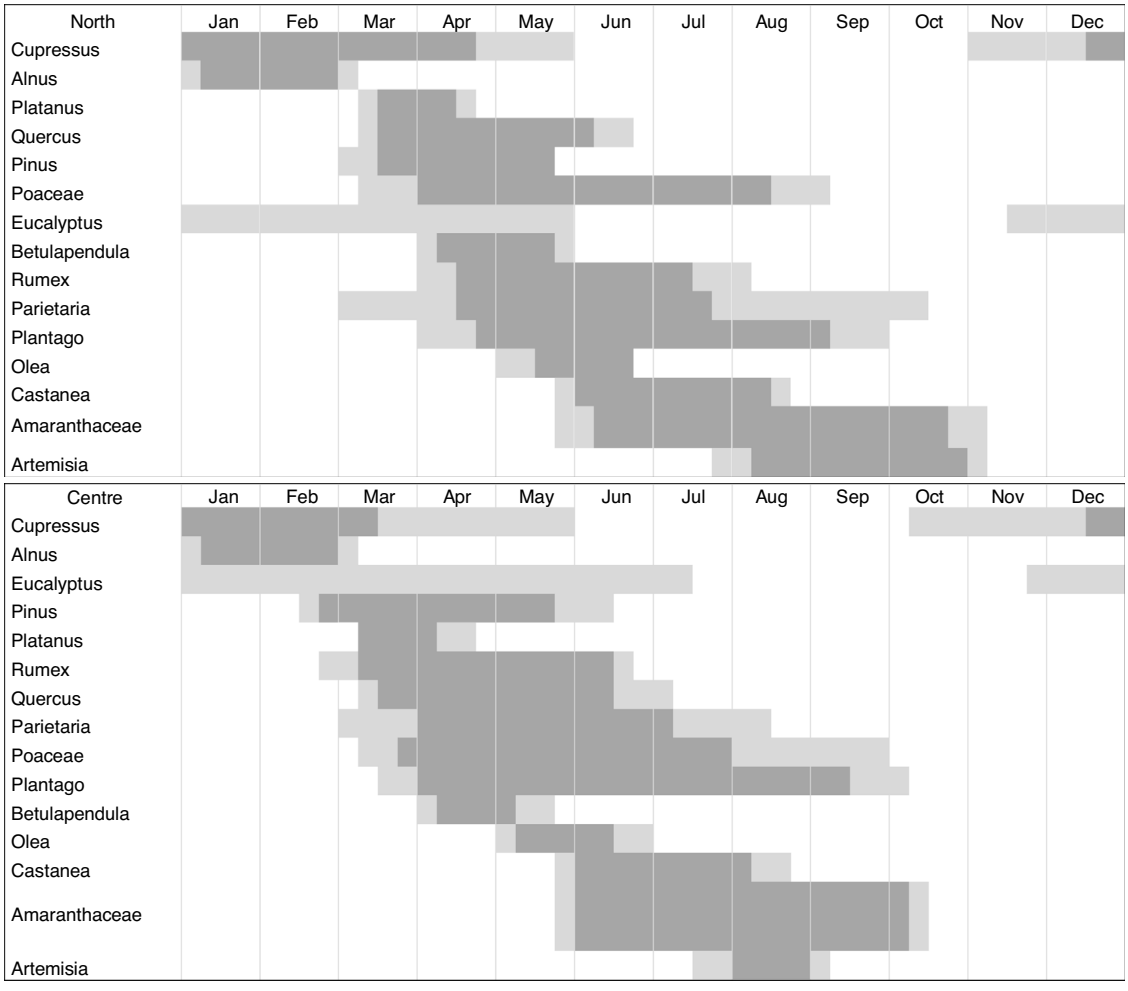

Figure 1 Pollen calendar of five Portuguese mainland regions and insular regions, Azores and Madeira. (Dark grey highlights the main pollen season)..

