Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Bioquímica

Tese de Doutoramento

Efeito de poluentes atmosféricos e caracterização da matéria particulada adsorvida a grãos de polén aerossolizados

Ana Catarina Galveias Jorge

Orientador(es) | Ana Rodrigues Costa
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A tese de doutoramento foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor do Instituto de Investigação e Formação Avançada:

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Évora 2022

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Agradecimentos

Apresento os meus agradecimentos à Universidade de Évora e ao Instituto de Ciências da Terra – ICT pelo acolhimento e apoio que permitiram a realização do trabalho apresentado nesta tese de doutoramento.

Este trabalho foi financiado maioritariamente pelo projeto **Pollensorb - Caracterização físicoquímica da matéria adsorvida aos grãos de pólen presentes na atmosfera** (PTDC/ATPEAM/0817/2014) também pelos projetos TOMAQAPA (PTDC/CTAMET/29678/2017) e CILIFO (0753_CILIFO_5_E) e pelo ICT-financiado por fundos nacionais através de projetos FCT (UIDB/04683/2020 e UIDP/04683/2020), que permitiram a obtenção dos bens materiais para desenvolver a investigação. Além disso, o projeto Pollensorb assegurou uma bolsa de investigação com a duração de 30 meses que permitiu o desenvolvimento do trabalho de doutoramento.

Agradeço também aos projetos **ALOP - Sistema de observação, previsão e alerta na atmosfera e em reservatórios de água do Alentejo** (ALT20-03-0145-FEDER-000004) e **NanoSen - Development and field validations of low cost-low energy consumption nanosensor system for real time air quality monitoring** (SOE2/P1/E0569) co-financiado pela União Europeia através do Fundo Europeu de Desenvolvimento Regional, enquadrado no Programa Interreg Sudoe. Neste projeto obtive, o apoio através de bolsas de investigação que permitiram a dedicação à investigação durante o tempo necessário para a elaboração desta dissertação.

Apresento, ainda, os meus agradecimentos à European Academy of Allergy and Clinical Immunology - EAACI pelo patrocínio dado à elaboração do PollenMap.

Agradecimentos Pessoais

Admito ser um espaço limitado para expressar tudo aquilo que estou a sentir e os devidos agradecimentos a todas as pessoas que estiveram envolvidas no meu percurso académico, no entanto, acredito que as palavras expressadas neste texto refletem todo o sentimento de gratidão e agradecimento a todos aqueles que de uma forma ou de outra se cruzaram comigo ao longo deste caminho.

Em primeiro lugar, quero agradecer aos meus pais e avós por todo apoio e ajuda nesta grande fase da minha vida, e que sem eles tudo isto seria impossível de se realizar. Obrigada por sempre apoiarem as minhas decisões, por sempre me ouvirem, por acreditarem em mim e pelos ensinamentos que me proporcionaram ao longo da minha vida. Nunca vou esquecer todo o vosso esforço e dedicação que fizeram em prol do meu futuro.

Às minhas orientadoras, Célia Antunes, Ana Costa e Helena Ribeiro, pelo conhecimento transmitido, pela ajuda, pelos ensinamentos ao longo deste percurso e pela oportunidade de realização deste trabalho. Foi um privilégio trabalhar convosco. Foram imprescindíveis para a minha evolução e formação académica e ser-vos-ei internamente grata por tudo.

Ao meu namorado, Luís Xavier Cordeiro, por todo o apoio emocional, foste imprescindível nesta etapa da minha vida. És uma pessoa incrível e o meu porto seguro.

Aos meus colegas de laboratório Mariana Marques e Carlos Gastalho, pelo bom ambiente de trabalho, pelo conhecimento partilhado e pela entreajuda.

Às técnicas do laboratório da fase III do Colégio António Verney, Esperança Alves e Deolinda Varela por todo o apoio no suporte de material solicitado e lavagem do mesmo, bem como, na disponibilidade do fornecimento de reagentes.

À minha amiga Teresa Morgado por todo o apoio e conversas.

A todos os intervenientes, Um mais sincero e enorme OBRIGADA!!

Resumo

O pólen é um bioaerossol atmosférico capaz de induzir reação alérgica respiratória em indivíduos suscetíveis. O objetivo deste trabalho foi estudar a disseminação, distribuição, transporte e alterações bioquímicas de alguns tipos polínicos alergizantes resultantes da interação com os parâmetros meteorológicos e com os poluentes atmosféricos.

Observou-se que os parâmetros meteorológicos influenciam a emissão, distribuição e transporte do pólen, afetando a época polínica e a integridade do pólen. Observou-se ainda que a composição da matéria particulada adsorvida ao pólen é variável entre regiões de acordo com as características topográficas e bioclimáticas. Confirmou-se que os poluentes atmosféricos provocam alterações químicas e bioquímicas (imunorreconhecimento e/ou conteúdo em alergénios) no pólen, com significativas implicações alergológicas.

Estes resultados contribuem para o conhecimento dos fatores que afetam a exposição a alergénios polínicos e, consequentemente, constituem uma ferramenta para o desenvolvimento futuro de sistemas de alerta que contribuam para melhorar o controlo da doença alérgica respiratória.

Abstract

Effect of air pollutants and characteristic of particulate matter adsorbed to pollen aerosols

Pollen is an atmospheric bioaerosol capable of inducing respiratory allergic reaction in susceptible individuals. The objective of this work was to study the dissemination, distribution, transport, and biochemical changes of some allergenic pollen types, related to the interaction with meteorological parameters and air pollutants.

It was observed that meteorological parameters influence emission, distribution, and transport, thus affecting the pollen season and integrity. It was also observed that the distribution of pollen-adsorbed particulate matter composition varied between regions according to topography and bioclimate characteristics. It was confirmed that atmospheric pollutants evoked pollen chemical and biochemical changes (allergen immune-recognition patterns and/or content), with significant allergological implications.

This work highly contributes to the knowledge of the factors affecting human exposure to pollen allergens and, consequently, constitute a tool for the future development of effective alert systems contributing to improve respiratory allergic disease control.

Abreviaturas, Acrónimos, Símbolos e Unidades

°C - Graus Celsius

DEP´s - partículas de escape de motores a diesel

GSSG – Glutationa oxidase

° - graus

Kj/m^2 - Kilojoules por metro quadrado

h – Horas

H_2O_2 – peróxido de hidrogénio

HBO_3 – Ácido Bórico

hPa- Hater Pascais

m^3 – metro cúbico

mg – miligrama

mg/L –miligrama por litro

mg/ml – miligrama por mililitro

$MgSO_4$ – Sulfato de magnésio

min – minutos

ml – mililitros

mm – milímetros

mM – milimolar

m/s - metro por segundo

MPS – Época polínica principal (*Main pollen season*)

NADPH – Fosfato de dinucleótido de nicotinamida e adenina

NBT – Cloreto de nitro azul de tetrazólio

nm – nanómetro

NO – Óxido nítrico

$-NO_2$ – grupo de nitrogénio

NO_2 – Dióxido de nitrogénio

NO_x – Óxidos de nitrogénio

NO_2+O_3 – Mistura de O_3 e NO_2

O_2 – Oxigénio

O_2^- – Anião Superóxido

O_3 – Ozono

PM – matéria particulada

% - percentagem

RH – Humidade relativa

ROS – Espécies reativas de oxigénio

SDS-PAGE – dodecil sulfato de sódio - eletroforese em gel de poliacrilamida

SOD – Superóxido dismutase

SO_2 – Óxido de enxofre

SEM – Microscopia eletrónica de varrimento (*Scan eletronen microscopy*)

$T_{máx}$ – Temperatura máxima

T_{mean} – Temperatura média

T_{min} – Temperatura mínima

1. *Introdução*

1.1. Doença alérgica respiratória

A Alergia foi descrita, pela primeira vez, em julho de 1906 por *Clemens von Pirquet*, médico pediatra de Viena, que a definiu como "*uma reação indesejada, inata ou adquirida do sistema imunitário humano a substâncias que geralmente não são consideradas nocivas"* (Linskens & Cresti, 2000). Era vista na altura como uma doença rara, porém, hoje em dia, sabe-se que esta doença é muito comum e que se encontra na comunidade humana e em animais domésticos (Martins, 2022) ganhando muita relevância no contexto de *One Health*.

Atualmente, a alergia é caraterizada pela ocorrência de uma reação de hipersensibilização imunológica do tipo I e IV, que ocorre principalmente em indivíduos predispostos geneticamente a desenvolvê-la, e para o qual já se conhecem alguns fatores que a desencadeiam (Calderón et al., 2012). Essa reação é desencadeada por moléculas antigénicas, os alergénios (Arosa, Cardoso & Pacheco, 2007). Os alergénios são proteínas que de acordo com as suas caraterísticas pertencem a diferentes famílias, e estão presentes em diversas partículas biológicas, como por exemplo fungos, ácaros, pólenes, entre outros (Arosa, Cardoso & Pacheco, 2007).

De acordo com os dados da Organização Mundial da Alergia (WAO), atualmente a alergia respiratória atinge 23,3% da população mundial e assiste-se a um aumento de 0,3% de incidência por ano, estimando-se que em duas décadas a prevalência alcance os 30% (Björkstén et al., 2008). Várias pesquisas mostram que, nos EUA, 2-10% dos residentes são afetados pela polinose. Na Europa, a incidência de doença alérgica aumentou de 1%, no início do século XX, para 20% e estima-se que nas próximas décadas suba para 35% (Burr, 1999).

As reações de hipersensibilidade podem distinguir-se pelo tipo de resposta imunitária envolvido e pelas diferentes moléculas efetoras geradas no desenvolvimento da reação (Kindt, Goldsby & Osborne, 2007). Existem 4 tipos de reação de hipersensibilidade, a reação de hipersensibilidade mediada por IgE (tipo I), reações de hipersensibilidade mediada por anticorpos (tipo II), reações de hipersensibilidade mediada por complexo imune (tipo III) e reações de hipersensibilidade mediada por células (tipo IV) (Kindt, Goldsby & Osborne, 2007). A reação alérgica dá-se por uma reação de hipersensibilidade do tipo I e de tipo IV, esta última descrita como alergia de contacto.

A reação de hipersensibilidade do tipo I ou também designada reação de hipersensibilidade imediata, é uma reação rápida que origina permeabilidade vascular aumentada, vasodilatação, e contração do músculo brônquico-visceral seguida de uma reação inflamatória em indivíduos alérgicos (Kindt, Goldsby & Osborne, 2007). Também pode originar reação cutânea, conjuntival ou digestiva (Regateiro & Faria, 2016).

A resposta alérgica é composta por mecanismos que compreendem duas fases: a fase de sensibilização ao alergénio que é caraterizada pela produção de IgE específica e a fase efetora caraterizada por ser a fase tardia quando ocorre um segundo contacto com o antigénio (Kindt, Goldsby & Osborne, 2007).

A mucosa respiratória é a primeira linha de defesa para a proteção dos pulmões e é formada por um conjunto de barreiras mecânicas e imunológicas (Georas & Rezaee, 2014; Hiemstra, 2015; Parker & Prince, 2011). As atividades da mucosa para o combate a agentes infeciosos incluem fluido de revestimento da superfície das vias aéreas com péptidos de defesa contra o agente hospedeiro, a produção de muco para a fixação dos corpos estranhos, ligação das células epiteliais para impedir a transição das partículas inaladas até aos pulmões, recetores inatos e vias de sinalização para o recrutamento de células do sistema imunitário (Huff et al., 2019). A camada de muco de revestimento das vias aéreas é a primeira barreira que as partículas poluentes inaladas encontram (Widdicombe, 2002).

Quando ocorre um primeiro contacto com o alergénio dá-se a fase de sensibilização (figura 1). Este alergénio, geralmente solúvel, de grandes dimensões ou haptenizado e com capacidade para desenvolver uma reação imunológica, entra em contato com o organismo através da mucosa respiratória ou oral. Ao entrar em contacto com a mucosa, o alergénio vai sofrer um processo de endocitose, principalmente, por células dendríticas e macrófagos, consideradas células apresentadoras de antigénio (APCs). As APCs tem aptidão de migrar até aos gânglios linfáticos e apresentar os antigénios às células moduladoras do sistema imunitário. Nos gânglios linfáticos, vão apresentar os péptidos ao complemento de histocompatibilidade (MHC) da classe II. Caso a apresentação do antigénio seja bem-sucedida, os linfócitos T_h com especificidade para esse antigénio vão reconhecê-lo. Como consequência desta apresentação, os linfócitos T_h vão proliferar, diferenciar-se e iniciar a produção de interleucinas (IL-4, IL-5 e IL-13). As IL-4 e Il-13 vão induzir a produção de IgE específica para o alergénio que entra em circulação, ligando-se a recetores de alta afinidade das membranas dos mastócitos e basófilos (figura 1). A IL-5 é um dos principais indutores de ativação dos eosinófilos (Kindt, Goldsby & Osborne, 2007; Regateiro & Faria, 2016).

A fase efetora ocorre quando existe um segundo contacto com o alergénio ao qual o indivíduo ficou sensibilizado (figura 1). É caraterizada por uma resposta rápida e imediata, uma vez que, tantos os anticorpos específicos como os mediadores dos grânulos dos mastócitos/basófilos estão pré-formados e disponíveis para iniciar a reação. Quando um alergénio é reconhecido por duas moléculas de IgE contíguas ligadas na superfície dos mastócitos e basófilos – "cross-link" – é induzida a desgranulação dessas células, com libertação de mediadores como a histamina, a heparina, enzimas proteolíticas e fatores quimiotáticos (Regateiro & Faria, 2016).

A fase efetora pode ainda ser dividida em fase imediata e fase tardia (figura 1). Na fase imediata existe a ligação do alergénio à molécula de IgE que se encontra na superfície das células e que resulta na ativação de mastócitos, originando processos de libertação dos mediadores ativos (Kindt, Goldsby & Osborne, 2007).

A fase tardia ocorre horas mais tarde e é originada pela síntese e libertação de mediadores, como leucotrienos, quimiocinas e citoquinas a partir de mastócitos ativados. Estes vão convocar leucócitos, entre eles eosinófilos, e células T_h2, que vão libertar para o exterior interleucinas (Kindt, Goldsby & Osborne, 2007).

As interleucinas libertadas, Il-4 e Il-13, vão ativar os linfócitos B e, por conseguinte, a síntese de IgE. A interleucina IL-5 vai ativar os eosinófilos que vão libertar mediadores responsáveis pela sintomatologia alérgica manifestada (Kindt, Goldsby & Osborne, 2007). Podem também ser formados mediadores responsáveis pelo aparecimento de sintomatologia alérgica tardia, como os leucotrienos e TNF-α. A sintomatologia alérgica é caraterizada por manifestações como o congestionamento nasal, broncoespasmo, urticária, angioedema e anafilaxia (Kindt, Goldsby & Osborne, 2007).

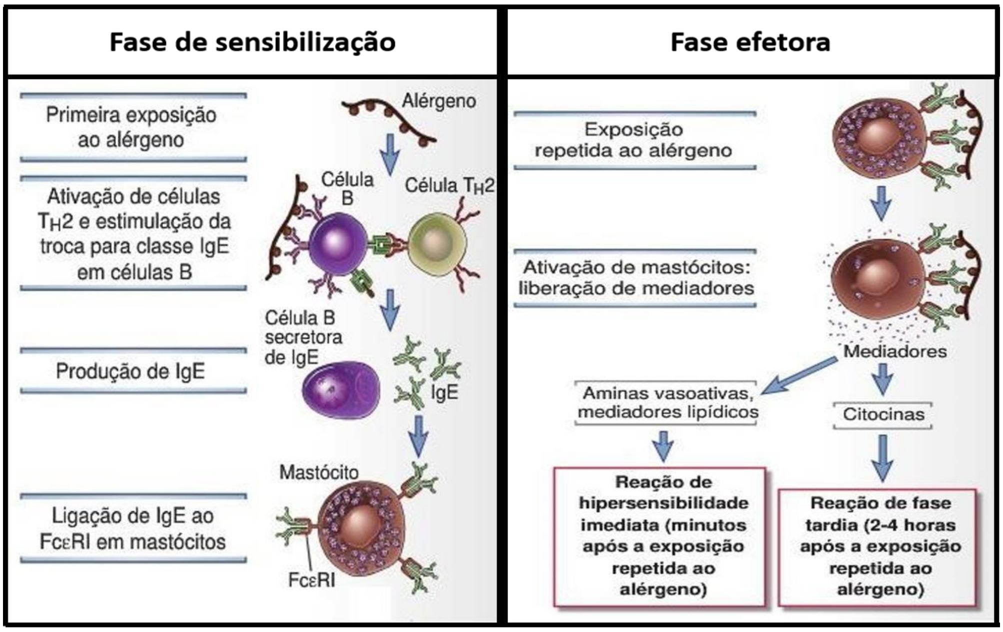


Figura 1: Reação de hipersensibilidade tipo I (retirado de Abbas et al., 2012).

A alergia a pólen designa-se de polinose. É uma afeção comum, também designada febre do feno, que pode ser causa de morbilidade, perda da qualidade de vida e mortalidade. A polinose foi descrita pela primeira vez no início do século XIX por Jonh Bostock (Bostock, 1819). Ele descreveu uma série de sintomas incomuns, com maior ou menor gravidade, que ocorriam desde o início a meio do mês de junho, com incidência anual (Bostock, 1819). No ano de 1831, Elliotson atribuiu os sintomas de febre do feno à "desagregação da flor de gramíneas". Blackley em 1873 foi o primeiro a demonstrar o papel da natureza no desenvolvimento da febre do feno (Blackley, 1873; Smith et al., 2014). O trabalho desenvolvido por Blackley foi notável e a sua abordagem metodológica foi aceite por diversos autores. William Dunbar, no ano de 1903, confirmou a teoria de Blackley sobre o papel dos grãos de pólen na saúde humana (Smith et al., 2014), e Alfred WolffEisner reconheceu que eram as proteínas do pólen que provocavam os sintomas alérgicos (Ring & Gutermuth, 2011). A alergia ao pólen está atualmente confirmada e sabe-se que é mediada por IgE. A maioria dos indivíduos que sofrem desta patologia apresentam sintomas ao nível do trato respiratório, espirros intensos, congestionamento nasal, prurido faríngeo e lacrimejo e frequentemente, tosse, que podem tornar-se mais grave com a exposição prolongada. A reação pode ocorrer em muitos locais, se o trato respiratório inferior for afetado, a reação clínica será a asma, no entanto, também pode afetar a pele, causando dermatite atópica e/ou urticária. A alergia é considerada um problema a nível mundial com elevada prevalência e impacto na população. Na Europa, um estudo realizado por Bauchau e Durham, 2004, demonstrou que países como a Bélgica era o que apresentava uma taxa de ocorrência de rinite alérgica na população mais elevada com 28,5%, seguido o Reino Unido com 26,0%, a

França (24,5%), a Espanha (21,5%), Alemanha (20,6%) e por último Itália que demonstrou ser o país com menor prevalência de rinite alérgica na população com apenas 16,9%. No conjunto de outros países a prevalência não chegou a 23 % (Bauchau & Durham, 2004).

Em Portugal, um estudo realizado a 6859 individuos, distribuídos de Norte a Sul do país, com idades compreendidas entre os 16-95 anos, observou que a prevalência da rinite alérgica era no total 26,1%. Neste mesmo estudo, foi ainda determinada a prevalência por regiões, observando-se que a região do Alentejo apresentava uma maior prevalência, cerca de 30%, seguido da região de Lisboa e Vale do Tejo (aproximadamente 29%), o Centro com cerca de 27%, o Norte com 24% e por último a região do Algarve com apenas 16% de prevalência desta patologia (Morais-Almeida et al., 2005; Todo-Bom et al., 2007).

A gestão clínica da polinose tem subjacente conhecer quais os agentes biológicos causadores da mesma, os seus padrões de emissão, bem como os parâmetros que interferem nesses padrões de emissão sazonais e/ou que podem afetar a sua alergenicidade. Assim, nos capítulos seguintes estes aspetos serão abordados em detalhe.

1.2. O Pólen

O grão de pólen é o gametófito masculino, estrutura biológica através da qual as plantas superiores com semente asseguram a proteção e dispersão dos gâmetas masculinos, mediante o processo de polinização (figura 2). São produzidos nos sacos polínicos das anteras da planta com flor como resultado da meiose da célula mãe do micrósporo (Amjad & Shafighi, 2012).

A fim de participarem na reprodução sexuada, os grãos de pólen são transportados para o estigma da mesma ou de uma flor diferente, por vários agentes bióticos ou abióticos. Nas plantas com processo de polinização anemófila, os grãos de pólen são libertados para atmosfera e a disseminação é efetuada pelo vento (Linkens & Cresti, 2000). Para o sucesso da fertilização, as plantas desenvolveram adaptações compensatórias, como a produção de grandes quantidades de pólen e produção de grãos de pólen com caraterísticas aerodinâmicas. Como consequência do processo de polinização anemófila, os grãos de pólen ao serem transportados pelo vento e dispersos por ação de correntes de convecção e turbulência geradas na camada limite atmosférica, podem depositar-se localmente ou então serem transportados por longas distâncias, dependendo das suas caraterísticas morfológicas e aerodinâmicas e das caraterísticas da atmosfera (figura 2) (D'Amato et al., 2007; Grewling et al., 2019; Rojo & Pérez-Badia, 2015).

Os grãos de pólen, sendo parte do aerossol atmosférico quando se encontram em suspensão, podem ser afetados por inúmeros fatores, dos quais, os parâmetros meteorológicos apresentam um papel importante (Linskens & Cresti, 2000).

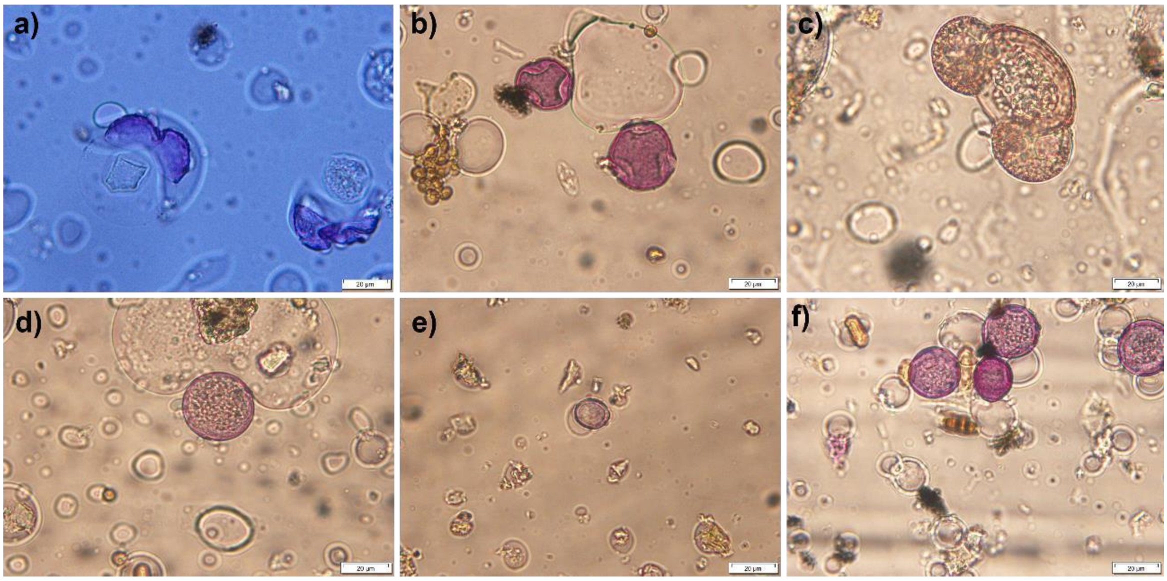


Figura 2: Fotografias em microscopia ótica, numa ampliação de 400x, dos tipos polínicos de Cupressaceae (a), *Quercus* spp (b), *Pinus* spp (c), Poaceae (d), *Platanus* spp (e) e Poaceae e *Olea* spp (f). Barras representa 20 µm.

Durante o processo de sedimentação, os grãos de pólen podem depositar-se diretamente sobre o gametófito feminino da mesma espécie ou de espécies diferentes. Se o alvo for o adequado, o gametófito feminino da mesma espécie, são ativados no pólen mecanismos para o desenvolvimento do tubo polínico para que seja cumprido o objetivo final, a fecundação da oosfera (Lord & Russell, 2002). Contudo, sendo a polinização anemófila pouco dirigida ao alvo, muitos grãos de pólen não chegam ao destino final. Alguns deles, devido à grande concentração na atmosfera, chegarão por meio da inspiração às mucosas do sistema respiratório dos humanos e, se os indivíduos forem suscetíveis, podem vir a desenvolver uma reação alérgica.

As proteínas alergénicas polínicas podem estar presentes na exina (parte externa da parede do grão de pólen constituída maioritariamente por esporopolenina), intina (parte interna da parede do grão de pólen) e/ou no citoplasma. Alguns destes alergénios participam no crescimento, reconhecimento e desenvolvimento do tubo polínico, como os inibidores da invertase ou as proteínas semelhantes à taumatina (TLP- “thaumatin-like proteins”), ou são proteínas de transporte, como as polcalcinas, que ligam cálcio, ou as LTP (LTP - lipid transfer proteins), que ligam lípidos, ou ainda são proteínas de resposta a stress, como a superóxido dismutase (Linskens & Cresti, 2000). Dos tipos polínicos mais alergizantes na zona mediterrânea, destacasse a *Olea europeae*, o *Cupresssus sempervirens*, o *Platanus hispânica* e várias espécies de gramíneas (Cariñanos, Casares-Porcel, & Quesada-Rubio, 2014), alguns dos quais pertencem a famílias que apresentam uma elevada emissão polínica, podendo, algumas espécies libertar mais de 10.000 grãos de pólen por antera (Molina et al., 1996).

O conhecimento da aerodinâmica destas partículas biológicas com potencialidade alergénica, faz com que exista junto da população uma maior sensibilização, tomando medidas preventivas e protetoras para a diminuição da ocorrência de reação alérgica, tais como, o uso de mascara de proteção na altura da estação do ano, a primavera, a evicção à exposição por parte dos indivíduos que sofrem de alergia (evitar, por exemplo, estar ao ar livre), o fecho de janelas durante o dia, podendo apenas arejar a casa depois das horas de maior calor (>16h) e/ou dar inicio a terapêutica farmacológica preventiva adequada (toma de anti-histamínicos, por exemplo) (Hoyte & Katial, 2011).

É assim muito relevante monitorizar e conhecer os padrões de dispersão polínicos destas espécies ou famílias. No capítulo seguinte iremos abordar este tópico.

1.3. Monitorização polínica

O principal tema da Aerobiologia é o estudo da origem, do transporte na atmosfera, e da deposição das partículas biológicas, tais como, grãos de pólen e esporos de fungos (Hirst, 1991). Aerobiologia como ciência multidisciplinar, foi introduzida por Meier nos anos 30, englobando o estudo da dispersão atmosférica das partículas biológicas e os seus impactos nos organismos e no meio ambiente. Ao longo dos anos, foram vários investigadores a contribuir para a definição de Aerobiologia. Gregory em 1973, definiu Aerobiologia como o estudo das interações das partículas bióticas com os agentes atmosféricos, propondo que esta ciência se encarregava do estudo de todas as partículas viáveis e não viáveis, transportadas de modo passivo pelo vento. Edmonds, em 1973, definiu Aerobiologia como a "ecologia da atmosfera", Pathirane em 1975 definiu esta ciência como multidisciplinar, que compreende o estudo desde a fonte de produção das partículas biológicas, à emissão, dispersão, transporte, deposição e ressuspensão das mesmas, bem como da sua incidência na atmosfera (Pathirane, 1975). Hirst em 1991, definiu esta ciência como aquela que "se ocupa do estudo do transporte de organismos e material biológico através da atmosfera" (Hirst, 1991). A visão mais atual chega-nos de Frenguelli que definiu a Aerobiologia como a ciência que se dedica "ao estudo de partículas biológicas, a sua libertação, dispersão, deposição e o impacto no ecossistema" (Frenguelli, 1998).

Desde há algum tempo, investigadores têm demonstrado interesse em conhecer e caraterizar o conteúdo de matéria particulada no ar e o seu transporte pelos impactos que tem na saúde pública, clima e ecossistema. Portanto, a monitorização da qualidade do ar foi um passo fundamental de invenção do homem para codesenvolvimento dos estudos aerobiológicos.

Os dados aerobiológicos têm vindo a ser utilizados por inúmeros especialistas em diversas áreas, tais como na gestão clínica de patologias alérgicas, designadamente da polinose, mas também no controlo de doenças infeciosas causadas por agentes bacterianos, virais ou fúngicos (Tummon et al., 2021). Mas não só na área clínica, os dados aerobiológicos são também importantes para a agricultura, como a sua utilização na fitopatologia para controle da dispersão de microorganismos prejudiciais às plantas, na disseminação de organismos geneticamente modificados e em previsão de colheita, na descoberta de plantas invasoras que podem condicionar e impedir o crescimento de outras espécies, no reconhecimento de plantações ilegais e por fim no controlo de ervas daninhas (Edmonds, 1979; Frenguelli, 1998). Os dados aerobiológicos são igualmente importantes para o estudo das mudanças climáticas, com a ocorrência de emissão de bioaerossóis (pólen e fungos) mais longas, com o aparecimento de novas espécies fúngicas em locais onde as temperaturas são mais elevadas, na fenologia das plantas, com o antecipar nas datas e épocas de floração mais extensas e intensas no estudo das mudanças climáticas, e de qualidade do ar pois elevadas concentrações de CO2 potencia a produção polínica (Tummon et al., 2021).

1.3.1. Métodos de amostragem aerobiológica

A monitorização ambiental da atmosfera também tem vindo a ser utilizada desde o início do século 19 com o objetivo de quantificar e caraterizar a composição de bioaerossóis na atmosfera. A amostragem de ar pode ser realizada para muitos fins e podem abranger uma monitorização qualitativa ou quantitativa. No primeiro caso, é determinado que tipos de espécies que estão presentes na atmosfera, a sua distribuição temporal e quais as variáveis que os influenciam, que pode ser complementado pela quantificação da concentração destas partículas no ar (Edmonds, 1979).

As técnicas de amostragem devem satisfazer o objetivo inicial do programa de amostragem, ser eficientes na captura e recolha das partículas de interesse e serem compatíveis com os métodos de contagem/analíticos. Assim, as escolhas dos métodos de amostragem dependem das caraterísticas das partículas aerotransportadas, tais como o seu tamanho, forma e estrutura da superfície (Edmonds, 1979). As partículas biológicas, como o pólen foram monitorizados, de forma contínua, pela primeira vez por Blackley no ano de 1870 (Blackley, 1873) e o primeiro registo foi efetuado no ano de 1943 no Reino Unido por Cardiff (Hyde, 1957).

Atualmente, existem inúmeras redes de monitorização polínica a nível mundial, nacional e regional, que fornecem regularmente informação acerca das concentrações polínicas no ar (Buters et al., 2018), procurando contribuir para o desenvolvimento de métodos de previsão polínica (Cotos-Yáñez et al., 2004), de modo a minimizar os riscos de ocorrência de reação alérgica (Hoyte & Katial, 2011). A nível internacional, conhecem-se, atualmente mais de 1000 estações de monitorização distribuídos por aproximadamente 53 países dos 5 continentes (Buters et al., 2018) o que revela um grande interesse nesta temática.

Inúmeros métodos são utilizados para o estudo de bioaerossóis. Os aparelhos de monitorização permitem a comparação de dados, independentemente das caraterísticas biogeográficas e climáticas do local de amostragem. Hoje em dia, os amostradores existentes funcionam com base em princípios físicos distintos, cada um com processos de amostragem igualmente distintos, quer na colheita quer no modo de deteção.

1.3.1.1. Métodos manuais

A monitorização de partículas polínicas tem tradicionalmente implicado a utilização de aparelhos cuja amostragem se baseia em deposição passiva das partículas, os gravimétricos ou na aspiração de volumes de ar conhecidos, os volumétricos.

Os métodos gravimétricos podem ser por exemplo, Placas de Petri, Método de Durham e armadilhas de Tauber. O **método Durham**, inicialmente, baseava-se na utilização de lâminas revestidas com uma substância adesiva ou de meios de cultura em agar. Anos mais tarde, em 1964, Durham, aperfeiçoou a técnica e padronizou-a. A lâmina revestida era colocada entre duas superfícies de metal que possibilitava a proteção contra condições meteorológicas adversas. A lâmina era trocada todos os dias e observada ao microscópio ótico. A maioria das partículas observadas eram depositadas por gravidade, resultado da sedimentação e da ressuspensão devido a turbulência de movimentos de massas de ar (Durham, 1946). Este instrumento de captação, tornou-se na altura, o mais utilizado por todo o mundo e atualmente ainda é bastante manuseado no Estados Unidos e no Japão, no entanto, apresenta algumas desvantagens, uma vez que, a sua localização condiciona a qualidade de bioaerossóis que são recolhidos, ou seja, se é colocado em altura capta maioritariamente pólen proveniente de árvores, pelo contrário, se colocado mais perto do solo, colhe maioritariamente pólen proveniente de plantas rasteiras. As Armadilhas de **Tauber** são semelhantes ao captador Durham, no entanto, foi cuidadosamente construído com uma tampa de modo a diminuir a turbulência aerodinâmica. É perfurado com um furo central de aproximadamente 5 cm de diâmetro (Tauber, 1974).

Por filtração em meio sólido, temos por exemplo o captador **Cour®,** baseia-se na captação dos grãos de pólen através de duas unidades filtrantes verticais. Estes amostradores, por ação de um intercetor de fluxo, orientam-se de acordo com a direção do vento, permitindo uma filtração omnidirecional das massas de ar. As unidades filtrantes são presas e mantidas em tensão totalizando uma superfície de exposição de 400 cm^2. Este tipo de unidades filtrantes permite a interceção de bioaerossóis, com um rendimento médio de filtração que varia entre 17 e 20% de acordo com a velocidade do vento (Cour,1974).

Os métodos volumétricos foram criados com o objetivo de ultrapassar as dificuldades sentidas na amostragem pelos métodos gravimétricos, designadamente, a pouca quantidade de bioaerossóis depositados e a baixa representatividade relativa à envolvente. São métodos que facilitam a obtenção de uma amostragem temporal de forma contínua. Apresentam 2 princípiosfísicos, amostragem por impacto ou por filtração. Na amostragem por impacto existem 3 variações, impacto em cascata (Andersen e ChemVol), inercial ou ciclónico (Rotorod e Cyclone®) e impacto por sucção (Hirst).

Os coletores de impacto em cascata, Andersen e ChemVol, possibilitam a monitorização de bioaerossóis e são compatíveis com metodologia de quantificação de alergénios. O captador **Andersen** é classificado como um amostrador de pequeno volume. Funciona sobre o princípio de impacto e consiste numa cascata de discos emparelhados e selados. Permite a estratificação de bioaerossóis pelo seu tamanho. A taxa de fluxo é de 28,3 L/min. Embora este tipo de captador tenha sido muito utilizado por inúmeras redes de monitorização distribuídas pela Europa, hoje em dia isso não se verifica. No entanto continua a ser muito utilizado nos Estados Unidos (Buters et al., 2018; Solomon, 1970). O captador **ChemVol** é um dispositivo de amostragem, composto por um tripé, um coletor estratificado, que colhe numerosas frações de partículas, e uma bomba de sucção de ar de alto volume. O ar é sugado pela seção superior e prossegue através de vários estágios no coletor. Possui uma plataforma que protege contra condições atmosféricas adversas. As partículas presentes na atmosfera são depositadas em dois ou mais filtros de acordo com as suas dimensões, podendo ter PM 10 μm, PM 2.5 μm ou PM 1.0 μm. A sua taxa de fluxo alcança os 800L/min (Demokritou et al., 2002). É utilizado maioritariamente para a monitorização de aeroalergénios (Buters et al., 2012; Buters et al., 2015).

O captador **Rotorod** é um amostrador de impacto inercial por rotação mecânica de pequenas dimensões, desenvolvido em 1950. Dispõe de dois braços em forma de U com uma substância adesiva. Por ação de um motor, gira a alta velocidade (2400 rpm) recolhendo as partículas do ar. É um captador de fácil transporte e a sua taxa de fluxo de ar é de 120 L/min (Solomon et al., 1980). O amostrador do tipo ciclónico é baseado no princípio descrito por Decker et al., 1969, em que as massas de ar são forçadas a moverem-se em rotação ou em espiral, fazendo com que as partículas sejam recolhidas pela força centrifuga gerada. A recolha pode ser efetuada em meio líquido, ou a seco, e as amostras de ar são posteriormente tratadas em laboratório (Decker et al., 1969).

O captador do tipo **Hirst** (figura 3 a)) é utilizado pela maioria das redes de monitorização a nível mundial (Buters et al., 2018/Capítulo I desta dissertação) e é o equipamento usado na colheita de pólen da metodologia considerada padrão para a monitorização polínica, recomendada pela IAA (*International Association of Aerobiology*) e ESA (*European Aeroallergen Society*). Este tipo de aparelho é constituído por 3 unidades, a unidade de impacto, um cata-vento, e uma bomba de ar. A unidade de impacto contém um orifício de entrada de ar com dimensões de 14x2mm, e um cilindro metálico que se encontra conectado a um relógio com um mecanismo giratório que possibilita um movimento numa razão de 2mm/h, permitindo assim dados diários e horários. Este cilindro dispõe uma fita de Mellinex® de 19mm, revestido por uma substância adesiva de silicone, o que facilita a captura das partículas em suspensão no ar. O cata-vento encontra-se no exterior da estrutura metálica e a sua função é manter o orifício de entrada de ar na direção dos ventos dominantes, tornando a captação mais eficaz. A bomba de vácuo permite uma sucção de volume de ar conhecido, regulável a partir de um sistema de ajuste que se encontra na estrutura metálica. O caudal de sucção é ajustado nos 10 L/min (Hirst, 1952) (figura 3b)).

Este equipamento apresenta uma autonomia de amostragem de 7 dias e a fita é posteriormente retirada e tratada em laboratório, onde são efetuadas preparações definitivas para contagem e identificação polínica (Gálan et al., 2007). Todas as medições são expressas em número de pólen por metro cubico de ar (pólen/m^3).

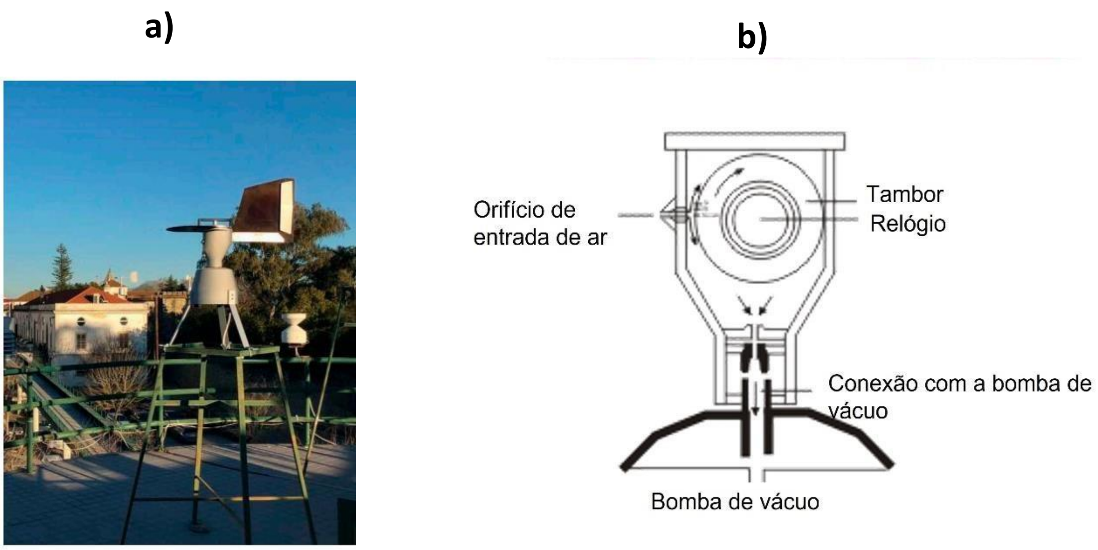


Figura 3: a) Amostrador Hirst-type localizado no Observatório de Ciências Atmosféricas (EVASO), ICT; b) Unidade de impacto do amostrador Hirst-type (adaptado de REA, 2021).

As desvantagens da utilização deste amostrador passam por um atraso na obtenção dos resultados da concentração polínica diária, relativamente à colheita. Geralmente fornece dados médios com um atraso de 3 a 10 dias (Galán et al., 2007) e é necessário muito esforço e tempo devido à natureza laboratorial da técnica, contagem e identificação microscópica.

É evidente que há uma necessidade geral por parte das redes de monitorização de obter relatórios polínicos mais rápidos e quase em tempo real das concentrações de pólen aerotransportado, e, portanto, a criação de métodos de monitorização em tempo real que permitam alcançar esse objetivo tem sido o foco principal da comunidade científica. Nesse sentido, uma nova geração de instrumentos automáticos está já operacional, que permite uma discriminação temporal mais fina.

1.3.1.2. Métodos automáticos

Os métodos automáticos são baseados em dois princípios de identificação, o reconhecimento por imagem utilizando as características da morfológica do bioaerossol (Boucher et al., 2002; Holt et al., 2011; Lagerstrom et al., 2015; Marcos et al., 2015; Vezey & Skvarla, 1990), e métodos espetroscópicos (Crouzy et al., 2016; Šauliene et al., 2019) (O´Connor et al., 2014).

Entre os equipamentos que utilizam métodos automáticos por reconhecimento de imagem podemos encontrar o Pollen Sense e BAA500. O **Pollen Sense** consiste no processamento de imagens retiradas a partir de sensores para contagem e identificação polínica. São sensores de baixa manutenção, e que libertam o técnico de fazer todo o procedimento laboratorial da amostra e consequente identificação dos diversos tipos polínicos. São instrumentos pequenos e práticos (“Automated Particle Sensors,” n.d.). **BAA500**, recentemente criado, é um sistema capaz de reconhecer, atualmente, 30 tipos polínicos. É um método que utiliza um algoritmo de reconhecimento de imagem de pólen. O sistema prepara automaticamente e de forma adequada lâminas para identificação visual das partículas atmosféricas. Com a utilização de uma câmara acoplada, este sistema de reconhecimento obtém dados de pólen em tempo quase real com um atraso médio de 3-6h (Oteros et al., 2015). Os erros deste instrumento automático podem ser parcialmente reduzidos com algum treino local (Oteros et al., 2015).

Os métodos com princípio de espetrometria de feixe de laser**, KH-3000-01** é um instrumento automático dirigido, maioritariamente, para contagem polínica utilizando um feixe de laser (Kawashima et al., 2007). **PA-300** consiste num instrumento de feixe de laser vermelho com comprimento de onda de 658nm e os dados de dispersão resultantes da medição são registados por dois foto-detetores. O sinal de espelhamento auxilia na caraterização física das partículas, como a sua forma, tamanho e superfície. Um segundo feixe ultravioleta (337nm) excita as partículas e o sinal de fluorescência é gravado com uma rede de difração e uma matriz de 32 foto-detetores. O ar é então bombeado para um detetor, a cerca de 2L/min. Para aumentar o nível de captação das partículas aerossolizadas, foi utilizado o princípio de impactação. A saída de ar foi conectada a uma segunda bomba com maior volume de sucção (30L/min) e com uma saída secundária de ar (Crouzy et al., 2016). Inicialmente para programar o aparelho para a identificação polínica foram efetuados cálculos do espalhamento geométrico de esferas, possibilitando que as amplitudes das mesmas sejam idênticas ao espalhamento calculado para as partículas aerossolizadas. A proporcionalidade foi estimada para 16 tipos polínicos conhecidos (Crouzy et al., 2016). **Rapid-E** foi o novo instrumento projetado e produzido pela Plair SA e é o sucessor do instrumento PA-300 anteriormente descrito. Rapid-E é um contador de partículas, ou seja, analisa todas as partículas individuais que são monitorizadas. É baseado em dois princípios físicos, o espalhamento do feixe de laser ultravioleta e o espalhamento de feixe de laser de fluorescência induzida por um laser de UV (Kiselev et al., 2013, 2013). O espalhamento multiangular é utilizado para determinação do tamanho e forma da partícula. O instrumento aspira constantemente ar na parte superior do painel com um fluxo de ar de 2.8 L/min e com a taxa de contagem de 4500 deteções de partículas por minuto. O ar entra no orifício, criando um feixe na zona de medição. As partículas interagem com um feixe de luz laser a 400nm e o espalhamento de luz é capturado por 24 detetores de resolução de tempo em diferentes ângulos. A informação química das partículas, que induz fluorescência, é obtida por um laser ultravioleta profundo, num comprimento de onda de 320nm. Toda esta informação é utilizada para a identificação das partículas (Šauliene et al., 2019).

O método automático **WIBS-4 (Waveband-Integrated Biological Sensor)** é desenvolvido pelo princípio da fluorescência utilizando um espetrofotómetro de fluorescência on-line. Este instrumento utiliza a fluorescência e espalhamento ótico para diferenciar partículas fluorescentes e não fluorescentes presentes na atmosfera. Tem capacidade de determinar a forma, o tamanho e a fluorescência das partículas ambientais com uma resolução de milissegundos. Contêm duas lâmpadas de flash de Xenon configuradas a comprimento de ondas de 280nm e 370 nm. Para determinação da forma e tamanho das partículas, utiliza um feixe de díodo com comprimento de onda de 635nm. Os valores das caraterísticas morfológicas são obtidos usando a proporção de luz espelhada com incidência em 4 quadrantes. Quando a intensidade de luz é semelhante em cada quadrante indica que a forma esférica da partícula, pelo contrário quando a intensidade da luz é diferente em cada quadrante, a partícula tem uma forma irregular (O´connor et al., 2014).

Para além dos métodos manuais e automáticos, acima descritos, ainda existe métodos de analise bioquímica e molecular (Buters et al., 2015; Buters et al., 2012; Kraaijeveld et al., 2015). Ambos com objetivos e finalidades diferentes e que vão ser descritos no ponto seguinte.

1.3.1.3. Métodos bioquímicos e moleculares

Os métodos bioquímicos tem vindo a ser utilizados para a deteção de alergénios no ar. Estão associados a um sistema de monitorização manual descrito no ponto 1.3.1.1. As amostras processadas em laboratório são previamente extraídas, liofilizadas e ressupendidas em volumes conhecidos de tampão. A técnica bioquímica para a deteção de alergénio no ar designasse ELISA (*Enzyme-Linked Immunosorbent Assay*). Esta técnica permite o reconhecimento de grupos de alergénios pertencentes a vários tipos polínicos e da obtenção de dados da quantificação de alergénio no ar (Buters et al., 2015; Buters et al., 2012; Gálan et al., 2013).

A monitorização molecular também se encontra em elevada expansão e talvez seja uma perspetiva de monitorização aerobiológica. Longhi et al., 2009, desenvolveu um método, estatisticamente comparável a outros métodos aerobiológicos, que permitiu quantificar os grãos de pólen. O método tradicional baseado em brometo de cetiltrimetilamónio foi modificado para o isolamento de DNA dos grãos de pólen. As sequências de DNA dos diversos tipos polínicos foram identificadas através de ampliação por PCR e técnicas de bioinformática. Com base nas sequências de DNA retiradas da análise de PCR, foram desenvolvidos sondas TaqMan complementares. O ensaio de PCR em tempo real foi desenvolvido para três espécies e apresentou resultados promissores na identificação e quantificação polínica, mesmo quando existiam várias espécies misturadas. Como perspetiva futura pretende-se desenvolver PCR multiplex em tempo real para a deteção simultânea de vários tipos polínicos no mesmo tubo da reação e a aplicação de métodos moleculares de alto rendimento (Longhi et al., 2009).

Devido ao surgimento acentuado de instrumentos automáticos, descritos anteriormente, é essencial que seja aproveitada a ocasião de estabelecer uma rede europeia padronizada que forneça informações que têm que corresponder às necessidades dos usuários e que evite duplicações desnecessárias ou esforços no futuro. Assim, foi criado o programa designado AutoPollen que consiste na criação de uma plataforma para o desenvolvimento de uma rede europeia de monitorização automática de pólen em colaboração com diversos países (Clot et al., 2020). O programa faz parte do agrupamento de serviços meteorológicos e hídricos europeus (EUMETNET), com o principal intuito de contribuir para a qualidade de vida da população alérgica. O objetivo do AutoPollen não é apenas estabelecer a monitorização automática a longo prazo, mas também fornecer a informação polínica e criar modelos de previsão para médicos, autoridades de saúde e público em geral (Clot et al., 2020). Existem outros programas paralelos ao programa AutoPollen, como por exemplo, COST Action ADOPT que consiste em novas abordagens na deteção de aeroalergénios e organismos patógenos, CA-18226. Atualmente reúne uma rede interdisciplinar de especialistas em monitorização ambiental de bioaerossóis com métodos padrões ou tecnologias futuras. O principal objetivo da ação COST é encorajar a pesquisa para o desenvolvimento de novos métodos de analise de dados (Clot et al., 2020).

É de notar que a comunidade científica se tem dedicado ao desenvolvimento de métodos e estudos sobre a distribuição e o efeito dos bioaerossóis na saúde humana bem como evoluir na monitorização ambiental sistemática. No entanto, ainda existem questões que necessitam de ser abordadas de forma eficaz, de modo a adotar as melhores estratégias para a proteção da população e serviços de informação mais rapidamente atualizáveis, com sistemas de alerta adequados e de fácil consulta e resposta, para aumentar a qualidade de vida dos cidadãos.

1.3.2. Ferramentas de informação geográfica e de disseminação aerobiológica

O ciclo de vida das plantas está interligado com o clima de cada região (Garcia-Mozo et al., 2009) e os seus padrões reprodutivos refletem o seu grau de adaptação (Chuine, Cambon, & Comtois, 2000). As observações fenológicas das plantas podem fornecer informação para investigar a relação entre a variação do clima e o desenvolvimento da planta. Sabe-se, até ao momento que, a acumulação de calor, impulsionado pela temperatura, influência a taxa de desenvolvimento anual da planta e, portanto, pode até ocorrer que a existência de uma dada espécie ou as observações fenológicas dela possam potencialmente ser utilizadas para caraterizar a evolução climática de uma determinada região (Spano et al., 1999).

