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Poaceae pollen as the leading aeroallergen worldwide: a review.

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Abstract

The Poaceae family comprises over 12,000 wind-pollinated species which release large amounts of pollen into the atmosphere. Poaceae pollen is currently regarded as the leading airborne biological pollutant and the chief cause of pollen-allergy worldwide. Sensitization rates vary by country, and those variations are reviewed here.

Grass pollen allergens are grouped according to their protein structure and function. In

Poaceae, although species belonging to different sub-families are characterized by distinct

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allergen subsets, there is a considerable degree of cross-reactivity between many species. Cross-reactivity between grass-pollen protein and fresh fruit pan-allergens is associated with the appearance of food allergies. The additional influence of urban pollution may prompt a more severe immunological response.

The timing and the intensity of the pollen season is governed by species genetics, but plant phenology is also influenced by climate; as a result, climate changes may affect airborne pollen concentrations. This paper reviews the findings of worldwide research which has highlighted the major impact of climate change on plant phenology and also on the prevalence and severity of allergic disease.

Introduction

The term “grasses” is traditionally applied to herbaceous species belonging to the Poaceae family. The origins of this botanical family date back to around 80-100 million years ago, and it now includes 12,000 species classified into 771 grass genera belonging to 12 subfamilies (Anomochlooideae, Aristidoideae, Arundinoideae, Bambusoideae, Chloridoideae, Danthonioideae, Micraioideae, Oryzoideae, Panicoideae, Pharoideae, Puelioideae, and Pooideae) [1]. Of these, the subfamilies Bambusoideae, Arundinoideae, Pooideae, Chloridoideae and Panicoideae comprise the largest number of species. Pooideae dominate temperate climate zones; Chlorodoideae cover the North American, African and Australian continents and Panicoideae grow in tropical and subtropical environments of Asia, Australia, Africa and South America [2,3].

Most Poaceae species are annual or biannual grasses with hollow stems. Young leaves grow from the base of the plant, rather than the tip. This strategy is reportedly an evolutionary response to predation. Further protection is achieved by the secretion of silica crystals within plant tissues. This highly-cosmopolitan group includes a number of major crop species (wheat, rice, maize, oats, rye, barley, etc.), as well as pasture grasses, sugar cane and bamboo. Apart from crops, thousands of Poaceae species are of considerable ecological importance, covering the land surface of most biomes worldwide. Grassland ecosystems alone cover around 20% of the land surface [4].

All Poaceae species are wind-pollinated, and most have dangling anthers. They produce large amounts of the same type of pollen, which is released into the atmosphere and is currently classed among the leading aeroallergens. Grass pollen grains are spheroidal to suboblate in shape, with a single circular pore surrounded by a distinct thickening of the outer and inner wall (annulus) (Fig. 1). Mean grain size is around 35x40 μm . Pollen grains larger than 45 μm and with robust annuli belong to cultivated species [5].

This paper reviews the influence of the biosphere (grass species) on the atmosphere (airborne pollen) and the anthroposphere (allergies). The timing and the intensity of the pollen season is governed by species genetics, but plant phenology is also influenced by climate; as a result, climate changes may affect airborne pollen concentrations. Research carried out in different parts of the world has highlighted the major impact of climate change on plant phenology and also on the prevalence and severity of allergic disease. The marked contribution of grass pollen to the prevalence of pollen allergy is a key environmental and health issue, and merits close attention.

This paper examines the main features of Poaceae pollen as a leading aeroallergen worldwide, focusing on some of the most significant recent research into grass pollen allergenicity and particularly on studies of spatial and temporal variations in grass phenology. Research based on aeroallergen detection and on field phenology observations provides valuable information both for allergy patients and for physicians; the latest work on modelling and forecasting the main features of the grass pollen season and the way these are influenced by climate change is thus of considerable interest. This paper also looks at pollen patterns, and examines the possible interactions between air pollution levels and allergic disease.

Grass pollen allergy

Poaceae pollen currently ranks among the leading aeroallergens worldwide, and is the main cause of pollen allergy in most developed countries, especially in North America and Europe [6]. The allergenic nature of grass pollen was first recognized by Charles Blackley, who traced the link between allergy symptoms and exposure to pollen; his skin and provocation tests, devised in 1873, confirmed the etiology of the disease [7]. Poaceae is the most prolific and diverse family growing in urban areas which are home to over half the world's population. A large number of grass species shed their pollen in high concentrations during the pollen season, leading to allergic symptoms ranging from seasonal rhinoconjunctivitis to bronchial asthma in susceptible individuals [8].

Grass pollen is present in the air in late spring and early summer, when it can give rise to allergic rhinitis, allergic conjunctivitis and asthma. Over 95% of allergy-relevant grass species belong to three subfamilies; Pooideae, Chloridoideae and Panicoideae. In the northern hemisphere, the most widespread allergy-causing genera are *Phleum* spp.,

Dactylis spp., *Lolium* spp., *Trisetum* spp., *Festuca* spp., *Poa* spp., *Cynodon* spp. and *Anthoxanthum* spp. Other species making an important contribution to the pollen spectrum in Europe are *Holcus lanatus* L., *Trisetaria* spp., *Elymus repens* L., *Sorghum halepense* (L.) Pers., *Vulpia* spp., *Anlhoxanthum odoratum* L., *Agrostis gigantea* Roth and *Piptatherum miliaceum* (L.) Coss. [9].

At present, the most effective option for preventing allergy symptoms is thought to be allergen-specific immunotherapy (AIT), commonly known as “allergy shots”; a long-term treatment which reduces the symptoms of allergic rhinitis, allergic asthma, and conjunctivitis. Since the aim is to lower the patient’s sensitivity to allergens, it is essential that both the dosage and the allergen composition of allergy shots are carefully controlled. One possible shortcoming of this therapy is the lack of specific allergens not identified during allergy testing [10]. A mixture of extracts from the most widespread and allergenic species is currently used for AIT, and this appears to be the best strategy for reflecting the natural exposure of the most polysensitized subjects [11].

Nevertheless, the commercial solutions available do not necessarily contain the specific allergen(s) to be found in a given patient’s area of residence, so it is important to take into account a broad botanical spectrum of pollen sources. Allergy diagnosis in patients exposed to multiple pollen species is complex, misdiagnosis often gives rise to unsuccessful specific immunotherapy [12]. The sensitization profile of individual allergens (major, minor and pan-allergens) in pollen-sensitized patients may vary depending on their exposure to different pollen types or even to pollen from different botanical or crop varieties.

Clinical data and sensitization

Sensitization to Poaceae allergens is usually detected by applying grass pollen extracts in skin prick tests, positive reactions indicating sensitization to the allergen tested. The degree and spectrum of sensitization are linked to plant distribution and pollen exposure. The prevalence of pan-allergenicity is higher in patients living in areas where pollen seasons overlap, and particularly where several major allergens appear simultaneously. A clear example is southern Europe, where grasses flower at the same time as other allergenic taxa, including olive, pellitory, *Plantago* and Chenopodiaceae [13]. By contrast, separate pollen seasons—such as grasses and birch in north and central Europe—favor sensitization to specific major and/or minor allergens. As a result, moreover, minor allergens that rarely sensitize allergy-sufferers become major allergens in areas with extensive exposure [14, 15].