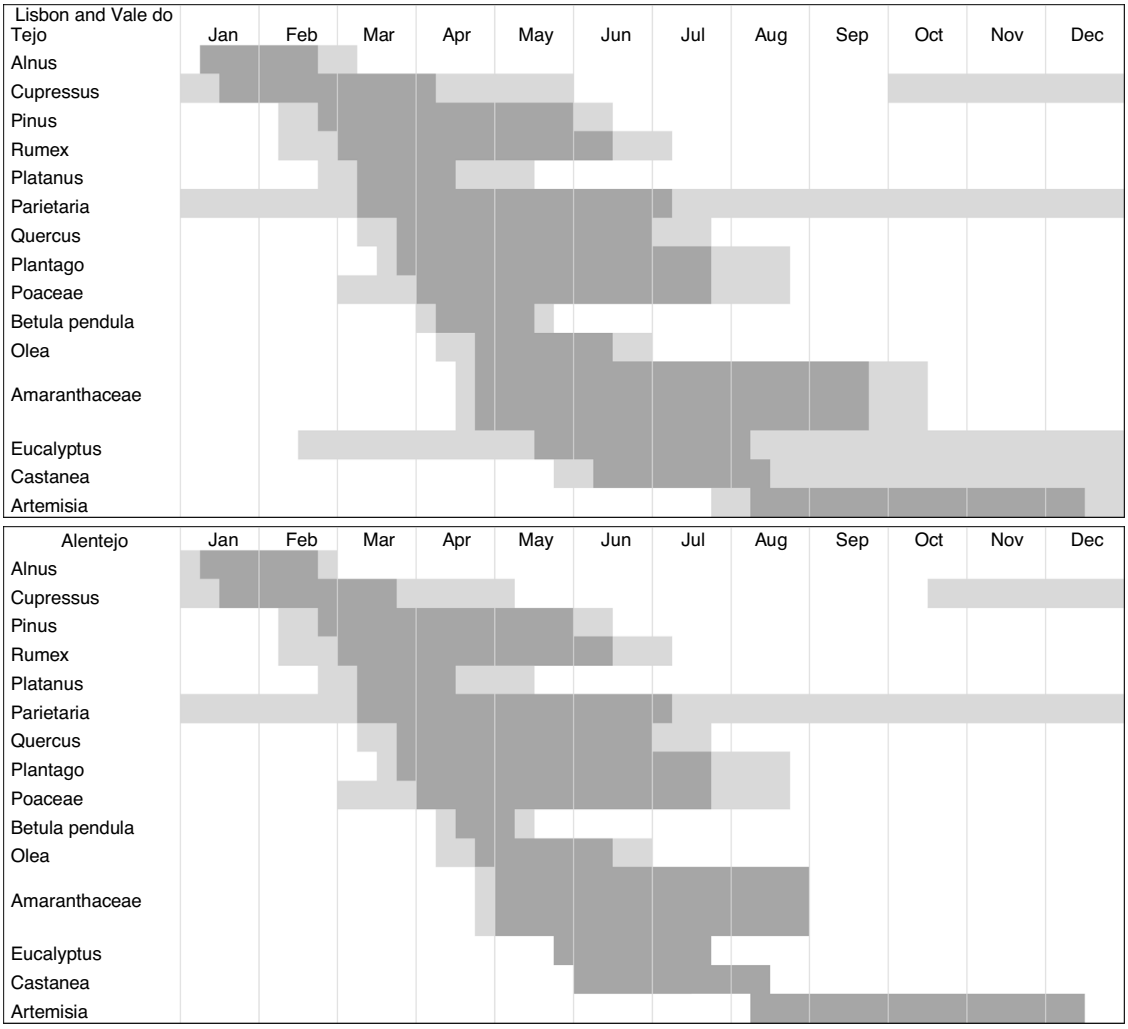

Figure 1 (*Continued*)

Overall, the peak pollen season of most allergenic taxa such as grasses and *Olea* were centred in April and July. In the Centre and Lisbon regions, the grasses pollen season started in March and declined in September whereas in the North, Alentejo and Algarve it started in April and ended in August-September. Grasses were recorded in the Azores mainly during May and August, and from March until July in Madeira.