De modo a efetuar um controlo, caraterização e interpretação da informação polínica de uma determinada região são construídos os calendários polínicos podendo ter em conta três variáveis, geolocalização, mapeamento geográfico das espécies e clima. Os calendários polínicos são representações gráficas da sazonalidade dos principais tipos polínicos alergizantes presentes na atmosfera num determinado local. Construídos a partir de dados de concentração diários ou mensais, que sintetizam de forma simples e direta a dinâmica anual do pólen, os períodos de polinização e os dias de maior e menor concentração polínica (Elvira-Rendueles et al., 2019) (figura 5). Os primeiros calendários polínicos foram criados no ano de 1873 por Backley com o objetivo de estudar a atmosfera de Manchester. Kenned (1953), foi também um dos autores que utilizou esta ferramenta de informação polínica a partir de dados obtidos com aparelhos gravimétricos (Kennedy & Edmonton, 1953). Para além dos calendários polínicos, é possível definir o período do ano de maior concentração polínica no ar, a época principal de polinização, bem como as suas caraterísticas. A época principal de polinização é o período em que os grãos de pólen se encontram na atmosfera em concentrações mais elevadas. Existem diferentes métodos para definir o início e o fim da época principal de polinização (Sabariego et al., 2012; Galán et al., 2017). O método da percentagem, método de ajuste logístico, método clínico, método do número de grãos de pólen e método de média móvel. O método da percentagem é comumente utilizado para definir a época polínica com base na curva cumulativa percentual da concentração polínica e eliminação de um intervalo de dados, correspondente a uma determinada percentagem, no início e no fim da época de polinização. Descrito por Nilsson e Persson, 1981 e Andersen, 1991, por exemplo, se a época polínica é baseada em 95% do pólen anual total, a data de início da época polínica é marcada no dia em que se atinge 2.5% do pólen total registado e a data final ocorre no dia que forem atingidos os 97.5% do total de pólen registado (Andersen, 1991; Nilsson & Persson, 1981).

O método de ajuste logístico foi desenvolvido por Ribeiro, Cunha & Abreu, 2007 e modificado por Cunha et al., 2015. É baseado no ajuste anual de um modelo de regressão logística não linear à curva diária cumulativa da concentração atmosférica de um determinado tipo polínico, através de uma curva sigmoide de 2 assimptotas, uma para o início e outro para o fim da época. A função logística e a derivada permite que seja calculada a data de início e de fim da época principal de polinização. É especialmente bem ajustada quando as quantidades de pólen são estáveis no início e no fim da curva acumulada (Cunha et al., 2015; Ribeiro, Cunha, & Abreu, 2007).

O método clínico proposto por Pfaar et al., 2017, é baseado na relação entre o risco de exposição e os sintomas alérgicos. Diferentes períodos podem ser considerados a partir deste método, tais como o intervalo de época principal de polinização e/ou os dias de maior concentração (Pfaar et al., 2017).

O método do número de grãos de pólen foi proposto por Gálan et al., em 2001, originalmente para o pólen de Oliveira, mas, atualmente, é utilizado para outros tipos polínicos. A data de início e fim da época polínica são determinados como um certo número de dias em que é excedido um certo limiar de pólen (Galán et al., 2001). Por exemplo, considera-se o início da época principal de polinização quando são detetados mais do que 5 grãos de pólen/m^3/dia e que a época se mantém enquanto a concentração de grãos de pólen não for, novamente, inferior a esse valor.

O método da média móvel foi proposto no âmbito da implementação da libraria em R - *AeRobiology,* uma extensão do programa *R* para organização, tratamento e análise de dados aerobiológicos. Neste caso, a definição da época principal de polinização é baseada na aplicação de um ajuste de média movel à serie temporal de dados de pólen, a fim de obter a sazonalidade na curva polínica, evitando a natural variabilidade diária. Assim a data de início e fim da época principal de polinização é estabelecida quando a curva da média movel atinge o limite de pólen (Rojo, Picornell & Oteros, 2019).

A seleção de cada método de análise da época principal de polinização depende do objetivo do estudo e o método aplicado deve ser sempre bem identificado (Galán et al., 2017). As métricas extraídas correspondem à data de início e fim da época, a sua duração, o índice polínico integral e anual, que se trata do somatório da concentração diária em um período de intervalo determinado. O dia do pico de concentração máxima bem como o seu valor também são calculados assim como a duração dos períodos pré-pico e pós-pico e concentração acumulada. No método clínico, ainda se pode identificar o início e fim da época em que as concentrações de pólen na atmosfera foram superiores a um valor de base. A partir deste estudo podemos caraterizar as épocas polínicas quanto à sua intensidade, observando o índice polínico integral e anual, a sua duração, se foi uma época mais longa ou mais curta e relacionar com os parâmetros meteorológicos (Andersen, 1991; Nilsson & Persson, 1981; Cunha et al., 2015; Ribeiro, Cunha, & Abreu, 2007; Pfaar et al., 2017; Galán et al., 2001; Rojo, Picornell & Oteros, 2019). Partindo destas informações fornecidas por todas estas ferramentas, é possível obter uma correlação entre o aparecimento de sintomatologia alérgica e a sua duração, auxiliar no diagnóstico rápido e correto por parte dos profissionais de saúde à população alérgica, e também, possibilitar que a população alérgica desenvolva medidas preventivas durante os períodos de maior risco polínico, com o intuito de reduzir a exposição nos dias de maior concentração polínica no ar. Deste modo é possível a disseminação da informação de forma assertiva.

Em Espanha, no ano de 1991, investigadores da Universidade de Córdoba e a Universidade Politécnica do Norte de Londres, demonstraram a importância de criar uma Rede de Monitorização Aerobiológica com a possibilidade de integração na Rede Europeia de Aeroalergénios (European Aeroallergen Network - EAN). No ano de 1992, realizou-se a primeira reunião, a qual, pela iniciativa do professor Eugénio Dominguez Vilches, assistiram diversos investigadores de várias universidades espanholas. Desde a primeira reunião, surgiu uma pequena rede com apenas três estações de monitorização com objetivo de ampliação para todo o país. Atualmente é uma rede constituída por 47 estações de monitorização. A **Rede Espanhola de Aerobiologia (REA)**, pública toda a informação relacionada com os níveis polínicos atmosféricos, através das estações de monitorização existentes por todo o país, divulgando os tipos polínicos que se encontram na atmosfera e os seus níveis em cada região - muito baixo, baixo, moderado e elevado. Esta classificação por níveis de risco faz-se com base na concentração de grãos de pólen por metro cúbico durante 24 horas e depende do grau de alergenicidade de cada tipo polínico (Gálan et al., 2007).

Em Portugal, neste momento operam duas plataformas, a **Rede Portuguesa de Aerobiologia (RPA)** pertencente à Sociedade Portuguesa de Alergologia e Imunologia Clínica (SPAIC). A Rede Portuguesa de Aerobiologia foi criada no ano de 2002, com o principal objetivo de monitorizar a nível nacional os níveis polínicos que se encontram no ar e divulgar a nível nacional ou regional informação qualitativa associada ao nível de risco. A consulta dos dados qualitativos é gratuita. Nas estações do ano de Inverno, Verão e Outono a informação polínica é divulgada através dos websites da SPAIC e RPA, no entanto, na Primavera essa informação é também divulgada através da comunicação social e redes sociais ("RPA - Rede Portuguesa de Aerobiologia," 2021).

A rede **Pólen Alert** é uma base de dados académica criada por investigadores das Universidades de Évora, Porto e Instituto Politécnico da Guarda, constituída, atualmente, por 4 estações de monitorização localizadas em Évora, Lisboa, Porto e Guarda. O seu principal objetivo é a divulgação do risco de exposição diário dos tipos polínicos mais prevalentes em cada região. Este risco é categorizado em baixo, moderado e alto, consoante a alergenicidade de cada tipo polínico. Possui também informação botânica e morfológica sobre os tipos polínicos mais abundantes. Esta página pode constituir uma ferramenta de consulta útil para investigadores, responsáveis municipais, profissionais de saúde, pessoas que sofrem de alergia a pólen e público em geral (Polen Alert, 2021). **Instituto Português do Mar e Atmosfera, IPMA**, também já tem disponível na sua página de divulgação um separador com os dados de pólen da região de Lisboa, Évora, Porto e Guarda. A consulta destes dados é gratuita (IPMA,2021).

1.4. Caraterização aerobiológica e climática da Península Ibérica

A Península Ibérica (34.625–45.075 N; − 15.125 O e 4.785 E) é considerada uma área de elevada biodiversidade, rica em espécies endémicas com elevado grau de extinção (Giménez et al., 2006).

Geograficamente, a península ibérica faz fronteira com o oeste, sudoeste e norte do Oceano Atlântico e pelo sudeste e leste do mediterrâneo. Na Península Ibérica situa-se a Andorra, o território ultramarino britânico de Gibraltar e os países Portugal e Espanha (figura 4 a)).

De um modo geral, apresentada um clima mediterrâneo (Csa) na metade do sul da Península com verões quentes e secos, e parte do norte com verões amenos, classificado como clima oceânico mediterrâneo (Csb) com verões amenos. O sudeste de Espanha com clima semiárido (Bsh). Possui também áreas montanhosas localizadas a norte, a centro e sudeste com clima maioritariamente oceânico (Kottek et al., 2006).

1.4.1. Clima, aerobiologia e potencial alergénico das regiões em estudo

1.4.1.1. Caraterização climática das regiões em estudo

A cidade de Évora, localizada a sul de Portugal (38° 340 N, 7° 540 W, 293 m acima do nível do mar), é caraterizada por um clima temperado com verões quentes e secos que podem ser descritos como clima mediterrâneo de verão quente, de acordo com a classificação climática de Köppen – Csa. De acordo com a norma climatológica (1981-2010), fornecida pelo Instituto Português do Mar e Atmosfera (IPMA), a temperatura média anual do ar é de 16,2 °C, com um valor mais alto no mês de agosto (23,7 °C) e o menor valor mensal no mês de janeiro (9,4°C). O período pluviométrico, que ocorre normalmente de forma sazonal entre os meses de outubro e abril, apresenta uma precipitação anual de 585,3 mm (figura 4b)), sendo que novembro é o mês com maior ocorrência de precipitação, cerca de 85,9 mm (https://www.ipma.pt/bin/file.data/climate-normal/cn_81-10_EVORA.pdf, último acesso 21 de novembro 2021). A direção do vento predominante nesta região é Noroeste (IPMA, 2021). Na classificação da ocupação do uso do solo para esta região, encontra-se destacado o tecido urbano, mas também ao seu redor, Zonas industriais, comerciais ou transportes, minas, depósitos de resíduos ou zonas de construção, zonas artificializadas não agrícolas com vegetação, terras aráveis, culturas permanentes, pastagens, zonas agrícolas heterogéneas, florestas, zonas de vegetação arbustiva e/ou herbáceas. Também se encontra prados naturais, matos, florestas ou vegetação arbustiva de transição e zonas húmidas interiores que dizem respeito a pântanos. A zona urbana encontra-se distribuída pelo centro histórico e o redor da cidade, no entanto, também se encontra numa extensa área zonas agrícolas heterogéneas, nomeadamente, montados de sobro e azinheira bem como pastagens (Batista, 2011).

A cidade da Guarda está localizada a norte de Portugal continental (40,53 ° N, 7,26 ° O), é considerada a região mais elevada de Portugal, a uma altitude de 1056 metros. A sua paisagem é essencialmente rural. É caraterizada por um clima mediterrâneo húmido de acordo com o índice de Emberger. Apresenta um clima quente e temperado, segundo a classificação climática de Köppen e Geiger (Csb) e de acordo com a norma climatológica 1981-2010, a temperatura média anual é de 11,4° C, sendo o mês de janeiro, o mais frio, com temperatura média de 4,2° C e o mês de julho, o mais quente com temperaturas a rondar os 20,4° C. A precipitação anual é de 885,0 mm, ocorre principalmente nos meses de inverno, e o mês de outubro é aquele que apresenta maior ocorrência de precipitação (132,4mm) (figura 4b) (https://www.ipma.pt/bin/file.data/climate-normal/cn_81-10_GUARDA.pdf, último acesso 21 de novembro 2021). Os ventos sopram principalmente no quadrante NW (IPMA, 2021). Ao nível da caraterização do uso do solo, a região da Guarda apresenta 12 classes principais que compreendem a ocupação da zona urbana dispersa e espaços intersticiais cujo uso do solo pertence a classes como florestas, vegetação, áreas ardidas e agricultura. A classe que abrange uma maior área ocupada é floresta, vegetação e áreas ardidas, equivalente a 38,71% do total da área, seguida da Agricultura com 25,44% e posteriormente o tecido urbano com 24,94% (Soares, Fonseca, & Ramos, 2016). Dentro das classes das florestas, vegetação e áreas ardidas destaca-se vegetações herbáceas em mosaicos com sistemas agroflorestais, matos ou vegetação esclerofila, florestas de *Pinus pynaster* A. e pontualmente, com florestas de *Quercus robur* L. e *Quercus Pyrenaica* W. (Ribeiro & Monteiro, 2014).

A cidade do Porto localiza-se a norte de Portugal continental (41.51° N, 8.61° W), é delimitada a oeste pelo Oceano Atlântico e a sul pelo rio Douro. É uma cidade bastante urbanizada. De acordo com o Sistema de Classificação Climática de Köppen, a cidade do Porto está incluída na categoria Csb-verões quentes e secos. Apresenta um clima mediterrâneo, mas com influência do Oceano Atlântico, o que provoca um decréscimo das temperaturas. A temperatura média anual é de 14,9° C (figura 4 b)). No mês de Janeiro é de 9,8° C e o mês de agosto, é considerado o mais quente com uma temperatura média de 20,0° C. A precipitação total anual é de 1139,5 mm (figura 4 b)) e ocorre principalmente nos meses de outono e inverno, sendo o mês de dezembro o que apresenta maior precipitação (168,8 mm) (https://www.ipma.pt/bin/file.data/climate-normal/cn_81-10_PORTO_PEDRAS_RUBRAS.pdf, último acesso 21 novembro 2021). Os ventos são predominantes dos quadrantes de O e NO no verão e E e SE no inverno (figura 4 b)) (Miranda et al., 2001; Kelcey, 2015; IPMA, 2021). A região do Porto carateriza-se por uma ocupação de solo classificado em Área histórica, correspondente a edifícios antigos, Área de frente continua I e II, Área de blocos isolados, Espaços de atividades económicas, no qual estão inseridas atividades económicas I e II, espaços verdes que se subdivide em área verde fruição coletiva, área verde associada a equipamentos, área verde lúdica-produtiva, área verde de proteção e enquadramento e área de frente ribeirinha e atlântica. Também apresenta classes referentes a espaços de uso especial e por fim espaços urbanos de baixa intensidade (Carvalho, Bento, Costa, & Santos, 2018).

A cidade de Granada, Espanha, é classificada como a região Csa de acordo com a classificação climática de Köppen. A temperatura média anual é de 15,1 °C, com um maior valor mensal no mês de julho (26 °C) e um menor valor mensal em janeiro (6,8 °C). O período de precipitação ocorre principalmente nos meses de outono a primavera, apresentando uma média de 357 mm (AEMET, 2021). As condições climatéricas nesta cidade espanhola são fortemente condicionadas pela Serra Nevada, uma das cadeias montanhosas mais alta da Europa, que fica apenas a 40 km de distância da cidade. A influência das montanhas/Serras é muito significativo no padrão de ventos vale-montanha, uma vez que, a atmosfera que se encontra em contacto com a superfície, no topo da montanha, é mais afetada pelas trocas de calor durante o dia, do que por exemplo a atmosfera sobre o vale, pois esta, está mais distante do solo. Durante a noite, o sentido é inverso, ou seja, é gerado ar frio do topo da montanha para o vale, uma vez que, o topo da montanha arrefece mais rápido. Sobre o vale o ar sobe, fecha a circulação, e dá lugar a brisa de montanha. O vento da brisa de montanha denomina-se vento catábico. Como consequência da dos ventos catábicos, existe com muita frequência inversões térmicas, que ocorrem principalmente durante o inverno, uma vez que, o ar frio fica fixado junto ao vale (Clements, 1999; Zamora & Pérez-Luque, 2016; IPMA, 2021). Ao nível da caraterização do uso do solo observa-se que na região de Granada são identificadas 11 tipologias que sintetizam as grandes diferenças províncias: **Alta montana**, esta tipologia compreende a Serra Nevada com 2000 metros de altitude e que inclui parques naturais com modelos glaciares e peri-glaciares. **Paisagem de caráter de serra** que engloba a maioria dos sistemas béticos e outras áreas de menor altitude. **Paisagem de caráter Alpino**, trata-se de zonas com escassez de vegetação e com áreas de relevos acentuados e abruptos. **Paisagem de caráter erosivo**, distingue-se por zonas onde a erosão é muito intensa e vegetação escassa. **Zonas litorais-tropicais**, caraterizam-se pela existência de uma mistura do uso de componentes de diversas paisagens. O que define, fundamentalmente, esta tipologia é a existência de relevos montanhosos até ao mar, embora também exista a formação de zonas costeiras típicas, como praias ou estuários. **Paisagem agrícola dominada pela agricultura de sequeiro** está amplamente distribuída por toda a região e carateriza-se por apresentar evidências de ação humana, como por exemplo, intenso espaço agrícola de sequeiro dedicado á plantação de cereais. **Paisagens agrícolas dominadas por olivais,** esta tipologia compreende espaços modificados pelo uso agrícola de olival intensivo, acompanhado pela presença de cereais ou carvalhos. **Paisagem agrícola de sequeiro dominada por mosaico de culturas**, esta tipologia é caraterizada por uma mistura de culturas que apresenta, por exemplo, culturas de regadio, olival, amendoal e vinhas. Embora seja caraterizada pelo cultivo, ainda existe a presença de vegetação natural. **Paisagem agrícola caraterizada pelo cultivo de regadio intensivo**, tal como a tipologia anterior, esta também é caraterizada pela presença de uma mistura de culturas, como pomares, árvores de fruto e choupos. **Paisagem agro-natural com agricultura de regadio** é uma paisagem com a presença de elementos naturais, agricultura desenvolvida em terraços com a presença de ambiente urbano adaptado ao meio ambiente no qual está inserido. **Paisagem urbana** são espaços altamente alterados pelo homem, pelo que, domina a presença de componentes antropogénicos. Estas caraterísticas só podem ser identificadas na capital de

Granada e nos municípios da sua região metropolitana, abrangendo uma parte da zona de Veja de Granada, que deixa de ser totalmente agrícola e rural para ser uma área urbana (Ferrer et al., 2000; Delegación de medio ambiente, 2022).

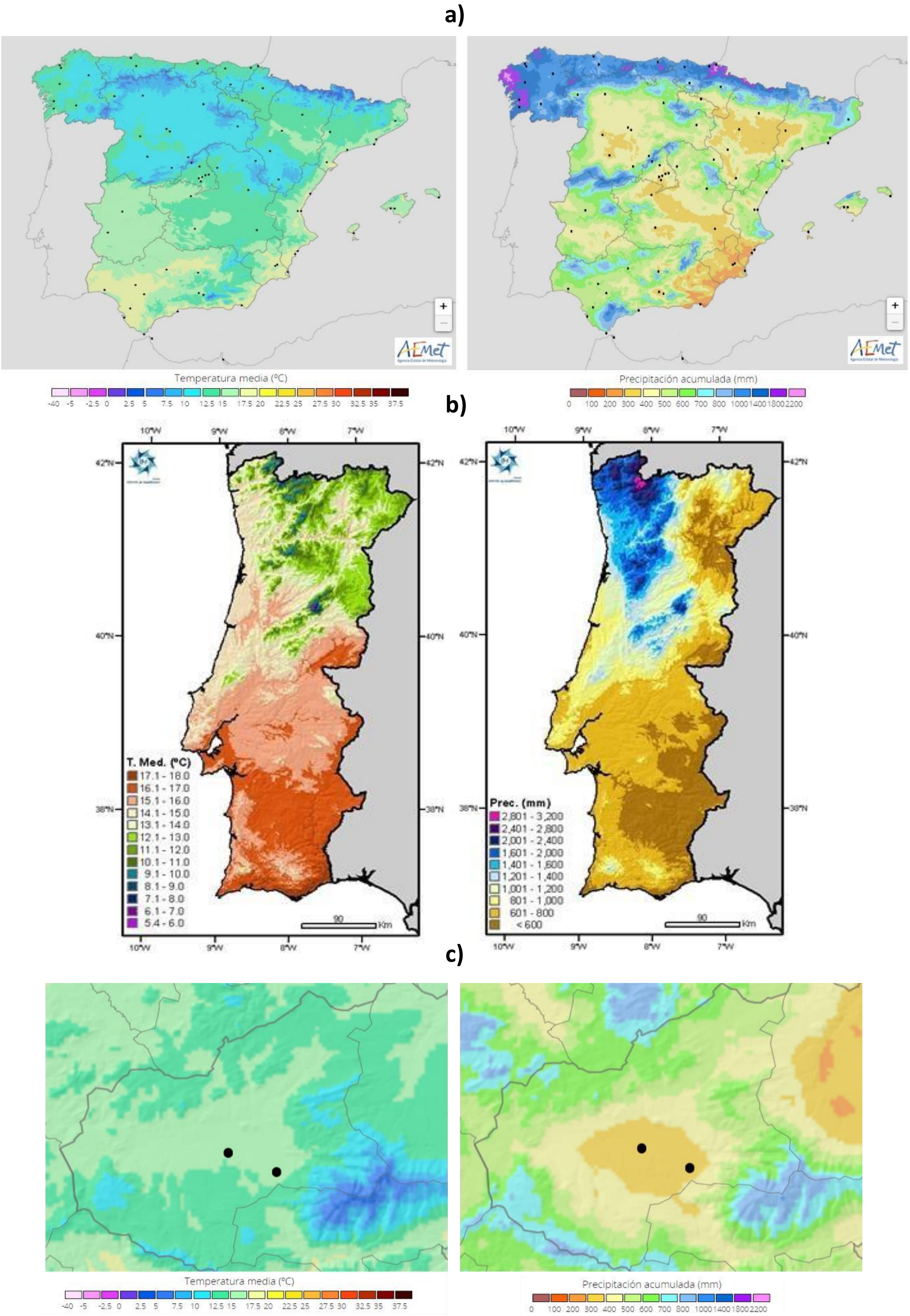


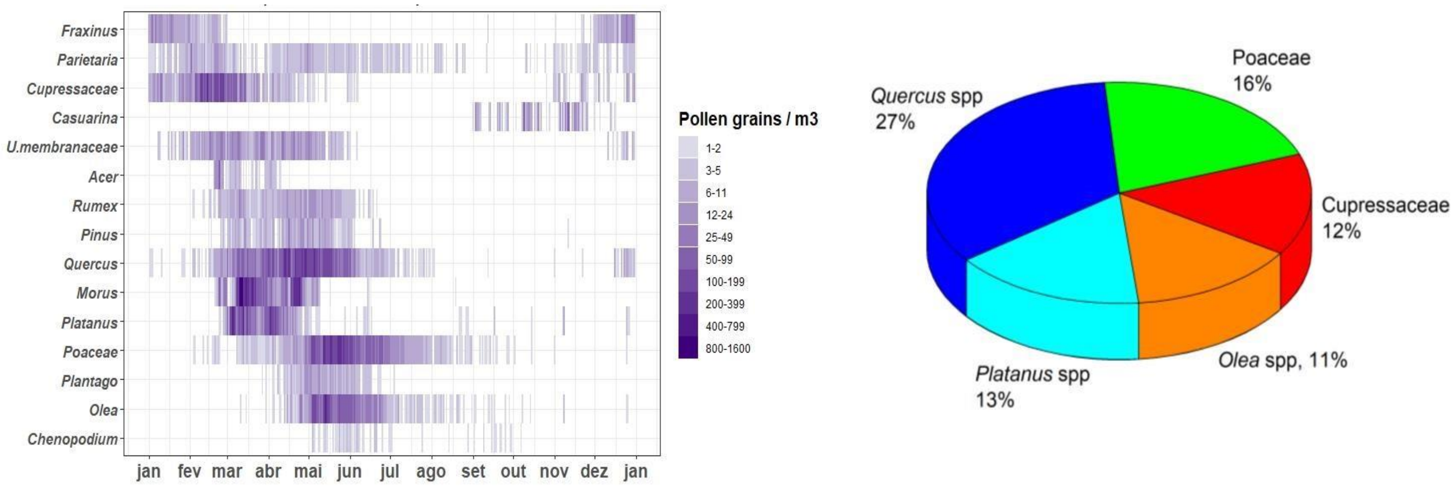
Figura 4: a) Mapa climático referente a temperatura media e precipitação para a Península Ibérica; b) Mapa climático de Portugal (a) e de Granada (c) para temperatura média e precipitação anual. Os pontos a preto representado nas figuras dizem respeito ao código de identificação de cada estação (retirado de IPMA, 2021 e www.aemet.es/es/serviciosclimaticos).

1.4.1.2. Caraterização aerobiológica das regiões em estudo

O ciclo de vida do pólen na atmosfera inicia-se com a libertação das anteras. Em árvores com polinização anemófila, a deiscência das anteras é resultado da desidratação, para a qual contribuem um conjunto de condições climatéricas como temperaturas elevadas, aumento da exposição solar, baixa humidade relativa e vento moderado (Linskens & Cresti, 2000). Uma vez libertados das anteras e dispersos na atmosfera, a força mecânica do fluxo do ar, induzido pelo vento médio ou por turbulência com a ocorrência de redemoinhos, torna-se o único processo físico que possibilita manter os grãos de pólen em suspensão na atmosfera. A elevação de movimentos turbulentos determina em grande parte a fração dos grãos de pólen libertados que são dispersos em larga escala (Sofiev & Bergmann, 2012). O vento médio torna-se a principal força de transporte dos grãos de pólen pelo ar, enquanto a turbulência os redistribui dentro e fora da camada limite da atmosfera. A dispersão polínica em escala regional, em distâncias de cerca de 100 quilómetros, apresenta desafios diferentes quando comparado com escalas locais (Sofiev & Bergmann, 2012).

A maioria dos tipos polínicos identificados nas regiões em estudo têm origem em plantas com polinização anemófila. Atualmente, na região de Évora, são identificados pelo menos, 15 tipos polínicos diferentes, cada um com o seu grau de predominância e com diferentes períodos de ocorrência. Nos meses de janeiro e fevereiro, são identificados tipos polínicos de *Fraxinus spp, Alnus spp, Parietaria spp, Urtica membranaceae e* Cupressaceae. O pólen de *Parietaria spp* está presente em praticamente todo o ano. Nos meses de março e abril é identificado pólen de Cupressaceae, embora já em concentrações minoritárias, *Parietaria spp*, *Urtica membranaceae*, *Acer spp*, *Rumex spp*, *Pinus spp*, *Quercus spp*, *Morus spp e Platanus* spp. Nos meses de maio e junho pode notar-se que estão presentes com maior abundância tipos polínicos de Poaceae, *Olea europaea* e *Quercus* spp (figura 5a)).

A figura 5 mostra a diversidade polínica na região de Évora, no entanto, os tipos polínicos mais prevalentes e relevantes do ponto de vista alergológico são: Cupressaceae (12%), Poaceae (16%), *Platanus spp* (13%), *Quercus spp (*27%) e *Olea europaea* (11%) (figura 5b)).



a) **b)**

Figura 5: Calendário polínico dos principais tipos identificados na região de Évora entre os anos 2017-2020 (a). Prevalência dos tipos polínicos na região de Évora entre os anos 2017-2020 (b).

Os índices polínicos dos pólenes anteriormente referidos variaram entre os anos 20172020. O pólen de Cupressaceae apresentou índices polínicos mais elevados nos anos 2017 e 2019, 5993 e 7824 grãos de pólen/m^3, respetivamente. O mesmo aconteceu para o pólen de *Quercus*, 13367 e 10818 grãos de pólen/m^3 respetivamente. Para o pólen de *Platanus*, os índices polínicos mais elevados ocorreram nos anos de 2017 e 2020 com um total de 5677 e 7069 grãos de pólen/m^3, respetivamente e por fim para o pólen de Poaceae o índice polínico foi mais elevado nos anos de 2018 e 2020, com concentração de 8829 e 8358 grãos de pólen/m^3, respetivamente. Em termos de média de duração da época polínica, o pólen de Cupressaceae encontrase no ar durante 95 dias e o pólen de *Platanus spp*, *Quercus spp* e Poaceae encontram-se no ar durante 43,69 e 82 dias, respetivamente. Estudos já realizados, corroboram estas observações, a região de Évora apresenta valores de contagens polínicas mais elevadas que outras regiões portuguesas, como por exemplo, Porto, Lisboa, Coimbra ou Portimão (Todo-bom, Nunes, & Caeiro, 2006).

Na caraterização aerobiológica da cidade da Guarda, os tipos polínicos mais comuns são *Quercus* spp, Pinaceae e Poaceae que perfazem cerca de 50% do pólen total observado. Outras espécies são encontradas, embora em concentrações minoritárias, tais como Cupressaceae, Urticaceae, Apiaceae, Oleaceae e Polygonaceae (figura 6). São registadas concentrações elevadas de pólen durante o período entre o início da primavera e início do verão, onde os meses de abril e maio são os que apresentam um maior registo de concentração mensal (Lisboa et al., 2016).

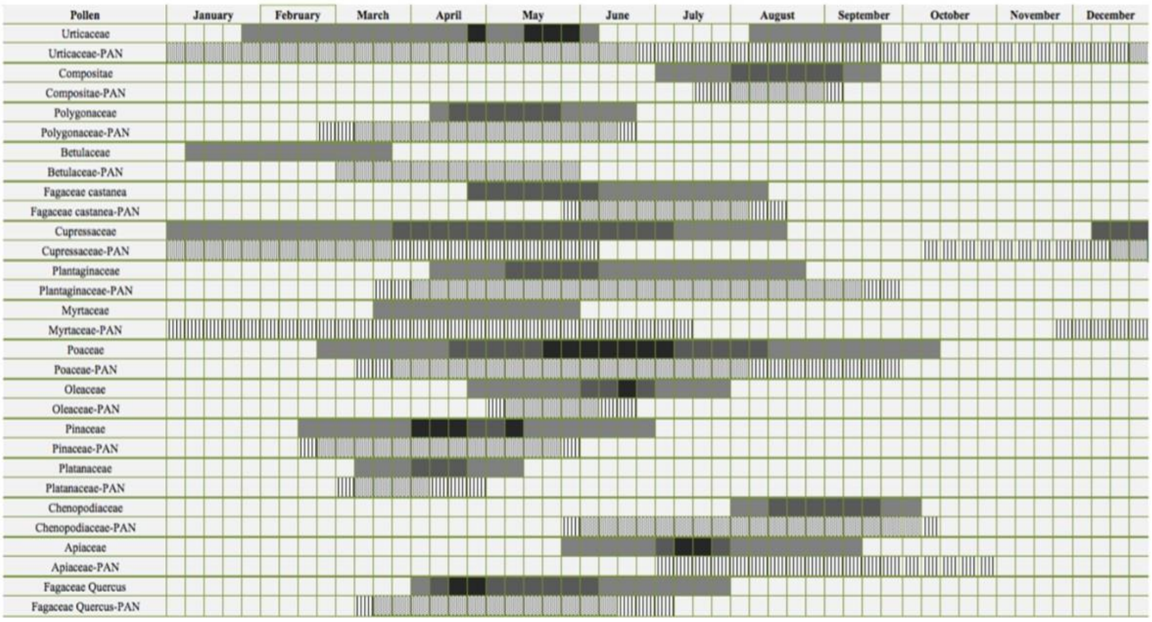


Figura 6: Distribuição dos tipos polínicos mais prevalentes no ar na cidade da Guarda, Portugal entre os anos 2013-2014. A escala de cor indica a concentração de pólen no ar (preto-maior concentração de pólen e cinzento-claro – menor concentração de pólen) (retirado de Lisboa et al., 2016).

Na região do Porto, os tipos polínicos mais prevalentes são *Acer* spp, *Fraxinus* spp, *Liquidambar*, *Pinus* pp, *Platanus* spp, *Populus* spp, *Prunus* spp, *Quercus* spp e *Tilia.* De herbáceas é mais comum encontra-se, *Plantago* spp, Poaceae, *Rumex* spp e Urticaceae. As concentrações de pólen no ar são mais elevadas nos meses da primavera (março, abril e maio) registando um total superior a 2000 grãos de pólen/m^3. Pelo contrário os meses de dezembro apresentam concentrações mais baixas. O pólen de Urticaceae e Poaceae encontra-se na atmosfera da cidade do Porto durante o todo o ano (Abreu & Ribeiro, 2005).

Para a cidade de Granada, Espanha, as espécies encontradas com maior abundância e que contribuem significativamente para o espetro polínico são *Olea* europaea com 35,5% do pólen total, Cupressaceae que representa 30% do pólen total, enquanto pólen de *Acer* spp, *Pinus* spp, *Platanus* spp, Poaceae, *Quercus* spp, Urticaceae e *Populus* spp representam, individualmente, <10 % do pólen total (Cariñanos et al., 2016).

1.4.2. Espécies alergologicamente mais relevantes nas regiões em estudo

Este capítulo carateriza as espécies comuns e mais alergénicas das regiões em estudo, dando destaca à sua distribuição ecológica, descrição da morfologia polínica e a sua constituição em alergénios. Os subcapítulos seguintes estão organizados por ordem decrescente de relevância alergológica.

1.4.2.1. Poaceae e o seu potencial alergénico

A família das Poaceae é constituída por espécies herbáceas com flor, tradicionalmente chamadas de gramíneas. A origem botânica desta família data de há 80-100 milhões de anos e atualmente existem 12000 espécies, distribuídas por 771 géneros, pertencentes a 12 subfamílias (Soreng et al., 2015). A maiorias das espécies da família das Poaceae são anuais ou bianuais com caules ocos. As folhas novas crescem diretamente da base da planta, estratégia adotada provavelmente para o combate a predadores. As Poaceae são de elevada importância ecológica, uma vez que cobrem 20% da superfície terrestre (García-Mozo, 2017). Todas as espécies desta família efetuam polinização anemófila, ou seja, pelo vento e a maioria tem anteras. Produzem grandes quantidades de pólen, classificados atualmente, como contendo alguns dos principais aeroalergénios. O pólen de Poaceae é esferoidal, monoporado. A sua exina é fina em quase todo a sua superfície, mas com elevada espessura ao redor do poro (figura 8) (García-Mozo, 2017). Este tipo polínico está presente no ar no final da primavera, início do verão e mais de 95% das espécies são relevantes do ponto de vista alergológico e pertencem a 3 sub-famílias: Pooideae, Chloridoideae e Panicoideae. No Hemisfério Norte, as espécies mais alergénicas pertencem ao género *Phleum* spp., *Dactylis* spp., *Lolium* spp., *Trisetum* spp., *Festuca* spp., *Poa* spp., *Cynodon* spp., e *Anthoxanthum* spp. (Prieto-Baena et al., 2003). Outras, devido a agricultura de regadio estão a aumentar, como é o caso do Trigo (*Triticum aestivum*), o arroz (*Orysa sativa*), e o Milho (*Zea mays*) estão a aumentar devido ao regadio (Cassman, 1999).

Em Londres, 10-50 grãos de pólen/m^3 deste tipo polínico na atmosfera era capaz de induzir o aparecimento de sintomas em indivíduos com sensibilização (Davies & Smith, 1973). Em Cardiff, 10% dos indivíduos sensibilizados a este tipo polínico apresentam sintomas quando as concentrações estão na ordem dos 10 grãos de pólen/m^3 (Hyde, 1972). Em Bilbao, Espanha, 100% dos indivíduos com polinose apresentam sintomas quando a concentração de pólen de gramíneas supera os 37 grãos de pólen/m^3 (Antepara et al., 1995). Em Turku, Finlândia, concentrações de 30 grãos de pólen/m^3 foi significativamente correlacionada com o aparecimento de sintomas na população sensibilizada (Rantio-Lehtimaki et al., 1991).

Atualmente, 13 grupos diferentes de alergénios são conhecidos por induzir resposta inflamatória, os quais, 11 pertencem à subfamília de Pooideae. Entre esses, os principais alergénios estão descritos na tabela 1.

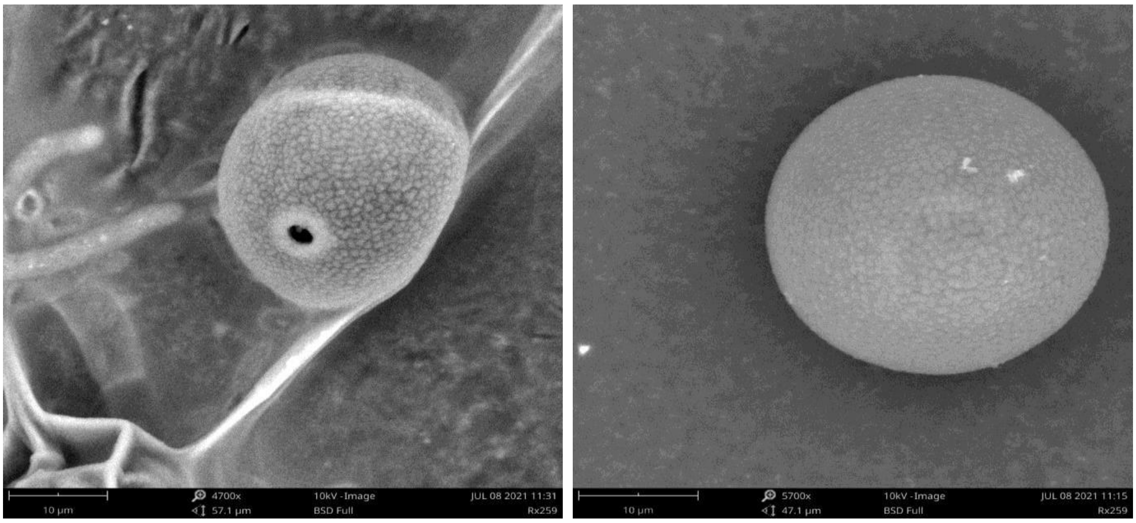


Figura 7: Fotografias em visão polar dos grãos de pólen de Poaceae em SEM a 10kV de resolução.

1.4.2.2. *Olea europeae* e o seu potencial alergénico

Olea europaea pertencem há família Oleaceae que inclui 400 espécies de arbóreas, arbustivas, lenhosas, lianoses e perenifólias. São árvores de folhas simples e opostas que podem atingir os 10 metros de altura e as suas raízes os 12 metros de profundidade. A planta apresenta uma polinização primária por insetos e uma polinização secundária pelo vento. A *Olea* adaptase bem a condições climáticas extremas e pode resistir a altas temperaturas e precipitação intensa (Minero & Candau, 1997). A distribuição de *Olea* ocorre na bacia do Mediterrâneo, espalhando-se pela Grécia, Itália, França, Espanha e Portugal (Maldonado, López & Caudullo, 2016). O pólen é esferoidal com tamanho pequeno a médio entre 16-25 µm. Numa visão polar apresenta uma forma triangular e na visão elíptica é circular sendo tricolporado, isopolar e mónada com ornamentação reticulada e exina com 3 µm de espessura (figura 9) (PalDat, 2021.; Liccardi, D´Amato M. & D´Amato G., 1996). O período de polinização da oliveira depende muito da latitude de cada região, no entanto, geralmente ocorre entre o final de abril e fins de junho com os picos de maior concentração no mês de maio (Liccardi, G.& D´Amato M., 1996). O período principal de polinização de *Olea* não é longo, pode durar cerca de 1 mês. O pólen de *Olea* apresenta um potencial alergénico importante, representando uma das principais causas de doença alérgica respiratória na região do Mediterrâneo (Berghi, 2014). O pólen de Oliveira contêm, atualmente, 15 alergénios descritos, ***Ole e 1*** a ***Ole e 15*** que se encontram apresentados na tabela 1. A taxa de sensibilização na região do mediterrâneo com áreas significativas de olival é comparável à taxa de sensibilização das gramíneas (Carnés & FernándezCaldas, 2002).

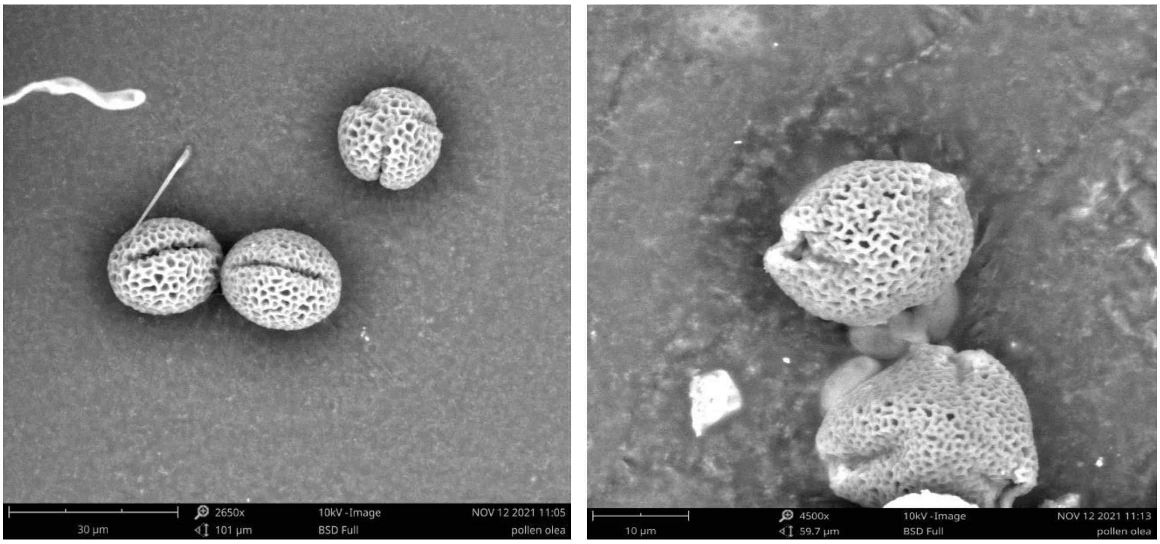


Figura 8: Fotografias em visão polar dos grãos de pólen de *Olea europeae* em SEM a 10kV de resolução.

1.4.2.3. *Platanus* spp e o seu potencial alergénico

Platanus spp*,* com nome comum plátano, pertence à família Platanaceae, são árvores caducifólias com crescimento rápido que podem atingir uma altura máxima de 35 metros. Apresentam ramas abertas que formam uma copa bastante ampla e brotos densos (Rocha Afonso, 1841). Plátanos são arvores encontradas, frequentemente, em cidades do oeste europeu (Spieksma et al., 1993; Castroviejo, 1995). A sua época de floração, na Europa, ocorre entre março e junho, dependendo do clima (Spieksma et al., 1993). São detetadas altas concentrações de pólen de *Platanus* em Espanha (Surinyach, 1974; Subiza,1980), Portugal, Grã-Bretanha, França, Suíça, Bélgica e Itália (Subiza et al., 1994). Em Portugal representa-se pela espécie *Platanus hybrida* Bro. O nome *hybrida* refere-se provavelmente ao cruzamento entre espécies euro-asiática (*Platanus orientalis*) e espécies norte-americana (*Platanus accerifolia*). São árvores plantadas em parques, jardins, praças, passeios, ruas e cursos água devido à sua elevada capacidade de tolerar ambientes poluídos. Com a sua polinização anemófila, liberta para a atmosfera enormes quantidades de pólen, com uma produção polínica estimada em $2X10^7$ grãos de pólen por inflorescência (Molina et al., 1996). O pólen de *Platanus* é um trizonocolpado, isopolar e radiosimétrico, com forma sub-triangular numa visão polar e forma sub-circular/elíptica numa observação equatorial. O seu tamanho varia entre 18-24 □m numa visão polar e diâmetro equatorial de 20-26 μm. A exina apresenta 2 μm de espessura. A superfície é reticulada com lúmenes pequenos e irregulares (figura 10). Apresenta aberturas simples, com colpos largos e curtos (figura 7) (2008 Atlas Aeropalinológico de España copia.pdf, 2021; PalDat, 2021).

As frações alergénicas deste tipo polínico possuem um tamanho entre 10-43 kDa e estão descritas na tabela 1. Normalmente indivíduos alérgicos a pólen de plátano apresentam também sensibilização a outros tipos polínicos, sendo mais frequente encontrar indivíduos polisensibilizados do que monosensibilizados. A maioria dos indivíduos polisensibilizados a *Platanus* apresentam sensibilização a gramíneas, *Olea europaea*, *Parietaria judaica*, *Artemisia vulgaris*, e Quenopodiáceas (Enrique et al., 2002; Valero et al., 1999).

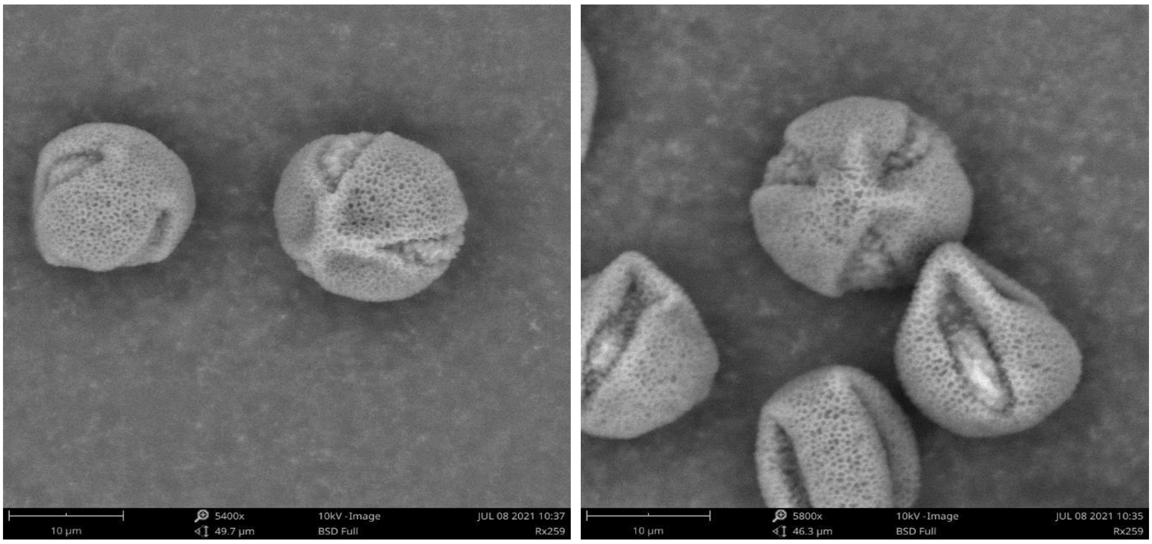


Figura 9: Fotografias em visão polar dos Grãos de pólen de *Platanus* spp em SEM a 10kV de resolução.

1.4.2.4. Cupressaceae e o seu potencial alergénico

Cupressaceae é uma família de plantas cosmopolita e a segunda família mais importante da classe das gimnospérmicas. É uma família constituída por 30 géneros e 160 espécies distribuídas por regiões temperadas e não temperadas do hemisfério norte e sul (Schulz, Knopf, & Stützel, 2005). São plantas coníferas, de porte médio, podendo atingir uma altura máxima de 30 metros. Cupressaceae é a única família de gimnospérmicas que apresenta um microsporofilo não espiral e cada microsporófilo tem na sua constituição 2-6 sacos polínicos (Christenhusz et al., 2011). Atualmente, esta família está distribuída por todos os continentes, exceto na Antártida. Na península Ibérica, as espécies do género *Juniperus* fazem parte da vegetação normal sendo considerado espécies autóctones, no entanto, espécies do género *Cupressus*, *Chamaecyparis* e *Platycladus* são consideradas alóctones, utilizadas em jardins e parques para fins ornamentais e reflorestamento. A sua principal função é atuarem como barreira de vento e ruido (San-Miguel-Ayanz et al., 2016). Em Portugal, várias espécies do género *Juniperus* e *Cupressus* estão amplamente distribuídas por todo o território nacional, sendo o género *Cryptomeria*, particularmente, as espécies *Cryptomeria japonica* bastante abundantes nos Açores (Instituto da Conservação da Natureza e das Florestas, 2016). Das espécies autóctones do género *Juniperus*, estão representadas *Juniperus communis* L, *Juniperus navicularis* Gand., *Juniperus oxycedrus* L. e *Juniperus turbinata* Guss (Flora-On | Flora de Portugal interactiva, 2021). O género *Cupressus* está representado por espécies *Cupressus sempervirens* L., *Cupressus macrocarpa* Hartweg, *Cupressus lusitanica* Miller. e *Cupressus arizonica* Greene. *Cupressus semprevirens* é encontrado sobretudo em zonas urbanas, *Cupressus macrocarpa* é encontrado na serra de Sintra, Buçaco e Gerês e por fim *Cupressus lusitanica* é encontrado em ambos os ambientes. *Cupressus arizonica* é desconhecida a sua existência em Portugal continental (Flora-On | Flora de Portugal interactiva, 2021; Jardim Botânico UTAD, 2021). A polinização é anemófila, ou seja, ocorre pelo vento entre o final do inverno e início da primavera, podendo durar mais do que um mês devido ao seu processo de maturação do microsporófilo, da base até ao topo do cone (Hidalgo, Galán, & Domínguez, 2003; Khanduri & Sharma, 2000). Os tipos polínicos não se distinguem de espécie para espécie dentro da mesma família. É um tipo polínico uniforme, subesferoidal ou esferoidal com um diâmetro na vista equatorial entre 15-36 μm. A sua superfície é microverrugada (Kurmann, 1994). É monoporado (Bortenschlager, 1990). A exina deste tipo polínico tem uma espessura de 0,3 a 0,9 μm (Kurmann, 1994) e o seu citoplasma é granular (figura 6). O seu tamanho varia entre 24-32 μm (figura 11) (Bortenschlager, 1990).

O elevado impacto epidemiológico induzido por esta família está, possivelmente, relacionado com abundância destas árvores em áreas urbanas e com a elevada produção e libertação de pólen para a atmosfera (Caeiro et al., 2020; Díaz De La Guardia et al., 2006; Polen Alert, 2021) que, por sua vez, possibilita o aumento da concentração de alergénio no ar e consequentemente o aumento do risco de exposição, tornando-se uma das principais causas de reação alérgica no inverno em países do sul (Boutin-Forzano et al.,2005; D'Amato et al., 2007; et al., 2010; Díaz De La Guardia et al., 2006; Guerra et al., 1996; Rodríguez et al., 2013). A taxa de sensibilização ao pólen de Cupressaceae é altamente variável, sendo que na população em geral encontra-se entre o intervalo de 2,4% a 9,6% e em indivíduos sensibilizados pode chegar a valores superiores a 30%, em zonas urbanas (Charpin et al., 2005). As frações alergénicas mais abundantes deste tipo polínico estão descritos na tabela 1.

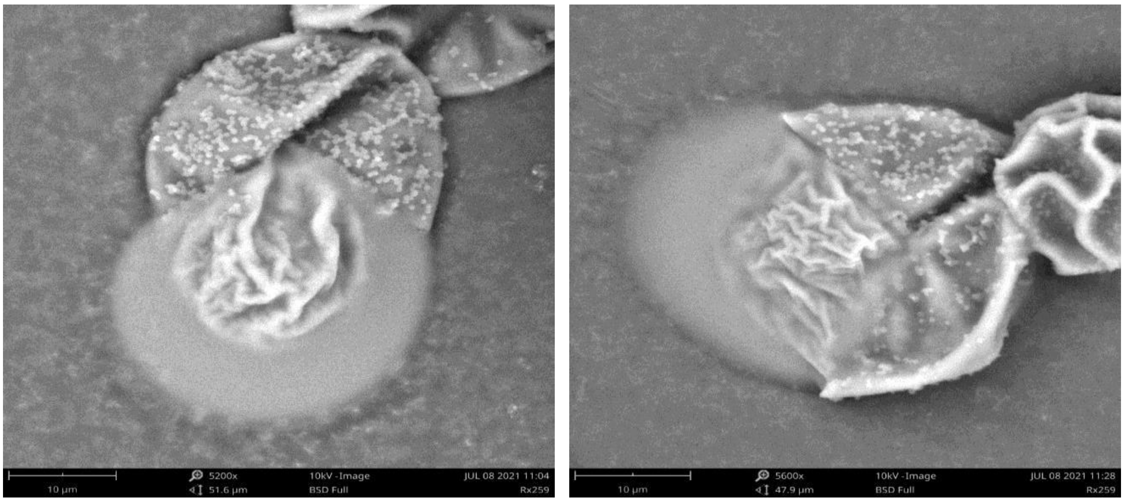


Figura 10: Fotografias em visão polar do grão de pólen de Cupressaceae em SEM a 10kV de resolução.