Data on sensitization rates to grass pollen pan-allergens are mainly available for Europe and North America; relatively little research has addressed this issue elsewhere. Although only 8% have been officially diagnosed, it is believed that around 20% of the United States population suffer from pollen allergy, while 15% are sensitized to grass pollen [16-18]. In Europe, too, 20% of the population are affected by grass pollen allergy [6, 17]. Nevertheless, available data show marked variations between and within countries [19, 20].

Around 50 million people in the United States suffer from nasal allergies, costing the U.S. healthcare system about \$17.5 billion per year [21]. For various reasons, including air pollution and climate change, the prevalence of allergic disease in the U.S. has risen dramatically since the mid-20th century. Today, asthma is the most common chronic childhood disease, affecting almost 9.6 % of American children [22]; it is also the leading cause of school absenteeism and hospitalization [23]. In the United States, 20% of the population are sensitized to *Lolium* allergens, the highest rates being recorded in the Western

states. Sensitization to the *Cynodon* group is around 15%. In Europe, sensitization rates in adults range from 10% in Poland to 30% in Germany [20].

In Australia, the seasonal prevalence of pollen-related asthma and hay fever is rising, with up to 10% of adults and 30% of children diagnosed with clinical respiratory disease [24, 25]. Fewer data are available regarding other continents, although a number of authors report that grass pollen allergens are among the major causes of allergy in Asia and Africa [26-28]; 50% of allergy-sufferers in Zimbabwe and 60% in Saudi Arabia are sensitized to *Cynodon* allergens. Tests performed elsewhere in Asia have shown that 12% of the population in India and 22% in Malaysia are sensitive to *Cynodon*, while *Lolium* sensitization rates range from 10% in Taiwan to 20% in Thailand.

Data for South America are relatively sparse, considering the large number of countries concerned and the wide distribution of Poaceae species; however, reports suggest that 30% of the population are sensitized to grass pollen allergens in Argentina, 20% in Chile and 20% in Brazil [29-32].

The worldwide incidence of allergic respiratory diseases and bronchial asthma appears to be increasing, and city-dwellers are more frequently affected than people living in rural areas [33]. Chemical pollutants resulting from human activity are present at ever-higher concentrations, and interact with biological pollutants (allergens), prompting an increase in morbidity rates associated with allergic respiratory disease. Lung function may be negatively affected by atmospheric pollutants in asthma-sufferers, especially children. Damage caused by air pollution to the mucous membrane lining the respiratory system may facilitate access of inhaled allergens to immune-system cells [34]. The percentage of the population living in urban areas is rising dramatically in the developing world, prompting an increase in the number and severity of allergic diseases, mostly linked to a combination of pollution and pollen.

Recent climate change, moreover, is believed to be having an adverse impact on the incidence of hay fever (allergic rhinitis) and asthma, via its effects on the concentration of pollens and other aeroallergens [35]. Potentially-influential atmospheric variables include CO₂, PM₁₀ and PM_{2.5} levels, temperature, rainfall, humidity, and wind speed and direction. With allergic diseases already being a significant public health issue in much of North America, Europe and Australia, the potential for any adverse impact resulting from climate change is of serious concern [36, 37].

Grass pollen allergens

Allergens may be classified in terms of their population-based IgE prevalence (i.e. into major or minor allergens), their physicochemical structure or their biological role in the organism. Major allergens are defined today as those to which over 50% of allergic patients react [38]. A given allergen source, such as pollen grains, generally contains one or two major allergens, while the number of minor allergens—i.e. those to which less than 50% of the sensitized population react—varies depending on species and variety. Pan-allergens are groups of homologous and structurally-related proteins (e.g. profilins, polcalcins, lipid-transfer proteins) responsible for cross-reactivity among different allergenic sources [39, 8]. Today, a number of methods are available for the molecular and immunological characterization of pollen pan-allergens, including screening, cloning, and chromatography [40].

In Poaceae, although species belonging to different sub-families (e.g. *Pooideae* and *Chloridoideae*) are characterized by distinct allergen subsets [41, 19], there is a considerable degree of cross-reactivity between many species [42, 43]. Sensitization to grass can predispose a subject to the oral allergy syndrome (OAS), and grass-pollen allergy in association with the so-called “fruit-pollen syndrome” may result in allergy to tomatoes,

potatoes and peaches. In general terms, cross-reactivity between grass-pollen protein and fresh fruit pan-allergens is associated with the appearance of food allergies.

Pollens are known to be a major cause of Type I allergies due to the presence of several allergens [44]. Over the last twenty years, the findings of research into the influence of minor allergens on allergic disease and the association between pan- and minor allergen sensitizations have enabled a considerable advance in the molecular characterization of pollen-derived allergenic proteins causing Type I allergy. Pollen allergens belonging to various protein families have been identified in a range of plant species. Profilins—a ubiquitous family of proteins prompting Type I allergy—control actin polymerization in cells; in particular, profilins are involved in the acrosomal reaction of animal sperm cells [45]. The findings of a 1992 study by Valenta et al. [46] suggested that profilins—hitherto unreported in plants—may play a similar role in pollens during plant fertilization and therefore act as allergenic components in almost all pollens. The presence of profilins and related proteins in most reported cross-reactions would account for Type I allergic reactions both to pollens and to food from species belonging to different plant families [47].

Grass pollen allergens are grouped by protein structure and function. The most widely-known grass allergens belong to the Lol, Poa and Phl groups, originally extracted from *Lolium perenne* (rye-grass), *Poa pratensis* (Kentucky bluegrass) and *Phleum pratense* (timothy grass), respectively.

Grass-pollen allergen groups, identified using the official nomenclature as defined by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) are shown in Table 1. Group 1 allergens are present in all Poaceae subfamilies, while Group 5 allergens are specific to the *Pooideae* subfamily. Due to their abundance and potency, these two groups are regarded as immunodominant among grass pollen allergens

[48]. By contrast, the pan-allergens profilin (Group 12) and polcalcin (Group 7) are responsible for 10-15% of reported cross-reactivity between grass, tree and other weed pollens. Attention is drawn to the structural homology of Poaceae pollen to other allergenic pollen types, including birch, plane, and *Olea*, which may account for their marked cross-reactivity even with food allergens [49, 50, 51], a factor that must be taken into account in the treatment and diagnosis of grass allergies [52]. The recent use of component-resolved diagnosis for the epidemiological analysis of allergies has provided a wealth of valuable information regarding the patient sensitization/ allergen exposure interaction, showing amongst other things that exposure to high airborne pollen concentrations is not necessarily associated with more severe allergic conditions, but rather is indicative of differential sensitization profiles.