Olea main pollination season started in April in the Lisbon, Alentejo and Algarve regions, and then in May in the North and Centre where it prevailed until June. This taxon was rarely observed on the islands.

In turn, *Parietaria* occurred mostly between March and July in the Lisbon, southern regions and Madeira, appearing later in April in the North, Centre and Azores.

Regarding the other allergenic taxa such as *Alnus, Cupressus, Castanea, Pinus* and *Platanus*, their peak pollen concentrations revealed a constant pollination pattern over the time in all the Portuguese regions, but in the case of *Amaranthaceae* and *Plantago* pollen types, the pollination season started earlier in the Centre and South regions. In contrast, *Eucalyptus* pollen peaked later in all regions, except in the North and in the Centre. At last, *Rumex* pollen attained peak levels earlier in the Centre, Lisbon and Algarve, while on the islands reached higher counts by May-June.

Discussion

The present aerobiological survey revealed that the pollen spectrum in Portugal has Mediterranean characteristics, with the predominance of Poaceae, *Quercus* spp., Urticaceae and Cupressaceae pollen types. Such pollen taxa, along with those belonging to the Betulaceae, Corylaceae, Fagaceae, Oleaceae, Salicaceae and Taxaceae families are considered as having allergological interest.[2] However, in the Portuguese pollen spectrum it is very rare the presence of *Ambrosia artemisiifolia* L., common short ragweed; the prevalence of sensitization to its highly allergenic pollen is increasing in Europe and reflects the expansion of its populations. Despite the highest levels of this pollen type being centred mostly in France, Northern Italy, the Pannonian Plain and Ukraine, their mean atmospheric pollen levels tend to decrease away from these centres, e.g. towards the Atlantic