1.4.2.5. *Quercus* spp e o seu potencial alergénico

Quercus spp é uma árvore, ou arbusto, angiospérmica e dicotiledónia. Pertence à ordem das Fagales, família Fagáceas, género *Quercus*. As árvores apresentam uma copa arredondada, podendo alcançar os 25 m de altura. As suas flores são unissexuais, as masculinas são amentilhos pendentes com 3-8 lóbulos e 6-12 estames (Plantas y Hongos, 2021). O género *Quercus* é constituído por 600 espécies localizadas nas zonas temperadas do Hemisfério Norte e contabilizando 22 espécies na Europa e 12 espécies na Península Ibérica. São consideradas um reservatório de grande biodiversidade.

Em Espanha, *Quercus* é representado por 13 táxons, que se encontram bem distribuídos em regiões atlânticas e mediterrâneas. Por exemplo, espécies como Sobreiro (*Quercus suber* L.), azinheira (*Q.ilex* subsp. Ilex L. e *Quercus ilex*), Carvalho (*Quercus robur* L.) (Pinto et al., 2011) são bastante predominantes nas florestas de Espanha e a sua presença é sinal de maturidade biológica, uma vez que tem como principal função manter as caraterísticas do ecossistema (Jato, Rodríguez-Rajo, & Aira, 2007; Rodríguez-Rajo, Méndez, & Jato, 2005; Recio et al., 2018; Wang et al., 2019). No noroeste de Espanha, o género *Quercus* é representado por 6 espécies, no qual *Quercus robur* L. é a espécie mais prevalente (Costa, Morla, & Sainz, 1998; Rivas Martínez et al., 1987). Em Portugal, encontra-se distribuída em grande parte do território nacional, com maior predominância no interior do país (Flora-On | Flora de Portugal interactiva, 2021).

O pólen de *Quercus* é semelhante de espécie para espécie. É isopolar, radiosimétrico e trizonocolporado. Os colpos são estreitos e a intina é frequentemente espessa sob os colpos. O citoplasma é granular. Em visão polar é circular com tamanho de 22,3 μm e em visão equatorial é hexagonal com tamanho de 24,7 μm (Figura 12) (Wien, 2021). As frações alergénicas, mais frequentes, deste tipo polínico estão representadas na tabela 1.

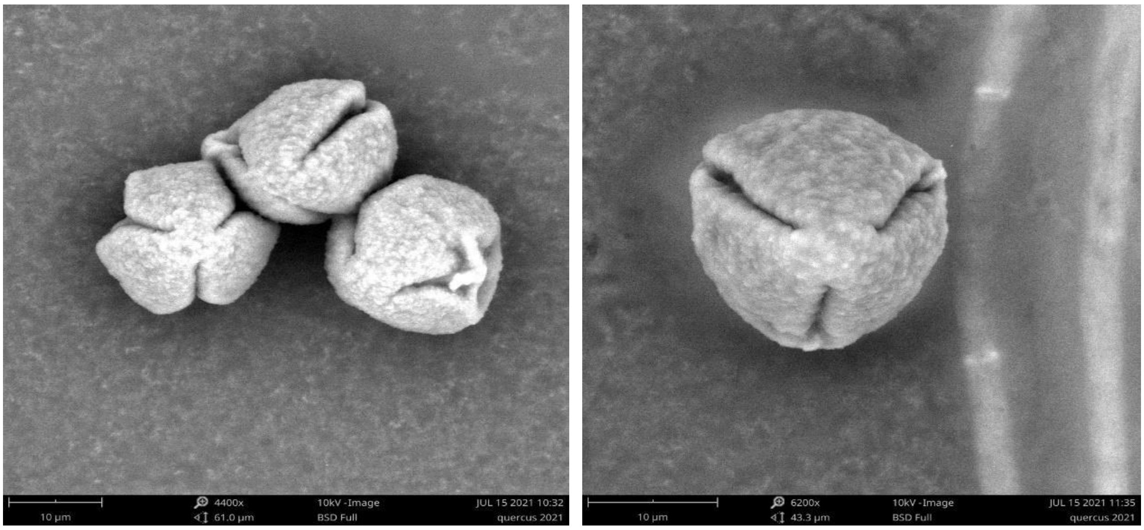


Figura 11: Fotografias em visão polar dos grãos de pólen de *Quercus* spp em SEM a 10kV de resolução.

Table 1: Caraterísticas das frações alergénicas dos tipos polínicos: Poaceae, *Olea europea*, *Platanus* spp., Cupressaceae e *Quercus* spp. MW - peso molecular; pI- ponto isoelétrico (allergome, 2021).

	Alergénio	MW (kDa)	pI	Função biológica	Observações
Poaceae	*Dac g 1*	32	6	Expansiva	
	Dac g 2	10	-	-	
	Dac g 3	14	9	-	
	Dac g 4	60	10.4	Enzimas ponte beberina	
	Dac g 5	26	-	Ribonuclease	
	Dac g 7	9	-	Pocalcina/Ca^{2+}BP	
	Dac g 12	14	-	Profilina	
	Dac g 15	60	-	Poligalacturonase	
Olea europea	*Ole e 1*	(20 kDa*) (18.5 kDa**)	-		*Forma glicosilada **Não glicosilada Alergénio major
	Ole e 2	15	-	Profilina com função de ligação à actina	-
	Ole e 3	9		Pocalcina (proteína de ligação ao cálcio)	-
	Ole e 4	32		Gluconase	-
	Ole e 5	16		Superóxido dismutase	-
	Ole e 6	10	-	-	-
	Ole e 7	9.5	-	Proteína de transferência lipidica (LPT)	-
	Ole e 8	21	-	Pocalcina	-
	Ole e 9	46	-	Glucanase	Proteína de resposta a patógenos
	Ole e 10	10.8	-	Glicosil-hidrolase	Reatividade cruzada com diversos tipos polinicos
	Ole e 11	37.4	-	Pectinesterase	-
	Ole e 12	37	-	Reductase de isoflavone	-
	Ole e 13	23	-	Taumatina	-
	Ole e 14	46.5	-	Poligalacturonase	-
	Ole e 15	19	-	Ciclofilina	-
***Platanus* spp.**	*Pla a 1*	18	-	Proteína glicosilada inibidora da invertase	Alergénio maioritário aparecendo em 92% dos individuos polisensibilizados e 83% dos individuos monisensibilizados
	Pla a 2	43	-	Poligalacturonase	84% dos individuos polisensibilizados
	Pla a 3	10	-	Proteína transportadora de lipídos (LTP)	Presente em individuos com alergia alimentar
	Pla a 8	-	-	Profilina	Panalergénios, apresentam reatividade cruzada com alimentos de origem vegetal
	Pla a TLP	-	-	Taumatina	.
Cupressaceae	*Cup a 1*	43	-	Pectato liase	.
	Cup a 2	-	-	Poligalacturonase	.
	Cup a 3	-	-	Taumatina like	.
	Cup a 4	-	-	Pocalcina	.
***Quercus* spp.**	*Que a 1*	17		Proteína de resposta a patogenos	-
	Que a 2	14	-	-	-
	Que a 4	9		Proteínas ligantes de cálcio	-
	Que ru1	-	-	Proteína de resposta a patogenos	-

A prevalência de sensibilização alérgica, na população em geral, está relacionada com diversos fatores. Em cada região, a sensibilização descrita reflete o nível de risco de exposição aos alergénios polínicos. Um estudo da sensibilização alérgica, realizado em Portugal e Espanha relatou que num grupo de 3384 indivíduos alérgicos (2155 identificados em Espanha e 1273 identificados em Portugal) foi observado para Espanha, 55% de sensibilização a pólen de gramíneas, 44 % de sensibilização em pólen de *Olea*, 24 % de sensibilização em pólen de Cupressaceae e 24 % em pólen de *Platanus*. Para Portugal, foi identificado 49% de sensibilização para o pólen de gramíneas, 20 % para o pólen de *Olea*, 10 % para o pólen de Cupressaceae e 15% para o pólen de *Platanus*. Quando divido por regiões, em Espanha, na região sul, ocorreu maior sensibilização alérgica nos tipos polínicos de *Olea* e gramíneas, 63% e 53% respetivamente. Em Portugal, observou-se que o pólen de gramíneas obteve maior sensibilização alérgica na região norte (53%) comparativamente à região centro e sul com 51 e 41% respetivamente (Pereira et al., 2006). *Olea europaea*, *Platanus acerifólia* e *Cupressus arizonica* mostraram o mesmo padrão de sensibilização nos dois países. Por outro lado, o pólen de Cupressaceae tem vindo a apresentar um aumento da percentagem de sensibilização de há uns anos a esta parte na Península Ibérica, o que está de acordo com o observado em outros países do sul da Europa (D'Amato et al., 1998; Pereira et al., 2006).

1.5. Influência dos parâmetros atmosféricos físicos e químicos no pólen aerotransportado

1.5.1. Fatores físicos

O pólen atmosférico quando disperso no ar pode ser influenciado por parâmetros físicos, os parâmetros meteorológicos de temperatura, precipitação, humidade relativa e vento, ou por inúmeros gases e/ou partículas de diferentes origens, antropogénica ou natural, que provocam modificações no pólen aerotransportado (Linskens & Cresti, 2000). Essas modificações podem alterar a alergenicidade do pólen, pois podem induzir uma maior exposição ou disseminação dos alergénios, podem induzir modificações químicas nas proteínas polínicas, alterando o seu reconhecimento imunogénico, e podem ainda modificar a produção dos alergénios polínicos (Taylor et al., 2004; Taylor & Jonsson, 2004). As partículas químicas e orgânicas também podem ficar adsorvidas na superfície dos grãos de pólen e, chegando às vias respiratórias dos seres humanos, contribuir para o aumento das manifestações alérgicas (Visez et al., 2020).

Os parâmetros meteorológicos são fatores importantes na fisiologia da planta e, consequentemente, na dinâmica do pólen (Linkens & Cresti, 2000). Como já referido anteriormente, os parâmetros meteorológicos podem afetar as concentrações de pólen no ar, a duração da época principal de polinização, a morfologia polínica e a alergenicidade. Embora as caraterísticas reprodutivas sejam mais estáveis do ponto de vista genético do que as caraterísticas vegetativas, as caraterísticas morfológicas do pólen, tais como o diâmetro, forma, volume, número de poros e ornamentação da exina podem ser influenciados pelas condições climatéricas (Stanley e Linkens, 1921). De um modo geral, a temperatura tem um papel importante na maturação e floração, sendo o fator que mais influencia o crescimento e desenvolvimento das plantas e consequentemente a produção polínica (Laaidi, 2001). A temperatura tem sido também associada à formação de grãos de pólen de tamanho elevado, no entanto, sucessivos arrefecimentos durante a microsporogénese, influencia a formação de pólen com um número variável de poros. Experiências em ambiente controlado em alguns tipos polínicos mostraram que o diâmetro do pólen é mais influenciado por variações nas temperaturas noturnas. Não observaram alterações na estrutura da exina, mas o diâmetro do pólen diminui em temperaturas noturnas > 20°C e <10 °C e em temperaturas diurnas > 30°C (Stanley e Linkens, 1921).

A baixa humidade relativa favorece o processo de antese e consequentemente a deiscência das anteras, controlando o processo de floração e emissão polínica (Linskens & Cresti, 2000). As condições de água são importantes nos estágios iniciais do desenvolvimento do pólen, no entanto, stress hídrico está associado à formação de grãos de pólen não viáveis (Stanley e Linkens, 1921).

Além desta ação das condições climatéricas na fenologia das plantas e na produção polínica, há efeitos dos parâmetros atmosféricos no pólen após a sua emissão, e são sobretudo esses que nos interessa discutir neste capítulo.

A precipitação tem um efeito de lixiviação da atmosfera, contribuindo para a redução do pólen no ar, induzindo a sua deposição (Fernández-Rodríguez et al., 2014 (b); Fernández-Rodríguez et al., 2014; Pérez, Gassmann, & Covi, 2009).

As mudanças meteorológicas podem causar a perda da integridade do pólen, alterando a sua estrutura. De facto, a exacerbação da asma tem sido associada a eventos climatéricos como tempestades/trovoadas. Packe e Aynes (1985) descreverem a ocorrência de um surto de asma coincidente com a ocorrência de tempestades na Grã-Bretanha em 26 indivíduos asmáticos. Outros surtos foram relatados em Melbourne, Austrália (Taylor & Jonsson, 2004; Thien et al., 2018). Em Londres, 100 indivíduos deram entrada nas urgências hospitalares, a maioria não apresentava diagnóstico de asma brônquica, no entanto, foram afetados por rinite sazonal que evoluiu para asma (Thien et al., 2018). Assim, é da maior importância conhecer de que forma as alterações meteorológicas condicionam a ocorrência destes fenómenos, bem como compreender as alterações ocorridas no pólen. Uma das hipóteses que tem sido levantada é a disrupção do pólen, sendo que Taylor et al. (2002) propõe que ciclos de humedecimento e secagem, seguido de perturbação por vento, provoquem rutura do pólen e originem partículas com tamanho de 0,12 a 4,67 µm que podem conter os alergénios. Nesse sentido, observou-se que o alergénio do pólen de oliveira *Ole e 1* foi detetado não apenas na fração do ar associada ao pólen (PM>10 µm) mas também na fração inferior 10<PM<2,5 µm (Gálan et al., 2013), evidenciando que os alergénios podem ser encontrados na atmosfera e que, sendo de menor dimensão, têm um maior potencial de dano ao sistema respiratório (Gálan et al., 2013; Buters et al., 2012; Buters et al., 2015).

Os fatores ambientais também podem influenciar a alergenicidade do pólen. No pólen de Bétula de montanha (*Betula pubescens*), verificou-se que a quantidade de alergénio de Bet v 1 aumentava em arvores cultivadas em regiões com temperaturas mais elevadas (D'Amato & Cecchi, 2008). Hjelmroos et al. (1995), observou diferenças na morfologia das proteínas polínicas de árvores localizadas em regiões onde a temperatura era mais alta (Hjelmroos, Schumacher, & Van Hage-Hamsten, 1995).

Para além dos parâmetros meteorológicos, os poluentes atmosféricos podem ter implicações nefastas no pólen, com alterações de estrutura, metabolismo, conteúdo proteico e modificação estrutural de proteínas alergénicas.

1.5.2. Fatores químicos

A poluição atmosférica tornou-se uma grande ameaça a nível global, afetando a qualidade do ar, principalmente em zonas urbanas (Davis, Bell, & Fletcher, 2002). Influenciada pela ação de fatores meteorológicos, temperatura, humidade relativa e luz ultravioleta (Huff, Carlsten, & Hirota, 2019) entende-se por ser uma *alteração da composição química natural do ambiente com propriedades físicas e químicas complexas*, de origem antropogénica (tráfego rodoviário, tráfego aéreo, construções, trabalho agrícolas, incêndios, entre outros) ou de origem natural (erupções vulcânicas, fogos florestais espontâneos, bioaerossóis) (Curtis et al., 2006; Koenig, 1988). Quimicamente estes poluentes podem ser apresentados sobre forma de vapores inorgânicos, como ozono (O_3), monóxido de carbono (CO), dióxido de nitrogénio (NO_2) e dióxido de enxofre (SO_2) ou como poluentes orgânicos, como é o caso dos hidrocarbonetos policíclicos aromáticos (PAH´s), hidrocarbonetos monocíclicos de benzeno, tolueno, xileno e produtos químicos alifáticos e a matéria particulada (PM) (Li et al., 2016) (Bloemsma, Hoek, & Smit, 2016). Na sua caraterização podem ser representados como poluentes primários, aqueles que são emitidos diretamente para a atmosfera, tal como, o SO_2, o NO_2, o CO e a PM, ou poluentes secundários que se formam no ar, resultam de reações químicas entre outros poluentes e gases, incluindo o O_3, óxidos de azoto (NOx) e outras partículas. A composição do ar é dinâmica e complexa mudando espacialmente (Chowdhury et al., 2018) e temporalmente (Becker et al., 2005). Atualmente, em áreas urbanas com elevado tráfego automóvel são detetadas elevadas concentrações de O_3, NO_2 e PM de origem orgânica e biológica (D'Amato et al., 2001).

O **O_3** é o um poderoso oxidante, incolor e o principal componente de oxidação fotoquímica, respondendo a 90% dos níveis totais de oxidantes nas cidades urbanas. O O_3 é gerado ao nível troposférico por reações fotoquímicas entre óxidos de azoto e compostos orgânicos voláteis emitidos pelo tráfego e/ou fontes industriais, na presença de luz solar e temperaturas elevadas (U.S. Environmental Protection Agency | US EPA, 2021). Devido ao seu potencial de sensibilizar a inflamação das vias aéreas ou danificar os tecidos pulmonares, pode causar muitos problemas respiratórios, incluindo tosse, respiração ofegante e dor no peito (Szyszkowicz et al., 2012;

Trasande & Thurston, 2005; Xu et al., 2011). Também pode aumentar a resposta imunológica a alergénios (Auten & Foster, 2011). A exposição das células epiteliais das vias aéreas humanas ao O_3 causam o aumento da libertação de citocinas pro-inflamatórias, como IL-8, IL-6, GM-CSF, potenciando o agravamento da resposta imunológica, em indivíduos asmáticos (Auten & Foster, 2011; Bell et al., 2004; Bayram et al., 2001). Aproximadamente 40–60% do O_3 inalado é absorvido pelas vias aéreas nasais, enquanto o restante pode atingir as vias aéreas inferiores (Bosson et al., 2007). As partículas de O_3 podem viajar milhares de quilómetros e perduram na atmosfera por mais tempo durante o inverno (1-2 meses) do que no verão (1-2 semanas) (Curtis et al., 2006). Para o O_3, o valor limiar para a proteção da saúde humana na Europa é de 120 $\mu g/m^3$, que não pode exceder mais de 25 dias, em média, por ano civil, num período de 3 anos (UE, 2008).

NO_2 é um percursor fotoquímico que pode ser encontrado no ar interior ou externo (Berhane et al., 2011; Bevelander et al., 2007; Latza, Gerdes, & Baur, 2009; Shima & Adachi, 2000). É um gás com uma cor castanho-claro originário do tráfego automóvel e indústria. Este gás pode reagir na atmosfera sob forma de ácido nítrico corrosivo, de nitratos orgânicos tóxicos ou como acidificante (Sabo, Popović, & Dordević, 2015). Em associação com hidrocarbonetos provenientes do tráfego automóvel resulta na produção de O_3. A exposição a NO_2 pode induzir alterações crónicas e agudas da função pulmonar, incluindo infiltração neutrófila brônquica, produção de citocinas pro-inflamatórias e resposta a alergénios inalados por indivíduos com asma (Delfino et al., 2006; Everard, 2006; Koening, 2015). A inalação de NO_2 é prejudicial para o pulmão, pois, por um lado, pode aumentar o grau de inflamação das vias aéreas e por outro, prolongar a resposta das vias aéreas induzida por alergénios (Poynter et al., 2006). O limiar de concentração de NO_2 para a proteção da saúde humana na Europa por ano civil é de 40 $\mu g /m^3$, não podendo exceder este valor 18x por ano civil (UE, 2008).

PM são uma mistura orgânica e inorgânica de partículas sólidas, líquidas ou multifase de diferentes origens, tamanhos e composição química. Podem ser emitidas de diversas fontes naturais ou antropogénicas, como por exemplo, chaminés de fábricas, motores a diesel, bem como da construção, mineração e atividades agrícolas (Chen et al., 2008; Rosenlund et al., 2009; Son, Bell, & Lee, 2011). Geralmente, PM é classificado de acordo com o seu diâmetro aerodinâmico, que vai desde nanometros até dezenas de micrómetros. As partículas com um diâmetro inferior a 10 μm são classificadas PM_{10} e definidas como poeiras grossas (Byeon et al., 2015). O PM_{10} pode atingir apenas o trato respiratório superior e causar ou agravar episódios de bronquite ou outras doenças respiratórias, reduzindo a capacidade do organismo de combater possíveis infeções. As partículas com um diâmetro inferior a 2,5 μm, $PM_{2,5}$, são definidas como partículas finas. Devido ao seu pequeno tamanho, podem penetrar profundamente no sistema respiratório inferior atingindo o trato-bronquial aumentando, possivelmente, o desenvolvimento de doenças de caráter respiratório e cardiovascular. As partículas menores, $PM_{0,1}$, são chamadas ultrafinas, têm diâmetros inferiores a 0,1 μm. Este tipo de partículas para além de afetar o trato alveolar, podem chegar ao sistema circulatório e provocar danos nos órgãos internos (Baldacci et al., 2015). O valor limite de PM_{10}, num dia, é de 50 µg/m^3, e num ano é de 40 µg/m^3. Para $PM_{2,5}$ os limiares numa média anual, são 25 µg/m^3. Para ambas as PM os valores limite não podem exceder mais de 35 vezes em cada ano civil (UE, 2008).

DEP´s, em zonas com elevado tráfego automóvel, são responsáveis pela maior parte das PM aerotransportadas (~90%) na atmosfera (Diaz-Sanchez et al., 1996; Riedl & Diaz-Sanchez, 2005; Takafuji et al., 1987; Takenaka et al., 1995; Sydbom et al., 2001; Nauss, 1995). É caraterizada por um núcleo carbonáceo, no qual, 18.000 compostos orgânicos diferentes de alto peso molecular são adsorvidos. Têm origem no tráfego automóvel e embora os motores a diesel libertem menos dióxido de carbono que os motores a gasolina, libertam 10 vezes mais dióxido de nitrogénio, aldeídos e PM respirável que os motores a gasolina e 100 vezes mais que os motores com conversores catalíticos (Nauss, 1995).

Diversos estudos relatam que a exposição a partículas de O_3, NO_2, SO_2 e PM têm efeitos adversos na mucosa respiratória e na resposta das vias aéreas (Duki et al., 2003; Zhao et al., 2020). Uma das consequências da elevada exposição a partículas poluentes em zonas urbanas é o comprometimento da função de proteção da mucosa respiratória e indução de mediadores inflamatórios (Huff et al., 2019). As mucinas segregadas são afetadas pela exposição a partículas poluentes, observando-se um aumento dos níveis de proteínas MUC5B e MUC16 em pacientes atópicos após a exposição a níveis de 300 mg/m^3 de $PM_{2,5}$ (Mookherjee et al., 2018). O aumento da exposição a partículas urbanas correlacionou-se positivamente com o aumento da expressão do gene MUC5AC (Xu et al., 2018).

Nos últimos anos, muitas pesquisas etiológicas foram realizadas com o objetivo de determinar as causas do aumento da alergia respiratória em zonas urbanas, e significativos avanços ocorreram para perceber o efeito da poluição do ar na saúde humana. Atualmente sabe-se que a poluição do ar aumenta a resposta das vias aéreas respiratórias, intensificando essa resposta quando existe exposição a alergénios em indivíduos suscetíveis. De facto, indivíduos que habitam em áreas urbanas de países industrializados, tendem a ser mais afetados por doença alérgica respiratória comparativamente aqueles que habitam em zonas rurais (Riedler et al., 2000).

Alguns episódios de exacerbação de asma relacionados com a poluição atmosférica são devidos a condições meteorológicas, como por exemplo, tempetura, velocidade do vento, humidade relativa e trovoadas, que favorecem a acumulação destas partículas ao nível troposférico (Cecchi et al., 2010; Viegi et al., 2006).

O aumento da doença alérgica respiratória em zonas urbanas pode, por um lado, ser devido à existência de interação entre os componentes de poluição e o pólen (Shafighi, 2003), ou então, as partículas adsorvidas ao pólen podem refletir a composição química geral da fração PM de aerossol e ter um efeito inflamatório na mucosa respiratória, aumentando a permeabilidade da sua membrana, facilitando a penetração do alergénio polínico, intensificando a sua ação e atuando com células do sistema imunitário, desencadeando a resposta alérgica e aumentando a resposta brônquica (Yoshizaki et al., 2010; D'Amato et al., 2005). Estudos experimentais demonstraram que DEP´s, causam problemas respiratórios em indivíduos predispostos a desenvolver doença alérgica respiratória, exercendo um efeito imunológico adjuvante na síntese de IgE, influenciando a sensibilização a alergénios aerotransportados (Diaz-Sanchez et al., 1996; Riedl & Diaz-Sanchez, 2005; Takafuji et al., 1987; Takenaka et al., 1995).

O pólen é um acumulador de todos os tipos de partículas poluentes, sejam elas, orgânicas, inorgânicas ou gasosas, uma vez que, apresenta caraterísticas de ornamentação da exina específicas e lipofilicidade que permite esta agregação (Garrec, 2006). A própria acumulação na superfície do pólen depende de processos físicos e químicos. Como consequência da exposição dos grãos de pólen a poluentes atmosféricos pode existir fragilidade da sua parede celular, alteração da forma, do tectum, da superfície externa e por vezes da cor. Como consequência da fragilidade da sua parede celular são formadas numerosas fissuras, possibilitando a libertação do seu conteúdo citoplasmático para o exterior (Chehregani et al., 2004). Os grânulos citoplasmáticos que se libertam podem provocar o aumento da ocorrência de reação alérgica, como já foi referido anteriormente. Estas alterações na estrutura morfológica do pólen já foram observadas em pólen de *Dactylis glomerata*, *Betula verrucosa e Chenopodium album* (Visez et al., 2020). Em pólen de Cupressaceae foi observado partículas iónicas de NO_3^-, SO_4^- e NH_4^+ adsorvidas à sua superfície (Wang et al., 2009).

A função reprodutiva do pólen também pode ficar comprometida. Na interação com poluentes atmosféricos pode existir alteração da sua viabilidade e germinação, prejudicando o crescimento do tubo polínico através da oxidação de biomoléculas, como proteínas, lípidos e ácidos nucleicos (Gottardini et al., 2004; Iriti & Faoro, 2008). Embora pouco significativo, observou-se que pólen de *Pinus sylvestris* e *Pinus nigra* colhido em zonas de elevado tráfego perde a sua capacidade germinativa, com tamanho de tubos polínicos mais curtos quando comparado com zonas sem tráfego (Gottardini et al., 2004). Em espécies como *Hedera helix L., Convolvulus sepium L., Cynodon dactylon (L.) Pers., Quercus ilex L., Dactylis glomerata L.*, *Daucus carota L*. e *Tilia cordata Miller* observou-se que a capacidade germinativa foi inversamente proporcional à poluição atmosférica. Pelo contrário, o pólen de *Parietaria difusa* aumenta a sua percentagem de germinação em zonas de elevado tráfego, adaptando-se bem a este tipo de condição ambiental (Iannotti et al., 2000).

A avaliação de modificações da exposição a poluentes no conteúdo proteico solúvel e nos alergénios dos grãos de pólen tem sido também alvo de estudo. A exposição dos grãos de pólen a componentes de poluição atmosférica pode causar a modificação pós-traducional de proteínas, promovendo a sua nitração, originando o aumento ou diminuição da sua alergenicidade (Franze et al., 2005; Gruijthuijsen et al., 2006). A função enzimática e equilíbrio redox está, igualmente, comprometido (Pasqualini et al., 2011), bem como, danos oxidativos na componente lipídica, proteica e de ácidos nucleicos (Iriti & Faoro, 2008). Em pólen de *Platanus acerifólia* quando exposto a O_3 ocorre a inibição da sua função proteica. Os aminoácidos de tirosina são oxidados, existindo a desnaturação de proteínas provocam modificações na sua estrutura secundária e terciária (Ribeiro et al., 2017; Cataldo, 2003).

Sendo os alergénios polínicos proteínas, também eles podem ser modificados pela presença de poluentes atmosféricos, embora ainda não se conheça bem todos os mecanismos moleculares envolvidos, curiosamente, cerca de ¼ dos alergénios de diferentes famílias polínicas são proteínas de resposta a stress, por fatores bióticos ou abióticos, como é exemplo a proteína superóxido dismutase (Conti et al., 2014). A nitração de alergénios também foi relatada aquando da exposição a NO_2 e O_3 (Gruijthuijsen et al., 2006; Karle et al., 2012). Em alergénios de *Betula pendula*, *Bet v 1*, é nitrado na presença de NO_2 e O_3, mas o grau de nitração é substancialmente menor quando alergénio é exposto apenas a NO_2, o que provavelmente indica que as espécies reativas formadas da interação destes dois poluentes podem ter um papel importante no processo de nitração. A nitração em *Bet v 1*, resulta na proliferação de linhas celulares T – específico de *Bet v 1*, e a ligação de IgE ao alergénio é maior em *Bet v 1* nitrado do que em não nitrado (Gruijthuijsen et al., 2006; Karle et al., 2012).

Em pólen de *Platanus acerifolia*, existiu uma maior reatividade de *Pla a 1* e *Pla a 2* para pólen exposto a NO_2 e O_3, mostrando que existiu uma modificação dos alergénios *major* desta espécie (Ribeiro et al., 2017). A perda de alergenicidade por parte de alguns tipos polínicos pela acidificação de alergénios também é relatado, existe alteração da sua estrutura, possibilitando a perda do seu reconhecimento por parte da IgE em indivíduos alérgicos (Rogerieux et al., 2007). O alergénio *Cup a 3* é expresso em pólen proveniente de zonas de elevado tráfego automóvel, no entanto, quando obtido de locais com baixos níveis de poluição, não existe expressão deste alergénio (Suárez-Cervera et al., 2008).

A absorção de partículas químicas no pólen, pode altera metabolismo, estimulando ou inibindo a síntese de aminoácidos (serina, alanina, glicina, treonina) (Mumford et al., 1972). Um dos mecanismos de defesa já observado é a biossíntese de flavonoides e de outras enzimas antioxidantes (Rezanejad, 2009).

2. *Objetivos*

O objetivo geral desta dissertação foi estudar a disseminação, distribuição, transporte e alterações bioquímicas resultantes da interação com os parâmetros atmosféricos e com poluentes atmosféricos de alguns tipos polínicos alergizantes nas regiões em estudo. Este objetivo geral dividiu-se em 3 objetivos específicos:

1. Identificação e elaboração de uma base de dados organizada e de consulta fácil e de acesso livre sobre as estações de monitorização por todo o mundo, contribuindo para promover a sua divulgação pública; os resultados são apresentados no Capítulo I- Monitorização ambiental de pólen atmosférico;

2. Estudo da influência dos parâmetros meteorológicos na disseminação, distribuição do pólen atmosférico e no seu potencial alergénico; neste âmbito foram efetuados 3 estudos cujos resultados são apresentados no capítulo II desta tese (Capítulo II- Influência dos parâmetros meteorológicos na época polínica: disseminação e potencial alergénico):

    2.1. Estudo do efeito dos parâmetros meteorológicos sobre a integridade do pólen de Cupressaceae e a influência destes sobre a libertação de alergénios para o meio ambiente; Este estudo foi efetuado na região de Évora, sul de Portugal, nos anos de 2017 e 2018, e procurou-se identificar os parâmetros meteorológicos responsáveis pelo fenómeno de disrupção polínica;

    2.2. Estudo da influência dos parâmetros meteorológicos na concentração de pólen de *Platanu s* no ar e a relação entre as concentrações atmosféricas de pólen e do alergénio *major Pla a 1*; Este estudo foi efetuado na região de Évora no ano de 2018, e procurou-se identificar a relação entre o alergénio *major Pla a 1* e as concentrações de pólen no ar, bem como identificar os parâmetros meteorológicos que afetam as concentrações de pólen no ar deste tipo polínico;

    2.3. Estudo da influência dos parâmetros meteorológicos nas concentrações de pólen de Cupressaceae no ar na época polínica principal; este estudo foi efetuado em duas regiões da Península Ibérica, Évora e Granada, nos anos de 2017-2019; Neste estudo procurou-se identificar os parâmetros meteorológicos determinantes da intensidade e duração da época polínica principal;

3. Estudo do efeito dos poluentes atmosféricos (gasosos e matéria particulada) na alergenicidade do pólen; os resultados estão apresentados no Capítulo III desta tese (Capítulo III: Efeito dos poluentes atmosféricos gasosos e matéria particulada na alergenicidade do pólen); neste âmbito:

    3.1. foram investigados os efeitos dos poluentes atmosféricos, O_3 e NO_2 isolados ou em combinação na capacidade germinativa do pólen e no seu conteúdo em alergénios; este trabalho foi efetuado em condições controladas, utilizando pólen Poaceae incubado em câmara ambiente controlado;

    3.2. foi efetuada uma caracterização física e química da matéria particulada adsorvida à parede do pólen de *Quercus* spp envolvendo 3 pontos de colheita no território nacional (Porto, Guarda e Évora); analisou-se também a influência dos parâmetros meteorológicos e as trajetórias de massa de ar no tipo e composição química da matéria particulada adsorvida ao pólen.

3. *Resultados*

3.1. Capítulo I- Monitorização ambiental

A conscientização pública sobre a elevada importância das alergias e de outras doenças de caráter respiratório levaram ao aumento do esforço científico para monitorizar com precisão e rapidez a qualidade do ar, com o intuito de prever as concentrações de pólen e de outros tipos de bioaerossóis na atmosfera. Os dados polínicos e outros bioaerossóis são variáveis de região para região de acordo com a geografia e o clima (Elvira-Rendueles et al., 2019). Embora, sejam úteis e frequentemente utilizados por especialistas de diversas áreas, designadamente pelos indivíduos que sofrem de polinose e por clínicos de várias especialidades, em particular imunoalergologistas, como indicador complementar no diagnóstico e no controlo da doença alérgica respiratória, na maioria dos países, ainda não existe legislação que obrigue a efetuar esta monitorização, sendo que muitas redes são académicas ou privadas (Pollen forecast - Met Office, 2021; REA, 2020), como é o caso de Portugal, apresentando, por isso dificuldades de sustentabilidade (Polen Alert, 2021; RPA - Rede Portuguesa de Aerobiologia, 2021). Assim, a obtenção de dados requer, por um lado, o conhecimento das redes que existem, e, por outro, a criação de condições para a construção e manutenção de estações de monitorização que avaliem a qualidade do ar das suas regiões.

Neste sentido, a primeira etapa deste trabalho teve como objetivo o inventário das estações de monitorização, a nível mundial, e a criação de um instrumento simples e de acesso livre que permitisse a divulgação da localização, características funcionais (ex., o tipo de captador utilizado, tipo de partículas monitorizadas, a resolução temporal da amostragem) bem como os contactos dos responsáveis das mesmas. Neste âmbito a criação de uma base de dados e de um mapa da distribuição das estações de monitorização por todo o mundo foi um avanço importante. O artigo científico *Pollen and spore monitoring in the world* (*Pollen Map*) publicado no *Clinical and Translational Allergy*, apresenta os resultados deste trabalho deste trabalho, desenvolvido no âmbito da Academia Europeia de Alergia e Imunologia Clínica (*European Academy of Allergy and Clinical Immunology - EAACI*), tendo sido financiado pela Task-Force 40108. A criação deste mapa permitiu fomentar a cooperação internacional possibilitando a construção de uma rede de contactos, com potencial para potenciar a troca de dados e realização de projetos e trabalhos científicos colaborativos diversos bem como contribuir para a divulgação e visibilidade das redes existentes.

O meu contributo para esta publicação foi contactar as redes de monitorização a nível mundial e compilar toda a informação e efetuar pesquisa bibliográfica relevante. As informações foram obtidas, na maioria, por e-mail, permitiram criar uma base de dados com as informações requeridas através de um questionário com os seguintes tópicos para preenchimento: i) Tipo de partículas são monitorizadas; ii) Início da monitorização; iii) localização (coordenadas GPS); iv) tipo e marca do captador; v) disponibilidade de dados e de controlo da qualidade; vi) dados de contacto do responsável da estação. O preenchimento conduziu à criação de um banco de dados (dados.csv), servindo de entrada para o mapa programado a partir do software R, permitindo a atualização fácil e regular dos dados e a sua divulgação no mapa interativo publicado em (Buters et al., 2018 and https://www.zaum-online.de/pollen/pollen-monitoring-map-of-theworld.html).

Buters *et al. Clin Transl Allergy (2018) 8:9*
https://doi.org/10.1186/s13601-018-0197-8

Clinical and Translational Allergy

BRIEF COMMUNICATION

Open Access

Pollen and spore monitoring in the world

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Abstract

Background: Ambient air quality monitoring is a governmental duty that is widely carried out in order to detect non-biological ("chemical") components in ambient air, such as particles of < 10 µm (PM_{10}, $PM_{2.5}$), ozone, sulphur dioxide, and nitrogen oxides. These monitoring networks are publicly funded and air quality data are open to the public. The situation for biological particles that have detrimental effects on health, as is the case of pollen and fungal spores, is however very different. Most pollen and spore monitoring networks are not publicly funded and data are not freely available. The information regarding which biological particle is being monitored, where and by whom, is consequently often not known, even by aerobiologists themselves. This is a considerable problem, as local pollen data are an important tool for the prevention of allergic symptoms.

Objective: The aim of this study was to review pollen monitoring stations throughout the world and to create an interactive visualization of their distribution.

Methods: The method employed to collect information was based on: (a) a review of the recent and historical bibliography related to pollen and fungal spore monitoring, and (b) personal surveys of the managers of national and regional monitoring networks. The interactive application was developed using the R programming language.

Results: We have created an inventory of the active pollen and spore monitoring stations in the world. There are at least 879 active pollen monitoring stations in the world, most of which are in Europe (> 500). The prevalent monitoring method is based on the Hirst principle (> 600 stations). The inventory is visualised as an interactive and on-line map. It can be searched, its appearance can be adjusted to the users' needs and it is updated regularly, as new stations or changes to those that already exist can be submitted online.

Conclusions: The map shows the current situation of pollen and spore monitoring and facilitates collaboration among those individuals who are interested in pollen and spore counts. It might also help to improve the monitoring of biological particles up to the current level employed for non-biological components.

Keywords: Allergy, Pollen, Moulds, Spores, Monitoring, Trap, Hirst, Durham, Cour, Automatic, Stations, Map

Background

Pollen and fungal spores have detrimental effects on health [1–7] and it is therefore logical that these airborne allergenic particles are monitored throughout the world. Monitoring the air quality of non-biological components in ambient air is commonplace and is undertaken worldwide, mostly on the basis of legal exposure limits. The airborne components such as PM_{10}, $PM_{2.5}$, SO_2, NO_X and O_3 that are monitored, often with standardized methods, may be different depending on the country that is carrying out the monitoring. The fact that this monitoring is carried out with public financing signifies that most of the data from these networks are available to the public at no cost and are often published with open access on the internet. Citizens can, therefore, easily assess the

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quality of the air that they breathe with a minimal lag time thanks to these online networks.

The situation of biological particles is, however, completely different. Only few countries like MeteoSwiss (Switzerland) and RNSA (France) have state-owned monitoring networks. Biological particles, including pollen and fungal spores (spores), were first monitored with medical purposes in 1870 by Blackley in the UK [8], and the oldest continuous pollen record dates back to 1943 in Cardiff, the UK, a station that has used a Hirst pollen trap since 1954 [9–11]. The volumetric Hirst-type pollen and spore trap is still one of the instruments most widely used for pollen and spore monitoring [12]. Records for non-biological components are more recent: for example, the CO_2 concentrations in ambient air at Mauna Loa, Hawaii, date back to 1958 [13]. Another difference between biological and non-biological air quality monitoring is that non-biological particles are collected by law as particles of < 10 μm or smaller (PM_{10}, $PM_{2.5}$), whereas biological particles are often > 10 μm [14–16]. Some pollen, such as *Urticamembranaceae* Poir (Urticaceae) [17], and some fungal spores are < 10 μm in diameter (e.g. *Aspergillus* or *Penicillium* [18]), but none are smaller than fine particles of < 2.5 μm. Non-biological air quality parameters are, therefore, either gasses or small particles, while biological air quality parameters are predominately very large particles.

Despite the large size of pollen, the imperfect capacity of common PM_{10} samplers to separate particles by size [19] signifies that up to 15% of birch (21 μm, range approx. 15–27 μm) or grass pollen (25–45 μm) falls into the PM_{10} fraction (particles of between 2.5 and 10 μm) [15, 20, 21], and the situation is similar for other pollen and moulds. The reason why very large particles are found in < 10 μm fractions of air is that the instruments used to collect particulate matter from ambient air do not completely separate them. These instruments, which are mostly impactors, have less efficient separation characteristics then their names suggest: PM_{10} implies that particles > 10 μm are separated from the smaller particles. This is not the case, and approximately 15% of pollen, and even pollen which is larger than 20 μm, can be detected in the fraction of air that should contain only particles of < 10 μm. Pollen and fungal spores are, therefore, collected with the existing air-quality monitoring networks, but very poorly and these technical limitations consequently signify that networks monitoring non-biological components are not useful for monitoring biological particles.

About 30% of the population suffers from some type of allergy to airborne pollen [6], which may have extreme effects on their health, including death [22]. However, few governments own pollen monitoring stations or run a monitoring network for pollen and/or fungal spores. Most pollen and spore networks are privately owned and the data they produce are not freely available.

For allergy sufferers, the location of pollen monitoring stations is unclear and mostly unknown: many Apps deliver pollen forecasts, but it is unclear how the data are obtained and are of little interest to the users, as they assume that sufficient data support the pollen flight prognosis. This is often not the case. For instance, Bavaria in Germany has 12 million inhabitants but only 3 non-public pollen monitoring stations are operating (www.pollenstiftung.de), despite the availability of many Apps. The quality of the pollen flight prognosis is consequently, questionable. Finding the location of pollen monitoring stations could, therefore, be useful for stakeholders. The creation of a map of pollen monitoring stations will improve access to local pollen data and will, hopefully, benefit those with allergies, the medical profession and, of course, aerobiologists. Our aim was, therefore, to a review pollen and spore monitoring stations throughout the world and to develop a practical visualisation method to disseminate their data.

Methods

The inventory of active pollen monitoring stations was created by: (1) reviewing the relevant bibliography and (2) contacting authors or the network managers of pollen networks. Information on pollen monitoring stations was obtained by phone, email or post. A small questionnaire was sent, which requested information on what was monitored, since when, at which location, using which collection method (Hirst-type, brand type Burkard or Lanzoni, automatic or another sampling system), the availability of quality control, and the station owner's most recent contact data. This questionnaire is also accessible in an interactive format in the map application. The questionnaire makes it possible to fill in a.csv database which serves as input data for the map.

The map was programmed in "R" [23]. Maps are instantaneously displayed at https://www.zaum-online.de/pollen-map.html. Stations that are currently running are displayed by default, but historic sites can also be made visible. New information for the map (changes of owner, a new station etc.) can be submitted to either the App-administrator or the map itself online via the "contact form (Modify info, add station, contact)" button. The information is then reviewed by the App-administrator and, if correct, entered in the database, after which the new information is displayed. It is not possible to make a modification without the App-administrator, thus preventing the misuse of the map.

Results and discussion

In order to clarify who is monitoring which biological particle and where, we created an up-dated inventory. We subsequently constructed a map of the pollen monitoring sites throughout the world. We received feed-back from 1011 sites. Because our old map [24] was already outdated upon publishing, we opted for an interactive on-line map. This map can be accessed at: https://www.zaum-online.de/pollen-map.html; http://www.eaaci.org/patients/resources/; https://oteros.shinyapps.io/pollen_map/ (see Fig. 1). Other websites also show information on pollen monitoring networks in the world (http://www.worldallergy.org/pollen/, accessed Jan 2018), but most information is incomplete or is limited to one country (https://www.uco.es/rea/infor_rea/estaciones.htm, accessed Jan 2018), or shows only links to certain national websites (https://www.polleninfo.org/country-choose.html, accessed Jan 2018). Access to this map was made open to the public in June 2017, and has already exceeded 250 h/month. The map is constantly updated and individuals who have, or know of, a station that is not currently on the map may use the contact or submission buttons to make that station visible to all. The map does not show any pollen or spore data, only where a station is located (zoom down to street level), what is monitored (pollen, spores or both) and who to contact in order to obtain information regarding that station (or pollen date). The map allows its users to search for specific stations, and also has a cluster view and filtering options for the monitoring features.

The map makes access to pollen and spore information freely available. As pollen and spores do not stop at borders, but measuring networks and their dissemination activities do, it was more complicated to obtain data from stations abroad in the past, simply because access was complicated. The new map only shows the contact information of all the data providers that agreed to be in. The map will foster international cooperation and enable policy makers to compare their local situation with that of the rest of the world. Patients who suffer from hay fever and members of the medical profession who are treating hay fever patients will profit by finding the most suitable

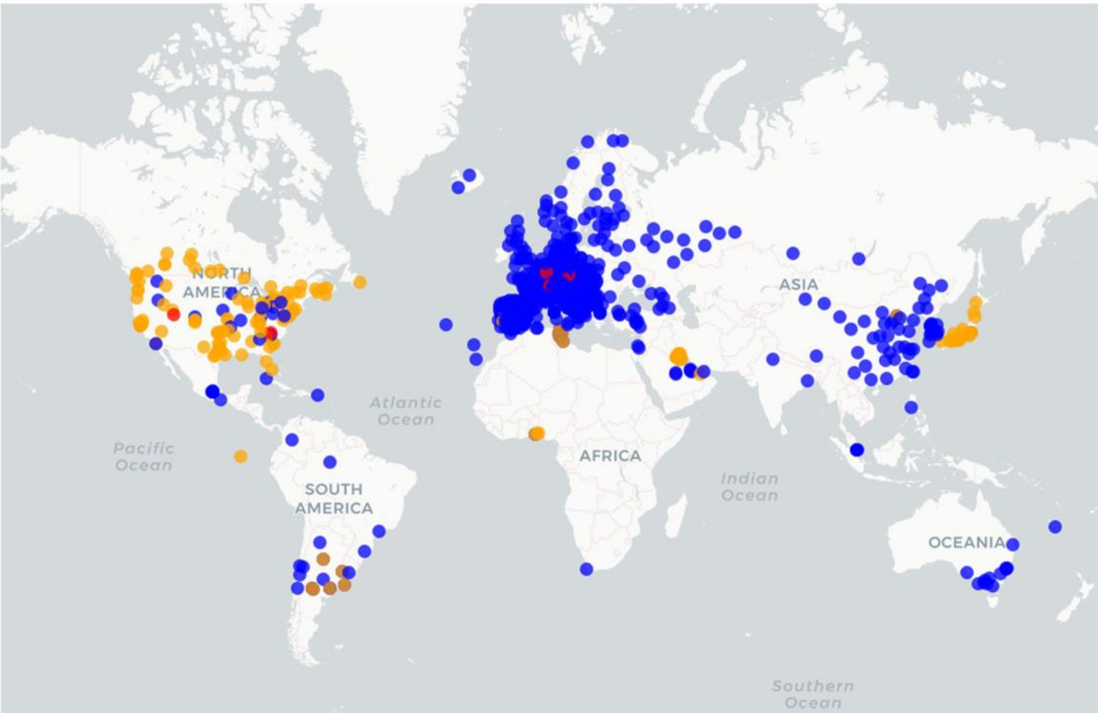

Fig. 1 Screen shot of the interactive map of pollen and fungal spore monitoring stations in the world. The map is web-based, zoomable down to street level, shows the contact information of each station, and can be searched and displayed according the users' needs. The map is constantly updated and is available at: https://www.zaum-online.de/pollen-map.html; http://www.eaaci.org/patients/resources; https://oteros.shinyapps.io/pollen_map (accessed 25-01-2018). Blue dots (Hirst trap), red (Automatic station), orange (other manual)

monitoring station from which to obtain pollen or spore data for comparison with the patient's symptoms. This could improve diagnosis.

Our review accounts for at least 879 open running pollen traps in the world from a total of 1020 records (Fig. 1). As can be observed, most of the traps are distributed in the northern hemisphere, the majority in Europe. The continent with the weakest coverage is Africa. The top six countries by number of active pollen stations are: Japan (143), Italy (88), USA (85), France (85), Spain (77) and Germany (44). The Environmental Agency of Japan owns the biggest network (120 automatic traps), while RNSA of France owns the biggest Hirst-based network (84). The inventory focused on currently operational stations. Some cities are densely monitored by several pollen traps e.g. Milan, Seoul, Tokyo, Toronto, Sydney, Madrid, Mexico DC or Paris. Discontinued historical locations were not specifically inventoried, although interested owners can include their stations on the map. Of those stations that are still open, 70% are based on the Hirst method (Table 1) [12]. Hirst is the prevalent method on all continents with the exception of America, where only 28% of the traps are Hirst and most are based on Rotorod technology. We observed a 50–50% equilibrium as regards the abundance of the two main Hirst trap brands: Burkard (http://www.burkard.co.uk/, http://www.burkardscientific.co.uk) and Lanzoni (www.lanzoni.it).

Japan is the pioneer in automatic monitoring, with 120 stations based on the KH technology [25]. Automatic monitoring in Japan is possible because the spectrum of pollen whose identification is of interest is very limited [26]. According to Pollen-Sense (http://pollensense.com/), there are only two automatic stations in the whole of the American continent. Europe is reported to have 8 automatic stations: 2 based on Plair PA-300 Rapid E [27] and 6 based on BAA-500 [28]. However, automation is increasing: a network is being built in Switzerland and a network of 8 BAA-500 is being built in Bavaria (Germany). The time resolution provided by automatic stations is between 1 and 3 h. Of the manual methods, Hirst allows the minimal time resolution of 2 h, which is provided by 50% of the stations. The other stations provide average concentrations of 24 h. About 50% of those stations that are open also monitor fungal spores in ambient air; the fungal species monitored depend on the country, but almost always include *Alternaria* spp. and often *Cladosporium* spp. Further statistics can be obtained from the online map site.

Conclusions

The majority of active monitoring stations are based on the Hirst principle (616 of 879 stations), but technological developments now allow automatic monitoring.

Table 1 Pollen and mould monitoring stations in the world

	Automatic	Hirst	Other	
Africa	0	6	3	9
America	2	43	106	151
Asia	120	38	24	182
Europe	8	517	0	525
Oceania	0	12	0	12
	130	616	133	879

Only information regarding those stations that were active in 2016 (879 stations) from the total inventory (1020 stations) is displayed

This map makes it clear where and by whom pollen and fungal spores are monitored in the world. It is similar to the publicly available maps created for non-biological (chemical) pollutants and makes it clear that, in our opinion, biological particle monitoring is a neglected aspect of air quality monitoring. We trust that the map will address this discrepancy.