It should be stressed that the airborne allergen load is attributable not only to pollen grains and fungal spores, but also to allergens present in submicronic and paucimicronic biological particles. Although these free allergens originate mainly from airborne pollen grains, some derive from other plant structures. In recent years, a number of projects have addressed the dynamics of these non-pollen airborne particles, among them the European HIALINE project [53]. In general, the comparison of pollen records with ELISA-based airborne allergen analyses suggests that, while the outdoor allergenic load is due mainly to pollen, monitoring of other airborne allergens might improve the assessment of allergen exposure.

The most abundant components of air pollution in urban areas (and particularly in the most densely-populated areas) include airborne particulate matter, especially PM 10 and PM 2.5 particles, nitrogen dioxide, and ozone; these prompt a more severe immunoglobulin (Ig)E-mediated response to aeroallergens in allergy patients [54]. D'Amato et al. [34] report that air pollution is giving rise to a greater prevalence of allergic respiratory diseases in polluted urban areas. In addition, the earth's temperature is increasing, mainly as a result of

anthropogenic factors (e.g., fossil fuel combustion and greenhouse gas emissions from energy supply, transport, industry, and agriculture), and climate change is altering the concentration and distribution of air pollutants, by prolonging the pollen season and thus extending the seasonal presence of airborne allergenic pollens [55]. The combined action of climatic change and pollutants leads to plant stress, which in turn gives rise to increased pollen production and thus to higher allergen concentrations [56].

Phenology

For a detailed understanding of airborne Poaceae pollen levels, it should be recalled that these grass species are mostly annual or biannual, and that their germination, growth and reproduction—though genetically determined—are also affected by climate. In genetic terms, members of this family are assigned to one of two major groups with differing biochemical pathways and different carbon fixation methods: C3 (“cool-season”) and C4 (“warm season”) grasses. C4 grasses are more drought tolerant, and tend to use water more efficiently thanks to their carbon dioxide fixation pathway. This biochemical feature, together with other biological factors including pest resistance, soil factors and age, influences plant physiology and phenology.

Both CO₂ levels and climate-related factors exert considerable influence on plant phenology. CO₂ has a particularly marked effect on plant reproduction and seed development: free-air CO₂ enrichment (FACE) experiments in grasses suggest that elevated CO₂ levels substantially increase photosynthesis, biomass and pollen and fruit production, and may also accelerate phenology [57, 58]. However, long-term exposure to elevated CO₂ concentrations may prompt a loss of sensitivity to this gas [59].

A number of climate-related parameters, including photoperiod, water availability, temperature, and even wind speed and direction, have been found to affect plant phenology.

In annual grasses, the key factor is the weather prevailing during the growing season: temperature, photoperiod and water availability are the most influential weather-related parameters [60]. The influence of these factors varies depending on the phenological phase.

In grasses, water availability exerts a major influence on vegetative development, and particularly on emergence [61], while temperature patterns govern the rate of phenological development [62]. Some cereals need to be exposed to a period of chilling temperatures before flowering, a process known as vernalization. Afterwards, the photoperiod remains decisive for flowering induction in grasses [63, 64].

Global climate change is affecting plants and animals worldwide, changing biological seasons, species distribution and ecosystems [65]. A number of authors have examined its potential impact on Poaceae species, and especially on cereals [66-69]. Climate changes are clearly prompting variations in grass phenology. A generalized advance in most phenophases has been observed, mainly during the spring. Oteros et al. [69] analyzed the phenological response of five crops at 26 sites growing in Spain (southern Europe): oats (*Avena sativa* L.), wheat (*Triticum vulgare*), rye (*Secale cereale*), barley (*Hordeum vulgare*) and maize (*Zea mays*). Analysis of data for the last 30 years revealed an advance in spring phenology in wheat and oats, the “Flag leaf sheath swollen” and “Flowering date” phenophases being brought forward by around 3 days/year and 1 day/year, respectively. Recent research in southern Spain has also shown an advance in the phenology of a number of wild grasses, including *Dactylis glomerata* and *Trisetaria panicea*, although other wild species (e.g. *Bromus* spp. and *Hordeum* spp.) shown no noteworthy variation [70, 71]. Temperature changes during the period prior to phenophase onset were identified as the cause of these phenological trends, though changes in rainfall patterns also exerted a significant effect.

Grass phenology displays year-on-year variations depending not only on environmental factors but also on the internal response of grass populations to the negative feedback mechanisms of population dynamics. These internal or endogenous processes are able to persist over time in a state of dynamic equilibrium with their environment [72-74]. Both feedback structure and exogenous factors interact in grass population dynamics. Knowledge of this interaction is fundamental in order to understand and predict grass phenology and its response to climate. In a recent paper, García de León et al. [75] disentangled the effects of feedback structure and climate on Poaceae reproductive phenology. Their findings highlighted the importance influence of first-order negative feedback as well as of temperature and rainfall on airborne Poaceae pollen dynamics.

Aerobiology

Poaceae species are wind-pollinate, i.e. they use the atmosphere as reproduction vector. Despite their large number, all Poaceae species share the same pollen type (Fig.1). Microscopic pollen identification does not allow the discrimination of pollen types at species or genus level [5, 76, 77], although a difference of mere micrometers is reported between members of certain genera, including *Zea* spp. or *Arundo* spp. Poaceae airborne pollen is present worldwide, especially during spring in temperate climates.

The reproductive phenology of these grasses determines the amount and timing of pollen release into the atmosphere, which is also influenced by other factors such as species, land use, latitude, altitude and climate [69, 78]. Pollen emission in anemophilous plants is a good bioindicator of reproductive phenology [79]. Aerobiological data, obtained from the continuous monitoring of airborne pollen, can be used as a bioindicator of plant phenological response. Apart from its ecological and environmental value, airborne pollen monitoring is of

primary importance for understanding allergy data, for the interpretation of symptoms and for planning therapy. Given its interdisciplinary nature, aerobiology can be applied in a range of science, including agronomy, allergology, climatology, environmental health, forestry, meteorology and phenology.

Aerobiological monitoring has traditionally been carried out using Hirst-type air samplers [80]. Current aerobiological research must comply with certain rules, including the use of standard monitoring methods, qualified training, and quality control assurance. All these aspects play a key role in building competence in aerobiological monitoring and research by laboratories belonging to the various national and international networks [81-83]. Publications in this field lay down minimum requirements regarding the reproducibility of pollen monitoring and analyses. The standardization of pollen monitoring enables comparison of data between sites, for example with respect to temporal and spatial variations in diurnal and daily concentrations, as well as seasonal characteristics (e.g. pollen season timing and intensity) and trends over time (e.g. in relation to land use and climate change). The production of comparable data also provides opportunities to construct models for predicting airborne pollen over large geographical areas [84-86].

Various surveys have revealed a strong correlation between high pollen peaks and the full flowering phenophase of certain grass species, observed in the field [70, 87, 88] (Cebrino et al., 2016; León-Ruiz et al. 2012; Zanotti & Puppi, 2000). These species tend to be the major contributors to the airborne pollen spectrum, due to their high pollen production, confirmed in the laboratory [9].