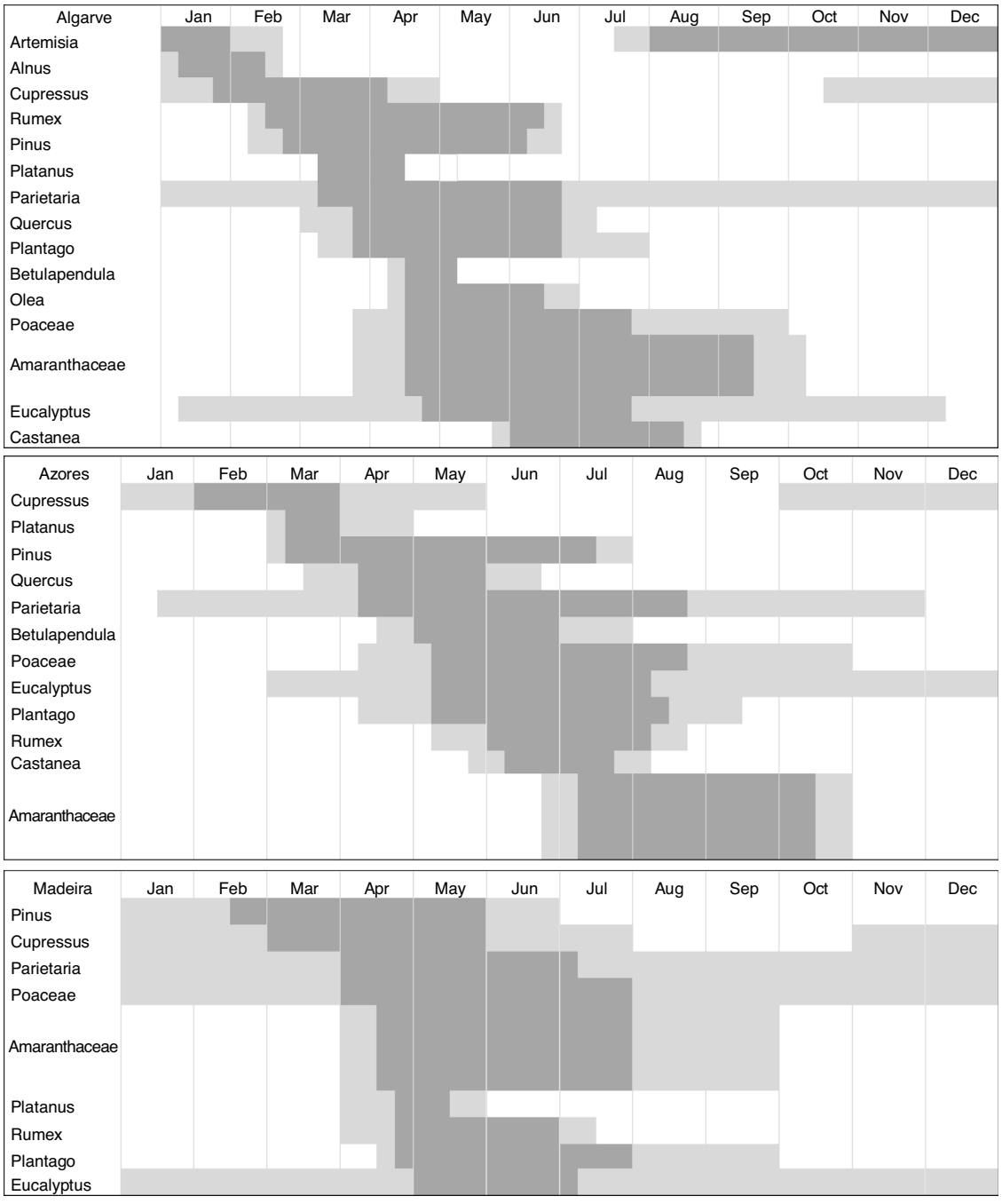

Figure 1 (*Continued*)

and Baltic coasts in the north, and the Mediterranean in the south.[24]

Tree pollen types such as Cupressaceae and *Quercus* spp. were predominant inland, in the Centre and Alentejo regions, while herbaceous pollen such as Urticaceae (*Parietaria*/*Urtica*) and Poaceae prevails in coastal (North and Lisbon) and insular regions. Poaceae pollen occurred in all regions of the country.

In this study it was found that the API was higher in the Alentejo and Lisbon regions and lower in Porto and insular sites. The overall taxa number of pollen types represented in the pollen calendars are in line with those documented for Andalusia[5] and Palma de Maiorca,[25] but comparatively lower to other Mediterranean regions, such as Nerja,[26] Salamanca[9] or Bratislava,[27] in part due to the insular and coastal nature of many of the sites.[13]

The greatest diversity of pollen types centred in March and July have also been observed in most European countries with bioclimatic similarities, with the main pollination period covering about half the year, from spring to autumn.[2] There is also a winter season (from November until February-March), dominated by Cupressaceae pollens, and by *Artemisia* in the case of Algarve and Lisbon regions (August–February).

Table 1 Mean annual pollen index of the allergenic taxa in Portugal. (The highest values obtained on each plant category are highlighted in bold).