Authors' contributions

Leading initiative and leading writing: JB; leading data collection: CA and AG; interactive map development and administrator: JO; database inventory and writing: JB, CA, AG, KCB, MT, CG, CS-W and JO. All authors read and approved the final manuscript.

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Acknowledgements

We would like to thank the many people who, with their personal contacts, helped us to collect all the necessary information regarding the pollen and spore monitoring stations, along with the EAACI (European Academy of Allergy and Clinical Immunology) for funding Task Force 40108. We also thank all participant who submitted their data to the map. We acknowledge Helmholtz ZentrumMünchen for the Postdoctoral Fellowship Program III 2017–2019.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Data are displayed at https://www.zaum-online.de/pollen-map.html.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

EAACI (European Academy of Allergy and Clinical Immunology) funding for Task Force 40108.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 2 November 2017 Accepted: 3 March 2018
Published online: 04 April 2018

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3.2. Capítulo II - Influência dos parâmetros meteorológicos na época polínica: disseminação e potencial alergénico

Nas últimas décadas tem-se observado uma tendência crescente na produção de pólen e por conseguinte nas concentrações de pólen atmosférico de certas espécies, incluindo de espécies consideradas mais alergénicas (Ziska et al., 2019). O aumento das concentrações de pólen tem como consequência o aumento do risco de exposição os alergénios polínicos (Buters et al., 2012 e 2015; Gálan et al., 2013) com drásticas implicações na saúde pública. No entanto, a exposição a alergénios polínicos, está também dependentes das oscilações diárias de temperatura, humidade relativa, precipitação, direção e velocidade do vento (Linskens & Cresti, 2000) e, que por sinal, são as variáveis que estão, por um lado, envolvidas na alteração da estrutura morfológica, mas também na emissão, dispersão e transporte do pólen para e na atmosfera.

Este capítulo compreende 3 publicações: i) *Cupressaceae Pollen in the City of Évora, South of Portugal: Disruption of the Pollen during Air Transport Facilitates Allergen Exposure* publicado no número especial 12, 64 da revista *Forests*; ii) *Dynamic and Behaviour of Plane Tree Pollen and Its Relationship with Pla a 1 Aeroallergen Concentration in Évora, Portugal* publicado na *EMJ Allergy Immunology; e,* iii) *Cupressaceae pollen prevalence in Southern Iberian Peninsula: the effect of surface meteorological factors on pollen season intensity, manuscrito em preparação e* em processo de revisão pelos co-autores, para submissão na revista *Environmental research*.

Nestes artigos o principal objetivo consistiu em estudar a influência dos parâmetros meteorológicos tanto nas concentrações de pólen no ar e nas características da época polínica bem como na ação destes sobre as características morfológicas do pólen aerotransportado.

O meu contributo para estes três estudos foi primariamente obter e tratar os dados das concentrações de pólen no ar, através da contagem e identificação polínica e efetuar as análises estatísticas necessárias e de correlação entre o pólen e os parâmetros meteorológicos, bem como elaborar os artigos.

forests

MDPI

Article

Cupressaceae Pollen in the City of Évora, South of Portugal: Disruption of the Pollen during Air Transport Facilitates Allergen Exposure

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Abstract: Research Highlights: Daily airborne Cupressaceae pollen disruption ranged from 20 to 90%; relative humidity (RH), rainfall and atmospheric pressure (AtP) were the major meteorological determinants of this phenomenon. *Background and Objectives:* Cupressaceae family includes several species that are widely used as ornamental plants pollinating in late winter-early spring and might be responsible for allergic outbreaks. Cupressaceae pollen disruption may favour allergen dissemination, potentiating its allergenicity. The aim of this work was to characterize the Cupressaceae pollen aerobiology in Évora, South of Portugal, in 2017 and 2018, particularly the pollen disruption, and to identify the meteorological parameters contributing to this phenomenon. *Materials and Methods:* Pollen was collected using a Hirst type 7-day pollen trap and was identified following the standard methodology. Temperature, RH, rainfall, global solar radiation (Global Srad), AtP, wind speed and direction were obtained from a weather station installed side-by-side to the Hirst platform. Back trajectories (12-h) of air masses arriving at Évora were calculated using the HYSPLIT model. *Results:* Cupressaceae pollen index was higher in 2017 compared to 2018 (>5994 and 3175 pollen/m^3, respectively) and 36 ± 19% (2017) and 64 ± 17% (2018) of the pollen was disrupted. Higher levels of disrupted pollen coincided with RH > 60% and rainfall. Temperature, Global Srad and AtP correlated negatively with pollen disruption. Wind speed and wind direction did not significantly correlate with pollen disruption. Intra-diurnal pollen pattern peaked between 9:00 am–2:00 pm, suggesting local origin, confirmed by the back trajectory analysis. Intra-diurnal pollen disruption profile followed hourly pollen pattern and it negatively correlated with AtP, temperature and Global Srad but was uncorrelated with RH. *Conclusions:* The results suggest that RH, rainfall and AtP are the main factors affecting airborne Cupressaceae pollen integrity and in conjunction with daily pollen concentration may be used to predict the risk of allergy outbreaks to this pollen type.

Keywords: Cupressaceae pollen; pollen disruption; meteorological parameters; back trajectories

Citation: Galveias, A.; Costa, A.R.; Bortoli, D.; Alpizar-Jara, R.; Salgado, R.; Costa, M.J.; Antunes, C.M. Cupressaceae Pollen in the City of Évora, South of Portugal: Disruption of the Pollen during Air Transport Facilitates Allergen Exposure. *Forests* **2021**, *12*, 64. https://doi.org/10.3390/f12010064

Received: 30 November 2020
Accepted: 2 January 2021
Published: 8 January 2021

1. Introduction

The Cupressaceae is a cosmopolitan family and the second most important family of Gymnosperms [1–3]. The family includes 30 genera and 160 species distributed by temperate or temperate-warm regions of Northern and Southern Hemispheres, although almost 3/4 of the species are present in the Northern hemisphere [1,3]. They are coniferous and heliophiles plants, with medium size, and can grow up to 30 m maximum. Cupressaceae is the only family that shows a non-spiral arrangement of microsporophylls and each microsporophyll bears a variable number (2–6) of pollen bags [2]. Currently, this family is distributed in all of Earth's continents, except Antarctica. In the Iberian Peninsula, species from the genus *Juniperus* form part of the natural vegetation and the scrubland and are considered autochthonous while species from the genera *Cupressus*, *Chamaecyparis* and *Platycladus*, widely used in parks and gardens as barriers against wind and noise, are considered allochthonous [4]. In Portugal, several species from the genera *Juniperus* and *Cupressus* are distributed throughout the continental territory, and *Cryptomeria*, particularly *Cryptomeria japonica*, is abundant in the Azores islands [5]. Among the several autochthones species from the genus *Juniperus*, the *Juniperus communis* L., *Juniperus navicularis* Gand., *Juniperus oxycedrus* L. and *Juniperus turbinata* Guss are well represented [6]. The genus *Cupressus*, is considered allochthonous in Portugal and is distributed throughout the country; the species *Cupressus sempervirens* L. is mainly found in urban environments, due to its use as ornamental plants, *Cupressus macrocarpa* Hartweg is usually found in mountains of Sintra, Buçaco and Geres, and *Cupressus lusitanica* Miller is found both environments; the distribution of *Cupressus arizonica* Greene is mostly unknown [6,7].

The pollination of *Cupressaceae* trees is anemophilous and occurs from late winter to early spring and may last for more than a month, due to its microsporophyll gradual maturation mechanism, from bottom to top of the flower [8,9]. Due to the large number of trees from these species in habitational areas or surroundings in Portugal, particularly the species *Cupressus sempervirens* and *Cupressus lusitanica* as a result of their use as ornamental plants [6,7], there is a high production and release of pollen into the atmosphere [8,10–12], which causes an increase in the Cupressaceae pollen allergen availability in breathing air [13–15] and an allergenic risk associated with these exposure [16,17].

Meteorological parameters are crucial factors influencing plant physiology and consequently pollen release dynamics. Temperature has an important role in maturation and flowering and is the factor that most influences the growth and development of plants and pollen production. Typically, anthers of the gymnosperm plants open when the relative humidity decreases, below 80%, and the temperature increases [18], allowing anemophilous pollen dispersal. Daily airborne pollen typically follows a dynamic pattern, that can be achieved by hourly accounts [19], with maximal concentrations in the atmosphere observed when temperature rises in the morning [10,20]. On the contrary, rain seems to develop a negative effect, contributing to a reduction of pollen in the atmosphere [21], probably by a washout effect, inducing the deposition of pollen [22].

Researchers have long believed that meteorological changes can cause loss of pollen and spore integrity, forming and releasing smaller particles to the surrounding environment. Pollen disruption in the anthers was observed in grasses and in birch inflorescences, caused by water or by a high humidity environment [23,24] as well as in atmosphere [24] but the meteorological factors affecting airborne pollen disruption is not yet fully understood. Moreover, to our knowledge the characterization of airborne pollen disruption on the Cupressaceae family has not yet been reported.

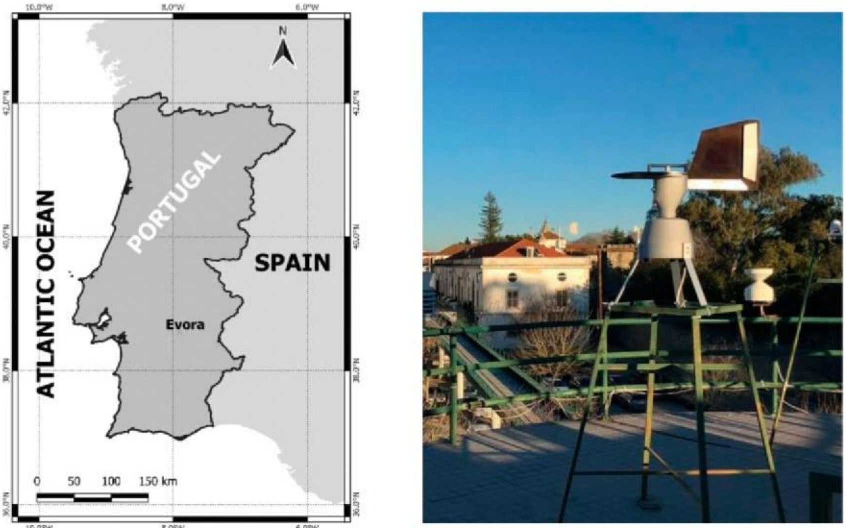
This phenomenon allows the release of the allergen content to ambient air enhancing its availability for inhalation. In fact, cycles of wetting and drying followed by wind disturbance originated aerosol particles in the size range 0.12 to 4.67 μm, where major allergens were identified [23]. Also, Ole e 1 allergen was detected not only in the PM > 10 μm fraction, associated with pollen, but also in the 10 μm > PM > 2.5-μm fraction [25], showing that small and allergenic particles are present in the atmosphere. Pollen disruption has already been associated with a significantly augmented risk for allergic asthma outbreaks [26]. Cupressaceae is main cause of winter allergic reactions in the southern countries [10] and pollen disruption in the atmosphere might be an important factor aggravating its allergenicity.

In this work we have investigated the disruption of Cupressaceae pollen in a real-world scenario, in the south of Portugal. Data from Cupressaceae airborne pollen and meteorological parameters were collected from the years 2017 and 2018, seeking to elucidate the effect of meteorological factors on airborne pollen integrity and to identify the most relevant parameters contributing to Cupressaceae pollen disruption in the atmosphere.

2. Materials and Methods

2.1. Study Area and Period

The study focuses on the city of Évora, located in the south of Portugal (Figure 1). Data was collected in the Évora Atmospheric Sciences Observatory (EVASO) of the Institute of Earth Sciences (ICT) at the University of Évora (UE) (38° 34′ N, 7° 54′ W, 293 m asl). Évora is characterized by a temperate climate with warm and dry summers that can be described as Hot-summer Mediterranean climate (Köppen Climate Classification—Csa). According to the climatological normal (1981–2010) provided by the Portuguese Institute for Sea and Atmosphere [27], national authority for climate, the annual mean air temperature is 16.5 °C, with the highest monthly value occurring in August (24.1 °C) and the lowest in January (9.6 °C). The rainfall period, occurring mostly seasonally between October and April, presents an annual precipitation of 585.3 mm.



(a) (b)

Figure 1. Map of Portugal illustrating the location of Évora (**a**) and photo of EVASO where the Hirst type pollen trap and the weather station are installed (**b**).

Cupressaceae pollen are typically detected in large amounts in the south of Portugal during late winter and early spring seasons, particularly, in the months of February and March. The periods selected for the present study comprise three weeks in 2017 and in 2018, from 22 February to 19 March.

2.2. Airborne Pollen Sampling

Airborne Cupressaceae pollen was collected with a Hirst-type volumetric spore trap (Hirst 1952) at EVASO (Figure 1), located on the roof of a building belonging to the University of Évora, approximately 10 m above ground level, since the 22 February 2017. The sampler sucks in air at a rate of 10 L/min, and pollen grains are trapped on an adhesive coated Melinex® strip. Sampling and slide analysis were carried out in accordance with the protocol developed by the European Aeroallergen Network (EAN) and Spanish Aerobiology Network (REA) [28,29]. Slides were analysed daily under a light microscope (400× magnification), with four longitudinal sweeps per slide, according to the standard method [28,29]. The daily or hourly airborne pollen concentrations were expressed as pollen per cubic meter of air (Pollen/m^3).

The main pollen Season (MPS) was determined by the method of percentage described for Nilsson and Persson, 1981, based on the elimination of a 2.5% percentage in the beginning and end of the pollen season [30,31].

2.3. Meteorological Parameters and Remote Sensing Data

The meteorological parameters used were obtained from the meteorological station installed at EVASO, namely the air temperature (°C), relative humidity (RH; %), precipitation (mm), global solar radiation (W/m^2), wind speed and direction (m/s; °) and atmospheric pressure (AtP; hPa). All variables were measured in 10 s intervals and subsequently averaged to yield hourly and daily values, except for precipitation that is accumulated during the period. The average of the wind direction is obtained applying the arctangent function to the ratio between the mean east-west and the north-south components of wind. The mean east-west and north-south wind components are computed using all the non-zero wind speed samples. This average is done according to the procedure described in the manual of the data logger used in the weather station (CR100) from Campbell Scientific (https://s.campbellsci.com/documents/br/manuals/cr1000.pdf, last accessed 21 December 2020) [32]. Maximum and minimum temperatures are obtained daily. All instruments are subject to periodic checks and maintenance procedures.

The ceilometer is an optical active remote sensing instrument that uses electromagnetic radiation to obtain information about the height where the atmospheric constituent backscatters the radiation. A VAISALA Ceilometer CL31 installed at EVASO since May 2006 provides measurements of the cloud base height up to three simultaneous layers and of the profile of the backscatter coefficient, which in the absence of clouds gives a good approximation of the qualitative aerosol boundary layer profile. This ceilometer has a measurement range from 0 to 7.5 km, with a maximum vertical resolution of 5 m and programmable measurement cycle (from 2 to 120 s). It uses an eye-safe laser InGaAs diode at 910 nm. The ceilometer backscatter measurements are used here as auxiliary data to detect low atmospheric aerosol layers that could be related with pollen occurrences reaching Évora.

2.4. Back Trajectory Analysis

Back trajectories of air masses arriving at Évora have been calculated using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT). The HYSPLIT model, developed by the National Oceanic and Atmospheric Administration (NOAA), is one of the most widely used and complete models for a simple calculation [33,34]. The meteorological data used for the back trajectory calculations is obtained from the Global Data Assimilation System (GDAS) at a spatial resolution of 0.5°. The vertical motion method used to compute the back trajectories is the model vertical velocity. The back trajectories were calculated for a 24-h period, for every hour of that period, at three different height levels of 100 m, 500 m and 1000 m. The back trajectories obtained for 10:00 UTC are presented in the manuscript, since they are representative of the situation during the rest of the day and in most of the cases a pollen peak is observed around this time. Additionally, the hourly back trajectories are presented in the Supplementary Materials.

2.5. Statistics Analysis

Statistical analysis including non-parametric tests and Pearson's correlation analysis (significance level of 5%) was used to study the relationship between meteorological parameters of maximum, minimum and mean air temperatures, precipitation, RH, global solar radiation, wind speed and direction and AtP (hPa) with disrupted pollen (%).

Statistical assessment of differences was performed using KrusKal-Wallis ($p < 0.05$ was accepted) [35]. Graphs were produced using OriginPro software (OriginLab corporation).

The principal component analysis (PCA) was applied to evaluate the association between the disruption pollen in the air with the meteorological parameters [36].

The mean values shown in the tables and the text represent mean ± standard deviation unless stated otherwise.

3. Results

3.1. Characterization of the Cupressaceae Pollen Season in Évora, South of Portugal

The Cupressaceae pollen was detected in large concentrations in the atmosphere of Évora during winter–spring seasons, particularly, in the months of February and March of 2017 and 2018 with a seasonal pollen index of >5994 (sampling begun on the 22 February 2017) and 3175 pollen/m^3, respectively. The pollen peaked on 25 February 2017 with a concentration of 1635 pollen/m^3 and on 26 February 2018 with a concentration of 644 pollen/m^3.

During the main season, the period of higher concentrations of Cupressaceae pollen occurred from 22 February to 19 March. During this period, 36 ± 19% (2017) and 64 ± 17% (2018) of the pollen that arrived at the collector was disrupted with exine wall rupture, dilatation of the intine wall and release of the cellular content to the environment (Figure 2a,b).

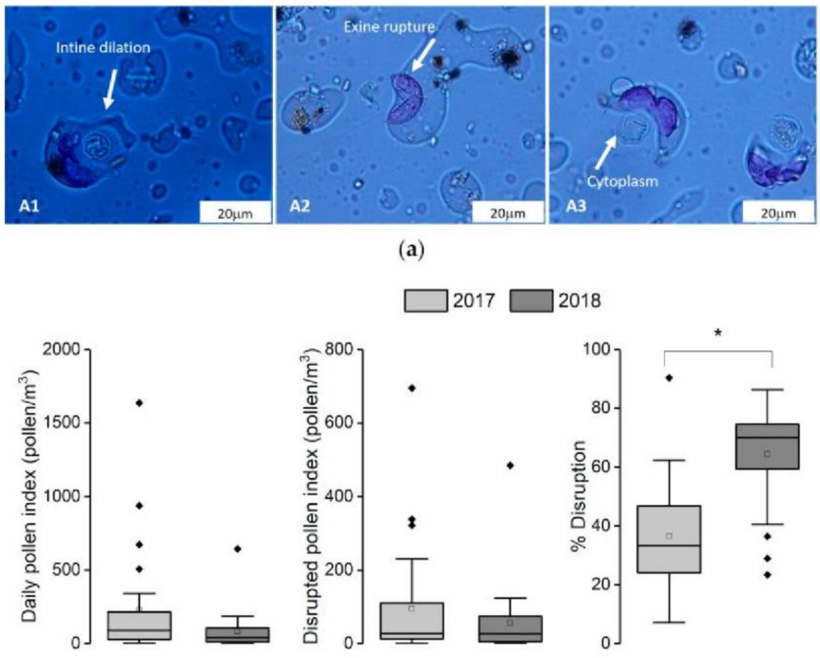


Figure 2. Loss of integrity of Cupressaceae pollen. (**a**) pollen micrographies (with microscopy camera using a 400X amplification). (**b**) daily pollen concentration and pollen disruption (%) in 2017 and 2018 in the studied period. * statistical significance at 5% range ($p < 0.05$).

A linear relationship was found between daily disrupted pollen (DRP) and daily pollen concentration (DPC); when taking the slope of the two years together, which is a measure of the mean ratio DRP/DPC which is 0.45 (0.41–0.48 at 95% confidence) while when considered separately, the slope is 0.42 (0.39–0.44 at 95% confidence) and 0.72 (0.66–0.77 at 95% confidence) for 2017 and 2018, respectively (see Supplementary Materials, Figure S1).

The year 2017 was extremely warm and dry from a climatological point of view, with the south of Portugal affected by drought conditions, which progressively reached severe and extreme drought according to the Portuguese Institute for the Sea and Atmosphere (IPMA; www.ipma.pt, last accessed on 20 December 2020). The situation lasted until the beginning of March 2018, when the occurrence of rainfall relieved the situation [37]. The meteorological conditions prevailing during both years are reflected in the conditions presented for the two periods of study in Table 1. The season was colder and moister in 2018 compared to 2017, as shown by the average seasonal temperatures 2 to 4 °C lower for the maximum (T_{max}), minimum (T_{min}) and mean (T_{mean}) temperatures accompanied by a lower RH in 2017 (Table 1).

Table 1. Values of meteorological parameters at Évora for the years 2017 and 2018 (averaged for the period 22 February to 19 March of each year, except precipitation). ** statistical significance (Stat. sig.) at 1% ($p < 0.01$).

Meteorological Parameters	2017	2018	Stat. Sig.
Maximum temperature, T_{max} (°C)	20.0 ± 3.9	16.43 ± 1.65	$p = 0.0002$ **
Minimum temperature, T_{min} (°C)	10.0 ± 2.2	7.9 ± 2.3	$p = 0.002$ **
Mean temperature, T_{mean} (°C)	14.3 ± 2.8	11.6 ± 1.8	$p = 0.0003$ **
Precipitation (accumulated, mm)	28.3	329.9	-
Relative humidity, RH (%)	63.5 ± 14.2	75.1 ± 15.7	$p = 0.001$ **
Global Solar radiation, Global Srad (W/m^2)	175.0 ± 61.7	130.1 ± 59.6	$p = 0.006$ **
Wind speed, WS (m/s)	2.2 ± 0.9	2.0 ± 0.5	$p = 0.811$
Wind direction, WD (°)	SW-NE	S-W	$p = 0.176$
Atmospheric pressure, AtP (hPa)	986.7 ± 4.3	973.0 ± 6.6	$p = 6.573 \times 10^{-9}$ **

In 2018, the average global solar radiation (Global Srad) was lower, and the precipitation was approximately 12 times higher (Table 1) compared to 2017, in the period considered. The mean wind speed (WS) was similar for both years, but the predominant wind direction (WD) was SW-NE in the year 2017 and S-W in 2018 and average atmospheric pressure (AtP) was slightly lower in 2018 (Table 1).

3.2. Daily Pollen Concentration and Daily Meteorological Parameters Affecting Pollen Integrity

The daily pollen concentration (DPC) was observed to be 2–3 fold higher in 2017 compared to 2018 and the daily ruptured pollen profile followed the DPI, but the level of disrupted pollen was higher in 2018. The number of days with >50% of ruptured pollen were 6 and 19 in 2017 and 2018, respectively (Figure 3a).

Concerning the meteorological parameters, the temperature, accompanied by the Global Srad, tended to increase during the season from February to March in 2017 but not in 2018; in 2018 the temperature and the Global Srad maintained fairly similar values throughout the season (Figure 3b,c). The AtP was above 970 hPa in 2017 during the period considered, while in 2018 it was usually below this value (Figure 3b). The daily RH varied from 50% to 85% in 2017 and from 50% to 95% in 2018 and the number of days with RH > 70% was 10 and 21 in 2017 and 2018, respectively (Figure 3c).

In 2018, rainfall occurred almost every day in the period under analysis (22/26 days), contrarily to 2017 when rainfall only occurred in 6/26 days (Figure 3a). The wind speed was usually below 2 m/s in both years except for a week from 11 to 18 March 2017 when the wind speed reached 5 m/s.

To evaluate the effect of the different meteorological parameters on the pollen disruption, a correlation analysis was performed. The ratio of pollen disruption showed a negative and significant correlation with temperature (T_{max}, T_{mean} and T_{min}), Global Srad and AtP while it was positively correlated with rainfall and RH (Table 2), where higher disruption is correlated with RH > 60% and with AtP < 980 hPa (Figure 4). Wind speed and wind direction did not significantly correlate with pollen disruption (Table 2). However, the pollen disruption ratio was higher when precipitation occurred (61.0 ± 15.8% and 33.6 ± 24.0%, respectively; $p < 0.05$) (Figure 4).

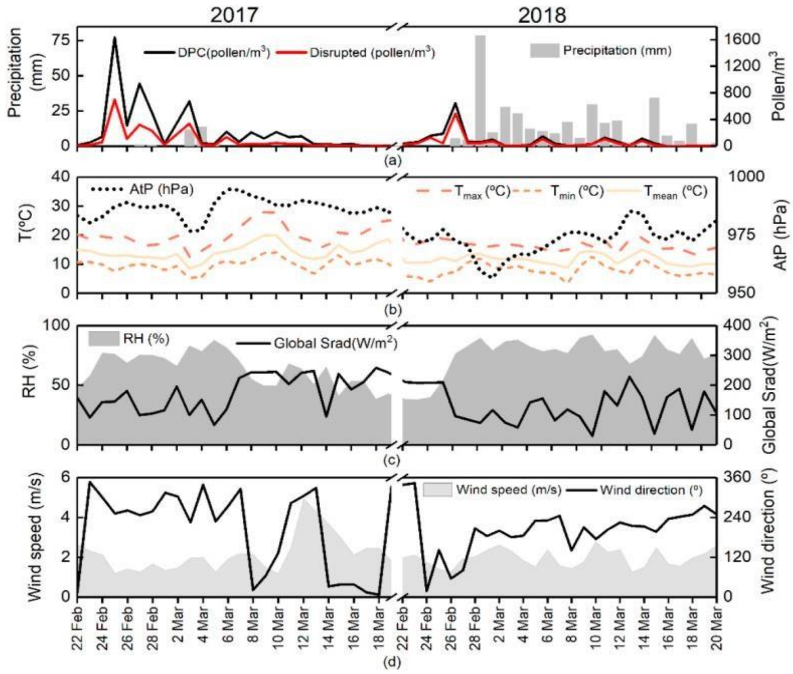


Figure 3. Daily meteorological parameters and daily pollen concentration (DPC), for the period 22 February to 19 March of 2017 and 2018. DPC and rainfall (**a**); daily averaged temperatures (T_{max}, T_{min}, T_{mean}) and atmospheric pressure (**b**); RH and Global Srad (**c**); and wind speed and direction (**d**).

Table 2. Pearson's correlation between meteorological parameters and pollen disruption in the years 2017 and 2018.

	T_{max} (°C)	T_{min} (°C)	T_{mean} (°C)	Precipt. (mm)	RH (%)	Global Srad (W/m^2)	AtP (hPa)	WS (m/s)	WD (°)	Pollen disr. (%)
T_{max} (°C)	1.000	0.371 *	0.768 *	−0.355 *	−0.573 *	0.672 *	0.549 *	−0.089	−0.360 *	−0.565 *
T_{min} (°C)		1.000	0.188	−0.047	0.010	−0.014	−0.212	−0.105	−0.25	−0.342 *
T_{mean} (°C)			1.000	−0.171	−0.282 *	0.338 *	0.639 *	0.131	−0.350 *	−0.484 *
Precipt.(mm)				1.000	0.535 *	−0.518 *	−0.634 *	−0.083	0.018	0.371 *
RH (%)					1.000	−0.800 *	−0.383	−0.114	0.426 *	0.448 *
Global Srad (W/m^2)						1.000	0.479 *	0.120	−0.214	−0.501 *
AtP (hPa)							1.000	0.048	0.008	−0.519 *
WS (m/s)								1.000	0.024	−0.077
WD (°)									1.000	0.136
Pollen disr. (%)										1.000

* Correlation is significant at 5% ($p < 0.05$); Pollen disr., Pollen disruption; Precipt., Precipitation.

Principal component analysis (PCA) showed that PC1 and PC2 explained approximately 42% and 17% of the total variability of the data, respectively. These two main components explain almost 60% of the variance. PC3 and PC4 allow the explanation of 22% of the total variance (Tables 3 and 4), thus with 4 components, approximately 81% of the total variability of the original data is explained (Table 3). To decide on the number of components to retain, the proportion of variance explained by the components and the eigenvalues of the sample correlation matrix were considered (Table 4).

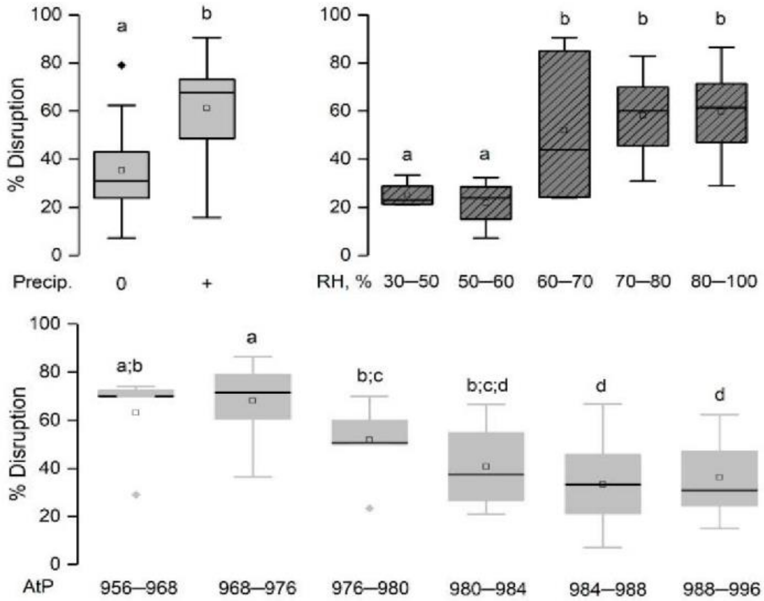


Figure 4. Influence of precipitation, RH and atmospheric pressure (AtP) on pollen disruption. The data was sectioned by the presence or absence of rainfall and by RH and AtP as mentioned. The different letters within the graphs indicate the statistical significance at 5% ($p < 0.05$) among homogeneous subsets.

Table 3. Own values and proportion of variance explained by components.

	Components Variances	Proportion of Variance	Cumulative Proportion
1	4.178	41.78	41.78
2	1.719	17.19	58.97
3	1.165	11.65	70.63
4	1.081	10.81	81.44
5	0.611	6.11	87.55
6	0.539	5.39	92.95
7	0.323	3.23	96.18
8	0.177	1.77	97.96
9	0.144	1.44	99.40
10	0.059	0.59	100

The first main component, PC1, is responsible for the greatest variability in the data. The variables that contributed most to the first component were T_{max}, T_{mean}, precipitation, RH, Global Srad and AtP. For the second component, PC2, the main meteorological parameters were T_{min}, precipitation and WD (Table 4 and Figure 5). All variables, except for WS, are determinant in the formation of the first two components. WS is the variable with the highest weight in PC4, thus it is the meteorological variable that least contributes to explain the pollen burst (component 4 only explains 11% of the data variability, respectively) (Table 3 and Figure 5). T_{mean}, RH, Global Srad and WD are the main contributors to PC3, however this component contributes to explain only 12% of the data variability. Pollen disruption (%) is associated mainly with PC1.

Table 4. Loadings of principal components.

	PC1	PC2	PC3	PC4
% Pollen disruption	**−0.362**	−0.106	−0.209	0.084
T_{max} (°C)	**0.421**	−0.271	0.170	0.111
T_{mean} (°C)	**0.353**	−0.128	**0.556**	−0.105
T_{min} (°C)	0.019	**−0.665**	0.119	0.070
Precipitation(mm)	**−0.315**	**−0.336**	0.236	−0.290
RH (%)	**−0.379**	0.0315	**0.494**	0.090
GlobalSrad (W/m^2)	**0.398**	0.035	**−0.326**	−0.070
AtP (hPa)	**0.369**	0.279	0.274	0.103
WS (m/s)	0.036	0.106	0.009	**−0.921**
WD (°)	−0.162	**0.499**	**0.346**	0.087

In bold: values are significant (>0.300).

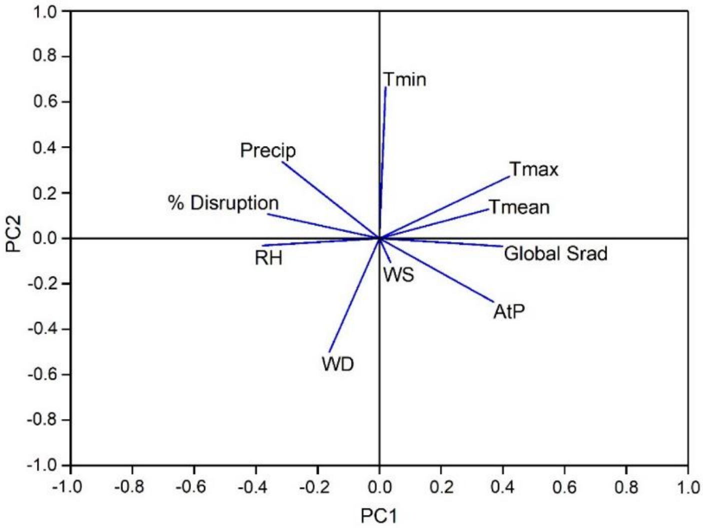


Figure 5. Diagram of the contribution of each variable for PC1 and PC2.

Figure 5 shows that precipitation and RH is positively associated with pollen disruption while AtP, Global Srad and T_{mean} are negatively associated with pollen disruption, according to the correlation analysis.

3.3. Intra-Diurnal Variations of Pollen Concentration and Meteorological Parameters

The diurnal variability of the hourly distribution patterns of Cupressaceae pollen and meteorological parameters were analysed on four days with high and low pollen disruption, within the period studied.

Table 5 depicts the detailed characteristics of the selected days in terms of DPI, pollen disruption, daily averaged temperatures, rainfall, Global Srad, AtP, WS and WD. All days selected have significant DPI and pollen disruption, verifying from 23% to 75%. T_{mean} and

T_{max} were not significantly different. T_{min} was also similar between days except for 27 February 2017; this day presented the lowest T_{max} and the highest T_{min}.

Table 5. Characterization of two days of high and low pollen disruption in the years 2017 and 2018 in Évora.

	25 February 2017	27 February 2017	25 February 2018	26 February 2018
DPC (pollen/m^3)	1635.0	938.0	185.0	644.0
Ruptured pollen (%)	42.5	34.3	23.2	75.3
T_{max} (°C)	18.9	16.9	19.0	18.2
T_{min} (°C)	7.5	10.1	6.7	7.3
T_{mean} (°C)	12.9	12.5	12.3	11.1
Rainfall (mm)	0.0	1.0	0.0	5.4
RH (%)	76.3	75.5	54.1	76.5
Global Srad(W/m^2)	145.1	99.7	209.4	96.0
AtP (hPa)	987.4	987.2	977.4	972.4
WS (m/s)	1.2	1.3	1.9	1.3
WD (°)	W-SW	W-SW	E	NE

RH was similar for three of the days (75.5–76.5%) but was lower on 25 February 2018. Global Srad was approximately twofold higher on dry days (25 February 2017 and 2018) compared to days with rainfall (27 February 2017 and 26 February 2018). In 2017 the AtP was slightly higher compared to 2018. The predominant wind direction was W-SW in 2017 and E and NE in 2018 and WS was mild for all the days, being more intense on 26 February 2018.

The diurnal pollen and meteorological profiles are depicted in Figure 6. The hourly pollen concentration presented in Figure 6 shows a diurnal cycle, starting to increase at about 07h00 UTC, with a peak between 10h00 14h00 UTC, followed by a diminution during the afternoon. The lowest pollen concentrations were detected during nighttime for all days and higher concentrations detected, predominantly, coincident with a diminution of solar radiation, thus decreasing atmospheric mixing and increasing particle concentrations near the surface.

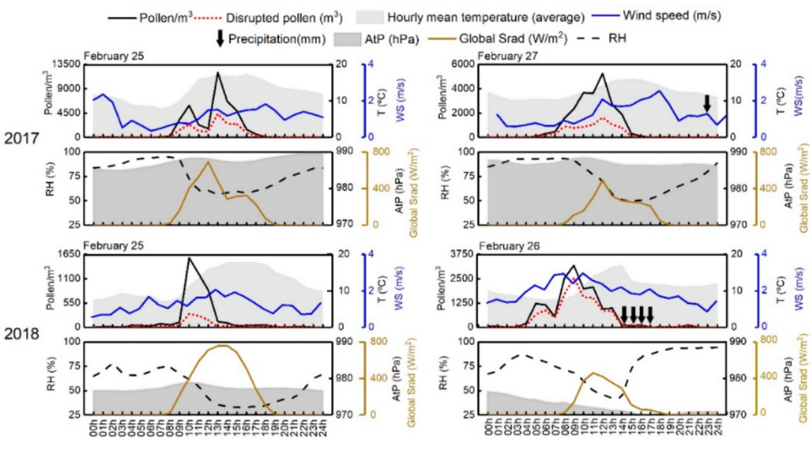


Figure 6. Hourly pollen concentration and meteorological parameters on 4 episodes of high pollen, 2 per year, with high and low pollen disruption indexes.

The maximum pollen peak on the 25 and 27 February 2017, was registered at 13h00 and 12h00, respectively, reaching 11,978 and 5247 pollen/m^3. The pollen concentration began to rise between 07h00 and 08h00 for both days. On 25 and 26 February 2018, the maximum pollen concentration (1568 and 3179 pollen/m^3, respectively) was detected at 10h00 and 09h00, respectively. On 26 February 2018, an additional peak was detected in the period from 04h00 to 07h00, suggesting a long-distance origin (Figure 6).

The variation of mean temperature was similar in the days of analysis, increasing from 9h00 until the middle of the day and then, decreasing again at approximately 19h00. RH was reversed with respect to the mean temperature. Precipitation occurred on 26 February 2018 from 14h00–17h00, coinciding with the decrease in the concentration of pollen in the air, and on 27 February 2017 at 23h00 (Figure 6, 1st and 3rd panels in the right). The atmospheric pressure was higher in 2017 compared to 2018. WS tended to be higher between 10h00 to 19h00, decreasing afterwards in all cases (Figure 6).

The hourly pollen concentration (HPC) was positively and significantly correlated with T and AtP while pollen disruption positively correlated with WS and negatively correlated with AtP (Table 6), suggesting that T and AtP contribute to pollen release and higher WS and lower AtP are factors contributing to pollen integrity loss. It is noteworthy, that the day with the lowest AtP (972.4 hPa) presented the highest disruption ratio (RH = 76.5%); on the contrary, for the day with the lowest disruption ratio the RH was 54.1%, although the AtP was 977.4 hPa (Table 5 and Figure 6).

Table 6. Pearson's correlation between hourly meteorological parameters and hourly disrupted pollen (%) hourly total pollen (m^3).

	T (°C)	RH (%)	Global Srad (W/m^2)	AtP (hPa)	WS (m/s)	Pollen Disruption (%)	HPI (Pollen/m^3)
T (°C)	1.000	−0.701 *	0.848 *	0.395 *	−0.218	−0.225	0.409 *
RH (%)		1.000	−0.785 *	0.262	−0.323	−0.007	−0.109
Global Srad (W/m^2)			1.000	0.147	−0.064	−0.222	0.286
AtP (hPa)				1.000	−0.714 *	−0.587 *	0.407 *
WS (m/s)					1.000	0.451 *	−0.227

* Correlation is significant at the 95% range ($p < 0.05$).

3.4. Remote Sensing and Transport Modelling of Cupressaceae Airborne Pollen: Contribution for Pollen Concentration

The HYSPLIT model was used to estimate the contribution of medium-long range transport of pollen for the DPI in the South of Portugal on the four days selected, at different altitudes (100, 500 and 1000 m). The back trajectories were calculated for every hour of the days analysed. The comparisons showed that for each day there were no significant differences between the results at different times. On the other hand, in most of the cases a pollen peak was observed around 10:00 UTC, therefore these back trajectories were deemed representative of the situation during the rest of the day and are presented in Figure 7. Nevertheless, the hourly trajectories are presented in the Supplementary Materials Figures S2–S5.

Remote sensing data presented aims at supporting the study through the analysis of ceilometer backscatter measurements presented in Figure 8 that are related with the vertical and temporal distribution of aerosols in the lower layers of the atmosphere, since, in the absence of clouds, these particles cause the backscatter sensed by the instrument. These measurements can be used to detect the presence of pollen particles in the atmospheric boundary layer, if there are no other concurrent aerosol events at the same atmospheric levels, such as for example desert dust transports that often occur in the area, which were not detected during the days considered here.

On 25 and 27 February 2017, it was observed from the back trajectory that the air mass came from northwest and west, respectively, passing through continental areas of central-south Portugal where these species are abundant, as the Sintra mountain and other mountain ranges slightly to the north (Figure 7).

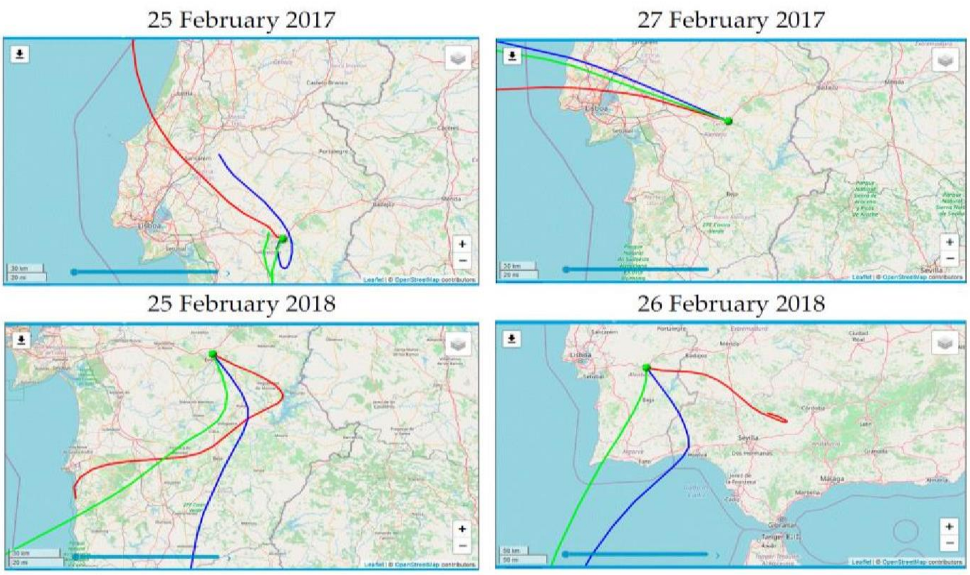


Figure 7. Air mass back trajectory for 2017 and 2018 at 10:00 UTC; height 100 m—red line, height 500 m—blue line and height 1000 m—green line.

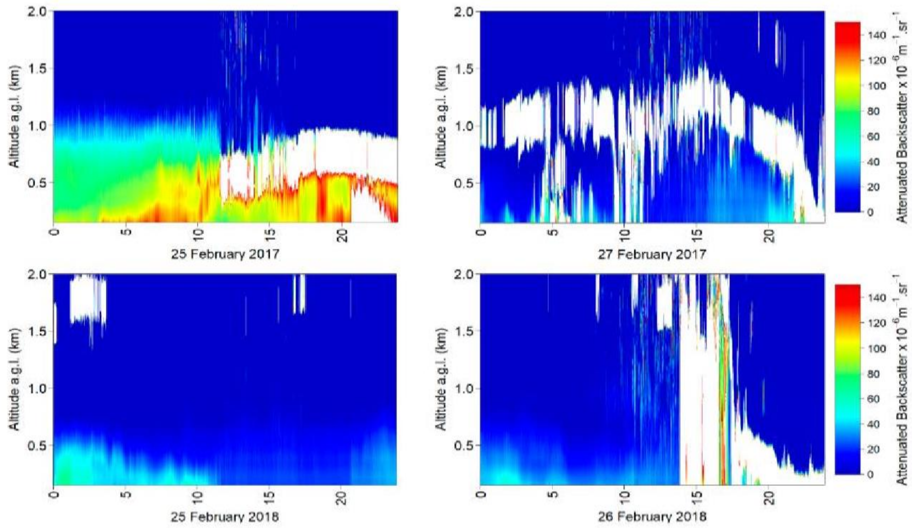


Figure 8. Ceilometer attenuated backscatter on 25 and 27 February 2017 and 25 and 26 February 2018. Clouds are represented in white and are observed over the observatory in the four days.

For the year 2018, on 25 February the back trajectory indicates that the air mass came from the south of Portugal while on 26 February the air mass originated in the southwest coming from south of Spain (Figure 7), where the Cupressaceae family is abundant. On 26 February 2018, a peak was detected between 04h00–06h00 coinciding with air masses originating from southwestern Spain (back trajectory not shown here). The nocturnal period of detection associated with the air mass origin, suggests that these pollens are not locally originated but regionally transported from Spain. Precipitation occurred in Évora starting at 14h00, coinciding with a decrease of concentration of pollen, due to wash-out and wet deposition.

During the morning (clear sky conditions) of 25 February 2017, only a low layer of particles is distinguished with a moderately high signal. AERONET observations at the same site (not shown here) present a mean aerosol optical depth at 500 nm of 0.31 and mean Ångström exponent of 0.83, typical of relatively large particles. This analysis indicates that the Cupressaceae pollen were probably transported at low levels, below about 1 km, and that their origin matches the regions where these species are mostly concentrated (Figure 8). After 12h00 on 25 February 2017 there were clouds present over the area (white color in the image), which attenuated the signal, hindering the analysis of the vertical distribution of particles in the atmosphere. On 27 February 2017, clouds were present, and the same problems occurred, thus not allowing obtaining a reliable signal of the distribution and intensity of the aerosols, as represented in Figure 8. On 25 and 26 February 2018, the increased backscattered signal at different times indicated the presence of particles at low levels, below about 1 km.

4. Discussion

Cupressaceae pollen, produced in large quantities (523 billion per plant) [38], are considered moderately allergenic [13] and because *Cupressus* species are widely used as ornamental plants, they are widely distributed in urban environments [15], thus being a common cause of winter allergy in urban environments [39], such as Évora, a middle-sized town situated in the South of Portugal. Cupressaceae pollen are present in the atmosphere of Évora during winter–spring, particularly, in the months of February and March (this paper and [11]). In the year 2017, higher concentrations were detected coinciding with higher air temperatures, solar radiation and atmospheric pressure and lower values of relative humidity with respect to 2018. These meteorological conditions can affect the density and viscosity of air [40,41] thus potentially affecting pollen dispersion, resuspension and transport in the atmosphere and, as a consequence the impact on respiratory health of the allergic population.

In this paper we describe a feature of the aerobiology of Cupressaceae pollen characterized by the exine rupture, dilation of intine and sometimes release of the protoplast, as observed in Figure 2a, thus causing the release of pollen allergens and possibly enhancing its allergenic activity. This feature is related to the Cupressaceae pollen morphology characterized for one pore with convex annulus, that has an important role on hydration and exine disruption [42,43]. This event is affected by physical factors during the dispersion process [44] and we have investigated the effect of meteorology factors on this phenomenon on a daily manner and on an intra-diurnal manner.

Concerning the daily observations, the rainfall and relative humidity, particularly above 60%, positively influenced the disruption of the pollen as shown in this paper (Figure 4 and Table 2) and by others. For instance, pollen rupture was reported for *Cupressus arizonica* pollen exposed to water [45], birch pollen exposed to high relative humidity or water [25], and grass pollen subjected to osmotic shock due to high relative humidity or rainwater [26,46] thus releasing the allergen content [47,48]. On the other hand, temperature, global solar radiation and atmospheric pressure are associated with the maintenance of pollen integrity. In fact, PCA analyses show that pollen disruption, rainfall and relative humidity vary in the same manner while atmospheric pressure, temperature and global solar radiation vary together opposing pollen disruption (Figure 5 and Tables 3 and 4). These parameters are all part of the first component that explains most of the variability of the data, in agreement with the correlation analyses. Temperature and global solar radiation relate to relative humidity inversely, indirectly contributing to reduce pollen hydration thus diminishing the probability of pollen disruption. The atmospheric pressure may contribute to counterbalance osmotic pressure, thus opposing the effect of relative humidity and maintaining the pollen integrity while in air suspension. In this paper, the diurnal pattern of the pollen concentration was evaluated for four selected days. The diurnal pollen profile showed a rise in the morning and a peak at the middle of the day (mostly at 10–12 am) following a pattern described by others [10,20], except for one case on 26 February 2018 where a peak during the night (4–7 am) was observed (Figure 6), coinciding with the thermal inversion period, increased wind speed and decreased relative humidity (Figure 4). These conditions allow anthers dehiscence, pollen emission and dispersion [49]. The increase of relative humidity and the decrease of temperature and wind speed reduce the atmospheric turbulence and enhance pollen deposition [50,51], coinciding with low concentrations of airborne pollen during the afternoon and night. Compared to the daily pollen concentration, the peaks of hourly pollen concentration are 5 to 8 times higher meaning. Hourly pollen concentration reveals that the threshold for allergy reaction might be reached during the day, despite the low daily pollen concentration, thus constituting a more representative human exposure and a better predictor of the risk for allergy outbreaks.

Analysing the effect of meteorological factors on intra-diurnal pollen disruption, while atmospheric pressure is linked to a lower disruption ratio, wind speed correlates with a higher disruption ratio, thus higher release of pollen allergens. Interestingly, hourly relative humidity did not correlate with pollen disruption, opposite of the observations on the daily parameters, nor with hourly pollen concentration. As expected, atmospheric pressure and temperature positively correlate with the hourly pollen concentration (Table 5). Wind speed and atmospheric pressure are correlated and vary inversely as lower surface pressure are frequently associated to low pressure systems where pressure gradients are typically more intense. Hence, the correlation of wind speed with disrupted pollen might be a coincidence following the atmospheric pressure changes. The disruption is a momentary event driven by a disequilibrium between osmotic pressure, generated by relative humidity upon hydration, and atmospheric pressure, pollen bursting occurring when the osmotic pressure overcomes the atmospheric pressure. In this sense, the pollen disruption is a function of the opposing effects of relative humidity and atmospheric pressure. During the four days analysed, the relative humidity was either mainly above or below 60% and atmospheric pressure varied between 972 and 988 hPa. Taking into consideration that 60% relative humidity and 980 hPa are critical values for pollen disruption (Figure 4), then the intra-diurnal variation of atmospheric pressure was the determinant factor for pollen burst, hence the lack of correlation with relative humidity. In fact, the day with the lowest atmospheric pressure associated with a high relative humidity presented the highest disruption ratio. On the contrary, on the day with the lowest disruption ratio the relative humidity was below 60%, despite a low atmospheric pressure (below 980 hPa). For the other days atmospheric pressure above threshold level seems to counterbalance the high relative humidity (Table 5 and Figure 6), supporting the findings on daily observations. Despite the low number of events analysed (4 days), hourly observations revealed a relevant interplay between the key weather factors on airborne pollen integrity loss and a wider research should be carried out in the future to further analyse this phenomenon. Furthermore, it suggests that the period of the day between 10 and 16h is commonly the one with higher risk for exposure to Cupressaceae allergens, as observed for other pollen types [10,20,52,53]. To our knowledge this is the first time that this analysis was performed, unravelling new features of the action of the meteorological factors on pollen behaviour in the atmosphere that might be taken into account for the interpretation of its allergenic potency.