Airborne pollen counts correlate strongly with the flowering intensity of wind-pollinated species growing at and around pollen monitoring sites. In the northern hemisphere, the parameters widely reported to influence flowering phenology—as well as the seasonal

and daily distribution of airborne Poaceae pollen—include maximum temperature, daily rainfall, humidity and wind speed [89-91]. Research in the southern hemisphere has shown that the effects of temperature and rainfall on grasses in northern Australia, which are adapted to heavy rainfall concentrated in the summer, differ from those recorded for southern Australian grasses under a winter rainfall regime [25].

Information on the major variables influencing airborne pollen dynamics enables aerobiologists to construct forecasting models based on data compiled over several years. These models, primarily used to predict the main features of pollen seasons, yield both long-term predictions (e.g. pollen-season start-date and Annual Pollen Index (API), and short term forecasts such as daily pollen counts [92]. Advance knowledge of these data is essential for clinicians and patients alike. Directly supplied with output from these models, allergy-sufferers can plan their medication and outdoor activities, while healthcare professionals can develop more accurate treatments and improve the scheduling of clinical trials. Forecasts are also of value to manufacturers and stockists of healthcare products [93].

Long-term aerobiological models enable pollen-season timing and intensity to be predicted well in advance using a range of statistical tools [84, 94]. Most traditional methods include calculation of the accumulated temperature required by each plant species in order for flowering to start [95, 96]; this is done using a number of biometeorological variables, such as the chilling units and growing degree days required to trigger the flowering phase [97-99]. Another type of modeling aims at predicting short-term concentrations, e.g. one or two days in advance, by analyzing the effect of meteorological variables on pollen concentrations; calculations are based on short-term series, correlations and regression analyses, and one-dimensional nonlinear equations are used [90]. Most models developed for Poaceae are based on linear parametric statistics [99, 100], although neural networks have more recently been tested for this purpose [101].

Variations in the pollen spectrum at any given location are influenced by local weather conditions, available nutritional resources for plants, and also changes in land use in surrounding areas [102-104]. These influences are more marked in annual species, including those making up most of the Poaceae family. Although there has been considerable research into the impact of climate change and weather variations on grass pollen patterns, relatively few studies address the impact of land cover changes on aerobiology [78, 105, 106]. Changes in land use and land cover are major components of global dynamic change, directly prompting alterations in habitat composition, biodiversity and the functioning of the ecosystem [107, 108]. Airborne pollen counts correlate with local plant cover, and one indirect consequence of changes in the landscape is the alteration of airborne pollen spectra and/or levels [78]. Moreover, pollen grains preserved in soil sediment provide a record of past vegetation and its response to climatic and land use changes [109, 110]. The study of sediments from the Quaternary Period provides valuable information regarding the ability of species to withstand future challenges.

In a recent study carried out in Córdoba city (Southern Spain), García-Mozo et al. [78] reported that the average annual increase in Poaceae pollen over the period 1996-2010 was around 1500 pollen grains above that of the Annual Pollen Index (API). This trend reflects the combined effect of two factors: the expansion of heterogeneous cropland, and an increase in the length of the grass pollen season, due to increasing temperatures and more intense rainfall prior to flowering. By contrast, the authors observed a significant decline in pollen from other herbaceous species growing in ruderal areas with more marked human activity, linked to changes in urban planning strategies in areas with higher building pressure.

Finally, it should be noted that the earth's climate is widely agreed to be changing, and that those changes are affecting temperature and also the amount and intensity of rainfall [111].

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These factors are influencing vegetation and plant phenology, particularly that of annual species [112]. Recent research suggests that trends in Poaceae flowering patterns derived from field phenological observations are similar to trends in aerobiological data for Mediterranean wind-pollinated species, indicating a tendency towards earlier foliation, flowering and fruit ripening [69, 113]. In natural species, an advance of up to 0.25 days/year has been observed for flowering and up to 0.15 days/year for fruit ripening. Statistical analysis shows that increased temperature is the principal reason for earlier foliation, flowering and fruit ripening, as well as for delayed leaf fall. Herbaceous species, including grasses, are reportedly more affected than trees by changes in rainfall patterns. This phenomenon is observed elsewhere in Europe, and also in the United States, in a number of species including Poaceae spp., the general conclusion being that the warmer the weather preceding the flower period, the earlier the plants start to emit pollen [114-116].

The literature reports a progressive global increase in the burden of allergic diseases across the industrialized world over the last half century. Clinical evidence reveals a general increase in both the incidence and the prevalence of respiratory diseases, including allergic rhinitis (common hay fever) and asthma. Such phenomena may be related not only to air pollution and changes in lifestyle, but also to an actual increase in the amount of airborne allergenic pollen.

Research into Poaceae pollen-season trends in Andalusia (southern Spain) over recent decades [117] has revealed an average advance in onset (-2.2 days/year), peak (0.5 days/year) and length (2.37 days/year), as well as an increase in the average API for the region (100 pollen grains/year); pollen-season severity (days>25 pollen grains/m³) has been found to increase by 1 day>25/year. These changes in grass phenology may be linked to changes in both climate and land use, and future consequence could include not only an increase in

pollen allergy symptoms due to higher pollen counts but also variations in pollen season timing and air pollution levels [35, 37].

One climate-related factor is the influence of the North Atlantic Oscillation (NAO). Smith et al. [118], analyzing the influence of the NAO on grass pollen counts in Europe, observed a significant correlation between the NAO and grass pollen season start-dates at 13 pollen-monitoring sites across the entire continent, although the strongest correlations were recorded close to the Atlantic coast. Moreover, significant correlations were also found between NAO winter averages and the severity of the grass pollen season. The role of the NAO in Spain (southern Europe) has recently been studied by Galán et al. [119]. Apart from the results indicated earlier for Andalusia, the authors observed a general increase in the API across Spain, although to a varying degree depending on the area: the increase in northern Spain is around 50 pollen grains/year, compared to around 100 in central Spain and 15 in eastern coastal areas. They also noted a negative correlation between airborne pollen concentrations and NAO winter averages. This study confirms that changes in rainfall patterns in the Mediterranean region, attributed to climate change, have a major impact on grass phenology.

Another factor affecting airborne pollen counts is the increase in atmospheric carbon dioxide levels. Experimental CO₂ enhancement has been shown to alter the amount and allergenicity of emitted pollen, but this effect has rarely been demonstrated in the wider environment [120]. Nevertheless, a Europe-wide study of airborne pollen counts by Ziello et al. [121] revealed an increasing trend in annual airborne pollen counts for many taxa, including Poaceae. The increase was more marked in urban than in semi-rural/rural areas, leading the authors to suggest that the rise in atmospheric CO₂ levels due to human activity may be the main influential factor.