Pollen-producing taxa	North	Centre	Lisbon and Tagus Valley	Alentejo	Algarve	Azores	Madeira
Trees	(grains/year/m^3) ($\pm$ standard deviation)						
Betulaceae (*Alnus* and *Betula*)	607 $\pm$ 535.6	427 $\pm$ 417.0	187 $\pm$ 187.2	198 $\pm$ 115.4	168 $\pm$ 115,.4	128 $\pm$ 117.1	31 $\pm$ 53.1
Castanea	510 $\pm$ 358.2	300 $\pm$ 241.8	178 $\pm$ 177.6	186 $\pm$ 129.5	115 $\pm$ 61.2	52 $\pm$ 54.4	42 $\pm$ 23.7
Cupressus	1070 $\pm$ 798.4	6583 $\pm$ 5274.3	5571 $\pm$ 5570.7	6614 $\pm$ 3404.8	1990 $\pm$ 873.0	527 $\pm$ 428.2	64 $\pm$ 180.1
Eucalyptus	338 $\pm$ 192.7	300 $\pm$ 203.8	717 $\pm$ 716.6	165 $\pm$ 97.4	196 $\pm$ 66.7	87 $\pm$ 59.8	44 $\pm$ 35.1
Olea	629 $\pm$ 350.8	2382 $\pm$ 1979.9	5155 $\pm$ 5155.0	6181 $\pm$ 3740.8	**13891** $\pm$ 4562.1	4 $\pm$ 2.7	1 $\pm$ 20.2
Pinus	1401 $\pm$ 651.5	2058 $\pm$ 1356.4	882 $\pm$ 881.7	670 $\pm$ 305.0	793 $\pm$ 238.1	639 $\pm$ 532.8	355 $\pm$ 108.8
Platanus	1401 $\pm$ 588.8	2058 $\pm$ 3517.8	882 $\pm$ 2345.2	670 $\pm$ 4100.4	793 $\pm$ 113.4	639 $\pm$ 103.7	37 $\pm$ 98.6
Quercus	2163 $\pm$ 1267.4	2588 $\pm$ 2636.2	5311 $\pm$ 5311.3	**20572** $\pm$ 11383.9	4942 $\pm$ 901.9	101 $\pm$ 95.0	14 $\pm$ 98.9
Weeds							
Amaranthaceae	114 $\pm$ 51.4	161 $\pm$ 97.7	690 $\pm$ 690.2	397 $\pm$ 160.7	**1218** $\pm$ 418.6	37 $\pm$ 24.6	17 $\pm$ 3.8
Herbs							
Parietaria	1583 $\pm$ 739.4	1952 $\pm$ 1228.9	4101 $\pm$ 4100.6	1664 $\pm$ 648.5	659 $\pm$ 189.1	540 $\pm$ 273.4	509 $\pm$ 112.7
Plantago	477 $\pm$ 260.3	331 $\pm$ 204.1	662 $\pm$ 662.1	1991 $\pm$ 965.4	1402 $\pm$ 503.0	211 $\pm$ 193.8	400 $\pm$ 237.0
Poaceae	2116 $\pm$ 895.7	2699 $\pm$ 1751.1	3760 $\pm$ 3760.4	**12227** $\pm$ 6866.9	4585 $\pm$ 1834.0	693 $\pm$ 693.4	903 $\pm$ 102.2
Rumex	186 $\pm$ 90.4	309 $\pm$ 192.9	514 $\pm$ 513.9	1527 $\pm$ 737.9	502 $\pm$ 172.4	23 $\pm$ 23.2	64 $\pm$ 24.4
Mean of the total pollen index by region (2002-2017)	19047	28129	41651	84931	39028	5113	2524

In Portugal, the risk for individuals with susceptibility to pollen allergens starts in January–February with the exposition to *Alnus* and Cupressaceae pollen, a pattern also reported in other European countries.[8,27] *Alnus* normally reveals a short pollination season, being one of the first in the spectrum of spring pollen in the European countries.[28] On the other hand, Cupressaceae pollen allergy has increased in the Mediterranean region in the last decades and represents one of the few causes of respiratory allergy of winter.[29]

The pollination of *Platanus* begins in March and its main pollen season lasts two months. However, the pollen from this ornamental tree is considered a known aeroallergen, capable of producing great quantities of pollen grains in a short period of time.[30] Birch pollen revealed a similar pattern, characterised by a sudden increase in airborne concentration during April and May, followed by a rapid decline. *Betula*, along with *Alnus*, represent important sources of allergic pollen in the temperate zone of the Northern Hemisphere, posing an enormous impact on the quality of life and productivity of allergy sufferers.[2]