Pollen grains can be deposited elsewhere away from the site that gave rise to it. The pollen grains are small in size and are lightweight and its transport in air masses from long distances was demonstrated for several pollen types [25,54–56] and the intra-diurnal pattern of pollen concentration is an indicator of the contribution of local emission and long range transport to the daily pollen concentration [19,20,53]. Although our results suggest that, for the days analysed, the pollen were mostly from local origin, it was shown that Cupressaceae pollen can be transported long distances in air masses, evidenced by the peak detected at night on 26 February 2018 (Figure 6), that was transported by air masses passing by the south of Spain, the Andalucia region where Cupressaceae is abundant [57], as suggested by the back trajectory analysis (not shown here). Hence the contribution of medium and long-range transport of Cupressaceae pollen for daily and hourly pollen concentrations cannot be excluded. Based on our results, pollen disruption associated with this transport was not different from the local pollen, but more data will be needed to confirm this observation.

Although clouds were present in all four days considered, in general it can be seen that the remote sensing observations agree with the pollen concentration intra-diurnal behavior, showing concordant daily patterns and backscatter values proportional to the pollen concentrations, which hints that ceilometer measurements may constitute a real-time proxy of the pollen concentration. Note that the remote sensing measurements are instantaneous, with a time resolution of 10 s, whereas the hourly pollen concentration corresponds to a period of one hour (previous 60 min). The case of 25 February 2017 when very high pollen concentrations were detected, with the hourly pollen concentration presenting a secondary peak at around 10:00 UTC and a primary peak at 13:00 UTC, is a good example. The corresponding ceilometer image shows the highest attenuated backscatter values in comparison to the other three days and although clouds are present from 12:00 UTC on (white color in the image), high backscatter values are detected in the lower layers of the atmosphere shortly after 05:00 until about 12:00, when clouds obstruct the particle backscatter detection. It is noteworthy, that on 25 February 2017, the pollen peak at 13h (Figure 6, upper panel) is consistent with a sudden diminution of solar radiation caused by clouds over the area (Figure 8), which induced changes in atmospheric turbulence and mixing, and a probable decrease of the atmospheric boundary layer height and increase of particles near the collector. In this period of analysis, no precipitation occurred. The temperature increased and the relative humidity was approximately 80% (Table 4). On 27 February 2017 the presence of clouds during the whole day does not allow for the analysis. On 25 (26) February 2018, the increased backscatter between 08:00 and 12:00 UTC (03:00 and 14:00 UTC) agrees well with the corresponding hourly pollen concentration shown in Figure 6. On 25 February 2018, the atmospheric boundary layer shows increased backscatter values shortly after midnight, which may be due to the lower thickness of this layer during nighttime with respect to daytime and the existence of non- biological particles [51].

This analysis agrees with findings of several authors in studies related to the detection of particles from remote sensing instruments, demonstrating that this technique can be used to detect the presence of aerosols, more specifically pollen in the atmospheric boundary layer [50,58,59]. As demonstrated by hourly pollen concentrations (this work and [60,61], the real-time pollen concentration to which the population is exposed during the day, is significantly higher than the daily estimates. Remote sensing combined with back trajectories and the other relevant meteorological parameters may in the future contribute to develop real-time methodologies for the detection of pollen in the atmosphere as well as allergy risk warning systems contributing to improve allergy management strategies.

In summary, the results shown in this paper suggest that relative humidity, rainfall and atmospheric pressure are the main factors affecting airborne Cupressaceae pollen integrity and in conjunction with the pollen concentration may be used to predict the risk of allergy outbreaks to this pollen type.

5. Conclusions

To our knowledge, this is the first report characterizing the considerable fraction of disrupted Cupressaceae pollen grains reaching the sampler, releasing pollen contents. It is expected that this disruption might significantly increase ambient free allergen, contributing to enhance ambient allergenic activity of this pollen type because of higher allergen availability. Moreover, this free allergen, contained most probably in small particles, readily inhaled, and might penetrate in the lower respiratory system thus evoking aggravated symptoms in sensitized individuals.

A better knowledge of the factors affecting pollen disruption of allergenic species will greatly contribute to improve allergy risk management. This work significantly contributes to a better understanding of this phenomenon, where relative humidity, atmospheric pressure and rainfall are key players. Being widely used as ornamental plants, *Cupressus* genera are widely distributed in urban environments or its surroundings, and this work also shows that Cupressaceae pollen was mainly from local origin, thus suggesting for a wise planning of urban gardens and green zones, contributing to diminish the loads of allergenic pollen.

Finally, the real-time pollen monitoring, namely by remote sensing, and the relevant meteorological parameters, may contribute to the development of pollen allergy forecasts and thus to a better management of allergy and asthma outbreaks.

Supplementary Materials: The following are available online at https://www.mdpi.com/1999-4907/12/1/64/s1, Figure S1: Regression analysis of the daily disrupted pollen in relation to the daily pollen in 2017 and 2018 together (**a**) and separately (**b**). The data corresponds to the number of pollen grains counted per day (N), Figure S2: Hourly air mass back trajectories for 25 February 2017; height 100 m–red line, height 500 m–blue line and height 1000 m–green line, Figure S3: Hourly air mass back trajectories for 27 February 2017; height 100 m–red line, height 500 m–blue line and height 1000 m–green line, Figure S4: Hourly air mass back trajectories for 25 February 2018; height 100 m–red line, height 500 m–blue line and height 1000 m–green line, Figure S5: Hourly air mass back trajectories for 26 February 2018; height 100 m–red line, height 500 m–blue line and height 1000 m–green line.

Author Contributions: All the authors actively and significantly contributed to this manuscript, as follows: Conceptualization, A.G. and C.M.A.; methodology, A.G., M.J.C. and D.B.; validation, A.G., A.R.C., C.M.A., M.J.C. and R.A.-J.; formal analysis, A.G., A.R.C., C.M.A., M.J.C. and R.A.-J.; investigation, A.G.; resources, C.M.A., A.R.C. and M.J.C.; data curation, A.G., C.M.A. and M.J.C.; writing—original draft preparation, A.G., A.R.C. and M.J.C.; writing—review and editing, C.M.A., D.B., M.J.C., R.S., R.A.-J.; visualization, A.G., A.R.C.; C.M.A., D.B., M.J.C.; supervision, C.M.A.; project administration, C.M.A., A.R.C. and M.J.C.; funding acquisition, A.R.C., C.M.A., D.B., M.J.C. and R.S. All authors have read and agreed to the published version of the manuscript.

Funding: The work is financed by national funds through FCT—Foundation for Science and Technology, I.P., within the scope of the projects UIDB/04683/2020 e UIDP/04683/2020 and also through projects TOMAQAPA (PTDC/CTAMET/ 29678/2017) and NanoSen-AQM (SOE2/P1/E0569)—EU-INTERREG-SUDOE; COST CA18226 "ADOPT- New approaches in detection of pathogens and aeroallergens" and COST CA18235—"PROBE-PROfiling the atmospheric Boundary layer at European scale". Supported by the European Commission, COST Office AUTOPOLLEN—EIG EUMETNET "Proof-of-Concept for a European automatic pollen monitoring network using high temporal-resolution real-time measurements".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Publicly available datasets of meteorological data were analyzed in this study. These data can be found here: [https://www.icterra.pt/g1/index.php/meteo-data/]; Pollen datasets are accessible either in [https://lince.di.uevora.pt/polen/index.jsp] upon request and within this article.

Acknowledgments: The authors gratefully acknowledge the NOAA Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model and/or READY website (https://www.ready.noaa.gov) used in this publication.

Conflicts of Interest: The authors declare no conflict of interest.

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Dynamic and Behaviour of Plane Tree Pollen and Its Relationship with Pla a 1 Aeroallergen Concentration in Évora, Portugal

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: This work received the support of the Portuguese Foundation for Science and Technology (FCT) through the ICT (Institute of Earth Sciences) project UIDB/04683/2020 (ref. POCI-01-0145-FEDER-007690), the Pollensorb Project (PTDC/ATPEAM/0817/2014), the Spanish Ministry of Science, Innovation, and Universities through the project SICAAP-CPI RTI2018-096392-B-C22, the UCLM through the UCLM Plan Propio, the Castilla-La Mancha Regional Government, the European Social Fund (ESF), and the Youth Employment Initiative (YEI) for the postdoctoral grant for Dr Lara.

Keywords: Airborne allergen, human respiratory allergy, Pla a 1, *Platanus* pollen, pollen allergy in urban environments.

Citation: EMJ Allergy Immunol. 2020;5[1]:xx-xx. Abstract Review No. ARX.

BACKGROUND AND AIMS

Platanus pollen is an important cause of allergy in many cities of western Europe, where this pollen is produced by the plane tree, *Platanus orientalis* L. var. *acerifolia* Dyand (Aiton), which is widely used as an ornamental species in parks, gardens, and other urban green areas,[1] favouring human exposure to its pollen. Its major allergen Pla a 1 is recognised by up 92% of monosensitised *Platanus* allergic patients and 83% of polysensitised patients, allergic to this pollen type, present 60% of IgE to its major allergen Pla a 1.[2] In this study, the authors studied this pollen type and the allergen Pla a 1 in the year 2018 in the atmosphere of Évora, Portugal. The aim was to analyse the aerobiological characteristics of the *Platanus* pollen and to study the relationship between the airborne concentration pollen and the major allergen Pla a 1. Furthermore, the influence of meteorological variables on the airborne concentration of this pollen type was investigated.

MATERIALS AND METHODS

Pollen and allergen sampling were performed using a Hirst-type spore trap (Lanzoni S.r.l., Bologna, Italy) and a high-volume cascade impactor ChemVol® (Butraco, Son, the Netherlands), respectively. Pollen was analysed following the procedure established by the European Aerobiology Society (EAS) and following the approach recommended in Galan et al.[3] Allergens were quantified using a specific ELISA method.[4] The main pollen season was calculated as 95.0% of total annual pollen, obtained after removing 2.5% of the start and end of total annual pollen integral.[5] Meteorological data were obtained from the Atmospheric Sciences Observatory (ICT), University Évora, Évora, Portugal.

RESULTS

The results indicate that the main pollen season of the *Platanus* pollen took place from March 28th to April 20th. The maximum concentration was recorded on April 1st with 619 pollen grains/m^3. There were 16 days of allergy risk (>50 pollen grains/m^3) for people with allergies, of which seven were considered as high-risk level (>200 pollen grains/m^3).[6] Regarding Pla a 1, the temporal profile coincided and a significant relationship between the concentration of airborne pollen and allergen was found (Spearman's R=0.632; p<0.01). The mean pollen potency[7] was 14.4±7.7 pg allergen/pollen.

Table 1: Spearman´s correlation analysis between pollen and meteorological variables considering different time lags.

Lag-time	Temperature maximum (ºC)	Temperature minimum (ºC)	Precipitation (mm)	Relative humidity (%)
t	0.123	0.083	0.027	0.110
t-1	0.211	0.082	0.009	0.184
t-2	0.209	-0.005	0.004	0.142
t-3	0.193	0.173	0.198	0.255
t-4	0.378*	0.333	-0.019	-0.026
t-5	0.485*	0.290	-0.188	-0.133
t-6	0.480*	0.295	-0.174	-0.285
t-7	0.413*	0.008	-0.430*	-0.577*
t-8	0.095	-0.009	-0.208	-0.353*

Lag-time is the number of days before the current day (t).
*p<0.05

The temperature, precipitation, and relative humidity (RH) were the meteorological variables that most influenced the airborne *Platanus* pollen in Évora; maximum temperature occurring 4–7 days prior to pollen release positively influenced pollen loads, while precipitation and RH, particularly 7–8 days prior to pollen release, had a negative influence (Table 1).

CONCLUSION

In summary, these results show that the allergenic load (Pla a 1) coincides with the presence and magnitude of the pollen concentration in the atmosphere. Only the meteorological conditions during 4–8 days prior to pollen release were significant, suggesting that the environmental conditions during the pollen maturation process in the anthers are key factors involved in the *Platanus* pollen and allergen emissions and, thus human exposure to its allergens. Finally, the results suggest that pollen counts are good indicators of the allergenic loads in the atmosphere and, together with meteorological conditions, are useful to design allergen forecasts and alert systems for the allergic population.

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Nota: Este manuscrito não inclui as revisões dos co-autores

Effect of meteorological conditions on Cupressaceae pollen prevalence in Évora and Granada, two cities from Iberian Peninsula

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Abstract

The *Cupressaceae* is considered a significant source of airborne allergens and allergy to its pollen is reported worldwide. The period of pollination for this species is the end of winter and beginning of spring, depending on meteorological factors. The objective of this study was to compare the aerobiology of *Cupressaceae* pollen and to analyse the influence of meteorological factors in the *Cupressaceae* pollination in two different sites: Évora and Granada.

Data were collected in two cities of southwestern Europe (Évora: 38.568542°N; 7.910526°W and Granada: 37.1041°N 3.3555°W). The *Cupressaceae* pollen was monitored using standard Hirst-type traps (2017-2019) and identified by optical microscopy, according to the standard methodology. The meteorological parameters were obtained from ICT/CGE platform and Agencia Estatal de Meteorología (AEMET).

Large concentrations of pollen were detected in both cities, 5-6-fold higher in Granada (Annual Pollen Integral, API, ranging from 2642-7568 pollen/m^3 in Évora and 15868-56593 pollen/m^3 in Granada). The peak date occurred between 25th February and 14th March in both cities and the pollen season duration was similar, ranging from 60-84 days. Granada presented twice the days >100 pollen/m3. During the season, temperature and solar radiation positively correlated with API while precipitation and relative humidity were negatively correlated. Considering meteorologic parameters from the autumn-winter previous to pollen season, maximum and

mean temperatures, as well as global solar radiation, seem to present similar influence over SPI in both cities, and with different trends in Sep-Nov or Dec-Jan months, and these meteorologic parameters might constitute indicators for the prediction of Cupressaceae pollen season intensity.

Introduction

Cupressaceae is the second most important Family of Gymnosperms, especially because of its forest interest. It includes 30 genera and 140 species (Schulz et al., 2005) distributed by temperate or temperate-warm regions of both hemispheres, although almost 3/4 of the species are present in the Northern Hemisphere (Mao et al. 2012; X.-Q. Wang & Ran 2014). The family includes trees or shrubs, sometimes stunted; of wood and leaves often resinous and aromatic, perennial (exceptionally deciduous in three genera), simple, spirally arranged, opposite or whorled, scaly, appressed and less than 1 mm in size, or linear and almost 3 cm in length. The bark it is often fibrous, detaching in long strips on mature trees. The Family Cupressaceae also includes some of the largest, tallest (eg. Sequoiadendron giganteum) and longest-lived (Sequoia sempervirens) plants on Earth (Simpsom, 2010.).

Cupressaceae is a cosmopolitan family, with species that grow in diverse habitats, from humid lands to dry soils, and from sea level to high elevations in mountainous regions (Pitterman et al., 2012.), Showing a high ecological plasticity under restrictive and changing environmental conditions. Although forest formations dominated by Cupressaceae are not frequent, in the Iberian Peninsula some forest formations dominated by the Genus Juniperus still persist, such as “enebrales” and “sabinares” in areas of great continentality, xericidad and in the high mountains (Blanco et al., 1997; Lorite, 2002), or the Araar formations, dominated by Tetraclinis, of which there are hardly any relict populations in the Western Mediterranean (Sánchez-Gómez et al., 2013). In addition to these, various species of Chamaecyparis, Platycladus and especially Cupressus, are widely cultivated in Spain and Portugal for forest and ornamental purposes (López-González, 1986; López-Lillo and Sánchez de Lorenzo Cáceres, 1999).

The great versatility and adaptability of various Cupressaceae species to urban microclimate conditions has facilitated its high presence as an urban tree (Pecero-Casimiro et al., 2020), where it frequently participates in elements of Green Infrastructure (IV) such as hedges, windbreaks, dividing lines, edges, and as components of the plant formations known as Trees outside the Forests (ToF) (Monroy-Colín et al., 2020; Falbitano et al., 2018). This high frequency is favored by the wide range of Ecosystem Services (ES) that the different species provide, such as their resistance to atmospheric pollution, a high mitigation capacity of gaseous and particulate pollutants (Nowak and Heisler, 2010), the high resistance to conditions changing climate and environmental stress (Baldi et al., 2010), the low resources they need for maintenance, the good tolerance to pruning, both thinning and topiary (Hendy and Wooster, 2015), or the soil holding capacity of their systems radical (Samson et al., 2017). To these we must add its close connection to the cultural landscape of Tuscany (Italy) and Provence (France), or to that of historical neighborhoods such as El Albayzin or La Alhambra in Granada (Spain) (Casares-Porcel, 2010; Moreira et al., 2006). Its use as a funerary tree is also notable, frequently linked to cemeteries, abbeys and medieval monasteries, where its sober needle-shaped bearing symbolizes the connection between earth and heaven (Farahmand, 2019).

In contrast to these ES, Cupressaceae species are also the cause of some Ecosystem Disservices (ED) (Lyytimäki and Sipilä, 2009; Cariñanos et al., 2017). Recently, several of the ornamental species are being attacked by both arthropod (Trisettacus pini cupressi Andre.) and fungal (Cupressobium cupressi) pests, parasites of different nearby genera, which are causing high mortality both in populations and in isolated individuals (Muñoz and Rupérez, 1980) The high content of essential oils in most species, in addition to extensive ethnobotanical use as a larvicide, acaricide, against diptera, or in folk medicine as an antiseptic, anti-inflammatory, antifungal or antibacterial, have a composition in which Biogenic Volatile Organic Compounds (BVOCS), of the type of monoterpene hydrocarbons (α-pinene, sabinene, myrcene), are abundant (Ibrahim et al., 2017; Owens et al., 1998), highly reactive in the formation of ozone (Paoletti, 2009). But if Cupressáceas are notable for a negative effect, it is due to the high allergenic capacity of their pollen grains (Charpin et al., 2005; Charpin et al., 2019). The high pollen production of all the members, derived from its anemophilous pollination strategy (Hidalgo et al., 1999; Hidalgo et al., 2003; Aboulaich et al., 2008), the presence of several allergenic molecules in its pollen grains (Shahali et al., 2010; Charpin et al., 2005), and its long flowering period (Díaz de la Guardia et al., 2006; Caeiro et al., 2020), position Cupressaceae pollen as one of the main allergens in the Mediterranean region, with figures of a 30% incidence in the population. It is also noteworthy that the sensitivity to pollen of Cupressaceae has been increasing in recent years, (Dominguez-Ortega et al., 2016; Pahus et al., 2018) and that the symptoms presented by sensitive patients is rated by specialists as severe, aggravated by the prevailing winter conditions during their pollination (Charpin et al., 2005; Charpin et al., 2019).

Cupressaceae have always been considered a typically winter flowering family, however, the high number of species it contains and the wide range of distribution it presents, makes this type of pollen to be recorded in aerobiological samplings for an extended period of time (Belmonte et al., 2000). This gives an idea of the enormous ecological plasticity of some of the genera of this family, adapted to highly variable climatic conditions, from very low temperatures, strong winds and snow in winter, to high temperatures and great summer mercy. Thus, in the case of Juniperus ashei (Mountain cedar), the main allergen in some southern states of the USA, low temperatures and winter rain usually inhibit the release of pollen (Leventin et al., 2011); while maximum temperatures, high RH and high wind speed are significant variables that favor the presence of pollen (Bunderson et al., 2014). For Cryptomeria japonica (Japanese cedar), the main allergenic Cupressaceae in Japan, high mean temperatures during the previous summer promote the formation of male cones and the differentiation of pollen (Ishibashi and Sakai, 2019). The Cupressus genus, one of the most represented in the Mediterranean region, has shown a great correlation with mean temperatures, peak temperatures, insolation, and cumulative precipitation (Malaspina et al., 2007; Díaz de la Guardia et al., 2006; Aboulaich et al.,2008). In addition to these meteorological variables, the pollen levels of Cupressaceae will also be dependent on other more local factors, such as the density of individuals of this family in the radius of influence of the sampler, as well as the orography of the terrain that such influence will have in microclimatic conditions (Katz et al., 2019). This generates that the pollen levels of very close locations can be very different (Belmonte et al., 2000; Caeiro et al., 2020).

In this work we have investigated the aerobiology of Cupressaceae pollen season on years 2017, 2018 and 2019 at Évora and Granada, two locations in the south of the Iberian Peninsula with different topographic and microclimatic conditions, and the studying the influence of meteorological conditions, maximum, minimum and mean temperature (°C), precipitation (mm), relative humidity (%), cumulative global solar radiation (KJ/m2), wind speed (m/s) and direction (°) and atmospheric pressure (AtP, hPa) on DPC (Daily Pollen Concentration), SPI (Season Pollen Integral) and pollen season duration (number of days).

We have also evaluated the influence of the preceding autumn weather conditions on the SPI in the following pollen season between the years 2017-2019 at Evora and Granada, representative of long-term effect of the weather conditions on the plant physiology.

Methods

Study area

Évora is characterized by a temperate climate with warm and dry summers that can be described as a Hot-summer Mediterranean climate (Köppen Climate Classification—Csa). According to the climatological normal (1981–2010) provided by the Portuguese Institute for Sea and Atmosphere (IPMA, 2020), the annual mean air temperature is 16.5°C, with the highest value occurring in August (24.1°C) and the lowest in January (9.6°C). The rainfall period, which occurs mostly seasonally between October and April, presents annual precipitation of 585.3 mm. The sampler was placed at the Évora Atmospheric Sciences Observatory (EVASO), located in the roof-top of the Science and Technology School of the University of Évora, about 10 m above the ground. The facility is located in the city center, surrounded by trees and buildings, with low road traffic in the neighborhood (Fig. SI-1). The most common pollen species in Évora are Cupressaceae, *Platanus* spp, *Quercus* spp, *Olea* spp and Poaceae. Other species are found but in minor concentrations as Rumex, Populus, Urticaceae, Chenopodium, Alnus, Acer, Casuarina, and Eucalyptus (IPMA, 2020).

Granada is classified as a Csa region according to the Köppen climate classification, similarly to Évora. The annual mean air temperature is 15.7 °C, with the highest monthly value occurring in July (26.0 °C) and the lowest in January (6.8 °C). The rainfall period, occurring mostly between autumn and spring, presents an average annual precipitation of 352 mm (www.aemet.es). Granada's weather conditions are strongly conditioned by its proximity to Sierra Nevada, one of the highest mountain ranges in Europe, which is just 40 km from the city. The influence of the Sierra is very significant in the pattern of valley-mountain winds (catabatic winds), and in the frequent thermal inversions that occur during winter, where cold air is trapped in the lowest layers of the atmosphere, and with he a high concentration of both biotic and abiotic contaminants (Clements, 1999; Zamora & Pérez-Luque, 2016). The most common pollen types in Granada are *Olea europeae* with 35.5% of total pollen, Cupressaceae represent in 30% of total pollen, however, *Acer* spp, *Pinus*, *Platanus* spp, Poaceae, *Quercus* spp, Urticaceae e *Populus* spp, represent, individually, <10 % of total pollen (Cariñanos et al., 2016).

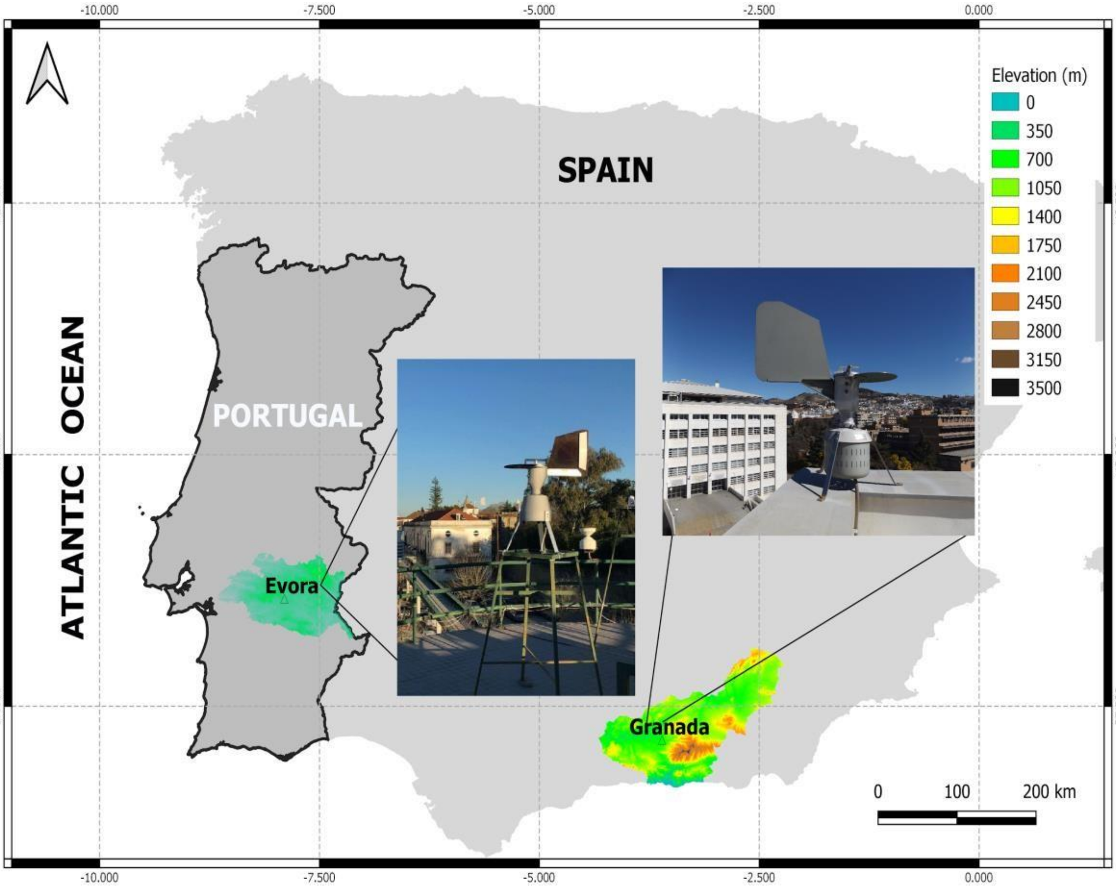


Figure 1: Maps of Évora and Granada (QGIS).

Airborne pollen sampling

Airborne Cupressaceae pollen was collected With Hirst-type volumetric spore trap (Hirst, 1952) at EVASO (Figure 1), located on the roof of a building belonging to the University of Évora, approximately 10 m above ground level, since the 22 February 2017. The aerobiological methodology followed in Granada was the same as in Evora, for a sampler located at the Faculty of Sciences, Fuentenueva Campus of the University of Granada (37 ° 10 ’50’ ’N, 3 ° 36’ 31’’W) (Figure 1).

Airborne pollen sampling was done using a Hirst-type 7-day volumetric spore trap (Hirst, 1945) that is a one stage impactor, design to sample air at a rate of 10 L/min, simulating the human inhalation. By passing through a narrow intake orifice (2x14mm) the sampled air impacts onto a clock driven drum rotating at an angular velocity of 2mm/h, taking 7 days to perform a complete turn. The drum is covered with a double-sided Melinex tape, where airborne pollen is retained (Galán et al., 2007). In our study, the Melinex tape was coated with a double-sided adhesive carbon tape, aiming at preserving the integrity of the pollen wall and allow the following analysis.

After exposure this tape is cut into seven 48mm long segments, representing each one day of sampling, that are mounted on a microscopic glass slide.

The main pollen Season (MPS) was determined by the method of percentage described for Nilsson and Persson, 1981, based on the elimination of a 2.5% percentage in the beginning and end of the pollen season (Nilsson and Persson, 1980; Andersen, 1991).

Pollen calendar

For the creation of the pollen calendars, the Aerobiology Package (Rojo et al., 2019) was used. With several hypotheses of pollen calendars, the authors decided to follow the *Violinplot* method. This pollen calendar is based on pollen intensity and adapted to the pollen calendar published by ORouke, 1990. Firstly, the daily averages of pollen concentrations are calculated, and these averages are represented using the *Violinplot*. It shows a relative comparison between the pollen intensity of the pollen types without scales and units. Average values below the level of the *th.pollen* argument will be removed from the pollen calendar (Rojo et al., 2019).

Seasonal Pollen Integral (*SPIn*)

SPIn is calculated by the sum of daily pollen concentrations on pollen season of Cupressaceae (Gálan et al., 2017). For Cupressaceae, being its pollen season between late winter and early spring, it was started in November/December for the two regions. In our study, the function of *calculate_ps* was used to calculate the main characteristics of the pollen season of Cupressaceae. The interval was used, to calculation SPIn, from October 1 to the end of May as reference.

Meterological parameters

The meteorological parameters used for Évora were obtained from the meteorological station installed at the Évora Atmospheric Sciences Observatory (EVASO) where the pollen sampler is also installed. The parameters measured were the air Temperature (°C), Relative Humidity (RH; %), Wind Speed and Direction (m/s; °). All variables were measured in 10 s intervals, and then averages were performed to originate hourly and daily data, except for precipitation that is accumulated during those periods. 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 December 21, 2020). All instruments are subject to periodic checks and maintenance. The time periods to evaluate the effect of the previous autumn were selected according to phenology of plants of *Cupressaceae*, where described the 5 phenophases of Cupressaceae (Hidalgo et al, 2003; Bouziane et al, 2008; Malaspina et al, 2007).

Statistics Analysis

The data did not follow a normal distribution, non-parametric tests were used. Spearman´s correlation analysis (significance level of 5% and 1%) was used to study the relationship between meteorological parameters of maximum, minimum, and mean air temperatures, precipitation, RH, global solar radiation, wind speed and direction and AtP with daily pollen concentration (DPC/m3). The pollen season was divided in two parts, the first one, before peak period and second one after peak period. Statistical assessment of differences was performed using KrusKal-Wallis (p< 0.05 was accepted) (Zar, 2007). Graphs were produced using Origin Pro soft-ware and Rstudio Software (OriginLab corporation and RStudio (Rstudio.com). The principal component analysis (PCA) was applied to evaluate the association between the DPC in the air with the meteorological parameters (Johnson and Wichern, 2002).

The variables were adjusted to Generalized Linear Models (GLM) using the Poisson distribution and the logarithmic link function. Various models were created and the most appropriated one was chosen to the Akaike Information Criterion (AIC) and deviance (D2). The multicollinearity of variables was studied and those with VIF values greater than >5 were discarded. Then the variables with the greatest influence were selected and therefore those that most influence these parameters (Ian M. Smith., Cook, & Smith., 2001). The GLM model was used for creating a model that better explains the relationship between pollen concentrations in the air and the meteorological parameters on pollen season of Cupressaceae for Évora and Granada.

Results and discussion

1. Characterization of the pollen season in Évora and Granada 2017-2019

Cupressaceae pollen is detected in large concentrations in the atmosphere of Évora and Granada during the winter-spring seasons, particularly in the months of February and March. For 2017 in Évora, the pollen monitoring began on 22 February, just in the middle of the Cupressaceae pollen season, but still in time to record its peak day (February 25), and the pollinic season ended on March 22. In 2018 and 2019 it was already possible to follow the entire pollinic season for Cupressaceae in Évora, which, for 2018, began on December 22 of the previous year and ended on April 3 and which, in 2019, began on the last day of December 2018 and lasted until March 12. In Granada, in the years 2017, 2018 and 2019, the pollen seasons also began in the final months of the previous calendar year, on 12 December 2016, on 23 November 2017 and 16 November 2018, respectively, having lasted until 24 April 2017, 28 April 2018 and 26 March 2019 respectively (Fig 2 and table 1).

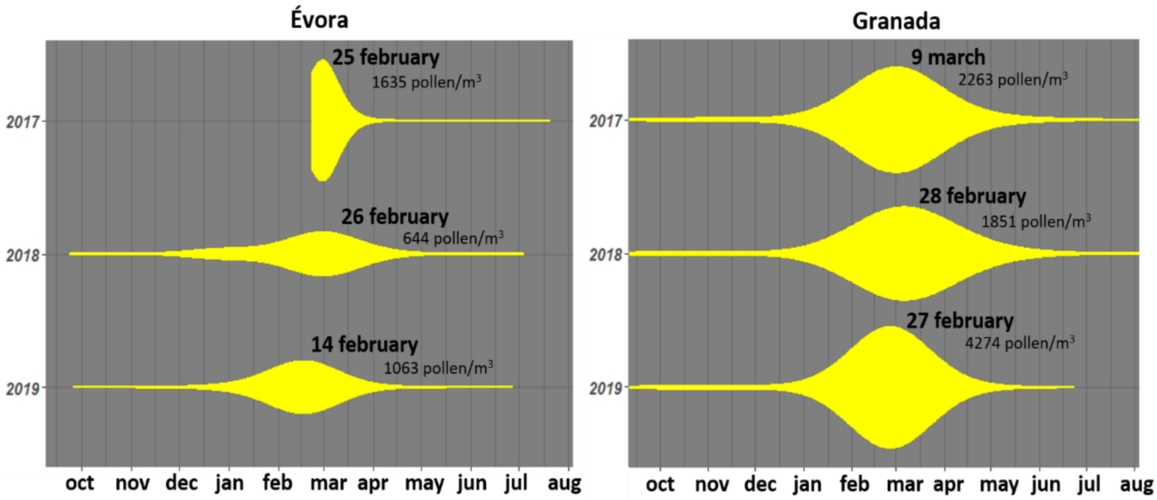


Figure 2: Pollen seasons in Évora and Granada in 2017-2019.

The SPI at Évora in 2018 and 2019 reached 3053 pollen/m3 and 7824 pollen/m3, respectively. In 2017, the total pollen in the period evaluated was 5993 pollen/m3, which was higher than SPI in 2018. In Granada the samplings yield 25580 pollen/m3, 16654 pollen/m3 and 57968 pollen/m3 for 2017, 2018 and 2019, respectively. Peak date for Evora was on February 25, 26 and 14 for the three years respectively and for Granada was March 9 and February 28 and 27, respectively (table 1).

Table 1: Characterization of the pollen seasons 2017-2019 in Évora and Granada.

	Évora			Granada		
	2017	2018	2019	2017	2018	2019
Start-date	<24-02-2017	22-12-2017	31-12-2018	12-12-2016	23-11-2017	16-11-2018
End-date	22-03-2017	03-04-2018	12-03-2019	24-04-2017	28-04-2018	26-03-2019
Duration of pollen season	>27*	103	72	134	157	131
Seasonal pollen integral, SPI	>5993*	3053	7824	25580	16654	57968
Mean Daily Pollen (Pollen/m3)	*	28	109	186	106	443
Peak-date	25-02-2017	26-02-2018	14-02-2019	09-03-2017	28-02-2018	27-02-2019
Peak value (pollen/m3)	1635	644	1063	2268	1851	4274
Pre-peak pollen integral (pollen/m3)	> 1744	1957	4094	18850	6941	35274
Post-peak pollen Integral (pollen/m3)	4175	1096	3729	6730	9713	22694
Number of days >100 pollen grains/m3		8	24	35	30	57

*Incomplete Pollen season (Beginning monitoring on 22 February 2017)

The year 2018 presented the lowest amount of Cupressaceae pollen, both in Évora and in Granada, which can be shown either by the SPI and by the concentration of pollen on the peak day. However, despite presenting a lower pollen concentration, 2018 presented a longer pollen season (in Évora only possible to compare with 2017). In addition, the post-peak period, which can be analysed from the two localities in the three years, was also longer in 2018, compared to 2017 and 2019 (25, 36 and 26 days in 2017, 2018 and 2019 for Évora, respectively, and 46, 59 and 27 days for Granada, for the same years). Also, the number of days with pollen > 100 pollen grains/m^3, considered days of high allergenic risk for this pollen type (“REA,” n.d.), was lower in 2018 in both localities (table 1).

Summarizing, Granada presented higher Cupressaceae pollen peak and higher SPI than Évora, in the years 2017-2019. Accordingly, the number of days with high allergenic risk associated with this pollen type was larger for each year in Granada, comparatively to Évora. Nevertheless, similar Cupressaceae pollen season duration, and a longer season in 2018, was observed in both cities.

Next, the influence of meteorological parameters on the Cupressaceae pollen season and amount of pollen, both in Évora and in Granada, will be studied. For this, we will begin describing the main characteristics of the meteorological parameters during the Cupressaceae pollen season for both localities, in the three years under study.

2. Effects of meteorological conditions during the Cupressaceae pollen season on daily Cupressaceae pollen concentration in Évora and Granada in 2017 – 2019

Seasonal average T_{max} during the Cupressaceae pollen season was similar of the years 2017, 2018 and 2019 (17.83±3.26 °C for Évora and 16.33±4.36 °C for Granada, average values for the 3 years), but T_{mean} and T_{min} tended to be lower in Granada, 9.36±3.36 °C and 2.52±3.52 °C, respectively. Precipitation was similar in almost every year, except in the year 2018, that presented more days with rain and with greater intensity, both in Évora and Granada with 433.2 mm and 368.8 mm, respectively.

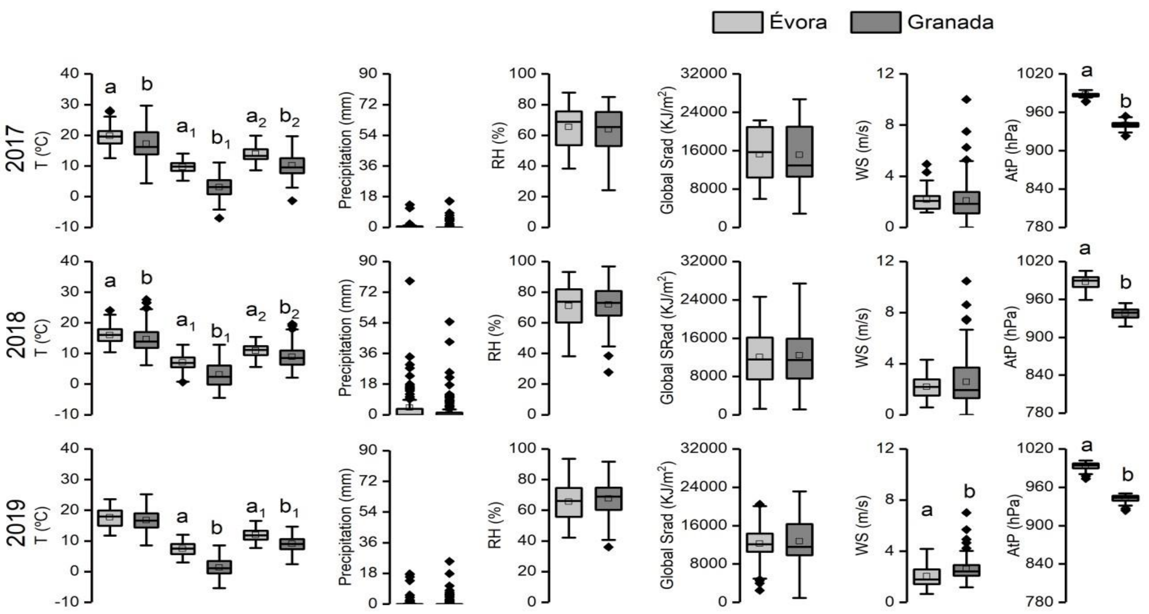


Figure 3: Bioclimatic characterization in pollen season of Évora and Granada in the years 2017, 2018 and 2019. Meteorological conditions of Temperature (T), Precipitation (mm), Relative Humidity (RH, %), Global Srad (KJ/m^2), Wind Speed (WS, m/s) and Atmospheric pressure (AtP, hPa).

The relative humidity and the global Srad were similar in both cities for all years (RH: 67.32±13.58 for Évora and 67.76±11.99 for Granada; Global Srad: 13155,61±4994,26 KJ/m^2 for Évora and 13393,91± 5976,10 KJ/m^2 for Granada). The wind intensity in Évora was lowest (2.14±0.84 m/s) compared Granada (2.42±1.36 m/s). Wind direction in Évora was mostly between Southwest and Northeast (SW-NE) for 2017 and between South-Northwest for the year 2018 and 2019. In Granada, the wind direction was mostly between the South-West and WestNorth coordinates (S-W; W-N) for 2017 and 2018. In 2019, the winds were mostly from a Southeast-South direction (SE-S). Average atmospheric pressure (AtP) was higher in Évora than in Granada (989.21±6.44 hPa, and 940.13± 6.24 hPa, respectively) (figures 3 and 4).

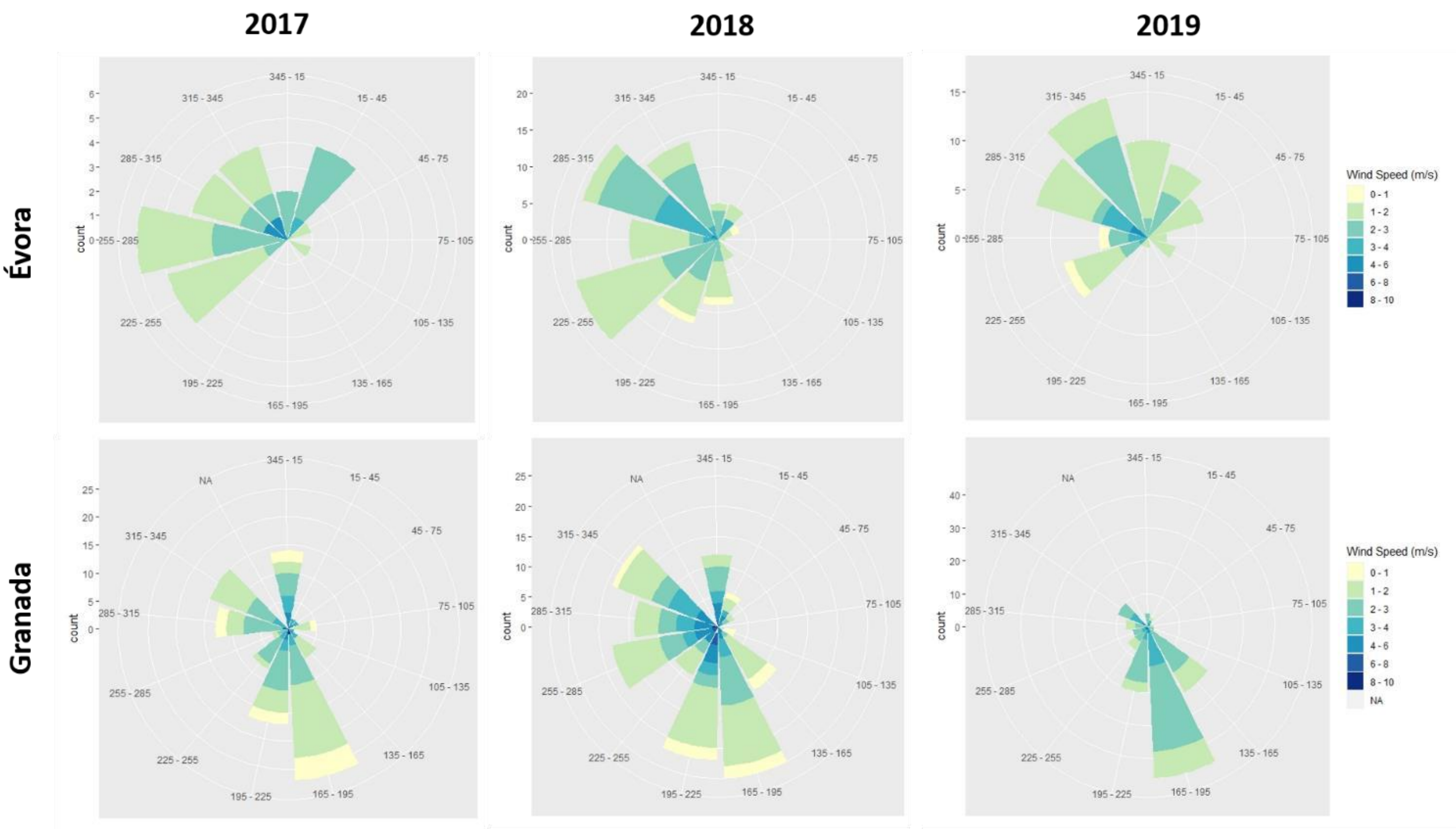


Figure 4: WS (m/s) and WD (°) during pollen season in Évora and Granada.

Concerning the meteorological parameters during the pollinic season for each year, whose daily variations can be observed in figure 5, additional differences between years and cities can be addressed. The precipitation, in Évora, in 2018, occurred in several days in the period under analysis (44/92 days), contrarily to 2017 when precipitation only occurred in 6/26 days and to 2019, occurring in 15/52 days. In Granada, in the years 2017, 2018 and 2019, precipitation occurred in 17/57 days, 41/94 days, and 12/85 days, respectively (Figure 5). Thus, 2018 presented the highest number of rain days and higher levels of precipitation in both cities, as already observed in figure 3.

The atmospheric pressure (AtP) was mainly higher in Évora than in Granada. Considering the values of the three years, in the evaluated period, jointly, AtP ranged from 958.3 to 1005.1 hPa and from 916.9 to 949.2 hPa in Évora and Granada, respectively. Days with lower AtP tend to be associated with precipitation occurrences (figure 5).

The daily maximum temperature varied between 10.4-26.9 and 4.4-29.7 °C in Évora and Granada, respectively, considering the studied periods of the three years together, showing, as already observed in figure 3, that maximum temperatures in this period of the year are similar between the two cities. Additionally, the daily variations in the maximum temperature are also not very different between years, nor throughout the pollen season of each year, except for 2019 in which, both in Évora and in Granada, there seems to occur a progressive increase for daily maximum temperatures in the period studied (Figure 5).

Considering the time periods of the three years together, the daily medium values vary between 0.6 – 14.02 °C and -7 – 12.8 °C in Évora and Granada, respectively. Regarding the minimum temperature, this tends to be lower in Granada in the period studied, in any of the years (as already shown in figure 3), which means that in this last city, the thermic amplitude tends to be larger. The year that presented an average minimum temperature lowest was 2018 and 2019, in Évora and Granada, respectively (figure 5).

The global solar radiation (global Srad) in both cities, tended to increase during the pollen season in any of the years studied. In Évora, global Srad ranged from 5927- 22322, 1242 24640 KJ/m^2 and 2516 – 21831 KJ/m^2 in 2017, 2018 and 2019, respectively. In Granada, it varied between 2850 – 26690 KJ/m^2, 1820 -29320 KJ/m^2 and 910 – 24990 KJ/m^2 in the same years. The year 2018, having presented many days with rainfall, is the one that presents the most days with lower values of global Srad, in both cities.

The daily RH varied from 50% to 90% in Évora for all years, and from 40% to 90% in Granada (Figure 5). For both cities, the year 2018 presented the higher % RH with 70.32 and 69.77 % for Évora and Granada respectively. For the years 2017 and 2019, for both cities, the % of relative humidity was <65%.

The wind speed was usually below 4 m/s in all years except for a week from 11 to 18 March 2017 when the wind speed reached 5 m/s, in Évora. In Granada, the wind speed tended to be more intense, with several days in each year with wind seed exceeding 5 m/s and reaching 8 m/s one day in 2017 and two different days in 2018 (Figure 5).

In summary, with regard to variations in meteorological parameters there are no significant differences in maximal and mean temperatures, solar radiation or relative humidity between sites, although minimal temperature tended to be lower in Granada, which can be due to the effects of Sierra Nevada, with cold catabatic winds during the nights (Pérez-Palazón et al., 2015). Additionally, the weather was dryer in Evora, showing lower amount of rain in the three years; nevertheless, 2018 was rainier than 2017 and 2019 in both places (fig 3 and 5) which can have contributed to the different characteristics of Cupressaceae pollen season (duration and pollen concentration) observed in both cities.

Regarding daily Cupressaceae pollen variations between 2017-2019, the daily pollen concentration (DPC) tends to be higher in Granada compared to Évora, as already suggested by the seasonal pollen integral (SPI) and peak pollen concentration for both cities, previously described. In 2017, only one day with pollen above 1000 pollen/m^3, in the studied period, was identified in Évora, occurring in Granada 6 days with these characteristics (Figure 5). In 2018, the year with the lowest pollen levels for both cities, daily pollen concentration never reaches 1000 pollen/m^3 in Évora, however, in Granada, it was possible to identify 5 days with daily pollen concentrations between 1000 and 2000 pollen/m^3 (Figure 5). In 2019, in Évora, two days with daily pollen concentration close to 1000 pollen/m^3 (bellow 1500 pollen/m^3) were identified, and in Granada there were 20 days with pollen above 1000 pollen/m^3, and on five of these days the daily value was even above 3000 pollen/m^3. Furthermore, on the peak day, 4000 pollen/m^3 were exceeded in Granada in 2019 (Figure 5).

With the aim of determining the effect of the meteorological parameters in the daily pollen concentration (DPC) of Évora and Granada, before and after peak, a non-parametric Spearman´s correlation was conducted.

The maximum and mean daily averaged temperatures (Tmax and Tmean) presented a positive correlation with the DPC, either in the period before the pollen peak, or in the post-peak period, in both cities, being the strongest associations observed for the Tmax parameter with the prepeak pollen levels (0,617 and 0,571, for Évora and Granada, respectively) (table 2). Tmin did not show association of DPC in the pre-peak pollen levels (table 2).

Global solar radiation (Global Srad) correlates positively with DPC in both cities, during the prepeak period, with the strongest correlation in Évora (0,427 and 0,258 in Évora and Granada, respectively). This parameter is not associated with DPC in the post-peak period (table 2).

The correlations between DPC and relative humidity (RH) and atmospheric pressure (AtP) were only significant in Granada, presenting contrary variations (a negative association with HR and positive with AtP), both before and after the peak, with stronger correlations in the post-peak period (RH: -0,222 and -0,288; AtP: 0,213 and 0,433; for pre- and pos-peak, respectively) (table 2).