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Finally, attention is drawn to the value of phenological and aerobiological models developed using large databases. Most models are sufficiently accurate to function using climate data extracted from future scenarios, thus enabling us to predict the effects of climate change under the different standardized scenarios proposed by the IPCC [122]. These represent contrasting temporal patterns of economic development and CO₂ emissions. Research carried out over the last few years predicts an increase in pollen levels due to a combination of variations in plant distribution and more intense flowering [78, 123, 124]. Recent studies suggest an increasing effect of airborne allergens on allergy-sufferers over this period, which may also imply a greater likelihood of their developing allergic respiratory disease as well as an exacerbation of patient symptoms [125]. The expected increase in airborne grass pollen levels ranges from 28.5% to 44.3% in Europe [75, 126].

Conclusions

All the findings reviewed here contribute to a greater understanding of grass allergens, sensitization and airborne pollen dynamics, and the potential impact of climate change on the future evolution of airborne Poaceae pollen concentrations and thus of related pollen allergies.

References

1. Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O. A worldwide phylogenetic classification of the Poaceae (Gramineae). *J Syst Evol* 2015;**53**(2):117-37.
2. Craine JM, Ocheltree TW, Nippert JB, Towne EG, Skibbe AM, Kembel SW, Fargione JE. Global diversity of drought tolerance and grassland climate-change resilience. *Nat Clim Change* 2013;**3**(1):63-67.

3. Vicentini A, Barber JC, Aliscioni SS, Giussani LM, Kellogg EA. The age of the grasses and clusters of origins of C4 photosynthesis. *Global Change Biol* 2008;**14**(12): 2963-2977.
4. Watson L, Dallwitz MJ. The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. 1992. Published on the Internet [<http://delta-intkey.com/grass>]
5. Erdtman G. Pollen morphology and plant taxonomy: angiosperms. Leiden, The Netherlands: Brill Archive, 1986.
6. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. *Allergy* 2007;**62**:976–990.
7. Taylor G, Walker J. Charles Harrison Blackley, 1820–1900. *Clin Exp Allergy* 1973;**3**(2):103-108.
8. Radauer C, Breiteneder H. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. *J Allergy Clin Immunol* 2006;**117**:141–147.
9. Prieto-Baena JC, Hidalgo PJ, Domínguez E, Galán C. Pollen production in the Poaceae family. *Grana* 2003;**42**(3):153-159.
10. AAAAI, American Academy of Allergy, Asthma and Immunology. The allergy report: Vol. 1: Science based findings on the diagnosis and treatment of allergic disorders. 2000; AAAAI, Milwaukee WI, USA.
11. Moingeon P, Hrabina M, Bergmann KC, Jaeger S, Frati F, Bordas V, Peltre, G. Specific immunotherapy for common grass pollen allergies: pertinence of a five grass pollen vaccine. *Int Arch Allergy Immunol* 2008;**146**(4):338-342.
12. Barber D, De la Torre F, Feo F, Florido F, Guardia P, Moreno C et al. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008;**63**(11):1550-1558.
13. D'Amato G, Lobefalo G. Allergenic pollen in the southern Mediterranean area. *J Allergy Clin Immunol* 1989;**83**:116–122.
14. Rodríguez R, Villalba M, Batanero E, Palomares O, Salamanca G. Emerging pollen allergens. *Biomed Pharmacother* 2007;**61**:1–7.
15. Valenta R., Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;**29**:896-904.
16. CDC 2014. Summary Health Statistics Tables for U.S. Adults: National Health Interview Survey.
17. Larsson O, Hellkvist L, Peterson-Westin U, Cardell LO. Novel strategies for the treatment of grass pollen-induced allergic rhinitis. *Expert Opin Biol Ther* 2016;**16**(9):1143-1150.

18. Blaiss M, Maloney J, Nolte H, Gawchik S, Yao R, Skoner DP. Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. *J Allergy Clin Immunol* 2011;**127**(1):64-71.
19. Davies JM. Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases. *Clin Exp Allergy* 2014;**44**(6):790-801.
20. Newson RB, Ree R, Forsberg B, Janson C, Lötvall J, Dahlén SE, Toskala EM, Baelum J, Brożek GM, Kasper L, Kowalski ML. Geographical variation in the prevalence of sensitization to common aeroallergens in adults: the GA2LEN survey. *Allergy* 2014;**69**(5):643-51.
21. Bernstein DI, Epstein T, Murphy-Berendts K, Liss GM. Surveillance of systemic reactions to subcutaneous immunotherapy injections: year 1 outcomes of the ACAAI and AAAAI collaborative study. *Ann Allergy Asthma Immunol*. 2010;**104**(6):530-535.
22. Hauptman M, Phipatanakul W. Year in review: pediatric allergy and asthma, excluding food allergy. *Ann Allerg Asthma Immunol* 2015;**114**(3):175–177.
23. O'Connell EJ. The burden of atopy and asthma in children. *Allergy* 2004;**59**(78S):7–11.
24. Australian Bureau of Statistics (1997) 1995–1996 National health survey – asthma and other respiratory conditions, Australia. Australian Government Publishing Service, Canberra
25. Green BJ, Dettmann M, Yli-Panula E, Rutherford S, Simpson R. Atmospheric Poaceae pollen frequencies and associations with meteorological parameters in Brisbane, Australia: a 5-year record, 1994–1999. *Int J Biometeorol* 2004;**48**(4):172-178.
26. Boral D, Chatterjee S, Bhattacharya K. The occurrence and allergising potential of airborne pollen in West Bengal, India. *Ann Agric Environ Med* 2004;**11**:5–52.
27. Chew FT, Lim SH, Shang HS, Dahlia MD, Goh DY, Lee BW, et al. Evaluation of the allergenicity of tropical pollen and airborne spores in Singapore. *Allergy* 2000;**55**:340–347.
28. Sibanda EN. Inhalant allergies in Zimbabwe: a common problem *Int Arch Allergy Immunol* 2003;**130**:2–9.
29. Baena-Cagnani, CE, Solé D, González-Díaz SN, Zernotti ME, Sisul JC, Borges MS, et al. Actualización de rinitis alérgica y su impacto en el asma (ARIA 2008). La perspectiva latinoamericana. *Revista Alergia de Mexico* 2009;**56**(2).
30. Caussade S, Valdivia G, Navarro H, Pérez E, Aquevedo A, Sánchez I. Prevalencia de síntomas de rinitis alérgica y su relación con factores de riesgo en escolares de Santiago, Chile. *Revista Médica de Chile* 2006; **134**(4):456-464.
31. Vanna AT, Yamada E, Arrudalk LK, Naspitz CK, Solé D. International Study of Asthma and Allergies in Childhood: validation of the rhinitis symptom questionnaire and prevalence of rhinitis in school children in Sao Paulo, Brazil. *Pediatr Allergy Immunol* 2001;**12**:95-101.
32. Vieira FAM. Polinose no Brasil. In: Negreiros EB, Ungier C. Alergologia clínica. São Paulo: Atheneu; 1995. p.106-11.