During March and June other tree allergenic sources revealed their highest pollen concentrations, particularly *Pinus*, *Quercus* and *Olea*. Their main pollination season normally lasts three months, being dominant in most inland regions of Portugal such as Alentejo, but also in Extremadura, Spain, as previously reported by Gonzalo-Garijo et al., 2006.[31] The genus *Pinus* is found from the Arctic to the southern hemisphere, but owing to the pollen size and weight, it is only occasionally implicated in allergy.[32] In contrast, tree pollen grains of the Fagales order which includes *Quercus* genus, are considered the main allergen source in the temperate zone of the northern hemisphere in the spring and during early summer in some European sites.[33] In the Oleaceae family, the most allergenic pollen is produced by *Olea europaea*, the olive tree, which in the Mediterranean area has also been recognised as being a major cause of seasonal respiratory allergy.[33]

During this period (March-June), the risk of exposure to allergenic pollen increases considerably, caused by the co-occurrence of several pollen produced by herbaceous plants. *Rumex*, *Parietaria* and *Plantago* pollen attained the highest pollen levels, as did Poaceae pollen, one of the most important causes of pollinosis throughout Europe, especially in the Mediterranean area.[2] In addition, *Amaranthaceae* was shown to have one of the longest pollen seasons among the weeds and herbs representatives, with its pollen prevalence in the atmosphere of Portugal occurring from April until November. These pollen types are common in the Mediterranean area, being especially abundant in semi-arid climates of the south-eastern Iberian Peninsula.[34] In addition, Amaranthaceae pollen might induce allergic diseases, and the clinical incidence of chenopod pollinosis has increased mainly in arid environments.[35,36]

In turn, Poaceae pollen prevailed at all the Portuguese regions, particularly in Alentejo. The grass pollen season is normally long, due to the simultaneous blossoming of several species of the family. In northern, central and eastern Europe the main grass flowering period usually starts at the beginning of May and ends in July. In the Mediterranean area, grass pollination occurs about 2–3 weeks earlier at sea level than in mountainous regions.[2]

A similar situation arose with *Parietaria*, the main allergenic genus of the Urticaceae family with a long persistence

in the atmosphere and high pollen levels, being responsible for a multi-seasonal symptomatology.[37] Moreover, the Myrtaceae family, which the *Eucalyptus* species belongs to, released pollen grains throughout the year, revealing one of the longest pollen seasons of Portugal.

By mid-July when most pollen taxa tend to disappear from the atmosphere, the summer-flowering season of Fagaceae (*Castanea*) begins. Immediately after, the occurrence of Mugwort (*Artemisia*) pollen starts, until November; it is an important allergenic taxon in the Asteraceae family, present in both urban and suburban areas and in the Mediterranean area.[38]

The pollen seasons of most allergenic taxa were centred from March to July, and peak concentrations occurred earlier in the South of the Portuguese territory and later in the North. In the Islands, the highest airborne pollen concentrations were detected slightly later (March to July). In fact, and in accordance with Zang et al.,[39] the start dates for the tree and grass pollen season depend strongly on latitude, with lower latitudes starting earlier.

This study presents the findings of a 15-year survey carried out by the RPA, showing in a concise and simply manner the spatial and temporal variations in the amount of airborne pollen recorded annually in the Portuguese territory. Data is comparable to earlier surveys performed in the country based on a 10-year historical database[13]; said time span corresponds to a stable reference period to chart a reliable pollen calendar.

Acknowledgments

The authors are grateful to the Portuguese Society of Allergology and Clinical Immunology (SPAIC) for the pollen data, help and financial support in this aerobiological study of the RPA, and thanks to their RPA collaborators too.

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