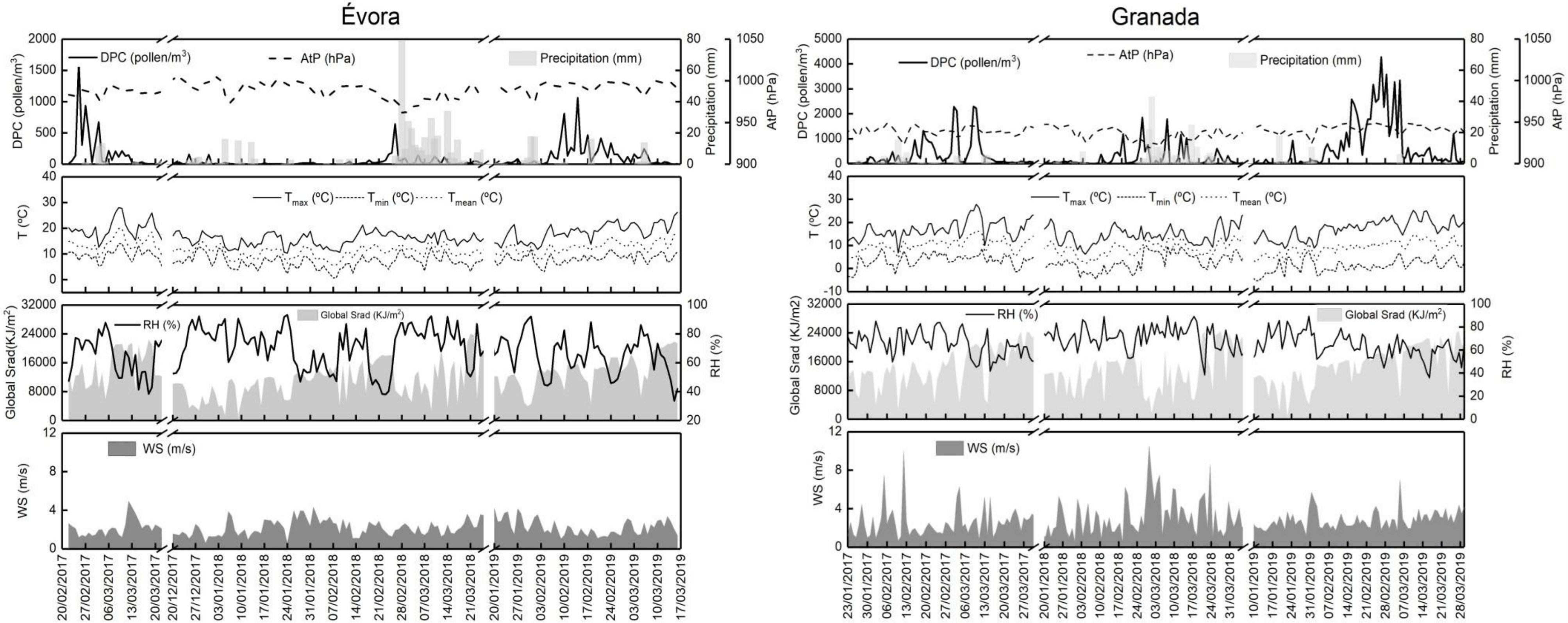


Figure 5: Time series of daily pollen concentration (DPC) and meteorological conditions for 2017-201 9. Pollen seasons for two sites, Évora and Granada. Daily temperature (T_{max} (°C); T_{min} (°C);T_{mean} (°C)), Precipitation (mm), average daily RH (%), daily cumulative Global Srad (KJ/m2) and AtP (hPa).

Table 2: Spearman's correlation coefficients between daily meteorological conditions (daily averaged T_{max} (°C), T_{min} (°C), T_{mean} (°C) and RH (%); daily cumulative Global Srad (KJ/m^2) and precipitation (mm) and daily pollen concentration at Évora and Granada for the years 2017, 2018 and 2019. For each year, the pollen season was divided into two parts: beginning of the season to the peak (Before peak) and after peak to its end (After peak). *Correlation is significant at the 95% range (p<0,05); ns – no significative correlation.

		Évora	**Granada**
Before peak	T_{max} (°C)	**0,617***	**0,571***
	T_{min} (°C)	ns	ns
	T_{mean} (°C)	**0,492***	**0,450***
	Precipitation (mm)	ns	ns
	RH (%)	ns	**-0,222***
	Global Srad (KJ/m^2)	**0,427***	**0,258***
	WS (m/s)	ns	ns
	WD (°)	ns	**-0,363***
	AtP (hPa)	ns	**0,213***
After Peak	T_{max} (°C)	**0,269***	**0,522***
	T_{min} (°C)	**0,217***	ns
	T_{mean} (°C)	**0,256***	**0,420***
	Precipitation (mm)	ns	**-0,262***
	RH (%)	ns	**-0,288***
	Global Srad (KJ/m^2)	ns	ns
	WS (m/s)	ns	ns
	WD (°)	**-0,199***	ns
	AtP (hPa)	ns	**0,433***

Furthermore, a principal component analysis (PCFA) was conducted to better understand the influence of the meteorological parameters in the DPC in both cities. The purpose of this analysis is to obtain a small number of linear combinations, of selected variables, that allow to explain the variability of the data.

Four components have been extracted for each city, before and after the pollen peak, since they had eigenvalues greater than 1, and they account for together between 71% and 81% of the variability in the original data (supplementary material- S1/S2). PC1, alone, is responsible for about one third of the variability in the data (30% - 41% of data variability) (supplementary material-S1/S2). Loadings of PCFA for each component are showed in table 3, and it can be observed that, before Cupressaceae pollen peak, for both cities, the variables which contributed most to the PC1 were T_{max}, Precipitation, RH and Global Srad (table 3). For Évora, T_{mean}, is also important, and for Granada, T_{min} and AtP also have significative scores. For the period after the peak, contribute the variables T_{max}, RH and AtP in both cities, and Global Srad and T_{mean}, are included in Évora group, and PP in Granada group of variables (table 3).

Table 3: Loadings of principal components of before and after peak in Évora and Granada, obtained of principal components analysis. In bold values are significant (>0.300).

		Évora				Granada			
		PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Before peak	T_{max} (°C)	**0,537**	0,127	-0,047	0,098	**-0,324**	**0,453**	0,172	-
	T_{min} (°C)	0,167	**0,613**	-0,068	0,214	**0,359**	**0,396**	0,264	-
	T_{mean} (°C)	**0,411**	**0,447**	-0,074	0,174	0,047	**0,590**	**0,307**	-
	Precipitation (mm)	**-0,313**	**0,403**	0,111	**-0,383**	**0,373**	0,124	-0,258	-
	RH (%)	**-0,381**	0,228	**-0,421**	0,099	**0,371**	-0,104	**0,431**	-
	Global Srad (KJ/m²)	**0,391**	-0,244	**0,401**	-0,086	**-0,461**	0,064	-0,102	-
	WS (m/s)	-0,151	0,173	**0,584**	0,250	0,238	0,274	**-0,505**	-
	WD (°)	-0,233	0,073	0,224	**0,469**	0,095	-0,190	**0,459**	-
	AtP (hPa)	0,035	**-0,313**	**-0,395**	**0,556**	**-0,402**	-0,083	0,265	-
	DPC (pollen/m³)	0,203	0,036	-0,299	**-0,399**	-0,217	**0,377**	-0,086	-
After peak	T_{max} (°C)	**0,484**	0,108	0,105	0,068	**0,432**	**0,305**	0,013	-0,034
	T_{min} (°C)	0,292	**0,446**	0,292	0,229	-0,205	**0,601**	0,243	-0,234
	T_{mean} (°C)	**0,440**	0,295	0,220	0,1683	0,283	**0,594**	0,141	-0,155
	Precipitation (mm)	-0,285	0,285	**0,476**	-0,090	**-0,384**	0,132	-0,049	0,118
	RH (%)	**-0,404**	0,284	-0,048	**0,379**	**-0,398**	0,066	-0,190	-0,121
	Global Srad (KJ/m²)	**0,355**	**-0,412**	0,154	-0,206	**0,403**	-0,184	0,088	-0,189
	WS (m/s)	-0,018	**-0,432**	**0,505**	-0,058	-0,178	-0,068	**0,745**	0,222
	WD (°)	-0,104	**-0,361**	-0,004	**0,635**	0,044	**-0,307**	**0,553**	-0,281
	AtP (hPa)	**0,319**	-0,090	**-0,426**	**0,370**	**0,417**	-0,105	-0,067	0,079
	DPC (pollen/m³)	0,064	0,211	**-0,408**	**-0,419**	0,121	0,163	0,088	**0,849**

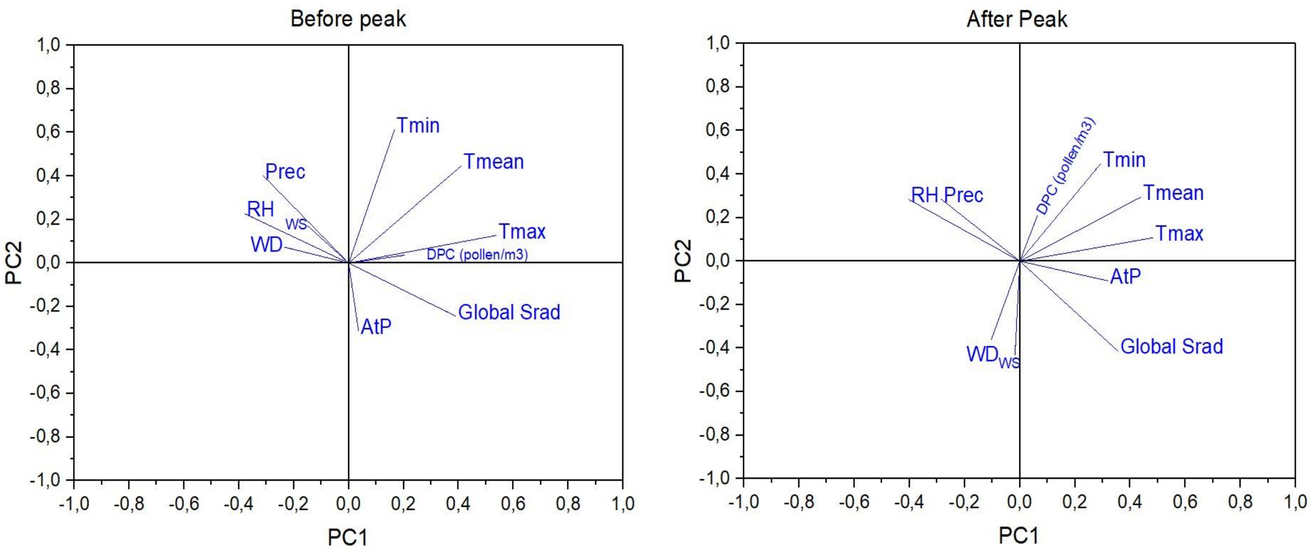
For the second component, PC2, for Évora, the main variables were, T_{min}, T_{mean}, precipitation, AtP (before the peak) and T_{min}, Global Srad, WS, WD (after the peak), that represented,

22% and 20% of variability of data, respectively. For Granada, before the Cupressaceae pollen peak, the variables that contributed most to the second component (explaining 24% of variability of data) were temperatures and DPC, and after-peak (explaining 17% of variability of data) were temperatures and WD (Table 3 and S2).

PC3 and PC4 present a data variability of less than 17% for each situation (Supplementary material-S1/S2).

To better understand the relationship between DPC and meteorologic parameters, a plot with the PC explaining the higher variability of data, PC1 vs PC2, was performed, and can be visualized in figure 6.

A B



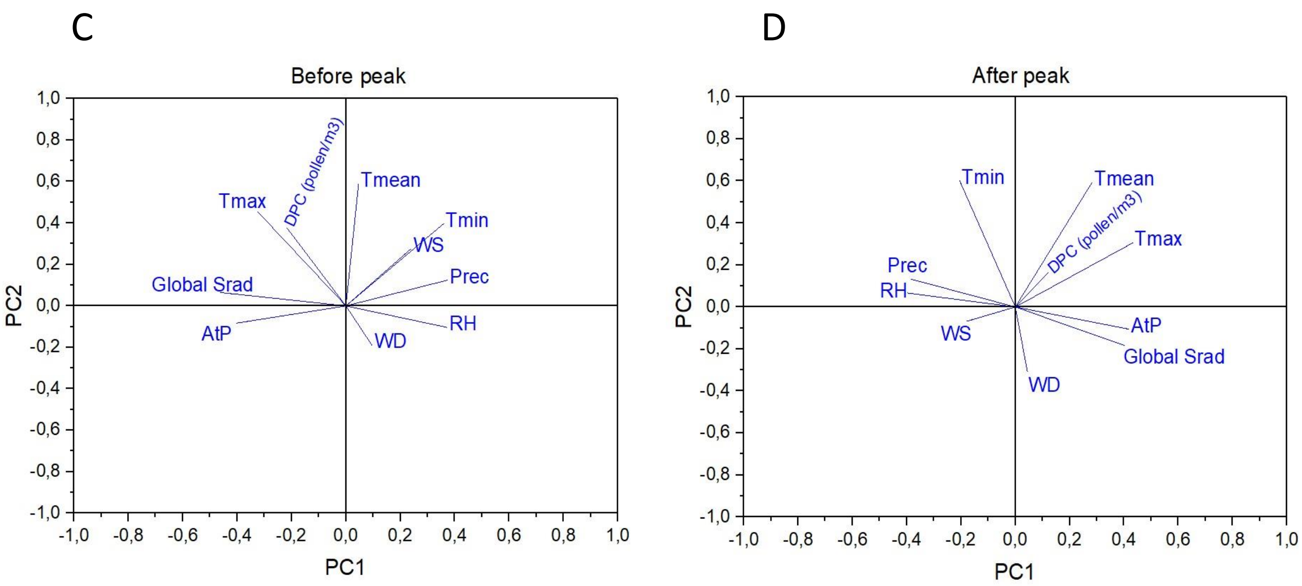


Figure 6: Diagram of the contribution of each variable for PC1 and PC2 in Évora (A and B) and Granada (C and D).

In Évora, before Cupressaceae pollen peak, DPC (pollen/m^3) is positively associated with T_{max}, T_{mean} and Global Srad and is negatively associated with precipitation, RH, WS and WD after peak. T_{max}, is the parameter with a strong influence.

In Granada, before peak, T_{max}, is also the parameter with the strongest influence over DPC, which is also positively associated with Global Srad and AtP, and negatively associated with Rain, RH, WS, WD and T_{min}.

After peak, in Évora, DPC is positively associated with T_{min}, T_{mean}, T_{max}, Global Srad and AtP, but is negatively associated with precipitation, RH and WD. In Granada, for the same period, DPC is also positively associated with T_{mean}, T_{max}, Global Srad and AtP, and is negatively associated with Rain and RH, and unlikely observed for Évora, DPC is negatively associated with WS and T_{min} (figure 6).

Table 4: GLM analysis for DPC and meteorological in pollen season of Évora and Granada. Coefficients and significance levels of the GLM between DPC and Meteorological Data. T_{max}: maximum temperature (°C); T_{min}: minimum temperature (°C); T_{mean}: mean temperature (°C), cumulative Precipitation (mm), Relative Humidity (%): RH, cumulative Global Solar radiation (KJ/m2): Global Srad and AtP (hPa).

	Variables	**Estimation**	**Std.Error**	**Z value**	**Pr(>|z|)**	**Signif. codes**
Évora	Precipitation	-0.036318229	0.002008417	-18.08	<2e-16	***
	RH	0.028763955	0.000961972	29.90	<2e-16	***
	Global Srad	0.000072229	0.000002261	31.94	<2e-16	***
	WS	-0.740743470	0.011585283	-63.94	<2e-16	***
	WD	-0.002615990	0.000086098	-30.38	<2e-16	***
	AtP	-0.027232555	0.001214872	-22.42	<2e-16	***
	AIC: 32042 **Null deviance:** 40172 **Residual deviance:** 31069 **D²:** 22.66% ***(< 0.001)					
Granada	Tmax	0.2375076876	0.0015025511	158.070	<2e-16	***
	Tmin	-0.0927578215	0.0013771456	-67.355	<2e-16	***
	Precipitation	0.0037004997	0.0010197400	3.629	0.000285	***
	RH	-0.0048338503	0.0004193750	-11.526	<2e-16	***
	Global Srad	-0.0001330235	0.0000009825	-135.390	<2e-16	***
	WS	0.2946208051	0.0025650349	114.860	<2e-16	***
	WD	-0.0016643514	0.0000463600	-35.901	<2e-16	***
	AtP	0.0649601817	0.0007828635	82.978	<2e-16	***
	AIC: 140948 **Null deviance:** 207945 **Residual deviance:** 139233 **D²:** 33.04% ***(< 0.001)					

The variables that are better correlated with DPC (pollen/m^3) before and after peak for both cities were adjusted to generalized linear model (GLM) by using Poisson distribution and logarithmic link functions.

In Évora, the model that performed best according to the Akaike Information Criteria (AIC) and once the lack of multicollinearity was verified using Variance Inflation Factor (vif>5), included variables as precipitation, RH, Global Srad, WS, WD and AtP. These parameters, explained by the model 22.66% of the variability of data (deviance). The variables precipitation, WS, WD and AtP have a negative sign. The variables selected by the model GLM to explain the DPC included those which it had a significant correlation (table 4).

In Granada, the model that performed best according to the AIC and vif>5, included, T_{max}, T_{min}, PP, RH, Global Srad, WS, WD and AtP. These parameters, 33.04% of variability of data explained by the model. The variables T_{min}, RH, Global Srad and WD have a negative sign (table 4).

Overall, Spearman correlation and PCA point out the relation between T_{max} and DPC, both in pre- and -post-peak periods, in both cities. Other studies show the importance of T_{max} in pollen concentration (Myszkowska & Piotrowicz, 2012; Ziska et al., 2019).

Also correlated with DPC, in pre-peak period, in both cities, is global Srad, which was also indicated as a relevant parameter by GLM analysis. Although global Srad can have different effects on different taxa (Majeed et al., 2018), this meteorologic parameter has been associated with pollen production and release into the atmosphere by several authors (Martínez-Bracero et al., 2019; Majeed et al., 2018).

T_{min}, in Évora, seems to be positively associated with post-peak DPC, as is showed in Spearman and PCA analysis. By the contrary PCA reveals that, in Granada, T_{min} seems to be negatively associated with DPC, both in pre- and post-peak periods (although Spearman doesn't show significative correlations), and accordingly, T_{min} is an important parameter in GLM model for Granada. Is already described the importance of minimum temperatures in phenology development of trees. The effect of high minimum temperatures may increase plant senescence but contribute to the decrease of plant capacity in pollen production (Hatfield & Prueger, 2015).

Relative humidity presented a negative association with DPC in Granada, both in preand post-peak periods. GLM and PCA also showed the importance of this parameter in DPC, in both cities. In literature, relative humidity has a role in pollen emission, that is, high relative humidity it makes it difficult to pollen emission for the atmosphere (González-Minero, Candau, & Marroquín-Santoña, 1993). Contrary, low relative humidity favours anthesis process, controlling flowering process, production, and emission of pollen grains (Linskens & Cresti, 2000).

Wind related parameters are also indicated as having important negative associations with DPC in GLM and PCA analysis. In fact, wind is known to be able to transport pollen grains to long distances (Gregory, 1978; Buurgeois et al., 1985). P. Cour collected pollen from Mediterranean vegetation located more 1000 Km to the north, in the middle of the Sahara Desert (González-Minero et al., 1993). WS is recognized as one of the most important factors (Ljungkvist, Bringfelt, & Fredriksson, 1977; McDonald, 1980; Traidl-Hoffmann, Jakob, & Behrendt, 2009). This meteorologcial parameter can fave three effects, transport, stronger winds should lower amount of pollen grains. Strong winds may dissipate the pollen grains of the sorroundings of the e emission points and strong winds can ressuspend the pollen grains already deposited (Ljungkvist, Bringfelt, & Fredriksson, 1977). A case study showed that allergic people to birch pollen in Sweden developed symptoms when the birch in the city were not yet blooming, and the explanation for that was found in the transport of pollen from the central Europe, where these swamps were already in pollination, by the action of the prevalent strong winds (Wallin et al., 1991).

WD is also an important factor when studying the decrease/increase in pollen concentrations in the air. In fact, the distribution of pollen emission sources and wind direction play an important role in pollen concentrations when sources are in the quadrants, on the contrary when they have great dispersion there is no relationship between pollen concentrations in the air and wind direction (Silva Palacios, Tormo Molina, & Muñoz Rodríguez, 2000). An example is what happens in coastal areas, where pollen concentrations increase when the wind directions is coming from the interior and decrease when winds come from the sea (González-Minero et al., 1993; McDonald, 1980).

3. Effects of meteorological conditions during the Cupressaceae pollen season on Cupressaceae pollen integral and pollen season duration, in Évora and Granada

The effect of average maximum, minimum and mean temperature during the Cupressaceae pollen season on pollen season duration and Cupressaceae seasonal pollen integral was studied. Figure 7 shows the relations between variables, and Pearson´s correlation coefficients are presented in table 5.

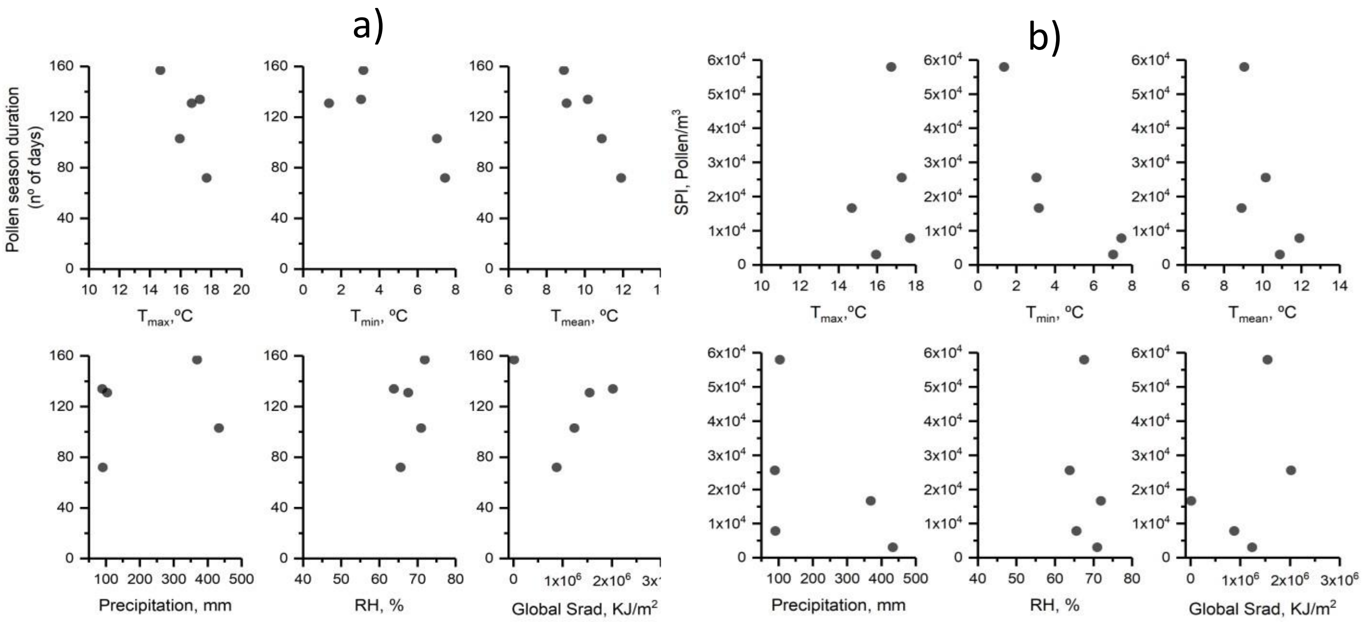


Figure 7: Influence of Tmax, Tmin, Tmean, Precipitation, RH and Global Srad on the Pollen season duration (a) and Seasonal Pollen Integral (b) of the year 2018-2019 for Évora and 20172019 for Granada

The maximum, and mean temperature (during the Cupressaceae pollen season) was inversely proportional to the pollen season duration, while seasonal pollen integral was inversely correlated with the minimum temperature (figure 7 and table 8).

Table 5: Pearson's correlation coefficients between daily meteorological conditions (season averaged) T_{max} (°C), T_{min} (°C), T_{mean} (°C) and RH (%); daily cumulative Global Srad (KJ/m^2) and precipitation (PP, mm), pollen season duration (PSD, days) and seasonal pollen integral (SPI, pollen/m^3) of the year 2018-2019 for Évora and 2017-2019 for Granada. *Correlation is significant at the 95% range (p<0,05); ns – no significative correlation

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	Tmax	Tmin	Tmean	Prec	RH	Srad	PSD	SPI
Tmax		ns	ns	**-0,830***	**-0,911***	ns	ns	ns
Tmin	ns		**0,898***	ns	ns	ns	**-0,824***	**-0,859***
Tmean	ns	**0,898***		ns	ns	ns	**-0,936****	ns
Prec	**-0,830***	ns	ns		**0,909***	ns	ns	ns
RH	**-0,911***	ns	ns	**0,909***		ns	ns	ns
Srad	ns	ns	ns	ns	ns		ns	ns

Other significative correlations were relative to the influence of meteorological parameters on each other, and a very hight significative correlation was observed between relative humidity and precipitation as well as negative correlations between maximal temperature and relative humidity and precipitation (figure 7 and table 5).

In fact, literature shows that the higher temperature can affect pollen season duration and consequently affect changes in duration of human exposure to airborne allergens (Ziska et al., 2019). Higher minimum temperature tend to decrease plant pollen production (Hatfield & Prueger, 2015).

4. Influence of the weather conditions previous to the Cupressaceae pollen season on Cupressaceae pollen integral and season pollen duration, in Evora and Granada

Averaged Tmax, Tmean and Tmin, from the in the autumn-winter (set – jan) period, previous to Cupressaseae pollination, tended to be lower in Granada comparatively to Évora (average Tmax, Tmean and Tmin: 19.78±5.95°C, 12.99±5.58°C and 6.22±5.21°C, for Granada, and 21.55±6.18°C, 15.58±5.01°C and 11.03±3.95°C, for Évora, respectively), presenting in both cities a decline from September to January.

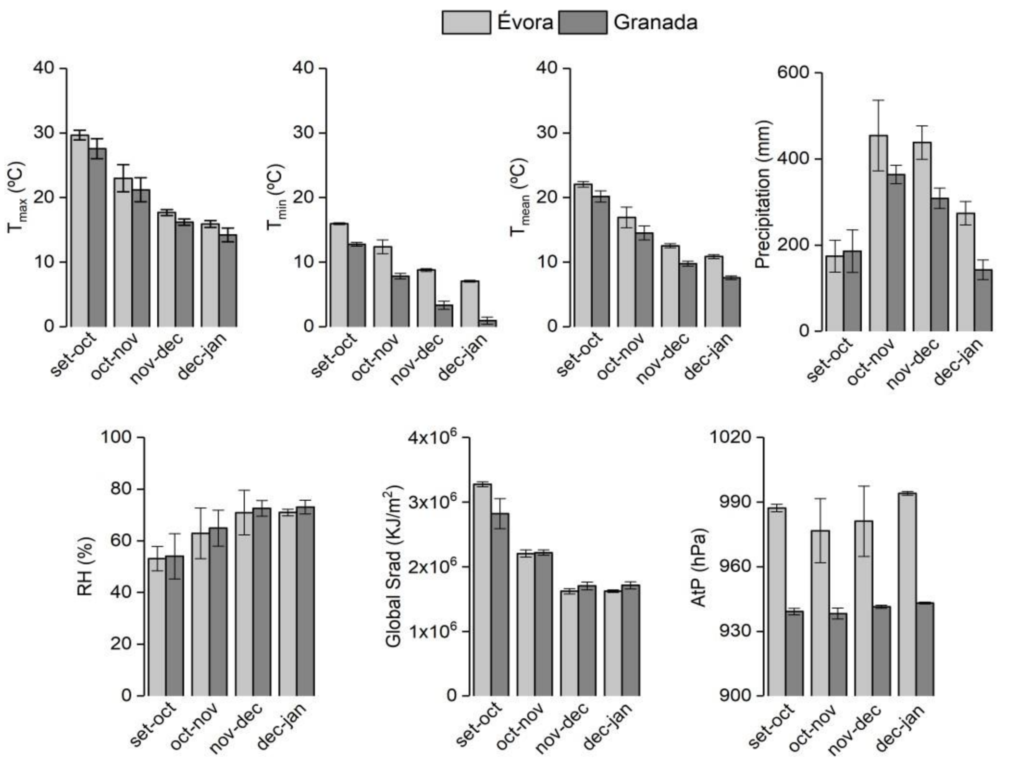

Figure 8: Bioclimatic characterization of Évora and Granada in the Autumn-Winter period (September to January) prior to the Cupressus pollen season (2017-2019). Meteorological parameters: Tmax, Tmin, Tmean, (°C); Cumulative precipitation (mm); RH (%), Global Srad (KJ/m2) and AtP (Pa).

Autumn was rainier in Evora than in Granada with cumulative precipitation reaching 1340 mm in Evora compared to 1000 mm in Granada. The RH was similar in both cities, with 64.46±8.49 % for Évora and 66.16±8.91 % for Granada, and cumulative Global solar radiation showed similar values in both cities with, 2.2x106±0.8x106 KJ/m2 for Évora and 2.1x106±0.5x106 KJ/m2 for Granada, declined, approximately, 3x between September-October and December-January. The AtP tend to be a higher in Évora with 984.76±7.53 hPa in all autumn, while for Granada average AtP was 940.49±2.21 hPa, as represented in figure 8.

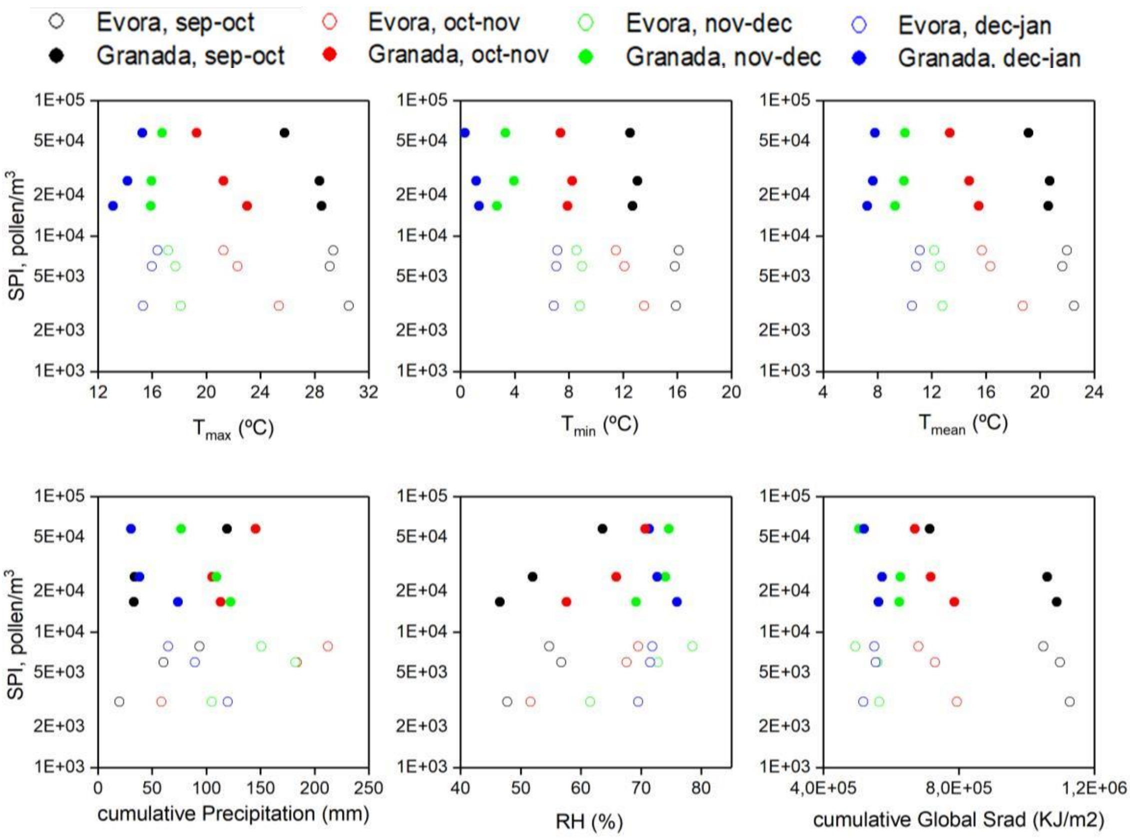

Figure 9: Influence of meteorological conditions of the Autumn-Winter period (averaged or accumulated from September to January), prior to the Cupressus pollen season, on season pollen integral (SPI), at Évora and Granada (2017-2019). Meteorologic parameters: Average Tmax, Tmin, Tmean (°C); cumulative precipitation (mm); average relative humidity (RH, %); Average global solar radiation (Srad, KJ/m2).

The influence of the meteorologic conditions from the Autumn-Winter period prior to Cupressus pollen season in the seasonal pollen integral can also be addressed annually. Plots representing the average, or cumulative data, for each of the meteorologic variables and seasonal pollen integral for each one the analysed years can be visualized in figure 9. Some features come out from the graphic representation of this data. In September-October and October-November periods, negative trends between maximum and mean temperatures and

SPI can be addressed and inversely, positive trends are observed for the period between December and January. Minimum temperatures present different relations with SPI, inconsistent between Évora and Granada.

A positive trend between relative humidity and SPI in September-October and OctoberNovember periods can also be visualised, and contrary relations can be observed in the same periods, for global solar radiation.

In short, it was observed that the meteorological parameters are related to pollen concentrations positively or negatively according to the period considered (Set-Nov or Nov-Jan). Therefore, during the autumn-winter period, monthly variations in meteorologic parameters should be taken into account, rather than considering the average values for the entire season.

Conclusions

This work showed a higher prevalence of Cupressaceae pollen in Granada, comparatively to Évora, which is probably due to the surrounding forests.

Although the differences in topography and microclimatic conditions between both cities, some similar meteorologic parameters could have been associated with Cupressaceae SPI, as T_{mean} and T_{max} and Srad during the pollen season. In the first half of the pollen season (before peak), meteorological parameters are important, especially temperature and global Solar radiation, since these parameters create convection currents that help in the development of the plant and make it possible to leave the dormancy state (Walck et al., 2011).

The second part of the pollen season (after peak), although the keep going high the temperature and solar radiation, the number of individuals of pollination is very low, decreasing the concentration of pollen grains in the air. In this period, it must be underlined the negative association of RH and precipitation with DPI, also a feature in both cities.

Despite the great differences in Cupressaceae pollen concentration between Granada and Évora, Cupressaceae pollen season duration was similar between location over the three years period, and 2018 season was the longest. The high duration of pollen season in 2018 is probably due to a higher precipitation occurring during the pollen season, a common feature in both cities.

Meteorological conditions are key factors affecting models for airborne pollen and for alert systems for the population concerning the risk of exposure to these allergens (Sabariego et al., 2012). Taking into account meteorologic parameters from the autumn-winter previous to pollen season, maximum and mean temperatures seem to present similar influence over SPI in both cities, and with different trends in Sep-Nov or Dec-Jan months. Nevertheless, longer temporal series of data must be used, in the future, in order to assure that maximal and/or mean temperature can be used as indicators to forecast the Cupressaceae pollen season intensity. But even with a short period evaluated, our results revealed the enormous importance to study the continuous variation of the meteorological parameters over time, rather than consider average values for the entire autumn-winter prior to Cupressaceae pollen season, information that should be taken into account in future studies.

Acknowledgements

This work is co-funded by the European Union through the European Regional Development Fund. included in the COMPETE 2020 (Operational Program Competitiveness and Internationalization) through the ICT project (UID/GEO/04683/2013) with the reference POCI-01-0145- FEDER-007690 and also through ALOP (ALT20-03-0145-FEDER- 000004) project.

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Supplementary data Principal component analysis Évora

Table S1: Own values and proportion of variance explained by components of before and after peak

		Components Variance	Proportion of Variance	Cumulative proportion
Before peak	1	3,04399	30.44%	30.44%
	2	2,14934	21.49%	51.93%
	3	1,72415	17.24%	69.17%
	4	1,01289	10.13%	**79.30%**
	5	0,97069	9.71%	89.01%
	6	0,54982	5.50%	94.51%
	7	0,2849	2.85%	97.36%
	8	0,17005	1.70%	99.06%
	9	0,07093	0.71%	99.77%
	10	0,02323	0.23%	100.00%
After peak	1	3,67887	36.79%	36.79%
	2	1,99238	19.92%	56.71%
	3	1,34745	13.47%	70.19%
	4	1,04769	10.48%	**80.66%**
	5	0,81099	8.11%	88.77%
	6	0,54752	5.48%	94.25%
	7	0,34196	3.42%	97.67%
	8	0,12942	1.29%	98.96%
	9	0,0848	0.85%	99.81%
	10	0,01894	0.19%	100.00%

Granada

Table S2: Own values and proportion of variance explained by components of before and after peak

		Components of variance	Proportion of variance	Cumulative proportion
Before peak	1	3,30185	33.02%	33.02%
	2	2,40165	24.02%	57.03%
	3	1,37299	13.73%	**70.76%**
	4	0,85745	8.57%	79.34%
	5	0,70422	7.04%	86.38%
	6	0,49865	4.99%	91.37%
	7	0,42732	4.27%	95.64%
	8	0,27842	2.78%	98.43%
	9	0,15745	1.57%	100.00%
	10	0	0.00%	100.00%
After peak	1	4,06818	40.68%	40.68%
	2	1,73307	17.33%	58.01%
	3	1,10957	11.10%	69.11%
	4	1,05969	10.60%	**79.71%**
	5	0,85176	8.52%	88.22%
	6	0,46205	4.62%	92.84%
	7	0,3324	3.32%	96.17%
	8	0,22827	2.28%	98.45%
	9	0,15501	1.55%	100.00%
	10	0	0.00%	100.00%

Table S3: Pearson's correlation coefficients between daily meteorological conditions (season averaged) T_{max} (°C), T_{min} (°C), T_{mean} (°C) and RH (%); daily cumulative Global Srad (KJ/m^2) and precipitation (PP, mm), pollen season duration (PSD, days) and seasonal pollen integral (SPI, pollen/m^3) of the year 2018-2019 for Évora and 2017-2019 for Granada. *Correlation is significant at the 95% range (p<0,05); ns – no significative correlation.

	Tmax	Tmin	Tmean	Prec	RH	Srad	PSD	SPI
Tmax	1	0.213	0.616	**-,830***	**-,911***	0.678	-0.656	0.136
		0.366	0.134	**0.041**	**0.016**	0.104	0.114	0.414
Tmin	0.213	1	**,898***	0.282	0.068	-0.194	**-,824***	**-,859***
	0.366		**0.019**	0.323	0.457	0.377	**0.043**	**0.031**
Tmean	0.616	**,898***	1	-0.147	-0.372	0.150	**-,936****	-0.655
	0.134	**0.019**		0.407	0.269	0.405	**0.010**	0.115
Prec	**-,830***	0.282	-0.147	1	**,909***	-0.538	0.224	-0.519
	0.041	0.323	0.407		**0.016**	0.175	0.359	0.185
RH	**-,911***	0.068	-0.372	**,909***	1	-0.703	0.328	-0.244
	0.016	0.457	0.269	**0.016**		0.093	0.295	0.346
Srad	0.678	-0.194	0.150	-0.538	-0.703	1	-0.142	0.390
	0.104	0.377	0.405	0.175	0.093		0.410	0.258

3.3. Capítulo III - Efeito dos poluentes atmosféricos gasosos e matéria particulada na alergenicidade do pólen

Atualmente, sabe-se que a poluição atmosférica tem um grande impacto quer sobre a saúde pública (Riedler et al., 2000) e sobre o ecossistema (Ochoa-Hueso et al., 2017). Vários estudos têm apontado para os efeitos adversos dos poluentes gasosos e da matéria particulada sobre grãos de pólen (Cuinica, Abreu, & Esteves Da Silva, 2014; Ribeiro et al., 2014; Sousa et al., 2012; Lu et al., 2014), no entanto desconhece-se quais os efeitos diretos sobre a alergenicidade do pólen e as consequentes repercussões na doença alérgica respiratória.

Este capítulo teve como objetivo o estudo dos efeitos dos poluentes gasosos e matéria particulada sobre as características químicas e bioquímicas do pólen, possivelmente afetando o seu potencial inflamatório, e compreende duas publicações: i) *Air pollutants NO_2- and O_3-induced Dactylis glomerata L. pollen oxidative defences and enhanced its allergenic potential*, publicado na revista *Aerobiologia*; e, ii) *Differential Quercus spp pollen-particulate matter interaction is dependente on geographical areas*, publicado na revista *STOTEN - Science of The Total Environment.*

No primeiro estudo foi, averiguou-se o efeito dos poluentes NO_2, O_3, isolados e em combinação, na fisiologia, designadamente, a resposta biológica ao stress oxidativo afetando a capacidade germinativa, e nas propriedades bioquímicas do pólen de *D. glomerata* que mais podem contribuem para a sua alergenicidade, designadamente a expressão de alergénios.

O segundo estudo teve como objetivo caraterizar a matéria particulada adsorvida na superfície dos grãos de pólen em três regiões com topografia e/ou bioclima distinto: Évora – planície com temperaturas médias na ordem dos 15 °C e com humidade relativa mais baixa em média (50%, aproximadamente), Porto – cidade costeira com temperaturas médias na ordem dos 15°C mas humidade relativa na ordem dos 80%, e Guarda – cidade em altitude com temperaturas medias mais baixas que as restantes cidades (12.5 °C) mas com humidade relativa aproximada aquela sentida na região de Évora, e avaliar o potencial efeito sobre da resposta alérgica ao pólen entre diferentes regiões.

O meu contributo para estes estudos foi efetuar o procedimento experimental que permitiu determinar as atividades enzimáticas, a capacidade germinativa do pólen e a expressão de alergénios (primeiro estudo) e a preparação das amostras recolhidas em Évora e toda a análise estatística sobre a matéria particulada aderente à superfície do pólen e relação com os parâmetros meteorológicos (segundo estudo), bem como elaborar os artigos.

Aerobiologia (2021) 37:127–137
https://doi.org/10.1007/s10453-020-09676-2

ORIGINAL PAPER

Air pollutants NO_2- and O_3-induced *Dactylis glomerata* L. pollen oxidative defences and enhanced its allergenic potential

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Received: 2 April 2020 / Accepted: 3 November 2020 / Published online: 27 November 2020

Abstract Air pollutants impact airborne pollen biochemistry. Oxidative damage to lipids, proteins and nucleic acids or protein nitration are among ozone (O_3) and nitrogen dioxide (NO_2) described deleterious effects possibly causing pollen physiology damage and enhanced allergenic activity, contributing to aggravate pollen driven respiratory allergy in urban areas. The goal of this research was to evaluate the effects of O_3, NO_2, alone and combined, on *Dactylis glomerata* pollen reactive oxygen species scavenging enzymes, on pollen germination and their potential contribution to the allergenicity. *D. glomerata* pollen was in vitro exposed to pollutants. Protein extracts were prepared and superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) activities were evaluated. Western blot with pooled sera or with IgG against group 5 allergens and profilin was performed. Pollen germination capacity was increased by NO_2 and was unaffected by O_3 or $O_3 + NO_2$ but showed longer pollen tubes in the latter. Exposure to O_3 did not affect SOD activity but induced a twofold increase in catalase activity. SOD activity was twofold higher in pollen exposed to NO_2. Exposure to $O_3 + NO_2$ induced a twofold and fivefold increase of SOD and catalase activities, respectively. Pollen GPx was unaffected by the pollutants. IgE-recognition of proteins in the molecular weight range of 42–57 kDa were amplified by NO_2 and $O_3 + NO_2$ and O_3 amplified proteins with molecular weight of 13 (profilin), 29 (Group 5), and 31 kDa. Taken together, these results show that pollen oxidative defences are activated by common air pollutants affecting both its germination capacity and its allergenic activity.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10453-020-09676-2) contains supplementary material, which is available to authorized users.

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Keywords Pollen germination · Superoxide dismutase · Catalase · Atmospheric pollutants · Aeroallergens · Allergenicity

Abbreviations

ROS Reactive oxygen species
SOD Superoxide dismutase
GPx Glutathione peroxidase
tBH *t*-Butyl hydroperoxide

1 Introduction

Motor vehicles emissions, from burning hydrocarbons, produce nitrogen dioxide (NO_2). With available UV light, photodissociation of NO_2 can produce nitrogen monoxide (NO) and atomic oxygen (O), which is very reactive and attaches quickly to oxygen (O_2) to form ozone (O_3). This way, tropospheric O_3 can be formed indirectly from pollution of NOx. The short half-life of O_3 causes its break down to $O_2 + O$, which in turn, can bind to natural nitrogen (N_2) or to NO to again form NO_2. Unlike naturally formed O_3, when O_3 is formed from smog, an "ozone cycle" begins that is difficult to break, where NO and NO_2 participate as catalysts, causing ozone to increase in our environment to unhealthy levels (Zumdahl 2009). Portugal has very intense solar radiation levels, so in the presence of the ozone precursors, which can be transport from the urban areas of Porto and Lisbon to the south and interior part of Portugal by the N–NW dominant winds, ozone levels can reach the threshold limit imposed by the EC directives, presenting risk for vegetation protection (Monteiro et al. 2007).

Airborne pollen during its transport comes in contact with a variety of atmospheric chemicals, including the common air pollutants O_3 and NO_2, which impact airborne pollen at several levels: affecting germination capacity (Gottardini et al. 2004) and viability (Cuinica et al. 2014; Pasqualini et al. 2011), morphology and cell wall structure (Chehregani et al. 2004) and protein content or release (Ribeiro et al. 2014), as well as inducing chemical modification of specific biomolecules. On one side, NO_2, alone or in combination with O_3, may contribute to protein nitration (Ghiani et al. 2016; Gruijthuijsen et al. 2006), affecting protein/enzymatic function. On the other side, the strong oxidizing potential of O_3 may affect pollen redox balance (Pasqualini et al. 2011), and oxidative damage to lipids, proteins and nucleic acids are among O_3 described deleterious effects (Iriti and Faoro 2007). Moreover, in nature it is expected that the airborne pollen will be exposed to both pollutants simultaneously and other synergistic effects may be expected, since the presence of O_3 increases NO_2 absorption (Chassard et al. 2015) favouring the nitration of proteins (Franze et al. 2005). Nevertheless, the effect of these pollutants on pollen, alone or in combination, is not fully elucidated and are, probably, pollen type specific.

The pollen of grasses is very abundant in the spring period in Portugal, corresponding to an increasing solar exposure (UV radiation), which induces, with the adequate catalysts present, elevated O_3 levels in the atmosphere (Bortoli et al. 2009). Additionally, the high humidity levels characteristic of the season, above 60% even in inland drier areas (Miranda et al. 2001), facilitate the uptake of pollutants by the pollen (Chassard et al. 2015). So, it is relevant to know the effects of atmospheric pollutants on this pollen type, particularly due to its high allergic potential.

Reactive oxygen species (ROS) are important for regulating many aspects of the life cycle and environmental response mechanisms of plants (growth, development, response to biotic and abiotic environmental stimuli). However, stress can lead to excessive ROS production, causing progressive oxidative damage. Whether ROS would serve as signalling molecules or could cause oxidative damage to the tissues depends on the delicate equilibrium between ROS production, and their scavenging (Bailey-Serres and Mittler 2006). In pollen, as in other plant tissues, ROS, directly or indirectly derived from the exposure to pollutants, are scavenged by enzymatic and non-enzymatic antioxidant defensive mechanisms. In this work, we investigate the action of superoxide dismutase (SOD), which constitutes the first line of defence against ROS, and catalyses the dismutation of the superoxide radical ($O_2^{-\cdot}$) into either O_2 or H_2O_2, which can also be damaging (causing lipids or proteins peroxidation) and is degraded by other enzymes such as catalase or glutathione peroxidase (GPx), which also will be investigated.

Environmental pollutants can affect allergic disease in several ways, for instance as adjuvant factors in sensitization or as triggering factors in allergic disease onset. Environmental pollutants can also affect allergen exposure features, influencing either sensitization or the onset and/or magnitude of allergic disease (Ring et al. 2001).

The goal of this research was to evaluate the effects of O_3, NO_2, alone and combined, on physiological aspects and on allergenicity of *D. glomerata* pollen. In order to perform this evaluation, we have investigated the effect of the above gaseous pollutants on: (i) pollen germination ability; (ii) the major defences against oxidative stress, the ROS scavenging enzymes SOD, catalase and GPx; (iii) the allergen expression patterns identified by IgE immunorecognition. *D. glomerata* pollen was exposed to the air pollutants in controlled conditions, using an environmental chamber.

2 Material and methods

2.1 Pollen collection

D. glomerata pollen anthers were collected during the flowering season (spring; June 2018) in a rural area of North of Portugal and were dried at 27 °C. Pollen was released by gently crushing the anthers and passed through 0.063 mm sieves to isolate the pollen samples that were stored at − 20 °C after exposure to the different treatments (Sect. 2.2). Figure 1 depicts the experimental design.

2.2 In vitro pollen exposure to O_3 and NO_2

Pollen samples were exposed to O_3, NO_2 or $O_3 + NO_2$ in an environmental chamber equipped with a Solar Simulator (Newport Oriel 96000 150 W), a fan (SUNON SF23080AF) to homogenize the air and temperature and relative humidity sensors (EBRO EBI20 sensor) (chamber and method details may be consulted in Sousa et al. 2012). The reproducibility of exposure conditions was demonstrated in Ribeiro et al. (2013). Pollen samples of 250 mg of dry weight (DW) were placed in a tube with both edges closed by a 23-μm pore length mesh (SEFAR PET 1000). This tube was then placed over a fan that impelled the air within the chamber to pass through. Pollen was exposed to pollutants during 6 h, at an average concentration equal to the current atmospheric limit value for the human health protection according to the European Union Directive 2008/50/EC of 21 May 2008 on ambient air quality and cleaner air for Europe (O_3 daily 8-h mean—120 μg/m^3; NO_2 1 h limit—200 μg/m^3). A pollen sample exposed under the same conditions to unpolluted air was used as control. The 6 h exposure time was used as a model for the period corresponding to the highest solar irradiation (around 12 o'clock).

2.3 In vitro pollen germination

The germination of the exposed and control pollen samples (4 batches from each condition) occurred at room temperature, in the dark, for 24 h, in an aqueous solution (Dafni 1992), using an optimized culture media (1% Saccharose, 0.6% polyethylene glycol 20 kDa and 2.5 mM $MgSO_4$, 8.3 mM $Ca(NO_3)_2$, 1.6 mM H_3BO_3, 1 mM KNO_3). At the end of the experiment, the samples were transferred to a slide, with a droplet of methylene blue. 200 pollen grains per slide were randomly counted using a light microscope (Olympus BX43, 400x) and the results were expressed as a percentage of germination. A pollen grain was

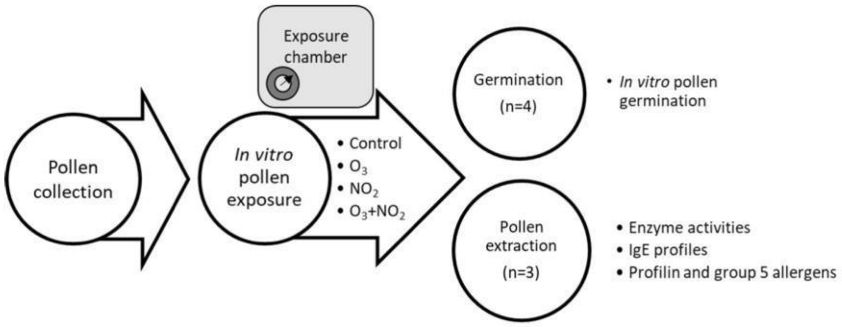


Fig. 1 Scheme of the experimental design

classified as germinated when the pollen tube was longer than the pollen size. Pollen tubes were classified as *long* and *short* when its size was higher or lower than twice the diameter of pollen, respectively.

2.4 Preparation of pollen extracts and total protein quantification

Pollen extracts from control or exposed to pollutants (O_3, NO_2 or $O_3 + NO_2$) (3 per treatment) were prepared in phosphate buffered (80 mM Na_2HPO_4, 20 mM de KH_2PO_4, pH = 7.8). Pollen grains were macerated (2.5 mg pollen/ml) in precooled buffer (4 °C) in a mortar. The pollen suspensions were then centrifuged at 10,000 *g* for 10 min, and the supernatants were recovered and frozen until analysis.