33. D'Amato G, Liccardi G, D'Amato M. Environmental risk factors (outdoor air pollution and climatic changes) and increased trend of respiratory allergy. *J Invest Allergol Clin Immunol* 2000;**10**:33-9.
34. D'Amato G, Cecchi L, D'Amato M, Liccardi G. Urban air pollution and climate change as environmental risk factors of respiratory allergy: an update. *J Invest Allergol Clin Immunol* 2010;**20**(2):95-102.
35. Shea KM, Truckner RT, Weber RW, Peden DB. Climate change and allergic disease. *J Allergy Clin Immunol* 2008;**122**(3):443-453.
36. Beggs PJ. Impacts of climate change on aeroallergens: past and future. *Clin Exp Allergy* 2004;**34**(10):1507-1513.
37. Beggs PJ. Impacts of climate change on allergens and allergic diseases: Cambridge University Press, 2016.
38. King TP, Hoffman F, Løwenstein H, Marsh DG, Platts-Mills T, W. Allergen nomenclature. IUIS/WHO Allergen Nomenclature Subcommittee. *Bull World Health Organ* 1994;**72**:797–806.
39. Chapman MD, Pomés A, Breiteneder H, Ferreira F. Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 2007;**119**:414–420.
40. Wopfner N, Gruber P, Wallner M, Briza P, Ebner C, Mari A, et al. Molecular and immunological characterization of novel weed pollen pan-allergens. *Allergy* 2008;**63**(7):872-881.
41. Andersson K, Lidholm J. Characteristics and immunobiology of grass pollen allergens. *Int Arch Allergy Immunol* 2003;**130**(2):87-107.
42. Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004;**59**(3):243-267.
43. Hejl C, Wurtzen PA, Kleine-Tebbe J, Johansen N, Broge L, Ipsen H. Phleum pratense alone is sufficient for allergen-specific immunotherapy against allergy to *Pooideae* grass pollens. *Clin Exp Allergy* 2009;**39**(5):752-759.
44. Zhang J, Wu LS, Fan W, Zhang XL, Jia, HX, Li Y, et al. Proteomic analysis and candidate allergenic proteins in *Populus deltoids* CL.“2KEN8” mature pollen. *Frontiers in plant science* 2015;**6**:548.
45. Bozicevic A, De Mieri M, Nassenstein C, Hamburger M. Pollen induced asthma—could small molecules in pollen exacerbate the protein-mediated allergic response? *Planta Med* 2016;**81** DOI: 10.1055/s-0036-1596159

46. Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, Kraft, D. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;**175**(2): 377-385.
47. Sette A, Schulten V. It's a lot of work to be nonallergic. *J Allergy Clin Immunol* 2016; <http://dx.doi.org/10.1016/j.jaci.2016.11.018>
48. Kleine-Tebbe J, Davies, J. Grass pollen allergens. In: Global Atlas of Allergy EAACI. Akdis, CA and Agache, I. (Eds.). 2014
49. Asturias JA, Ibarrola I, Bartolome B, Ojeda I, Malet A, Martinez A. Purification and characterization of Pla a1, a major allergen from *Platanus acerifolia* pollen. *Allergy* 2002;**57**:221–227.
50. Weber RW. Cross-reactivity of pollen allergens: Impact on allergen immunotherapy. *Ann Allerg Asthma Immunol* 2007;**99**:203–212.
51. Boye JJ, Achouri A, Raymond N, Cleroux C, Weber D, Koerner TB, Hucl P, Patterson CA. Analysis of Glabrous Canary Seeds by ELISA, Mass Spectrometry, and Western Blotting for the Absence of Cross-Reactivity with Major Plant Food Allergens. *Agric Food Chem* 2013;**61**:6102–6112.
52. Gangl K, Niederberger V, Valenta R. Multiple grass mixes as opposed to single grasses for allergen immunotherapy in allergic rhinitis. *Clin Exp Allergy* 2013;**43**:1202–1216.
53. Buters JM, Galán C, Thibaudon M, Smith M, Brandao R, Antunes C, et al. The HIALINE Project: allergen release from pollen across 10 European countries. *Proceedings of the 9th International Congress on Aerobiology* 2010, Buenos Aires, Argentina.
54. Buters JTM. Impacts of Climate Change on Allergenicity. In: Beggs PJ, ed. Impacts of climate change on allergens and allergic diseases. Cambridge University Press, 2016.
55. Ziska LH. Impacts of Climate Change on Allergen Seasonality In: Beggs PJ, ed. Impacts of Climate Change on Allergens and Allergic Diseases. Cambridge University Press, 2016.
56. Menzel A, Jochner S. Impacts of Climate Change on Aeroallergen Production and Atmospheric Concentration. In: Beggs PJ, ed. Impacts of Climate Change on Allergens and Allergic Diseases. Cambridge University Press, 2016.
57. Karnosky DF, Zak DR, Pregitzer KS, Awmack CS, Bockheim JG, Dickson RE, et al. Tropospheric O₃ moderates responses of temperate hardwood forests to elevated CO₂: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Funct Ecol* 2003;**17**(3):289-304.
58. Ainsworth EA, Long, SP. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol* 2005; **165**(2):351-372.
59. Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans, R. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Env* 1999;**22**(6):683-714.

60. White JF, Bernstein DI. Key pollen allergens in North America. *Ann Allergy Asthma Immunol* 2003;**9**:425–435.
61. Morellato LPC, Camargo MGG, Gressler, E. A review of plant phenology in South and Central America. In: Schwartz MD ed. *Phenology: An Integrative Environmental Science* Springer Netherlands, 2013; 91-113.
62. Wielgolaski FE. Starting dates and basic temperatures in phenological observations of plants. *Int J Biometeorol* 1999;**42**(3):158-168.
63. Trevaskis B, Hemming MN, Dennis ES, Peacock WJ. The molecular basis of vernalization-induced flowering in cereals. *Trends Plant Sci* 2007;**12**(8):352-357.
64. García-Mozo H, Galán C, Belmonte J, Bermejo D, Candau P, Díaz de la Guardia C, et al. Predicting the start and peak dates of the Poaceae pollen season in Spain using process-based models. *Agric For Meteorol* 2009;**149**(2):256-262.
65. Sparks TH, Menzel A. Observed changes in seasons: an overview. *Int J Climatol* 2002;**22**(14):1715-1725.
66. Olesen JE, Carter TR, Diaz-Ambrona CH, Fronzek S, Heidmann T, Hickler T, et al.. Uncertainties in projected impacts of climate change on European agriculture and terrestrial ecosystems based on scenarios from regional climate models. *Climatic Change* 2007;**81**(1):123-143.
67. Trnka M, Rötter RP, Ruiz-Ramos M, Kersebaum KC, Olesen JE, Žalud Z, Semenov M. A. Adverse weather conditions for European wheat production will become more frequent with climate change. *Nat Clim Change* 2014;**4**(7):637-643.
68. Vanuytrecht E, Raes D, Willems P. Global sensitivity analysis of yield output from the water productivity model. *Env Modell Softw* 2014;**51**:323-332.
69. Oteros J, García-Mozo H, Botey R, Mestre A, Galán C. Variations in cereal crop phenology in Spain over the last twenty-six years (1986–2012). *Climatic Change* 2015;**130**(4):545-558.
70. Cebrino J, Galán C, Domínguez-Vilches E. Aerobiological and phenological study of the main Poaceae species in Córdoba City (Spain) and the surrounding hills. *Aerobiologia* 2016;**32** (4):595–606.
71. Tormo R, Maya JM, Silva I, Fernández S, Gonzalo A. Flower production and phenology in *Dactylis glomerata*. *Aerobiologia* 2015;**31**(4):469-479.
72. Crone E. Desynchronization and re-synchronization of reproduction by *Astragalus scaphoides*, a plant that flowers in alternate years. *Ecol Res* (2013);**28**(2):133-142.
73. Ferrero R, Lima M, González-Andújar JL. Spatio-temporal dynamics of maize yield water constraints under climate change in Spain. *PLoSOne* 2014;**9**:e98220.
74. Lima M, Navarrete L, González-Andujar JL. Climate effects and feedback structure determining weed population dynamics in a long-term experiment. *PloSOne* 2012;**7**(1):e30569.

75. García de León D, García-Mozo H, Galán C, Alcázar P, Lima M, González-Andújar J L. Disentangling the effects of feedback structure and climate on Poaceae annual airborne pollen fluctuations and the possible consequences of climate change. *Sci Total Environ* 2015;**530**:103-109.
76. Erdtman G. Pollen morphology and plant taxonomy. *GFF* 1952;**74**(4):526-527.
77. Perveen A. A contribution to the pollen morphology of family *Gramineae*. *World Appl Sci J*, 2006;**1**(2):60-65.
78. García-Mozo H, Oteros JA, Galán C. Impact of land cover changes and climate on the main airborne pollen types in Southern Spain. *Sci Total Environ* 2016;**548**:221-228.
79. Schwartz M. Phenology: An Integrative Environmental Science. Springer, 2013..
80. Hirst J. An automatic volumetric spore trap. *Ann Appl Biol* 1952;**39**(2): 257-265.
81. Galán C, Cariñanos P., Teno P, Dominguez E. Spanish Aerobiology Network (REA): management and quality manual. Servicio de publicaciones de la Universidad de Córdoba, Spain, 2007.
82. Galán C, Smith M, Thibaudon M, Frenguelli G, Oteros J, Gehrig R, and the EAS QC working group. Pollen monitoring: minimum requirements and reproducibility of analysis. *Aerobiologia* 2014;**30**(4):385-395.
83. Oteros J, Galán C, Alcázar P, Domínguez E. Quality control in bio-monitoring networks, Spanish Aerobiology Network. *Sci Total Environ* 2013;**443**:559-565.
84. García-Mozo H, Chuine I, Aira MJ, Belmonte J, Bermejo D, Díaz de la Guardia C, Elvira B, Gutiérrez M, Rodríguez-Rajo J, Ruiz L, Trigo MM, Tormo R, Valencia R, Galán C. Regional phenological models for forecasting the start and peak of the *Quercus* pollen season in Spain. *Agric For Meteorol* 2008;**148**:372-380.
85. Sofiev M, Belmonte J, Gehrig R, Izquierdo R, Smith M, Dahl Å, Siljamo P. Airborne pollen transport. In: Sofiev M., Bergmann KC editors. Allergenic pollen. Springer Netherlands 2013;127-159.
86. Zhang Y, Bielory L, Cai T, Mi Z, Georgopoulos P. Predicting onset and duration of airborne allergenic pollen season in the United States. *Atmos Environ* 2015;**103**:297-306.
87. León-Ruiz E, García-Mozo H, Domínguez E, Galán C. The use of geostatistics in the study of floral phenology of *Vulpia geniculata* L. Link. *The Scientific World Journal* 2012; doi:10.1100/2012/624247
88. Zanotti AL, Puppi G. Phenological surveys of allergenic species in the neighbourhood of Bologna (Italy). *Aerobiologia* 2000;**16**(2):199-206.
89. Jato V, Rodríguez-Rajo FJ, Seijo MC, Aira MJ. Poaceae pollen in Galicia (NW Spain): characterisation and recent trends in atmospheric pollen season. *Int J Biometeorol* 2009;**53**(4):333-344.
90. Norris-Hill J. The modelling of daily Poaceae pollen concentrations. *Grana* 1995;**34**(3): 182-188.

91. Norris-Hill, J. The influence of ambient temperature on the abundance of Poaceae pollen. *Aerobiologia* 1997;**13**(2):91-97.
92. Larsson KA. Prediction of the pollen season with a cumulated activity method. *Grana* 1993;**32**(2):111-114.
93. Emberlin J, Mullins J, Corden J, Jones S, Millington W, Brooke M, Savage M. Regional variations in grass pollen seasons in the UK, long-term trends and forecast models. *Clin Exp Allergy* 1999;**29**:347-356.
94. García-Mozo H, Yaezel L, Oteros J, Galán C. Statistical approach to the analysis of olive long-term pollen season trends in southern Spain. *Sci Total Environ* 2014;**473**: 103-109.
95. Caramiello R, Siniscalco C, Mercalli L, Potenza A. The relationship between airborne pollen grains and unusual weather conditions in Turin (Italy) in 1989, 1990 and 1991. *Grana* 1994;**33**(6):327-332.
96. Clot B. Airborne birch pollen in Neuchâtel (Switzerland): onset, peak and daily patterns. *Aerobiologia* 2001;**17**(1):25-29.
97. Andersen TB. A model to predict the beginning of the pollen season. *Grana* 1991;**30**(1):269-275.
98. Jato V, Dopazo A, Aira MJ. Influence of precipitation and temperature on airborne pollen concentration in Santiago de Compostela (Spain). *Grana* 2002;**41**(4):232-241.
99. Laaidi M. Forecasting the start of the pollen season of Poaceae: evaluation of some methods based on meteorological factors. *Int J Biometeorol* 2001;**45**(1):1-7.
100. Chuine I, Belmonte J. Improving prophylaxis for pollen allergies: predicting the time course of the pollen load of the atmosphere of major allergenic plants in France and Spain. *Grana* 2004;**43**(2):65-80.
101. Sanchez-Mesa JA, Galán, C, Hervás, C. The use of discriminant analysis and neural networks to forecast the severity of the Poaceae pollen season in a region with a typical Mediterranean climate. *Int J Biometeorol* 2005;**49**(6):355-362.
102. El-Ghazaly G, El-Ghazaly PK, Larsson KA, Nilsson S. Comparison of airborne pollen grains in Huddinge and Stockholm, Sweden. *Aerobiologia* 1993;**9**(1):53-67.
103. Galán C, Emberlin J, Domínguez E, Bryant RH, Villamandos F. A comparative analysis of daily variations in the *Gramineae* pollen counts at Córdoba, Spain and London, UK. *Grana* 1995;**34**(3):189-98.
104. Sanchez-Mesa JA, Smith M, Emberlin J, Allitt U, Caulton E, Galan C. Characteristics of grass pollen seasons in areas of southern Spain and the United Kingdom. *Aerobiologia* 2003;**19**(3-4):243-250.
105. Maya Manzano JM, Fernández Rodríguez S, Vaquero Del Pino C, Gonzalo Garijo Á, Silva Palacios I, Tormo Molina R et al. Variations in airborne pollen in central and south-western Spain in relation to the distribution of potential sources. *Grana* 2016;**11**:1-2.