The soluble protein concentration in the supernatants was measured using the dye-binding Bradford protein assay (Bradford 1976), using bovine serum albumin as the standard.

2.5 SOD, catalase and GPx enzymatic activities

Total SOD (EC 1.15.1.1) activity quantification was based on the inhibition of the riboflavin-induced reduction of nitro blue tetrazolium (NBT), since superoxide anion is scavenged by SOD. The assay solution (pH = 7.8) contained 70 mM phosphate buffer, 10 μM EDTA, and 0,003% Triton X-100, 2 μM riboflavin and 10 μM NBT. NBT reduction was triggered by cool white fluorescent light (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and reduced NBT was measured at 560 nm, every 5 min, for 40 min. Reduction rates obtained in the presence (v) or absence (V) of pollen extract were used to calculate SOD enzymatic activity (U) as 1-(v/V), according to (Beauchamp and Fridovich 1971). Specific activity was expressed as U per mg of protein.

Catalase (EC 1.11.1.6) activity was determined as previously described by (Bailly et al. 2004) by spectrophotometrically following H_2O_2 consumption at 240 nm. Assay solution (pH = 7) contained 50 mM phosphate buffer and 10 mM H_2O_2. The results were expressed as specific activity, i.e. as μmol H_2O_2 decomposed/min/mg of protein.

The GPx (EC 1.11.1.9) activity was measured using *t*-butyl hydroperoxide (tBH) and reduced glutathione (GSH) as substrates, and the product, oxidized glutathione (GSSG), is then recycled back to GSH, employing glutathione reductase (GR) and NADPH, in a coupled reaction (assay solution, pH = 7.2: phosphate buffer 50 mM, 2.5 mM GSH, 0.5 mM NaN_3, 0.3 mM EDTA, 0.1 mM NADPH, 0.5 U GR, 0.4 mM tBH). Enzymatic activity of GPx is expressed as NADPH consumption, at 320 nm, μmol/min/mg of protein (Mannervik 1985).

Enzyme activity was assessed in all the pollen extracts prepared (3 per treatment). Technical replicates (4–5) were performed. For each extract, the enzyme activity was estimated as the mean of the technical replicates.

2.6 IgE-profiles and immunoblotting

Protein pollen extracts from the different samples (50 μg/lane) were separated in 12% polyacrylamide gels under reducing conditions (Laemmli 1970). For immunoblotting analysis, the proteins were electroblotted onto PVDF membrane (Westran, Whatman™, GE Healthcare). The membranes were saturated during 2 h in a blocking solution (5% non-fat dry milk (w/v) prepared in Tris–HCl buffered saline solution with Tween (TBST, pH = 7.6: 25 mM Tris–HCl, 150 mM NaCl and 0.1% Tween).

For IgE profiles, the membranes were incubated overnight (OV) at 4 °C with pooled sera from non-sensitized and sensitized patients to *D. glomerata* pollen diluted 1:20 in TBST. This study was performed under the Portuguese laws for Personal Data protection, DL 67/98 and DL 12/2005 and authorization GD/44721/2015 of the Ethics Committee for Health Science Research of the University of Évora; participant's signed informed consent forms before enrolment in the study. Bound specific IgE was detected with mouse anti-human IgE antibody (I6510, Sigma) diluted 1:10.000, incubated for 2 h, at room temperature (RT) and subsequently by a rabbit anti-mouse IgG horseradish peroxidase (HRP) conjugate (A9044, Sigma), diluted 1:30.000 and incubated for 2 h, RT. Colorimetric revelation was performed with TMB (T0565, Sigma), and a photographic image was taken.

For the identification of grasses group 5 allergen, biotinylated anti-Phl p5 monoclonal antibody (Allergopharma Joaquim Ganzer KG), 1:100, OV, 4 °C, was used, followed by streptavidin-peroxidase (S5512—Sigma) diluted 1:250, for 2 h, at RT. TMB colorimetric revelation was performed. Profilin was detected using a rabbit anti-profilin antibody (kindly provided by Probelte Pharma, 500x diluted), OV, at 4 °C, followed by a second detection step using an anti-rabbit IgG-alkaline phosphatase conjugate (NIF1317, GE Healthcare), incubated for 2 h, at RT; fluorescent substrate (RPN5785, GE Healthcare) was used for revelation. Image was acquired using Gel-Doc (Bio-RAD) under UV light. Two independent experiments were performed for each evaluation.

Image processing, involving spatial and density measurements, were performed using the software *ImageJ* (https://imagej.nih.gov/ij/index.html). Only bands presenting density variation above 10% were considered different from the control.

2.7 Data analysis

The results were expressed as mean ± standard error of the mean (SEM) unless stated otherwise in the figure legends. Data normality was confirmed by Kolmogorov–Smirnov test and QQ-plots and homogeneity of variance was assessed by Levene test. Statistical assessment of differences between mean values was performed using One-Way ANOVA ($p < 0.1$ or $p < 0.05$ were accepted) (Zar 2014). SPSS was used for statistical analysis (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp, IBM Corp. Released 2017).

Graphs were produced using OriginPro software (OriginLab corporation).

3 Results

The *D. glomerata* pollen in vitro germination presented rates from 3.6 to 11.3%. Pollen germination capacity was threefold increased by NO_2 exposure and was not significantly affected by O_3 or $NO_2 + O_3$ (Fig. 2a). When the length of the pollen tubes was taken into consideration, the percentage of germinated pollen with long tubes was twofold higher in pollen exposed to $NO_2 + O_3$ (Fig. 2b).

Enzymatic activities of ROS scavenging enzymes are presented in Fig. 3.

The SOD activity was 1.7-fold higher and 2.2-fold higher in pollen exposed to NO_2 ($p < 0.1$) or to $NO_2 + O_3$ ($p < 0.05$) compared to controls (280 ± 56, 463 ± 69 and 604 ± 102 U/mg protein, for control, NO_2 and $NO_2 + O_3$ groups, respectively). O_3 alone did not significantly affect SOD activity (Fig. 3a).

The catalase activity was 0.06 ± 0.02 µmol H_2O_2/min/mg protein in the controls and was unaffected by NO_2 exposure. In the contrary, the catalase activity was enhanced, respectively twofold and fivefold in pollen exposed to O_3 (0.13 ± 0.05 µmol H_2O_2/min/mg protein; $p < 0.1$) or to $NO_2 + O_3$ (0.33 ± 0.12 µmol H_2O_2/min/mg protein; $p < 0.1$) (Fig. 3b).

The GPx activity was similar among groups ($6.9 \times 10^{-5} \pm 4.4 \times 10^{-5}$, $9.2 \times 10^{-5} \pm 3.6 \times 10^{-5}$, $9.2 \times 10^{-5} \pm 5.4 \times 10^{-5}$ and $10.7 \times 10^{-5} \pm 7.4 \times 10^{-5}$ µmol NADPH/min/mg protein, for

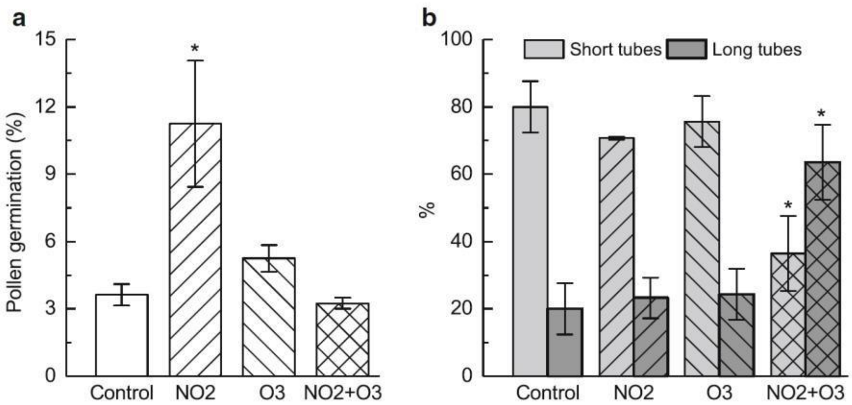


Fig. 2 Pollen germination in vitro. **a** Percentage of germination; **b** Percentage of short (1–2*Dp) and long (> 2*Dp) tubes of germinated pollen. 4 batches of pollen from each treatment were incubated in germination media. Each column represents the mean ± SEM. Statistical significance was assessed by One-Way ANOVA (*$p < 0.05$, relatively to control). Dp = Diameter of the pollen grain. See Appendix A, Fig. A1, for supplementary information

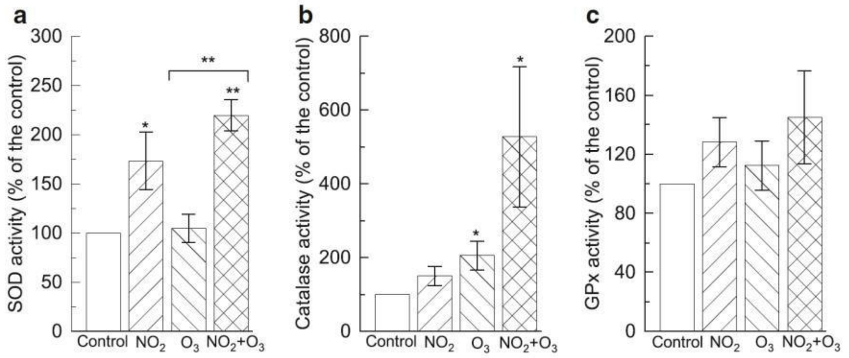


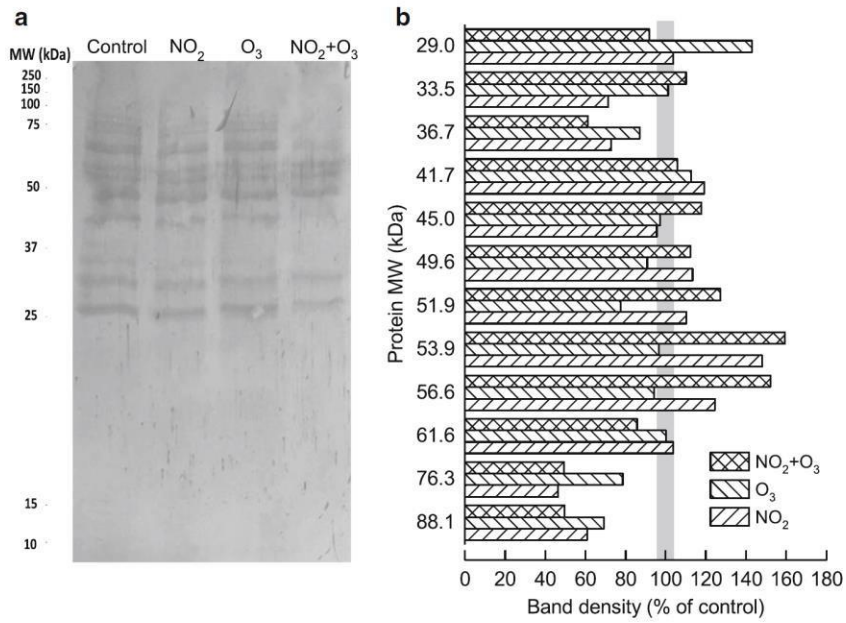
Fig. 3 ROS scavenging enzyme activities: **a** SOD (inhibition of the riboflavin-induced reduction of NBT—U/mg); **b** Catalase (consumption of H_2O_2/min/mg protein); **c** GPx (consumption of NADPH/min/mg protein). Extracts were prepared from batches of pollen (3 per treatment). To determine the enzyme activity for each pollen extract, 3–4 technical replicates were performed and the enzyme activity was estimated as the mean value of the technical replicates. Each column represents the mean ± SEM of the estimated activity for the 3 different extracts per treatment. Statistical significance was accessed by One-Way ANOVA (*$p < 0.1$, **$p < 0.05$)

control, NO_2, O_3 and $NO_2 + O_3$ groups, respectively) (Fig. 3c).

Protein bands, using SDS_PAGE, were observed ranging 10–90 kDa. The IgE reactivity profile of *D. glomerata* pollen, control and the IgE reactivity profile of *D. glomerata* pollen, control and exposed to pollutants, was investigated using a pool of sera from grass-sensitized patients (Fig. 4a). Several protein bands with similar molecular weight (MW) were identified in all groups (range 14.2–88.1 kDa). The bands with MW 29.0 ± 0.9, 33.5 ± 0.5, 41.7 ± 0.4, 49.6 ± 0.3, 53.9 ± 0.5, 56.6 ± 0.5 and 61.6 ± 0.7 kDa presented the highest density (≥ 5%) (Fig. 4a). A negative control, using a pool of sera of IgE-negative patients, was also performed, in the same conditions, and no bands were detected (data not shown).

The treatments differentially affected the band density (% of control; Fig. 4b); The bands with MW

Fig. 4 Exposure of *D. glomerata* pollen to atmospheric pollutants induced changes in allergenic profile. **a** IgE profile (representative of two independent experiments); **b** Relative Density (% of control) of the IgE reactive bands (cut off 2%). See Appendix B, Table B1, for supplementary information. See Appendix A, Fig. A2, and Appendix B, table B1, for supplementary information



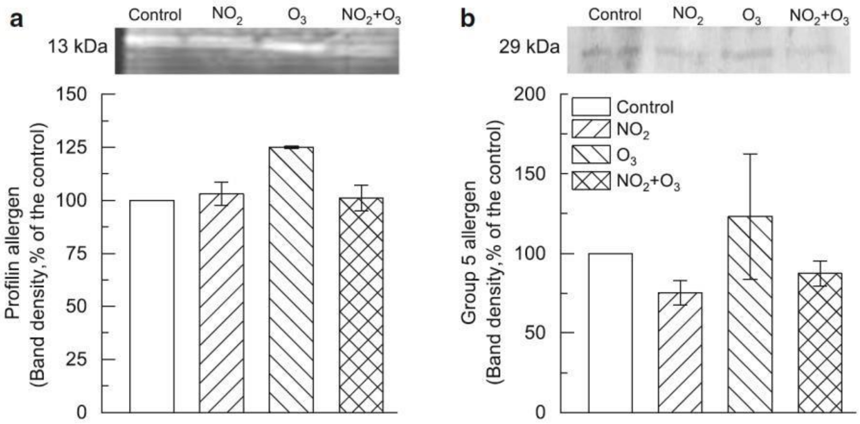


Fig. 5 Immunoblots and relative density (% of control) for profilin (**a**) and group 5 allergen (**b**). Specific IgG antibodies were used. Data represent the analysis of two independent experiments

41.7, 45.0, 49.6, 51.9, 53.9 and 56.6 kDa from NO_2 treated pollen presented augmented relative density (11–49%) while the bands with MW 36.7, 76.3 and 88.1 kDa presented diminished relative density (29–55%).

O_3 exposed pollen showed an enhancement of the bands with MW 29.0, 31.0 and 41.7 kDa (42%, 50% and 13%, respectively) and a reduction of the relative density of bands with MW 36.7, 51.9, 76.3 and 88.1 kDa (21–32%).

Finally, the bands with MW 45, 46,7, 51,9, 53.9 and 56.6 kDa were intensified (13–61%) by the exposure to both pollutants while the bands with MW 36.7, 61.6, 76.3 and 88.1 kDa were diminished by 15–52%.

The expression of profilin and grass group 5 allergens was assessed by immunoblot (Fig. 5). Profilin was identified as a peptide with 13 kDa and was induced 1.2-fold by O_3 treatment. Group-5 allergen presented a molecular mass of 29 kDa, which is compatible with the IgE-reactivity for the band 29.0 ± 0.9 kDa and was 1.6-fold induced by O_3 exposure.

4 Discussion

In this work it is shown that the pollutants NO_2 and O_3, which can be derived from vehicle traffic emissions, evoked differentiated effects on *D. glomerata* pollen. Differential effects caused by these agents in other pollen types have been described in multiple studies, either after in vitro exposure (Cuinica et al. 2014; Lu et al. 2014; Ribeiro et al. 2014; Sousa et al. 2012), as in this work, or during environmental exposure (Cortegano et al. 2004; Ghiani et al. 2012; Gottardini et al. 2004).

Starting with an overview of the most relevant results obtained in each test condition and our interpretation based on current knowledge, we seek to discuss the real-world implications of pollen exposure to traffic related pollutants.

4.1 Effects of pollen exposure to NO_2

Exposure to NO_2 induced a threefold increase in the germination rate of *D. glomerata* pollen, despite the low germination rate observed probably related with the storage conditions of the pollen samples since Cauneau-Pigot (1991) showed for this pollen species a reduction in germination after storage at − 20 °C compared with the fresh pollen.

A contradictory effect of NO_2 was observed in pollen germination from *Betula pendula* R., *Ostrya carpinifolia* S. and *Carpinus betulus* L. (Cuinica et al. 2014) and from *Acer negundo* L. pollen (Sousa et al. 2012) after NO_2 fumigation. Structural and/or biochemical characteristics of different pollen types may explain this opposing response. Indeed, at the seed germination level, for instance, the effects of NO_2 are also inconsistent where both impairment or promotion of germination have been described, depending on the species analysed (Beligni and Lamattina 2000; Keeley and Fotheringham 1997).

It has been shown that the uptake of NO_2 by *Phleum pratense* L. pollen is potentiated by water content, inducing a subsequent acidification (Chassard et al. 2015), as a result of NO_2^- (Bright et al. 2009; Chassard et al. 2015) and NO_3^- (Chassard et al. 2015) formation. Additionally, NO_2^- promoted synthesis of intracellular NO, an important signalling molecule in the process of pollen germination (Pasqualini et al. 2015). NO synthesis, favoured by NO_2 exposure, could explain the increase in the germination rate observed in this work.

At the level of the scavenging enzymes of ROS, catalase and GPx, there were no significant changes induced by NO_2. However, SOD activity recorded after exposure to NO_2 was higher than the control suggesting some oxidative stress in pollen, driven by superoxide anion, generated by NO_2 exposure. The activity of the catalase necessary to eliminate the H_2O_2 generated by cell metabolism, or by the action of SOD, consistently presented an average activity above the control. The physiological levels of ROS play important roles in germination signalling (Pasqualini et al. 2015), thus the ROS imbalance suggested by enzymatic activities measured in this work may partially explain the increase in germination rate observed.

Proteomic shifts have been observed previously in pollen exposed to pollutants, possibly affecting its allergenic potential. For instance, a higher IgE recognition of some protein bands after NO_2 fumigation (in vitro, 0.039 and 0.065 ppm, 6 h) was described for *B. pendula*, *O. carpinifolia and C. betulus* pollen (Cuinica et al. 2014) while a diminished IgE binding to some allergens (Phl p 2, Phl p 5b and Phl p 6) was described after *P. pratensis* pollen exposure to NO_2 (in vitro, 2 ppm, 2 h) (Rogerieux et al. 2007).

In our experiments, pollen exposure to NO_2 modified the immune reactivity of several proteins, five became over- and four became under-recognized. Among the proteins showing decreased immunoreactivity is a band of molecular weight 33.5 kDa, compatible with the grass group 1 major allergen (Kleine-Tebbe 2014). The grass group 5 major allergen is either unchanged or slightly diminished while five minor allergens with MW ranging 41–56.6 kDa that may include Group 4 and Group 13 are increased. Moreover, SOD activity, an allergen with MW 23 kDa recently described in *P. pratense* (Conti et al. 2014) is elevated in pollen exposed to NO_2. Whilst not ruled out, when taken together it is unclear whether the pollutant NO_2 induces a significant increase in *D. glomerata* pollen allergenicity.

4.2 Effects of pollen exposure to O_3

The exposure of the pollen to O_3 did not affect the germination rate and pollen tubes length as both were not significantly different to the control values. However, the duplication of catalase activity in pollen exposed to O_3, denotes oxidative stress generated by this gas, leading to increased production of H_2O_2 (Pessarakli et al. 2019). Since SOD activity was not changed by the exposure to this pollutant, the H_2O_2 might have been generated by a SOD-independent mechanism, possibly the induction of NAD(P)H oxidase, a mechanism described by Pasqualini et al. (2011) for *Ambrosia artemisiifolia* pollen exposed to O_3. GPx activity did not significantly change, suggesting that GSH-dependent pathway was not preferentially used to deal with O_3-induced oxidative stress.

At the level of allergen expression, there were several changes to the IgE-recognition pattern, with diminished recognition of four proteins (MW of 36.7, 51.9, 76.3 and 88.1 kDa) and increased recognition in three proteins (MW of 29, 31 and 13 kDa); two of the latter were identified by Western blot as grass group 5 (EACCI 2014) and profilin (grass Group 12), a pan-allergen presenting high homology between plant cells, responsible for cross-reactivity between pollen types (Hauser et al. 2010). Diminished IgE-binding to Phl p 5b have been reported by Rogerieux and colleagues (Rogerieux et al. 2007) probably resulting from different pollen exposure protocols; in fact, the kinetics and dose-response of the effects of pollen exposures to O_3 are yet unknown.

O_3 seems to cause an augmented allergenicity of grass pollen, characterized by the increased immune recognition of one of its major allergens and a pan-allergen that may be responsible for important cross-reactivity thus aggravating allergic symptomatology.

4.3 Effects of pollen exposure to both to NO_2 and O_3

Pollen germination rate was unaffected by simultaneous exposure to both pollutants. Nevertheless, pollen tubes of the germinated grains were longer, revealing cell signalling changes favouring the process of pollen tube formation.

The increased enzymatic activities of SOD and catalase enzymes suggest oxidative stress, resulting from additive effects of both pollutants, with positive effects on the pollen tube growth driven by reactive oxygen species (Pasqualini et al. 2015) without significantly affecting the germination. Due to a synergistic effect, the presence of O_3 increases NO_2 absorption (Chassard et al. 2015) and strongly favours the nitration of proteins (Franze et al. 2005) thus generating amplified SOD and catalase activity and altered IgE recognition patterns compared to O_3 or NO_2 alone. Indeed, in the IgE recognition profile six bands were intensified (MW in the range 41.7–56.6 kDa) and three have shown lower IgE-reactivity (MW of 36.7, 76.3 and 88.1 kDa). It is noteworthy that these bands correspond to the same ones identified in pollen fumigated with NO_2 alone and that the effect of NO_2 was amplified by the presence of O_3, either for increased or for decreased immune reactivities. Additionally, in accordance to NO_2 treatment, the increased SOD activity suggests an increase of the SOD allergen (Conti et al. 2014), compatible with a low intensity band of MW 22.6 ± 0.2 kDa detected in our allergograms.

As for NO_2 treatment, the major allergen and the pan-allergen profilin were unchanged thus the effect over allergenicity remains unclear.

4.4 An integrated overview

This work suggests that common air pollutants evoke a modified pollen allergenicity, potentially contributing to a higher incidence of respiratory allergic diseases in urban areas (D'Amato et al. 2016) with high vehicle traffic emissions.

Our results have shown that pollen oxidative defences are activated by common air pollutants, affecting both its germination capacity and its allergenic activity. Concerning the pollen germination, it is remarkable that NO_2 alone stimulated the germination, resulting from NO signalling (Pasqualini et al. 2015), while O_3 did not affect germination. On the other hand, $NO_2 + O_3$ induced the formation of longer pollen tubes, possibly due to increased ROS formation (Pasqualini et al. 2015), without affecting germination; in this condition, ROS formation might be highly favoured due to synergistic effect of the pollutants together, as suggest the increased SOD and catalase activities, and possibly lower NO formation compared to NO_2 alone, due to the skewing of the equilibrium to the protein nitration (Ghiani et al. 2012; Gruijthuijsen et al. 2006). From an allergenic point of view, when comparing the effects of NO_2 with the effect of NO_2 and O_3 together, an amplification of the NO_2 effect was observed. Additionally, the O_3 stimuli, separately, seems to be more harmful, at least in the case of grass species, as shown by our results. In fact, differential allergenic potency of airborne grass and olive pollen depending on the year and geographical location has been reported (Buters et al. 2015; Galan et al. 2013). The results shown in this work may contribute to explain these differences.

Modified immunoreactivity may be due to either a change in protein expression levels in response to the induced stress or to a change in antigen–antibody recognition patterns as result of chemical modification such as nitration of proteins epitopes (Gruijthuijsen et al. 2006), or by the combination of both aspects. Subsequent studies of post-translational modifications of proteins will be necessary to distinguish the contribution of each of these factors to the changes shown in this and other works. However, regardless of the pathway underlying immunoreactivity modification, the amplification of pollen allergenicity is expected to induce increased allergic reactions and aggravation of allergic symptoms.

Considering the elevated values of O_3 reached in the Alentejo region during the main grass pollen season (end of April to beginning of June) (Bortoli et al. 2009), an increased allergenic risk is expected, particularly if associated with high-vehicle traffic environments.

Acknowledgements This work was funded by the European Union through the European Regional Development Fund, framed in COMPETE 2020 (Operational Programme Competitiveness and Internationalization), through the ICT project (UID/GEO/04683/2013) with reference POCI-01-0145-FEDER-007690, and by National Funds by the Portuguese Foundation for Science and Technology (FCT) through the projects PTDC/ATP-EAM/0817/2014 and UIDB/04683/2020—ICT (Institute of Earth Sciences). The authors thank Russell Alpizar-Jara, CIMA (Research Centre for Mathematics and Applications) and Department of Mathematics, School of Science and Technology, University of Évora, for reviewing the statistical analysis.

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Science of the Total Environment 832 (2022) 154892

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Differential *Quercus* spp. pollen-particulate matter interaction is dependent on geographical areas

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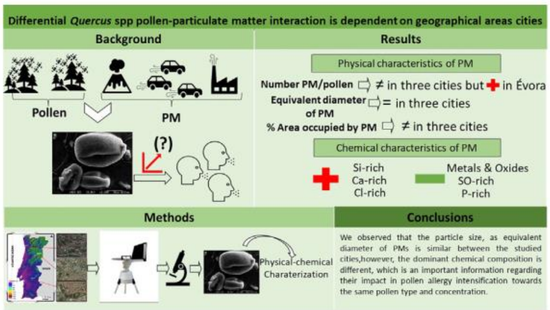
HIGHLIGHTS

- It was studied the properties of PM adhered to pollen in cities with contrasting environments.
- Airborne pollen was sampled, analyzed by SEM-EDS and air masses back trajectories were determined.
- Adhered particles size is similar among regions, with most having an equivalent diameter < 3 μm.
- Particles chemical composition was dominated by Si-rich, Ca-rich, Cl-rich and SO-rich, associated with other elements.
- Exposome impact on pollen allergies should consider PM concentration and chemical composition.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Hai Guo

Keywords:
Electron probe microanalysis
Particulate matter
Pollen
Respiratory allergies

ABSTRACT

Particulate matter (PM) and pollen interaction, either airborne or at the respiratory mucosa needs further clarification, as allergic reaction intensification can be related to the PM physical characteristics and toxicity. This study aimed to investigate the physical-chemical properties of PM that can adhere to the pollen wall during its transport or inhalation, using *Quercus* spp. as a model, in three Portuguese cities with different geographical locations, meteorological influence and urbanization levels. Possible sources were evaluated through air masses trajectory analysis using the HYSPLIT model and correlation with meteorological factors. The sampling was performed using a 7-days Hirst-type volumetric sampler, and the pollen grains were observed using a Field Emission Electron Probe Microanalyser for PM analysis. A secondary electron image of each pollen grain was taken, to determine the adhered particles characteristics and energy dispersive x-ray spectroscopy (EDS) spectra were obtained for individual particles. A total of 484 pollen grains was observed, with 7683 particles counted and 1914 EDS spectra analyzed. The particle's equivalent diameter ranged from 0.3-16 μm, with most having a diameter < 3 μm. For the three cities, there were significant differences in the number of particles per pollen and the % area occupied by the particles. Particles adhered were mainly

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http://dx.doi.org/10.1016/j.scitotenv.2022.154892
Received 29 November 2021; Received in revised form 24 March 2022; Accepted 25 March 2022
Available online 1 April 2022

Si-rich, but variations in other dominant groups were observed. For Évora and Guarda, Ca-rich, SO-rich were second and third more representative, while Porto were Organic and Cl-rich. Metals&Oxides were found in all cities with the highest number in Porto. P-rich particles were only found in Évora. Sea salt particles were observed in Évora, coincide with air mass trajectories possible carrying them from the Mediterranean Sea. In conclusion, the PM physical characteristics are similar between the studied cities, however, the dominant chemical composition is different, certainly impacting the exposome influence and pollen-allergy intensification towards the same pollen type and concentration.

1. Introduction

Pollen allergy is growing exponentially, particularly in urbanized environments, affecting between 10 and 30% of the world population (Ring, 2012). In the second half of the 20th century the pollen allergy was already very common in Western Europe. It was considered known as a rare disease, however, the prevalence has been increasing, and it is expected that by the year 2025, 50% of the European population will be affected by this pathology. Currently, 300 million people suffer from asthma and 400 million from rhinitis (Pawankar, 2014). The cause of this increase has been associated with several factors, including urbanization, industrialization, agriculture, air pollution, climate change and decreased biodiversity. The interaction between airborne pollen grains, air pollutants, and the immune system have been often described as the main trigger (Ring, 2012).

Among air pollutants, atmospheric particulate matter (PM) is one of the highest concerns, particularly related to human health. PM is a mixture of solid and liquid particles suspended in the atmosphere and may have an anthropogenic origin (car traffic, combustion, agriculture, or industry) or a natural origin (volcanic eruptions, forest fires, ocean-derived and bioaerosols) (Koenig, 2000). Generally, PM is classified according to their size or diameter and composition, ranging from nanometers (nm) to tens of micrometers (μm). Particles with less than 10 μm are classified as PM_{10} and defined as inhalable particles (Byeon et al., 2015; Ott et al., 2008). PM_{10} can reach only the upper respiratory tract and may cause increased severity of bronchial asthma attacks, cause or aggravate states of bronchitis or other lung diseases and reduce the body's ability to fight infections. Particles with a diameter below 2.5 μm ($PM_{2.5}$) are defined as fine particles, which can penetrate deep into the human respiratory system, reaching the tracheobronchial tract, increasing the specific risk of mortality, particularly from cardiovascular diseases. The smaller particles, $PM_{0.1}$, called ultrafine, have diameters of less than 0.1 μm and can affect the alveolar tract and reach the circulatory system, causing damage to internal organs (Baldacci et al., 2015; Zhao et al., 2020). In Portugal in 2008, there were between 21 and 40 deaths per 100,000 inhabitants derived from air pollution (WHO, 2011). Estimates from the World Health Organization showed that in 2012, seven million people died due to atmospheric pollution from both indoors and outdoors, which are now considered an individual risk to human health (WHO, 2014).

The link between PM and pollen allergy or bronchial asthma is widely documented in the literature (González-Díaz et al., 2016; Kim et al., 2013; Schiavoni et al., 2017; Kim et al., 2015; Knox et al., 1997), pointing to an adjuvant role of PM in modifying the environment of the respiratory mucosa in which pollen allergens are released, facilitating the access of these allergenic particles to antigen-producing cells, intensifying their response. Phosri et al. (2017) found that on days of high concentrations of PM, the number of medical consultations and hospital admissions by pollinosis increased. It was also verified that the number of admissions to the hospital emergency room due to asthma coincides with the increase in the concentration of PM (Kim et al., 2015). In urban areas, the high concentration of diesel exhaust particles (DEP's) may intensify the development of an immune allergic response (Riedl and Diaz-Sanchez, 2005; Saxon and Diaz-Sanchez, 2005).

The intensification of the allergic reaction may be related to the physical characteristics of PM and its toxicity (Le Souef, 2009; Traidl-Hoffmann et al., 2009). PM may induce cellular stress and toxicity, depending on particle size, chemical composition, and bound molecules on their surface (Kim et al., 2015). PM containing more transition metals, such as iron, increase the production of reactive oxygen species (ROS), causing genotoxic effects (Gilli et al., 2007; Kadiiska et al., 1997). In addition, associated with PM it may be found endotoxins that cause inflammation of the airways (Löndahl et al., 2006).

The airborne interaction between PM and pollen can be explained by coagulation processes, forming a pollen-particle complex (Schiavoni et al., 2017; Gottardini et al., 2004). Pollen grains from polluted areas, when compared to pollen from areas with low pollution, are more exposed to PM and therefore more likely to transport them on their surface. Duque et al. (2013) observed significant differences between the elemental composition of airborne pollen wall compared to that of pollen harvested directly from the plant. Guedes et al. (2009) observed the presence of diesel exhaust particles in pollen of the species *Chenopodium album*, harvested from areas with high road traffic. In addition, since PM presents physical and chemical characteristics that vary by location and have different degrees of toxicity for the environment and public health (Calvo et al., 2013; Sénéchal et al., 2015), likely the PM adhered to airborne pollen could reflect the overall chemical composition of the aerosol fraction of a site at a given moment. Furthermore, when humans breathe, the air inhaled enters the respiratory system in a whirlwind manner, which facilitates the PMs adhesion to the sticky pollen wall. Also, the meteorological parameters can influence the aggregation of PM to pollen grains. The high air temperature and consequently low relative humidity can be associated with lower agglomeration capacity of PM's (Anastasio and Martin, 2001). The wind speed can be associated with the high dispersion and possibly the low adsorption capacity of PM to the pollen surface. Cichowicz et al., 2020 showed that the lower wind speed is associated with higher concentration of PM in the air (Cichowicz et al., 2020). Wind direction is another condition that can influence the adherence of PM to the pollen surface, because studies have shown that the distribution of sources is very important for the detection of biological and organic particles (Palacios et al., 2000).

Therefore, the goal of this study was to characterize the physical (size, surface, particle number) and chemical (inorganic and organic) properties of the PM that can adhere to the pollen wall during its transport or inhalation, using *Quercus* spp. as a model, in three cities with different geographical positions, Porto (Atlantic coastal city), Guarda (high altitude city), and Évora (small size city located at a flat rural region). Also, it investigated the relation between meteorological parameters and back trajectories air masses analysis to ascertain changes in the PM characteristics. *Quercus* pollen was used due to its high abundance in Portugal.

2. Methods

2.1. Study area

The study was conducted in the cities of Évora, Guarda and Porto, located in Portugal (Fig. SI-1). The city of Évora (38.568°N; 7.912°W, 293 m asl), with about 50,000 inhabitants is located in the vast region of Alentejo, the largest Portuguese region with an area corresponding, to one-third of the country territory, approximately. The town is nestled in a vast mainly flat and rural region, dominated by holm (*Quercus rotundifolia* or *Quercus ilex*) and cork oaks (*Quercus suber*) with underlying semi-natural vegetation, constituting an important agro-silvo-pastoral system termed Montado (Godinho et al., 2016), where in the last years the agriculture pressure is also steadily increasing (Palma et al., 2021). The main sources of local pollution are related to traffic and, in the cold season, biomass burning used for domestic heating (Malico et al., 2017). Sometimes, long-range transport of anthropogenic or natural aerosols occurs, as urban/industrial (Santos et al., 2008, 2013), forest fire (Sicard et al., 2019; Salgueiro et al., 2021), desert dust (Bortoli et al., 2009; Valenzuela et al., 2017) and bioaerosols (Galveias et al., 2021). According to Köppen Climate Classification, Évora is characterized by a temperate climate with warm and dry summers that can be described as a Hot-summer Mediterranean climate (Table 1) (www.ipma.pt last accessed February 16, 2022). The most common pollen species in Évora are Cupressaceae, *Platanus*, *Quercus*, *Olea* and Poaceae. Other species are found but in minor concentrations as *Rumex*, *Populus*, Urticaceae, *Chenopodium*, *Alnus*, *Casuarina* and *Eucalyptus* (Camacho et al., 2020; https://www.ipma.pt/pt/saude/polens/ last accessed September 3, 2021; https://lince.di.uevora.pt/polen/index.jsp, last accessed September 3, 2021). 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. The facility is located in the city center, surrounded by trees and buildings, with low road traffic in the neighborhood (Fig. SI-1).

The city of Guarda (40.53° N, 7.26° W, 1056 m asl) with 42,541 inhabitants, is the highest in mainland Portugal at an altitude of 1056 m. It is the capital of the Guarda district, which is in the country central region in the sub-region of Beiras and Serra da Estrela. The landscape is essentially rural where the existing industry is not responsible for the emission of significant air pollution. The main source of gas emissions into the atmosphere comes from car traffic within the city and the surrounding highways, especially the A23 and A25, and from the combustion of biomass and natural gas used to heat the buildings. Also, some pollution originating in industrial areas in the coastal cities of the country can be transported to inland areas. Guarda has a warm summer and temperate climate according to Köppen Climate Classification (Table 1) (www.ipma.pt last accessed February 16, 2022). The most frequent pollen types are *Quercus* spp., Pinaceae and Poaceae corresponding to about 50% of the observed total pollen. Other pollen types are found, although in minority concentrations, Cupressaceae, Urticaceae, Apiaceae, Oleaceae and Polygonaceae (Lisboa et al., 2016). The sampling point was set on the roof of the Municipal Theater building, 20 m above ground level (Fig. SI-1), close to the main streets of the city center with some traffic during the day and nearby residential and service areas, in the vicinity of public gardens and the forest of the old sanatorium and current hospital Sousa Martins.

The city of Porto (41.51° N, 8.61° W, 115 m asl), the second-largest Portuguese city with about 238,000 inhabitants and a population density of 5736 inhabitants per km^2, is limited on the west by the Atlantic Ocean and the south by the Douro River. It is quite urbanized, with stationary sources of anthropogenic atmospheric pollutants like oil, petrochemicals, incineration units and shipping port activities, also presenting a high level of road traffic. The common species of trees found are *Acer spp, Fraxinus spp, Liquidambar spp, Quercus spp, Pinus spp, Platanus spp, Populus spp, Prunus* spp. *and Tilia* spp. Regarding herbaceous plants, Plantago, Poaceae, Rumex or Urticaceae predominate. Porto has a warm summer and temperate climate according to Köppen Climate Classification (Table 1) (www.ipma.pt last accessed February 16, 2022). The sampling point was set on the roof of the Faculty of Sciences in Porto, 20 m above ground level (Fig. SI-1), located near a residential area, near the ocean and the Douro River and very close to a road with high highway traffic, considered one of the main entrances of the city, where in rush hours there are frequent traffic jams.

Table 1
Annual values of meteorological parameters at Évora, Guarda and Porto, according to the climatologic normal (1971–2000) provided by the Portuguese Institute for Sea and Atmosphere (https://www.ipma.pt/pt/oclima/normais.clima/1971-2000/; Miranda et al., 2001).

	Tmean, °C	Tmax, °C	Tmin, °C	Prec, mm	RH, %	WD, °	Köppen Climate Classification
Évora	15.9	20.7	11.0	609.3	76	NW	Csa
Guarda	10.9	14.7	7.0	882.0	77	NW	Csb
Porto	14.7	19.2	10.2	1253.5	82	W-NW (summer) and E-SE (Winter)	Csb

2.2. Airborne pollen sampling

Airborne pollen sampling was done using a Hirst-type 7-day volumetric spore trap (Hirst, 1952) that is a one stage impactor, designed to sample air at a rate of 10 L/min, simulating the human inhalation. By passing through a narrow intake orifice (2x14mm) 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 Melinex tape, where airborne pollen is retained (Solvedilla et al., 2007). In our study, the Melinex tape was coated with a double-sided adhesive carbon tape, aiming at preserving the integrity of the pollen wall and allowing the following analysis. After exposure, this tape is cut into seven 48 mm long segments, representing each one day of sampling, that is mounted on a microscopic glass slide.

Four consecutive days were sampled in April 2017 (21st to 24th) (Fig. SI-5), which corresponded to the only period when peak pollination of *Quercus* spp. was concomitant in all three cities.

2.3. Meteorological data

The meteorological data used for Évora were obtained from the meteorological station installed at the Évora Atmospheric Sciences Observatory (EVASO) where the pollen sampler is also installed, for Porto by a meteorological station located 7 m apart from the pollen sampler at approximately the same altitude and Guarda from an IPMA automatic climatological station, located 1000 m apart from the pollen sampler. The parameters measured were the air Temperature (°C), Relative Humidity (RH; %), Wind Speed and Direction (m/s; °). All variables were measured in 10 s intervals, and then averages were performed to originate hourly and daily data, except for precipitation that is accumulated during those periods. 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 (Scientific Campbell CR1000 Measurement and Control System) (https://s.campbellsci.com/documents/br/manuals/cr1000.pdf last accessed December 21, 2020). All instruments are subject to periodic checks and maintenance.

2.4. Pollen analysis

Scanning Electron microscopy (SEM) with energy dispersive x-ray spectroscopy (EDS) is commonly used for single particle studies (Sobanska et al., 2006), as this method provides useful information on the morphology, elemental composition, density, and origin of aerosols (Kushwaha et al., 2012). In our study, a Field Emission Electron Probe Microanalyser (JEOL JXA-8500F) was used to investigate the physical-chemical characteristics of the particulate matter adhered to the surface of airborne pollen. This equipment combines high SEM resolution with X-ray analysis of submicron areas and is equipped with an EDS spectrometer and 5 WDS (Wavelength Dispersive Xray Spectroscopy) spectrometers (Oxford INCA X-Act) that allowed the simultaneously analysis of 5 elements WDS + 16 elements EDS as well as collecting images derived from backscatter and secondary electron detectors.

The microscopic slides were carbon coated at the same thickness for conductivity and reduction of electron charge. Slides were scanned along 4 to 6 equidistant traverse lines, depending on pollen concentration and *Quercus* spp. pollen was randomly selected and visually identified. A total of 484 pollen grains was observed.

For each pollen grain, a secondary electron image (SEI) was taken (magnifications varied between 2300 and 3300 times) to perform PM physical characterization, and an EDS spectrum (10 kV, 10 nA, 15 s) for all possible individual particles adhered to the pollen surface was obtained (Ribeiro et al., 2015). A total of 1914 EDS spectra was analyzed and used to chemically classify the particles based on the most prominent element peak intensities. This methodology showed image resolution restrictions concerning ultrafine PM, which are certainly adhered to the pollen, but was not possible to measure particles below 1 μm in diameter.

All SEI images were analyzed to determine the size parameters of the different pollen and particles adhered to the pollen wall. For this, pollen, and particle equivalent projected area diameter (diameter of a circle having the same area as the projection area of the particle), the number of particles counted per pollen, percentage of pollen area occupied by particles and particle size distribution were determined using ImageJ software. A total of 7683 particles was counted.

2.5. Statistical analysis

Since data did not followed a normal distribution (assessed by the Kolmogorov-Smirnov test), non-parametric Independent Samples Median test ($p < 0.05$ was accepted) was applied to compare if the number of particles per pollen, the equivalent diameter of pollen and the percentage of pollen area occupied by the particles varied significantly between the three studied cities.

Spearman correlation coefficients ($p < 0.05$ and $p < 0.01$ was accepted) were determined to study the non-linear relationship between the daily average meteorological parameters (mean temperature, relative humidity and wind speed) and daily average physical and chemical characteristics of particulate matter attached to the *Quercus* spp. airborne pollen in each city. For the wind direction, each hourly value of the data was coded according to 8 quadrants: N, NE, E, SE, S, SW, W, NW, then becoming a nominal variable. For each day, the frequency of hourly average wind direction in each quadrant was calculated, and these values were used in the correlation analysis. Graphics and the statistical analysis were done using IBM SPSS statistics v.21 software.

2.6. Backward trajectory of air masses

Backward trajectories of air masses arriving in Évora, Guarda and Porto were calculated using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT). This model, developed by the National Oceanic and Atmospheric Administration (NOAA), is one of the most widely used and complete models for simple trajectory calculations (Rolph et al., 2017; Stein et al., 2015). The meteorological parameters used to calculate the back trajectories are obtained from the Global Data Assimilation System (GDAS) at a spatial resolution of 0.5°. The vertical motion method used to calculate the back trajectories is the vertical velocity. The back trajectories were calculated for 48 h, for every hour of that period, at the arrival levels of 50, 250 and 500 m. The backward trajectories every three hours for the three levels are presented as supplementary material.

3. Results

3.1. Bioclimatic characterization of Évora, Guarda and Porto

Fig. 1 shows the boxplots of the meteorological parameters measured at the meteorological stations of the three sites (Évora, Guarda and Porto, from 21 to 24 April 2017).

The average temperature was fairly similar between the cities of Évora and Porto, however, in the city of Guarda the temperature was approximately 5 °C lower. The Relative Humidity (RH) was in turn quite similar between Évora and Guarda, while on Porto it was in average higher and presented more variability. The wind speed was higher in the Évora and Porto regions compared to Guarda and the wind direction was variable between *E*-W in Évora, while in Guarda and Porto it varied between S-SW (Fig. 1). There was no precipitation during the period of study.

3.2. Quercus spp. pollen equivalent diameter analysis

A total of 245 pollen grains were observed for the city of Évora, 155 pollen grains for Guarda and 84 pollen grains for Porto, with average equivalent diameters of 23.58 ± 4.01 μm, 22.74 ± 4.16 μm and 21.83 ± 3.27 μm, respectively. However, in all study cities, it was possible to divide the *Quercus* pollen into two groups, according to their size (Fig. SI-2), one with pollen with less than 25 μm and another between 27 and 35 μm (Fig. 2). Most of the pollen presented size of less than 25 μm, with the largest dimension group accounting for 30% in Évora, 26% in Guarda and only 10% in Porto. The median equivalent diameter of pollen from Évora was not significantly different from the one in Guarda but both were significantly larger than the one from Porto ($p = 0.008$ and $p = 0.023$, respectively) (Fig. 2).

To analyze if there were significant interdaily differences in the pollen equivalent diameter, an non-parametric independent samples median test was applied. According to the results described in Table 2, there were statistically significant differences in pollen size between the study days for the city of Évora ($p = 0.005$) and Guarda ($p = 0.015$). A clear increase in the average pollen size along the study period was observed in Évora while in Guarda the largest average size was observed on the 22nd and the smallest on the 24th of April. In the city of Porto, there were no significant differences between the days under analysis (Table 2).

3.3. Physical characterization of PM adhered to Quercus pollen

All 484 pollen grains accounted were analyzed for the number of particles per pollen and the percentage area occupied by the particles. Significant statistical differences were observed between the median PM/pollen of the three cities (Fig. 3). The number of pollen grains free of particles varied also between study places, with Guarda presenting most of the observed *Quercus* pollen grains with no particles adhered to its wall

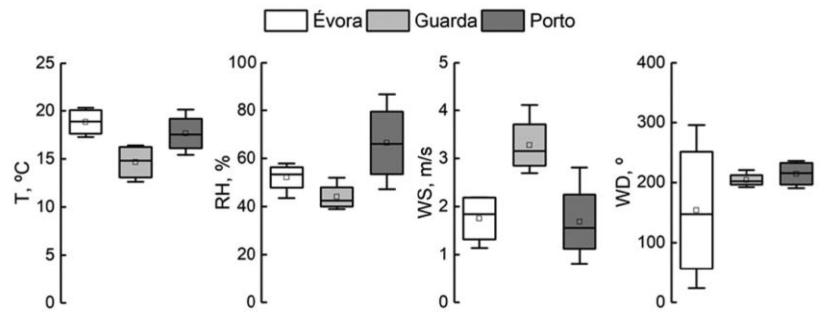

Fig. 1. Boxplots showing the variation of the meteorological parameters in Évora, Guarda and Porto during the days 21–24 April 21 of the year 2017. T: Temperature; RH: Relative Humidity; WS: Wind Speed; WD: Wind Direction.

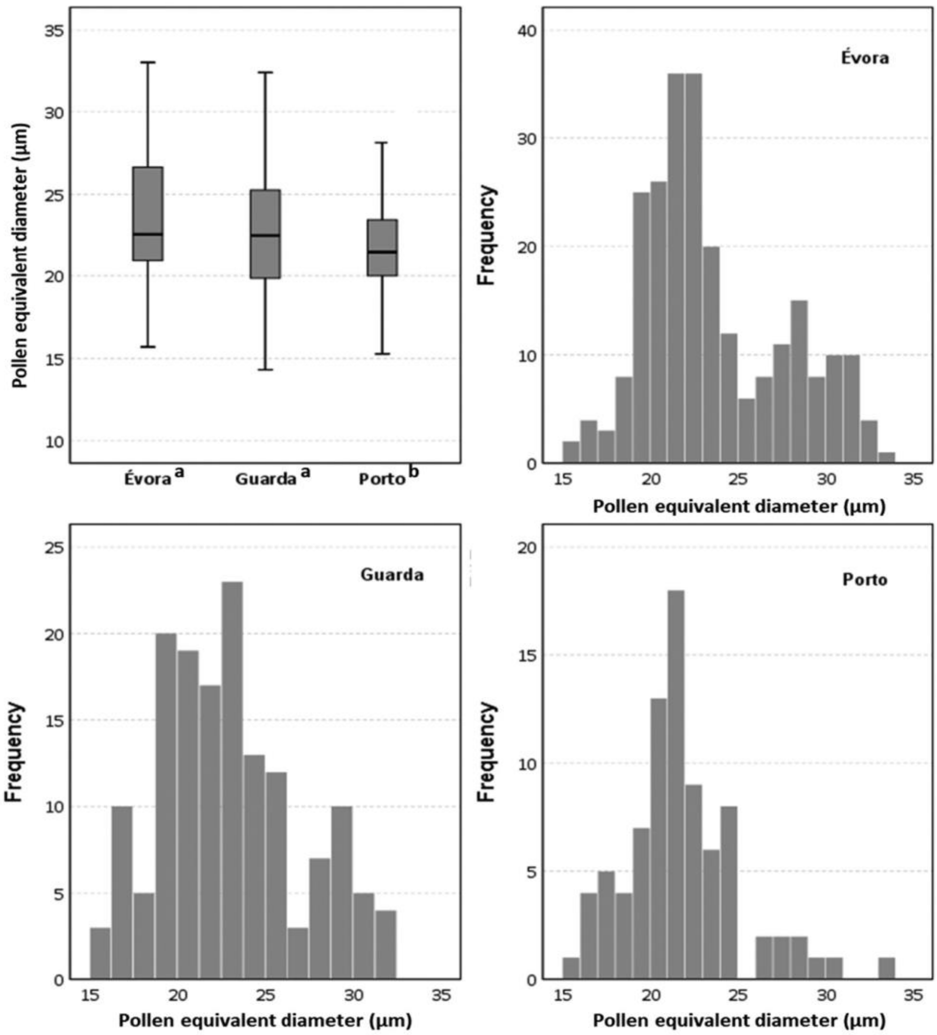

Fig. 2. Pollen equivalent diameter (µm) from Évora, Guarda and Porto (Boxplot and respective histograms). Different letters indicate statistically significant differences given by the Independent-samples median test followed by pairwise comparisons (*p*-value <0.05).

(66%) in opposite to Porto where 52% of the pollen grains had at least one particle and Évora where most of the pollen grains (72%) presented from 1 up to 19 adhered particles. In the city of Évora, the average number of particles per pollen (PM/pollen) was 3.6 ± 3.9, 3-fold higher than those registered in Guarda (1.4 ± 2.6 PM/pollen) and Porto (1.6 ± 2.0 PM/pollen) (Fig. 3).