106. Haberle SG, Bowman DM, Newnham RM, Johnston FH, Beggs PJ, Buters J, et al. The macroecology of airborne pollen in Australian and New Zealand urban areas. *PloS One* 2014;**9**(5):e97925.
107. Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, et al. Global consequences of land use. *Science* 2005;**309**(5734):570-574.
108. Turner BL, Lambin EF, Reenberg A. The emergence of land change science for global environmental change and sustainability. *P Natl Acad Sci* 2007;**104**(52):20666-20671.
109. Davis MB. Palynology after Y2K-understanding the source area of pollen in sediments. *Annu Rev Earth Pl Sc* 2000;**28**(1):1-18.
110. Seppä H, Bennett KD. Quaternary pollen analysis: recent progress in palaeoecology and palaeoclimatology. *Progress in Physical Geography* 2003;**27**(4):548-579.
111. Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, et al. Climate change 2014: synthesis Report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change (p. 151). IPCC 2014.
112. Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, et al. European phenological response to climate change matches the warming pattern. *Glob Change Biol* 2006;**12**(10):1969-1976.
113. García-Mozo H, Mestre A, Galán C. Phenological trends in southern Spain: a response to climate change. *Agric For Meteorol* 2010;**150**(4):575-580.
114. Darrow LA, Hess J, Rogers CA, Tolbert PE, Klein M, Sarnat SE. Ambient pollen concentrations and emergency department visits for asthma and wheeze. *J Allergy Clin Immunol.* 2012;**130**(3):630-638.
115. Frei T. The effects of climate change in Switzerland 1969–1996 on airborne pollen quantities from hazel, birch and grass. *Grana* 1998;**37**(3):172-179.
116. Zhang Y, Bielory L, Georgopoulos PG. Climate change effect on *Betula* (birch) and *Quercus* (oak) pollen seasons in the United States. *Int J Biometeorol* 2014;**58**(5):909-919.
117. García-Mozo H, Galán C, Alcázar P, Díaz de la Guardia C, Nieto-Lugilde D, Recio, M, et al. Trends in grass pollen season in southern Spain. *Aerobiologia* 2010;**26**(2):157-169.
118. Smith M, Emberlin J, Stach A, Rantio-Lehtimäki A, Caulton E, Thibaudon M., Jato, V. Influence of the North Atlantic Oscillation on grass pollen counts in Europe. *Aerobiologia* 2009;**25**(4):321-332.
119. Galán C, Alcázar P, Oteros J, García-Mozo H, Aira MJ, Belmonte J, et al. Airborne pollen trends in the Iberian Peninsula. *Sci Total Environ* 2016;550:53-59.
120. Rogers CA, Wayne PM, Macklin EA., Muilenberg ML, Wagner CJ, Epstein PR, Bazzaz FA. Interaction of the onset of spring and elevated atmospheric CO₂ on ragweed (*Ambrosia artemisiifolia* L.) pollen production. *Environ Health Persp* 2006;**114**(6):865-869.

121. Ziello C, Sparks TH, Estrella N, Belmonte J, Bergmann KC, Bucher E, et al. Changes to airborne pollen counts across Europe. *PLoS One* 2012;**7**(4):e34076.
122. Nakicenovic, N., & Swart, R. Special report on emissions scenarios. Special Report on Emissions Scenarios, Edited by Nebojsa Nakicenovic and Robert Swart, pp. 612. ISBN 0521804930. Cambridge, UK: Cambridge University Press, July 2000.
123. García-Mozo H, Galán C, Jato V, Belmonte J, de la Guardia CD, Fernández D, et al. *Quercus* pollen season dynamics in the Iberian Peninsula: response to meteorological parameters and possible consequences of climate change. *Ann Agric Environ Med* 2006;**13**(2):209-224.
124. Shafer SL, Bartlein PJ, Thompson RS. Potential changes in the distributions of western North America tree and shrub taxa under future climate scenarios. *Ecosystems* 2001;**4**(3):200-215.
125. Burr ML. Grass pollen: trends and predictions. *Clin Exp Allergy* 1999;**29**(6):735-738.
126. Cecchi L, D'Amato G, Ayres JG, Galan C, Forastiere F, Forsberg B, et al. Projections of the effects of climate change on allergic asthma: the contribution of aerobiology. *Allergy* 2010;**65**(9):1073-1081.

Allergen group	Biochemical character	Molecular weight [kDa]	Member in <i>Phleum pratense</i>	Features	IgE reactivity
1	β-expansin	27-35	Phl p 1	Glycoprotein, major and ubiquitous grass pollen allergen	>90%
2	Unknown	11	Phl p 2	Highly homologous to groups 1 and 3	40-60%
3	Unknown	11-14	Phl p 3	Highly homologous to groups 2 and 1	57-67%
4	Oxidoreductase	50-60	Phl p 4	Glycoprotein, Berberine bridge enzyme family member, plant pathogen response system	45-88%
5	Unknown	27-35	Phl p 5	Found in Pooideae grass species, submicronic cytoplasmic starch particles	65-85%
6	Unknown	12-13	Phl p 6	Homologous to internal group 5 sequences, only in <i>Anthoxanthum odoratum</i> , <i>Phleum pratense</i> and <i>Poa pratensis</i>	60-70%
7	Polcalcin, Ca ⁺⁺ -binding protein	8-12	Phl p 7	Panallergen, dimer assembly in grass pollen, broad pollen-related crossreactivity	5-35%
11	Ole e 1-related protein	16-20	Phl p 11	Glycoprotein, similar structure to pollen allergens from olive tree pollen (Ole e 1) and lamb's quarter (Che a 1)	18-56%
12	Profilin	13-14	Phl p 12	Panallergen, highly conserved, broad pollen and plant food-related crossreactivity	10-40%
13	Polygalacturonase	45-60	Phl p 13	Glycoprotein, susceptible to protease degradation	30-40%

Table 1. Grass pollen allergen groups. Modified from Kleine-Tebbe and Davies (2014). Due to their taxonomic and biochemical relationship, many grasses have similar allergens, grouped according to shared amino acid sequences. Specific allergens from *Phleum pratense* are given as examples.



Figure 1. Poaceae pollen grain. Average size: 35x40 μm .