Regarding the percentage of the *Quercus* pollen area occupied by the particles, in the city of Évora was registered the pollen with the highest area covered by particles, followed by Porto and finally by Guarda with 4.0 ± 5.2, 2.30 ± 3.5 and 2.3 ± 5.7%, respectively. There were significant statistical differences in the median value between the three studied cities (see dash line in Fig. 3).

Table 2
Equivalent diameter of the *Quercus* pollen from Évora, Guarda and Porto in each day analyzed.

Pollen equivalent diameter (mm)															
	Évora					Guarda					Porto				
	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max
21st	87	22.2 ± 3.1^{a}	21.5	15.7	31.4	36	$22.3 \pm 3.9^{a,b}$	22.3	14.7	31.9	19	22.1 ± 2.7	21.5	17.7	29.3
22nd	61	$23.3 \pm 3.8^{a,b}$	22.5	16.8	33.0	57	24.1 ± 4.0^{a}	23.3	14.3	32.4	32	22.0 ± 3.3	21.9	15.3	30.9
23rd	46	$24.6 \pm 4.3^{b,c}$	23.5	16.0	32.0	36	$22.7 \pm 4.8^{a,b}$	22.2	14.8	30.8	25	21.7 ± 3.8	20.9	16.8	33.4
24th	51	25.3 ± 4.5^{c}	25.5	15.9	32.9	26	20.4 ± 2.7^{b}	20.1	16.3	26.2	8	21.1 ± 3.2	20.9	16.7	26.6
p-value		**0.005**				**0.015**					0.670				

p-value associated with the Independent Samples Median test; different letters indicate statistically significant differences (Independent Samples Median test, *p*-value <0.05).
SD: Standard deviation; Min: Minimum; Max: Maximum; NP: number of pollen grains. Bold values indicate the statistical significance at 5% (p-value <0.05).

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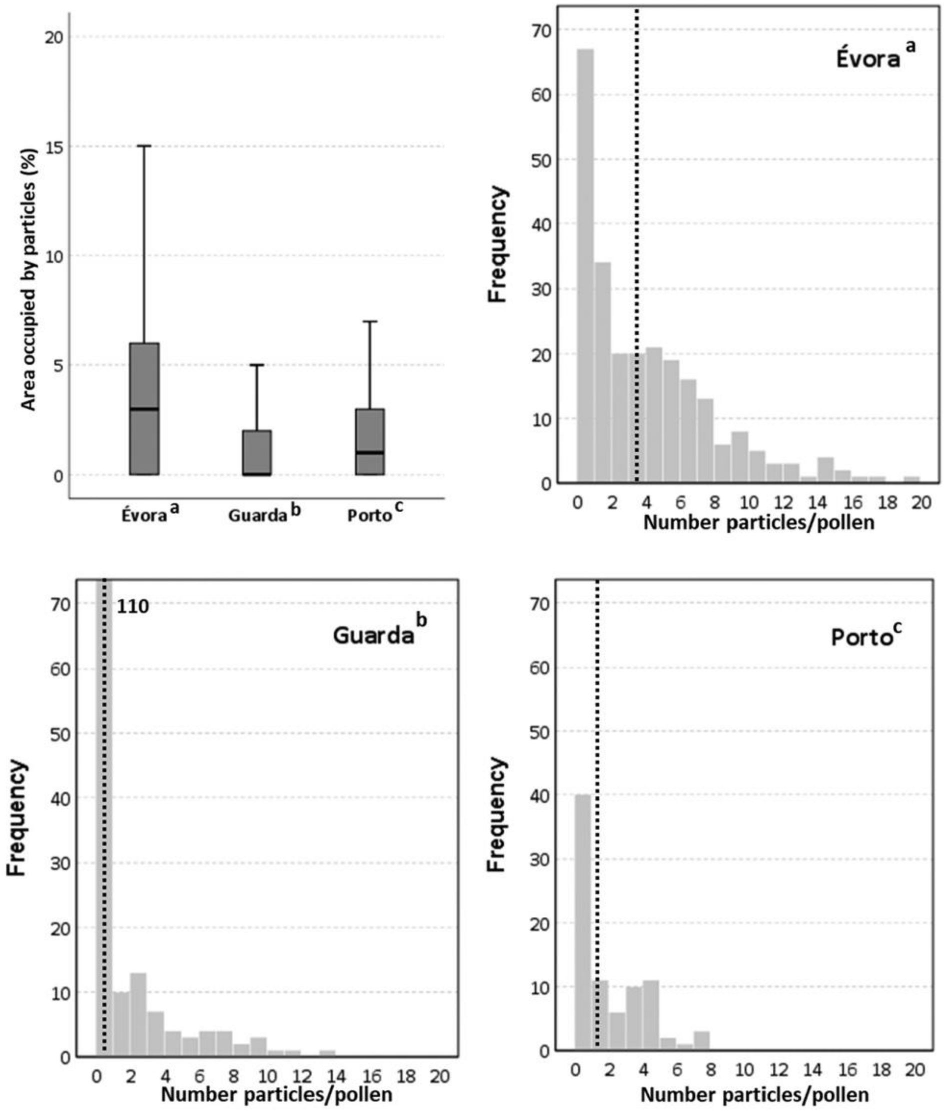

Fig. 3. Percentage of *Quercus* pollen area occupied by particles (boxplot and histograms of the number of particles per pollen) in Évora, Guarda and Porto. Different letters indicate statistically significant differences given by the Independent-samples median test followed by pairwise comparisons (*p*-value <0.05). The dashed line represents the median value obtained for each site. The different letters within the graphs indicate the statistical significance at 5% (p-value <0.05).

Considering the physical characterization of individual particles adhered to *Quercus* pollen wall, a total of 890 particles were analyzed for Évora, 217 for Guarda and 142 for Porto. The particles size distribution was very similar between the three cities, with average particle sizes of 2.16 ± 1.23 μm for Évora, 2.39 ± 1.61 μm for Guarda and 2.28 ± 1.28 μm for Porto. The equivalent diameter values ranged between less than 1 μm up to 16 μm, with the majority of the particles presenting a diameter lower than 3 μm (83% in Évora, 81% in Guarda and 75% in Porto) (Fig. 4).

An interdaily comparison analysis of the number of PM per pollen, the equivalent diameter of particles adhered to the pollen wall and percentage area of pollen occupied by particles was carried out for the three cities, between April 21st and 24th (Table 3). There was no significant differences between the sampling days for Évora and Guarda, $p < 0.179$ and $p < 0.220$, respectively. However, in Porto, there are significant differences between days, where the 21st of April had a significantly lower number compared to the other days ($p < 0.001$) (Table 3). As for the particle equivalent diameter, no significant differences exist between the days in Évora ($p < 0.327$), Guarda ($p < 0.070$) or Porto ($p < 0.236$). For the percentage area occupied by the particles, there was no significant differences between the sampling days in Évora ($p < 0.058$) and Guarda ($p < 0.220$) while in Porto, the day 21st of April had a significantly lower percentage than the other days ($p < 0.001$) (Table 3).

The non-linear correlation coefficients between daily average physical characteristics of particulate matter attached to airborne *Quercus* pollen and daily average meteorological parameters were computed for the study period. Overall, it was observed that wind direction is the parameter with the highest significant correlation with particles physical characteristics (Table 4). Wind speed has a negative significant correlation with the number of particles/pollen ($p < 0.043$) but no correlation with the other physical characteristics. Wind direction from E has a negative significant correlation with the number of particles per pollen, as well as NW winds with the equivalent diameter of particles (maximum) and the maximum

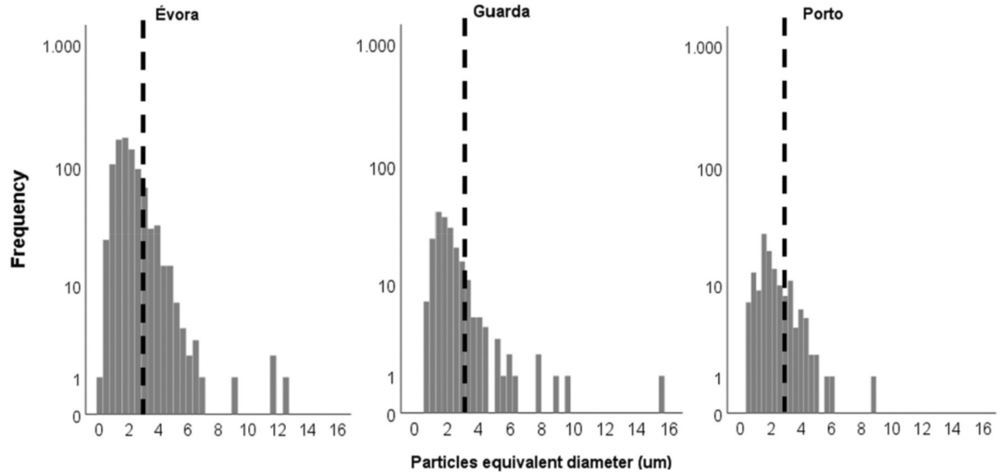

Fig. 4. Size distribution of the equivalent diameter of measured particles adhered to airborne *Quercus* pollen wall in Évora, Guarda and Porto (dashed line marks value with 3 μm size). The Independent Samples median test (*p*-value <0.05) shows that particle equivalent diameter between Évora, Guarda and Porto is not significantly different.

area occupied by particles. Opposite, winds from the WS quadrant have a positive and significant correlation with the number of particles per pollen (maximum) and the equivalent diameter of particles.

Average temperature and Relative humidity did not show a correlation with particles physical characteristics (Table 4).

3.4. Chemical classification of PM adhered to Quercus pollen

The chemical classification of 846 individual particles was analyzed and described in Table 5, corresponding to 585 particles for the city of Évora, 150 for the city of Guarda and 111 for the city of Porto (Fig. SI-3).

Si-rich was the most abundant group in the three regions, reaching values at Évora, Guarda and Porto of 64%, 89% and 65%, respectively (Table 5). The elements associated with the Si-rich group that were observed in higher concentration were AlSi rich, abundance 62% in Évora, 50% in Guarda and 39% in Porto, and SiO with an abundance of 18%, 18% and 25% in Évora, Guarda and Porto, respectively.

Other groups were present, and variations between the studied cities relative to the dominant ones were observed. For the city of Évora and Guarda, Ca-rich (most abundant association Ca-C-O), SO-rich (most abundant association SO-Ca) were second and third more representative while for Porto were particles with an organic origin, corresponding to almost 12% and Cl-rich. In Évora, particles with organic-origin account for less than 5% and Cl-rich around 4%.

The Metals & Oxides group could be found in all cities with the highest number in Porto followed by Évora. Sea salt particles were observed in Porto and Évora, in similar amounts, but were absent in Guarda while P-rich particles were only found in Évora.

It is interesting to notice that among the same chemical group there were differences observed in the particle size distributions between the study regions (Fig. 5). Although most of the particles belong to the $PM_{2.5}$ fraction, for instance, the size of the Si-rich particles at Guarda is greater than 1 μm, compared to the other cities that contain particles with sizes lower than 1 μm. A similar situation happens in Évora for the SO-rich

Table 3
Daily values of the pollen equivalent diameter and measured physical characteristics of particulate matter adhered to the *Quercus* spp. pollen wall in three cities, Évora, Guarda and Porto.

	Évora					Guarda					Porto				
	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max
Number of Particles/pollens															
21st	87	3.2 ± 3.4	2	0	19	36	1.4 ± 2.5	0	0	9	19	0.4 ± 1.2^{a}	0	0	5
22nd	61	3.9 ± 3.5	3	0	14	57	1.0 ± 2.1	0	0	10	32	1.4 ± 1.6^{b}	1	0	5
23rd	46	2.7 ± 3.8	1	0	15	36	1.3 ± 2.6	0	0	13	25	2.2 ± 1.8^{b}	2	0	6
24th	54	4.9 ± 4.8	4	0	17	26	2.5 ± 3.6	0	0	11	8	3.4 ± 3.2^{b}	3	0	7
p-value	0.179					0.220					**0.001**				
Equivalent diameter of each particle (μm)															
21st	276	2.1 ± 1.5	1.8	0.3	12	52	2.2 ± 1.5	2.0	0.7	8	7	1.7 ± 0.7	1.7	0.8	3
22nd	239	2.2 ± 1.3	1.9	0.4	12	55	2.5 ± 2.2	1.9	0.8	16	46	2.4 ± 1.1	2.3	0.6	5
23rd	127	2.2 ± 1.1	1.9	0.7	6	46	2.2 ± 1.2	1.8	1.1	6	54	2.3 ± 1.6	1.8	0.4	9
24th	248	2.2 ± 0.9	2.0	0.6	6	64	2.6 ± 1.4	2.3	1.0	10	35	2.2 ± 1.1	1.8	0.7	5
p-value	0.327					0.070					0.236				
Area of pollen occupied by particles (%)															
21st	87	4.2 ± 5.7	2.2	0	31.5	36	1.8 ± 4.0	0	0	18.9	19	0.3 ± 0.9^{a}	0	0	3.7
22nd	61	4.7 ± 5.9	3.1	0	33.7	57	2.0 ± 6.9	0	0	46.8	32	2.0 ± 2.8^{b}	0.9	0	11.0
23rd	46	2.7 ± 3.8	0.1	0	13.9	36	1.3 ± 2.8	0	0	10.2	25	3.6 ± 4.6^{b}	2.3	0	20.9
24th	54	4.1 ± 4.1	3.8	0	16.8	26	4.8 ± 7.4	0	0	22.2	8	4.2 ± 4.1^{b}	4.1	0	11.0
p-value	0.058					0.220					**0.001**				

p-value associated with the Independent Samples Median test; different letters indicate statistically significant differences (Independent Samples Median test, *p*-value <0.05). SD: Standard deviation; Min: Minimum; Max: Maximum; N: number of pollens/number of particles. Bold values indicate the statistical significance at 5% (p-value <0.05).

Table 4
Spearman correlation coefficients between daily average physical characteristics of particulate matter attached to airborne *Quercus* pollen, daily average meteorological parameters in Évora, Guarda and Porto for the days 21–24 April 2017. Values presented correspond to the coefficient's correlation of the Spearman test.

		T, °C	RH, %	WS, m/s	WD, °							
					N	NE	E	SE	S	SW	W	NW
N° PM	Mean	ns	ns	−0.592*	ns	ns	−0.802*	ns	ns	ns	ns	ns
	Max	ns	ns	ns	ns	ns	ns	ns	0.868**	−0.796*	ns	ns
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
PED	Mean	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.876**	ns
	Max	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.862**
	Min	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
% área occup.	Mean	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Max	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.895**

N° PM: number of particles per pollen; PED: particle equivalent diameter in μm; Min: minimum; Max: maximum; T: temperature; RH: relative humidity; WS: wind speed; WD: wind direction.
** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

particles that are present in this city in sizes lower than 1 μm, which does not occur in Porto and Guarda. Most particles of organic and metals & oxides nature in Évora have a size lower than 1 μm (Fig. 5).

The daily variations in the chemical composition of the particles adhered to *Quercus* wall pollen wall are represented in Fig. 6. Si-rich particles are the dominant particles in the days analyzed for the three regions. The daily concentrations over the 4-days period are very similar, both in Évora and Guarda, whereas for Porto there is an abrupt variation from the first day considered (21st April) presenting 100% of Si-rich particles, down to about 58% on the 24thApril.

Interdaily differences in the particle chemical composition are also observed for the other group-types. In the city of Évora, Cl-rich, SO-rich and organic particles presented a great decrease in concentration on the 23rd while particles of sea salt origin were only found on this day. Metals & Oxides particle group had a daily stable presence but at minor concentrations. In Guarda, on 21st, in addition to the Si-rich group, only particles of Metal & Oxides origin were observed. Ca-rich, SO-rich and Cl-rich particles presented interdaily variations, with Ca-rich being higher on the 22nd, while SO-rich was on the 24th. On the 22nd it was observed the greater decrease in the number of Si-rich particles and the only day with particles of organic origin. In Porto, the 22nd of April presented the most diversity in the particle chemical composition, with all accounted groups observed. Although Porto is a coastal city, particles of sea salt origin were only present in this day. Organic and Cl-rich particles have the greatest representation on the 23rd and Ca-rich particles were only found on this day. Particles of Metal & Oxides origin were the most present on the 24th.

The HYSPLIT model was used to estimate the possible source contribution of particles adhered to the surface of pollen grains (Fig. SI-4). For the city of Évora on 21st the air masses originated from the East during the morning, turning slightly to the southeast throughout the afternoon (supplementary material). The dominant chemical composition of the particles on this day was Si-rich, Ca-rich, Cl-rich and SO-rich. The back trajectories are shown in Fig. 7 for the selected time of 15:00 and arrival level of 50 m, whereas a more complete set of back trajectories can be found as supplementary material. On the 22nd, the air mass arriving at Évora continued from the southeast during the night, gradually moving to the east in the early morning and maintaining this origin for the rest of the day; the dominant particles were from Si-rich, Ca-rich, SO-rich with the increase of other particles and organic material compared to the previous day. On the 23rd the presence of sea salt particles is observed, coinciding with trajectories from the east, some coming from the western Mediterranean (Fig. SI-4) that arrived at Évora on the night and morning of 23rd April. On 24th the lowest air mass originated from the northwest, passing the industrialized coastal areas of Lisbon and Setúbal, whereas the highest (at 500 m) rotates during the day from northeast/east in the early hours, to northwest in the evening, observing the increase of Mixed particles, Organic particles, and Metals & Oxides, nonetheless, in a small percentage.

For the city of Guarda, on 21st, the air masses originated from the east, sometimes slightly northeast throughout the day. The dominant chemical particles were Si-rich particles. A small percentage of Metals & Oxides particles were also detected, which did not happen on the remaining sampling days. On 22nd, air mass originated from the east until the end of the afternoon, turning northwest. There was an increase in particles of organic origin, Ca-rich particles and SO-rich. On 23rd, air mass maintained their origin from the east, and only Si-rich and Ca-rich particles were detected. On the 24th, the trajectory seems to be local most of the day, extending from the

Table 5
Chemical classification, based on the element peak intensities of EDS spectra and frequency of particulate matter adsorbed to the airborne *Quercus* pollen.

Groups	Elements	Frequency (%)			Groups	Elements	Frequency (%)		
		Évora	Guarda	Porto			Évora	Guarda	Porto
Si-rich	Si-O; Ca-Si; Ca-K-Si; K-Si; Mg-Si; Na-Si; Cl-Si Na-Mg-Si; Ca-Mg-Si Al-Si-Fe-O; Al-Ti-Si; Al-Si; K-Al-Si; Ca-Al-Si; Mg-Al-Si; Zn-Al-Si;	63.6	89.3	64.9	Metals & Oxides	Fe-rich; Fe-O; Fe-Ni; Ti-O	1.4	0.7	3.6
					P-rich	P-O; P-O-Ca; P-O-Mg;	0.9	–	–
SO-rich	SO, SO-Na; SO-Ba; SO-Ca-K; SO-Fe; SO-Sr SO-K; SO-Ca; So-Cu SO-Ca-Na;	7.7	4.0	3.6	Cl-rich	Cl-Mg; Cl-Na; Cl-Na-Mg; Cl-K; Cl-Na-K; Cl-Ca-K Cl; Cl-Ca	4.1	–	5.4
Ca-rich	Ca-rich; Ca-C-O; Ca-Mg-C-O;	9.1	4.7	3.6	Sea salt misc	Na,Mg,Cl,Ca,K, S,Si, P; SO-Ca-Cl-Na;	1.2	–	1.8
Organic	C-rich (elemental & organic)	4.8	1.3	11.7	Mix	–	7.4	–	5.4

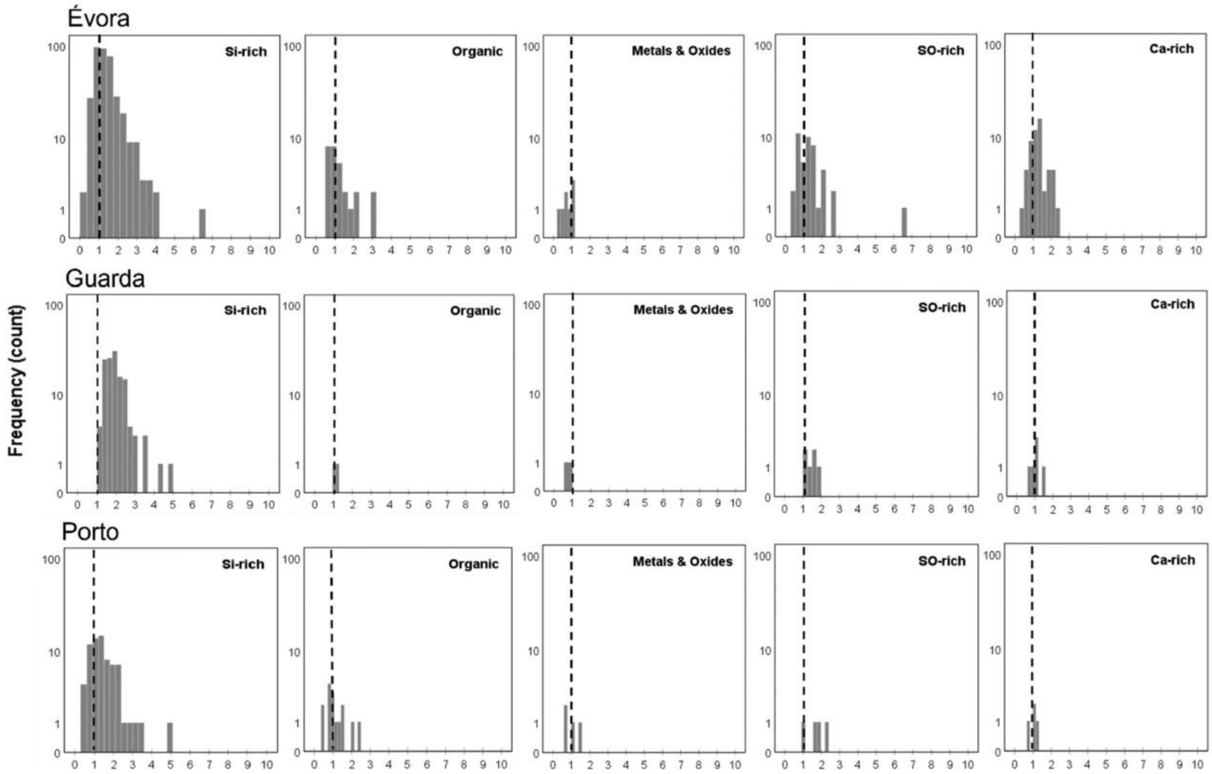

Fig. 5. Size distribution of the equivalent diameter of dominant chemical groups of particles adsorbed to the pollen wall of airborne *Quercus* spp. Dashed lines correspond to particles with a size of 1 μm.

Atlantic Ocean coastal regions in the late afternoon and evening, with an increase in SO-rich particles and the existence, albeit in small concentrations, of Cl-rich particles (Figs. 6 and 7).

For the city of Porto on 21st the air mass originated from east/southeast throughout the day, where only Si-rich particles were found. On 22nd the air mass would originate from the southeast, however, around 15:00 the back trajectory passes through the coastal zone, taking a short turn before reaching the end. This trajectory is coincident with the appearance of sea salt particles (Figs. 6 and 7). On 23rd the air mass originates from the northeast passing through the coastal zone near Galicia. During the afternoon

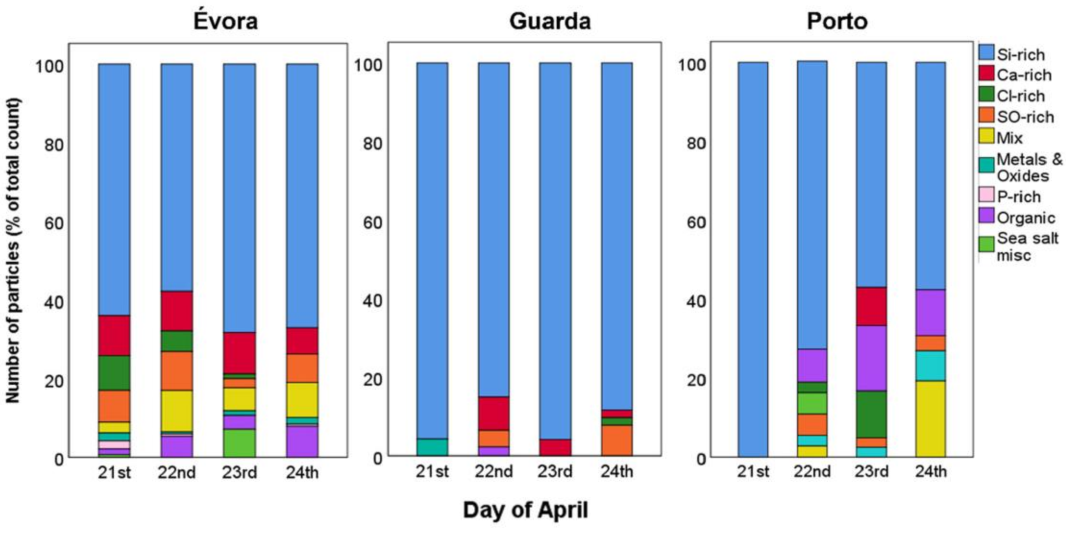

Fig. 6. Daily variation (% of total counts) of the chemical composition of particles adsorbed to the pollen wall of *Quercus* spp.

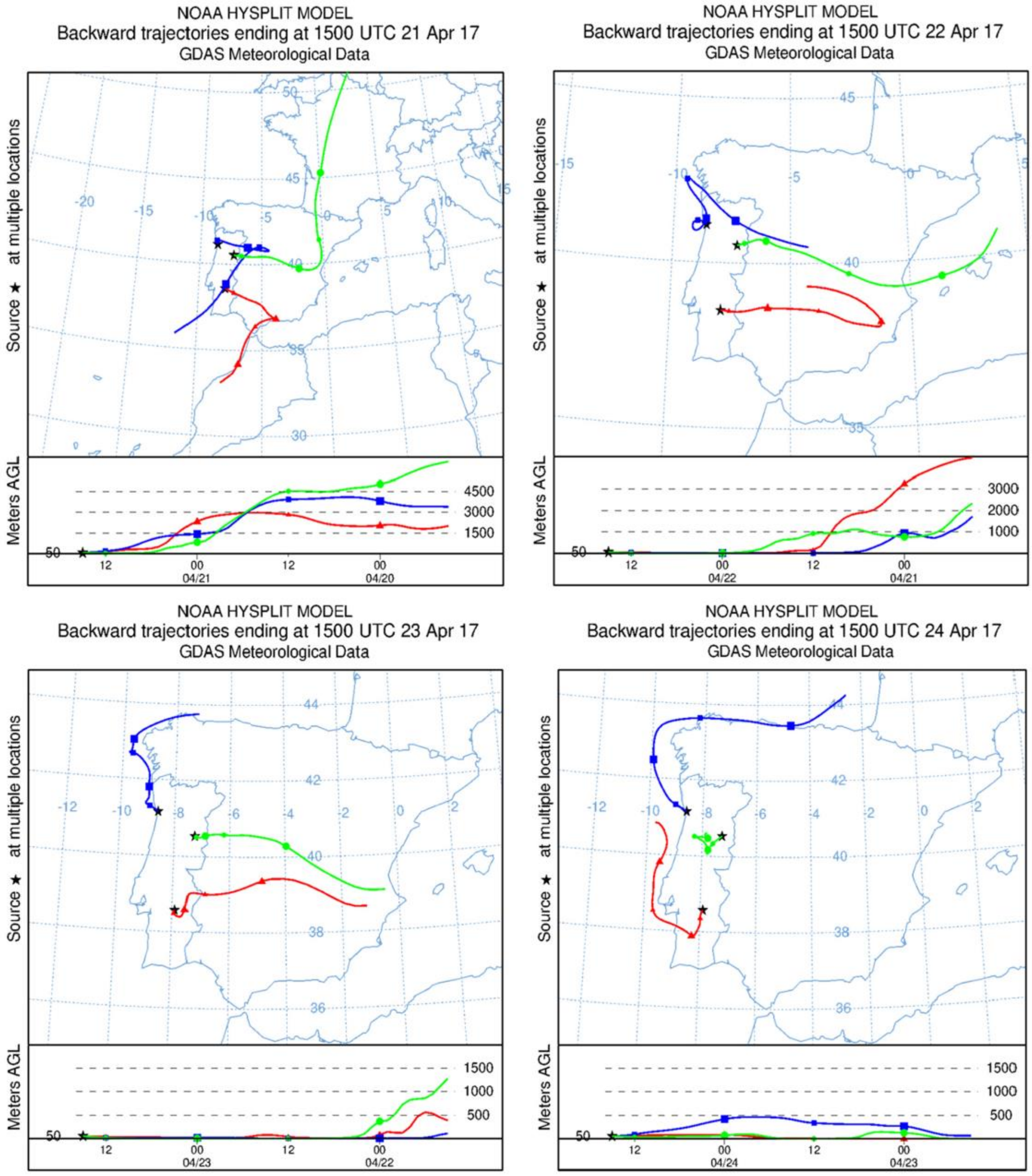

Fig. 7. Air mass backtrajectories arriving at Évora (line red), Guarda (line green) and Porto (line blue) from 21 to 24 April 2017, ending at a level of 50 m a.g.l.

there seems to be mostly local circulation. On this day it was observed, in addition to Si-rich particles, the increase of Cl-rich particles and the appearance for the first time of Ca-rich particles. On 24th the trajectory is maritime throughout the day, nonetheless, Si-rich, organic and metals particles were found (Figs. 6 and 7).

4. Discussion

Pollen grains when airborne can be in contact with numerous particles (Visez et al., 2020) of different origins and their synergy can be related to the probability of occurrence of 4 processes. The first one is pollen being polluted by particles before pollination and directly at the dehiscence of the anther; another plausible to consider is the pollen grains during the pollination process colliding with aerosol leading to the process of particle strains; contaminated in the soil through the resuspension process where relative humidity and wind speed are important factors or else during the human inhalation or in the respiratory mucosa where PM collide with pollen initiating the inflammatory process, creating an adjuvant chemical microenvironment for allergic reaction upon pollen grains deposition (Saxon and Diaz-Sanchez, 2005). This last process can be simulated by our sampling equipment.

In our study, significant differences were observed in the equivalent diameter of *Quercus* pollen between the three studied cities. In all study cities, it was possible to divide the *Quercus* pollen in two groups, reflected in the distribution of *Quercus* pollen size being characterized by a bimodal pattern, more pronounced in the city of Évora and less in Porto, which is reflected in the average equivalent diameter, larger in Évora, followed by Guarda and finally Porto (Fig. 2). This observation can be explained by the airborne pollen sampled belonging to distinct species of *Quercus* that have part of the pollination periods overlapped (March–April). In the city of Évora, there are two dominant species *Quercus rotundifólia* L. with pollen size between 21 and 25 μm, and *Quercus suber* L., with pollen size of 22–40 μm. In Guarda, the most prevalent species is *Quercus robur* L., its pollen being approximately between 31 and 35 μm while *Q. suber* is present but less representative (www.PalDat.org, last accessed February 16, 2022; www.flora-on.pt last accessed March 24, 2022). In Porto, and due to its high urbanization level, *Quercus* trees are less frequent and there is a greater variety of species, with *Q. suber* almost absent at the expense of other species with less economic importance. Also, there were interdaily statistically significant differences in the pollen size in Évora and Guarda but not in Porto. These differences may reflect the transition between pollen seasons of different species of *Quercus*, representing the possible end of *Q. rotundifólia*, *Q. robur* species and the beginning of *Q. suber* species. Probably, another possibility will be the transport of air masses from other locations, transporting pollen over long distances. In fact, it is verified that in Évora, on 24th, the air mass trajectory differs from the previous days, coinciding with the day when the equivalent diameter of pollen was greater (Table 1 and Fig. 7). The same occurs in the city of Guarda, on 24th, the air mass trajectory is of local origin, coinciding with the day when the equivalent diameter of pollen was smaller. It is possible that these events may contribute to the presence of other species of genera *Quercus*.

A greater number of particles per pollen grains of *Quercus* was observed in the city of Évora compared to the city of Guarda and Porto. In fact, for Évora it was shown that the number of particles adhered on the pollen wall may be influenced by the pollen size, as more area is exposed in the larger pollen grains and able to aggregate more particles during the inhalation process. The number of particles per pollen may also be related to the source of emission. Porto, although more urbanized, therefore expected to have finer PM present in the atmosphere and more particles adhered to the pollen, is a coastal area, affected by marine particles that are coarser in size with respect to pollution, with nonspherical shape (Dubovik et al., 2002; Silva et al., 2002). Nevertheless, in all cities, the average percentage of the pollen area occupied by the particles was less than 3% and therefore most of the *Quercus* pollen was clean or with few adhered particles (Fig. 3).

Considering the results obtained in this study regarding the equivalent diameter of the adsorbed particles, most particles belong to the fine fraction of PM with a diameter below 3 μm. This is the fine fraction of PM with sizes between 1 and 5 μm, which in addition to being able to penetrate the tissue of the respiratory mucosa, compromising its function, can also reach the bronchial respiratory system and alveolus where gas exchange occurs (Löndahl et al., 2006). Possibly they can escape into the bloodstream and cause significant health problems (Kim et al., 2015). Among the three cities represented in this study, there are no significant differences in the equivalent diameter of the particles, and therefore their size may not be a problem in allergic exacerbation when different sites are compared for the same pollen type, *Quercus* pollen.

It was observed a negative significant correlation between the number of particles per pollen and wind speed. This may be possible as a result of wind speed influence on the turbulence of the atmosphere since high wind speed is associated with high dispersion and possibly the low adsorption capacity of PM to the pollen surface. Some authors have demonstrated that the lower the wind speed, the higher the concentration of PM in the air (Cichowicz et al., 2020). Wind direction is another factor influencing the adherence of PM to the pollen surface because studies have shown that the distribution of sources is very important for the detection of biological and organic particles (Palacios et al., 2000). Wind direction proved to be important for the number of particles per pollen in the winds from S, contrary to winds of E, SW and NW. When winds come from NW it is not associated with the maximum equivalent diameter and percentage of area occupied by particles.

Considering the chemical classification of the adhered particles, the dominant groups for the 3 cities were Si-rich, SO-rich, Ca-rich, organic, and Cl-rich. Aluminosilicates associated with Fe, K, Mg, Zn and Ca, constitute the majority of particles in the group of silicates. The presence of Cl-rich particles with associated elements of Na and Mg in pollen grains may be related to coastal influence (Moreno et al., 2004) since these elements constitute 90% of the salinity of seawater. Sea salt particles can contain sulfates, potassium and sodium (Gieré and Querol, 2010), like the most abundant SO-rich particles observed in our study. In this study, it was also observed, for the three cities, the presence of particles of Metals & Oxides, composed mainly of Fe-rich, Ni and Ti elements, and organic particles, that usually have emission sources associated with automobile traffic (Moreno et al., 2003; Moreno et al., 2004). The physical and chemical characteristics of the particles vary depending on the location of the sampler (Calvo et al., 2013; Santos et al., 2008) because there were chemical groups observed in the city of Porto that were not observed in other cities. In the city of Évora, most of the influence is rural, with some contribution from sea salt particles and a smaller contribution from anthropogenic sources as road traffic or transport from different sources. The contributions are dominated by Si-rich and Ca-rich particles, constituents of the earth's crust (Haynes, 2016). Particularly, Ca-rich particles may originate from erosion of carbonate minerals as calcite and dolomite, which are widely present around the region of Évora with several active quarries where calcitic and dolomitic marbles are mined (Menningen et al., 2018). Desert dust is the main source of atmospheric phosphorus (P) and P-rich particles that are only detected at Évora on the 21st April, consistent with the back trajectory originating in north Africa (Stockdale et al., 2016). Sea salt particles present a moderate contribution for the total, with Cl-rich and Sea salt misc. classes. It is hypothesized that also a great part of the sulfates is from sea salt, as most of them present sizes above 1 μm (Ghahremaninezhad et al., 2016). Organic aerosols may be of local origin, also with contributions from more distant sources to the east. To note that on the 24th of April, when the organic fraction is slightly higher, the back trajectory shows the airmass crossing the coastline at the surface level in the area of Sines, an important industrial complex in Portugal.

In the city of Porto, chemical particles of the Organic group composed of CFe may have also anthropogenic local origin as the sampling sites is very close to an entry point and exit of the city, with high motor traffic in the morning and at the end of the day, there is internal combustion of oil, tire wear and fuel (Moreno et al., 2004; Ribeiro et al., 2015).

In the city of Guarda, it was observed a prevalence of particulate matter of the Si-rich group and little concentration of other types of particles present in the city of Évora and Porto, which may indicate that the city of Guarda, is perhaps, an environment with lower toxicity (Lisboa, 2014).

Like pollen grains, particles can be deposited locally or far from their source, particularly smaller particles, due to medium-long range transport (Galan et al., 2013; Mohanty et al., 2017). An example of these phenomena could be observed in our study for the inland city of Évora (day 23) when sea salt particles, probably originated in the Mediterranean Sea, were detected adhered to the pollen grains, as corroborated by the air mass trajectories. Another example was observed in Porto, on the 23rd a considerable increase in the amount of Cl-rich particles was observed coinciding with dominant air masses from NE, consistent with the results from the correlation analysis, and passing through the coastal zone. In fact, NaCl or MgCl can be associated with coastal influence (Moreno et al., 2004) as they account for 90% of the seawater salinity (Lide, 2007). Another example, on the 23rd in Porto, is the percentage increase of organic composition particles which coincides with the change in air masses to the Northeast passing through the coastal area.

On the other hand, the number of particles of Metals & Oxides origin can also correlate with a local origin, since its compounds e.g., FeO, Zn, Ni, V, Ti, can be assigned to road traffic and industrial sources associated with combustion processes (Moreno et al., 2003; Moreno et al., 2004; Calvo et al., 2013; Gieré and Querol, 2010). The close location of the sampling points to roads with frequent high traffic volume can explain the increase in organic-derived particles.

Si-rich particles originate mainly from geological formations (Calvo et al., 2013; Čupr et al., 2013; Pachauri et al., 2013). In our study, more than 50% of the Si-rich particles identified and adsorbed to airborne pollen were composed mainly of Si. These particles can originate from sediments that can be transported naturally by the wind. In fact, during the period of our study, there were events of desert sand particles felt in Évora but not observed for Guarda and Porto. In the case of Guarda city, the Si-rich particles can be originated from the gravel that is present on the roof of the municipal theater.

5. Conclusions

It was shown that most particles belong to the fine fraction with a diameter below 3 μm.

The particle size, as the equivalent diameter of PM, is similar between the studied cities, however, the dominant chemical composition is different, possible due, environmental conditions, for example, proximity of the sea, traffic and industry, which is important information regarding their impact on pollen allergy intensification towards the same pollen type and concentration. In future studies, when considering the exposome influence on pollen allergies, it would be important besides the concentration of PM in the air also take into account its chemical composition and how these characteristics could affect allergen release from pollen, its allergenicity, possibly allergens molecular structures and influence on the human respiratory tract.

CRediT authorship contribution statement

Ana Galveias: Data curation, Formal analysis, Writing – original draft. **Helena Ribeiro:** Funding acquisition, Conceptualization, Formal analysis, Writing – review & editing. **Fernanda Guimarães:** Methodology, Writing – review & editing. **Maria João Costa:** Methodology, Writing – review & editing. **Pedro Rodrigues:** Writing – review & editing. **Ana R. Costa:** Writing – review & editing. **Ilda Abreu:** Writing – review & editing. **Célia M. Antunes:** Writing – review & editing.

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.

Acknowledgements

The work was funded by National funds through FCT - Fundação para a Ciência e Tecnologia, I.P. (projects UIDB/04683/2020 and UIDP/04683/2020) and also through projects PTDC/ATP-EAM/0817/2014, TOMAQAPA (PTDC/CTAMET/29678/2017) and CILIFO (0753_CILIFO_5_E).

The authors gratefully acknowledge the NOAA Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model and/or READY website (ready.noaa.gov) used in this publication and Pereira J. for her help with SEM-EDS analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154892.

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4. Considerações finais

Neste estudo foram apresentados 4 artigos publicados (Buters et al., 2018; Galveias et al., 2020; Galveias et al., 2021; Galveias et al., 2022), e um manuscrito em preparação para submissão (Capítulo II, página 76-110), cujos os resultados foram já apresentados num congresso internacional (Galveias et al., 2020), e que contribuem, no seu todo, para melhorar o conhecimento sobre a influência dos parâmetros meteorológicos e poluentes atmosféricos na dinâmica aerobiológica dos grãos de pólen aerossolizados, nas alterações químicas e bioquímicas a que o pólen está sujeito, tendo em vista as possíveis implicações ao nível da doença alérgica respiratória.

Pela sua enorme importância ao nível da saúde pública, em particular da doença alérgica respiratória, os dados aerobiológicos têm vindo a ser gerados e utilizados por inúmeros especialistas em diversas áreas. Antes de abordarmos aspetos relativos à previsão e modulação das concentrações de pólen no ar, optou-se nesta tese por discutir avanços recentes na monitorização e disseminação de informação aerobiológica (Buters et al., 2018). No passado era difícil a obtenção desta informação, pois o seu acesso era restrito e existia um desconhecimento profundo sobre a implementação de estações de monitorização aerobiológica e a sua distribuição geográfica, impossibilitando contacto entre investigadores e retirando potencial de impacto aos dados gerados mundialmente. Nesse sentido a criação de ferramentas que possibilitem a consulta dos pontos de monitorização foi um passo importante. O mapa polínico (*Pollen Map*), apresentado no capítulo I desta tese, não apresenta dados polínicos, mas apresenta todas as caraterísticas das redes de monitorização, qual a metodologia de amostragem usada em cada um deles, qual o tempo de resolução do captador, que tipo de partículas monitorizam e qual o responsável de cada estação de monitorização, entre outras. Este mapa, apresentado numa página de internet de consulta aberta e gratuita, possibilita o conhecimento da distribuição geográfica de todas as estações de monitorização aerobiológica fomentando a cooperação internacional. Uma outra consequência da criação deste mapa foi estabelecer uma rede de contactos, que pode útil a imunoalergologistas e a individuos que sofrem de polinose pois desta forma podem encontrar informação e contactar a estação de monitorização geograficamente mais próxima e obter dados polínicos que possibilitem uma melhor gestão da doença alérgica respiratória (Capítulo I/Buters et al., 2018).

Os capítulos II e III da tese têm por base dados aerobiológicos obtidos maioritariamente na estação de monitorização de Évora inserida no Observatório de Ciências Atmosféricas do Instituto de Ciências da Terra, ICT, mas também de outras cidades, fruto de colaborações estabelecidas com outros laboratórios de investigação de outras cidades portuguesas e espanholas, fomentada pelo Pollen Map. Estes dados são fundamentais para, a partir deles, estudar o efeito de parâmetros físicos (Capítulo II) e químicos (Capítulo III) na disseminação, distribuição e potencial alergénico do pólen.

As concentrações de pólen atmosférico têm tido uma tendência crescente desde as últimas décadas até esta parte (Ziska et al., 2019). Este aumento tem como consequência o agravamento do risco de exposição a alergénios com graves implicações na saúde pública. As concentrações de pólen estão dependentes das oscilações dos parâmetros meteorológicos como temperatura, precipitação, humidade relativa e radiação solar, que por sinal são fatores importantes na emissão e dispersão polínica (Linskens & Cresti, 2000), que se torna ainda mais relevante num cenário de alterações climáticas discutido pela organização *Intergovernmental Panel on Climate Change* (IPCC) (IPCC — Intergovernmental Panel on Climate Change, 2022). De facto, tem sido observado um aumento da temperatura média da terra, bem como a ocorrência de eventos extremos de calor/frio, de precipitação intensa, entre outros. Uma das consequências das alterações climáticas é a mudança na distribuição de muitas espécies de florestas tropicais e temperadas para altitudes mais elevadas. As espécies tropicais são as mais sensíveis ao aumento da temperatura, no entanto, são aquelas que estão a acompanhar as mudanças de temperatura comparativamente a espécies de zonas temperadas (Freeman et al., 2021).

Nesse sentido, o estudo inserido no capítulo II relativamente ao efeito dos parâmetros meteorológicos no pólen de Cupressaceae nas cidades de Évora e Granada, ambas do sul da Península Ibérica embora com caraterísticas topográficas e microclimáticas diferentes, veio demonstrar que a temperatura e a radiação solar são os parâmetros que influenciam positivamente as concentrações de pólen no ar antes do pico de polinização principal, enquanto a precipitação e humidade relativa demonstraram o efeito contrário. Este estudo permitiu ainda averiguar que a temperatura mínima e média são fatores importantes na duração da época polínica afetando-a negativamente.

Tendo em conta os parâmetros meteorológicos de outono-inverno, a estação prévia à época polínica, estes influenciam o índice polínico de Cupressaceae. A temperatura máxima e média apresentam influência semelhante nos dois locais do estudo e com tendências diferentes nos meses de setembro-novembro ou dezembro-janeiro, constituindo possivelmente, indicadores de predição da intensidade da época polínica de Cupressaceae. No entanto, séries temporais mais longas devem ser analisadas para assegurar este resultado.

Tal como Cupressaceae, *Platanus* apresenta, igualmente uma polinização anemófila, e segundo o nosso estudo representado no capítulo II é igualmente influenciada pelos parâmetros meteorológicos. Foi observado que a temperatura máxima nos 4-7 dias anteriores ao início da polinização influencia positivamente as concentrações diárias de pólen, contrariamente ao observado para os parâmetros de precipitação e humidade relativa, que apresentam uma relação negativa com as concentrações de pólen nos 7-8 dias antes (Capítulo II/Galveias et al., 2020).

O conhecimento dos parâmetros meteorológicos que afetam as caraterísticas da época polínica, o seu início, a sua duração e a sua intensidade, é muito importante pois permite o desenvolvimento de ferramentas matemáticas de previsão bem como de análise de risco, uma vez que, os níveis polínicos afetam determinantemente a saúde respiratória dos indivíduos sensíveis. Tal acontece porque a um maior nível de pólen está associado um maior nível de alergénio, como mostram vários trabalhos na bibliografia (Buters et al., 2012; Buters et al., 2015; Gálan et al., 2013) e os nossos resultados corroboram, uma vez que, foi demonstrada uma correlação positiva entre a concentração de *Pla a 1*, um dos alergénios mais prevalentes do *Platanus hybrida* e as concentrações de pólen desta espécie (Capítulo II/Galveias et al., 2020).

Os parâmetros meteorológicos **podem afetar** não só a época polínica, mas também a disseminação de alergénios no ar, ao condicionarem a integridade morfológica do pólen (Taylor et al., 2004; Taylor & Jonsson, 2004). Nesta tese foi avaliada a fração de disrupção de grãos de pólen de Cupressaceae que atinge o amostrador e foram estudados os fatores meteorológicos que possam ter estado associados a este acontecimento. Mostrou-se que a humidade relativa alta e a pressão atmosférica baixa são os parâmetros que mais influenciam a rutura do pólen de Cupressaceae (Capítulo II/Galveias et al., 2021). Assim, na avaliação de risco para este tipo polínico devem ter-se em conta não só os totais de pólen, mas também as condições meteorológicas locais, uma vez que o rompimento do pólen pode promover o aumento significativo do conteúdo de alergénios no ar, ampliando o potencial alergénico do mesmo.

Quando o pólen está na atmosfera para além de ser afetado pela variação dos parâmetros meteorológicos, entra em contacto também com agentes químicos gasosos ou particulados presentes na troposfera. Estes agentes podem ter origem natural como as poeiras do deserto ou antropogénica como gases gerados pelos veículos automóveis, designadamente óxidos de azoto e ozono troposférico, bem como muitos tipos de material particulado (Moreno et al., 2003; Moreno et al., 2004). Independentemente da origem destes agentes, ao entrarem em contacto com o pólen no seu trajeto pela atmosfera podem provocar-lhe alterações morfológicas, estruturais ou bioquímicas com potenciais implicações alergológicas (Cuinica et al., 2014). Num dos estudos apresentados no capítulo III carateriza-se a matéria particulada adsorvida na superfície do pólen de *Quercus*, captado em três cidades portuguesas com caraterísticas climáticas e geográficas diferentes, Évora, Guarda e Porto, nos dias 21 a 24 de abril de 2017.

Na caraterização da matéria particulada, o tamanho das partículas observadas nas três cidades portuguesas em estudo foi semelhante, no entanto, o número de partículas por grão de pólen e a composição química das partículas foi diferente. O pólen de *Quercus* foi mais abundante na cidade de Évora e também nesta cidade foi registado um maior número de partículas adsorvido por grão de pólen. Quanto à constituição química das partículas podemos observar que a grande maioria tem origem natural, maioritariamente areias e partículas de sal marinho, estas últimas trazidas por massas de ar provenientes do oceano, que chegam não só ao Porto, mas também a Évora, como comprovado pelos estudos de trajetórias. Contudo parte do material particulado tem natureza antropogénica, nomeadamente óxidos metálicos (Capítulo III/Galveias et al., 2022. Estes compostos, ao serem transportados pelo pólen podem chegar as vias respiratórias dos indivíduos onde podem desencadear processos inflamatórios (Bayram et al., 2001), ou podem atuar no pólen como agentes de stress, havendo necessidade por parte do pólen de desenvolver processos que lhe permitam defender-se destes agentes. Nesta resposta biológica natural podem ocorrer alterações bioquímicas que modifiquem a alergenicidade do pólen (Sousa et al., 2012).

A fim de estudar alterações induzidas por poluentes de natureza antropogénica no pólen, realizou-se um estudo em que pólen de *Dactylis glomerata* foi exposto a O_3 e NO_2, isolados ou em combinação, numa câmara de ambiente controlado. Os resultados do nosso estudo mostram que estes gases afetaram a capacidade germinativa do pólen. Subjacente a este efeito parecem estar associadas alterações bioquímicas de resposta antioxidante, tendo sido observado alterações nas atividades enzimáticas da superóxido dismutase e catalase. Neste trabalho foi ainda demonstrado que as alterações na proteómica do pólen tem implicações ao nível do reconhecimento antigénico, havendo alergénios mais imunorreativos em pólen exposto a poluentes ambientais (Capítulo III/Galveias et al., 2021).

No seu conjunto este trabalho contribui para evidenciar a importância da monitorização polínica e qualidade do ar, bem como, do conhecimento dos fatores que afetam a emissão, distribuição e integridade do pólen para a construção de melhores ferramentas de previsão das épocas polínicas, da sua intensidade e do risco de desenvolvimento de patologia alérgica respiratória a ela associado. Neste trabalho fica ainda claro que existem fatores físicos e químicos que afetam o pólen. No seu percurso pela atmosfera o pólen está exposto não só variações meteorológicas que podem afetar a sua integridade, mas também a gases poluentes e material particulado que podem afetar a forma como o pólen interage com as vias respiratórias. Para além de ser portador destas partículas, estes agentes com os quais o pólen contacta, podem ainda modificar a sua carga alergénica, quer por modificarem a sua estrutura, por rebentamento por exemplo, quer por induzirem alterações bioquímicas que se traduzem em alterações proteómicas ou de modificação química de proteínas do pólen, com significativas implicações alergológicas.

Nota: Esta lista de referências não se aplica aos artigos científicos desta tese

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