Cluster analysis of variations in the diurnal pattern of grass pollen concentrations in Northern Europe (Copenhagen) and Southern Europe (Córdoba)

Diurnal pattern of grass pollen

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Abstract

From an allergological point of view, *Poaceae* pollen is one of the most important type of pollen that the population is exposed to in the ambient environment. There are several studies on intra-diurnal patterns in grass pollen concentrations, and agreement on the high variability. However, the method for analysing the different patterns is not yet well established. The aim of the present study is therefore to examine the method of pattern analysis by statistical clustering, as well as relating the proposed patterns to time of season and meteorological variables at two highly different biogeographical locations; Córdoba, Spain and Copenhagen, Denmark.

Airborne pollen is collected by Hirst type volumetric spore traps and counted using an optical microscope at both sites. The counts were converted to two-hours concentrations and a new method based on cluster analysis was applied with the aim of determining the most frequent diurnal patterns in pollen concentrations and their dependencies of site, season and meteorological variables.

Three different well defined diurnal patterns were identified at both locations. The most frequent pattern in Copenhagen was associated with days having peak pollen concentrations in the evening (maximum between18h-20h), whereas the most frequent pattern at Córdoba was associated with days having peak pollen concentrations in the afternoon (maximum between 14h-16h). These three patterns account for 70% of days with no rain and pollen concentrations above 20 grains m^{-3}. The most frequent pattern accounts for 40% and 57% of the days in Córdoba and Copenhagen respectively. The analysis clearly shows the great variation in pollen concentration pattern, albeit a dominating pattern can be found.

It was not possible to explain all the differences in the patterns by the meteorological variables when examined individual. Clustering method is estimated to be an appropriate methodology for studying aerobiological phenomena with high variability.

Keywords: Poaceae pollen, bioaerosols, clustering, hourly, aerobiology, meteorology, air quality

Introduction

Grass pollen is one of the most important from an allergological point of view, being the most important casue of pollinosis in extensive areas of the World like Europe (D'Amato et al., 2007). It is the most wide-spread pollen (Skjøth et al., 2013a) and may be considered as the most important cause of pollinosis in Europe due to a long season (Smith et al., 2014), its wide-spread distribution (Skjøth et al., 2013b) and the generally very high number of sensitizations (Burbach, 2009; D'Amato et al., 2007). Numerous species contribute to the concentration of this pollen (Kraaijeveld et al., 2015). Different grass species flower at different times of the year (Beddows, 1931; Jones, 1952) and day, this may affect the diurnal patterns in pollen from this family. As an example, *Agrostis* and *Festuca* flower at midday whereas *Anthoxanthum* and *Holcus* flower in the morning or late afternoon (Hyde and Williams, 1945; Peel et al., 2014). A number of studies have demonstrated an afternoon maximum in the concentrations of grass pollen (e.g. Goldberg et al., 1988; Simoleit et al., 2015). Nevertheless, variations in airborne grass pollen concentrations are not solely related to time of anthesis. Due to their transport and dispersion in the air, pollen concentrations also depend on atmospheric conditions like urban atmospheric stability and local breezes (Puc, 2012; Pérez-Badia et al., 2011; Muñoz Rodríguez et al., 2010; Kasprzyk, 2006). Every day, it happens the upward moving of thermals with pollen grains to higher elevation and when convection currents cease at the end of the afternoon, suspended particles are subject to gravitational settling which lead to increasing pollen concentrations at lower height.

Airborne grass pollen in Copenhagen (Denmark) and Cordoba (Spain) are likely to have different diurnal patterns, and no previous studies have reported multiple diurnal patterns for either site. This difference is caused by considerable differences in climate and species composition as described by base maps used in the habitat directive (e.g. http://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe). Also differences in local relief could affect the patterns, as Cordoba is highly affected by the surrounding mountains, and Copenhagen is located at a flat coastal position.

Grass pollen concentrations originate from an amalgam of species (García-Mozo et al., 2010). However, in Cordoba (Spain) it has been shown that just four Poaceae species are the dominating contributors to pollen concentrations (León-Ruiz et al., 2011; Cebrino et al., 2016) while other regions, such as Leiden in Northern Europe, have different profiles (Kraaijeveld et al., 2015). Meteorological effects on the diurnal profiles for grasses can vary considerable between years, e.g. potentially limiting flowering of specific grass species responsible for the early season profile, as the one observed by Peel et al. (2014). Long term studies and interregional comparisons are therefore important. Previous studies conducted in Cordoba have showed that the diurnal pattern in grass pollen concentration was homogeneous throughout the study years. These patterns showed an increase early in the morning, a moderate decrease in the afternoon, and stable values throughout the night (Galán et al., 1989; Cariñanos et al., 1999; Alcázar et al., 1999). A previous study in Copenhagen has shown the maximum frequency of pollen peak concentrations during the afternoon (Goldberg et al., 1988), whereas a recent study showed seasonal variation in the profile (Peel et al., 2014).

Determining the actual concentrations of pollen including the diurnal pattern is an important element in providing advice to patients on allergen avoidance during peak hours in pollen concentrations (Sommer et al., 2009). These patterns can vary over the season and can be specific to the geographical region. A single unified pattern therefore has limitations. The diurnal pattern and its potential variations are of importance for patients suffering from allergy as well as for doctors that are studying allergic rhinitis or treating and guiding patients. Until now primarily seasonal averaged diurnal pattern in pollen concentrations have been available in literature and the interest in bringing this a step forward and provide diurnal pattern as function of time of season and meteorological conditions is the background for the presented work.

The aim of this paper was to analyse the variation in diurnal patterns of grass pollen concentrations in Copenhagen (Denmark) and Cordoba (Spain). The diurnal patterns in two-hours pollen concentrations are examined by a new method based on statistical clustering to objectively reveal groups solely based on pattern and relate these to meteorology and time of season.

Material and Methods

This study investigates the measured two-hours pollen concentrations (concentration of pollen during periods of two hours) from two pollen traps in the cities of Copenhagen and Cordoba (Fig.1). Continuous monitoring of pollen in the air is carried out from Hirst type volumetric spore traps (Hirst, 1952). Air is sucked into the trap at a rate of 10 L/min through a 2mm×14mm orifice. Behind the orifice, the air flows over a rotating drum that moves past the inlet at 2 mm/h. The drum is covered with an adhesive coated, transparent plastic tape, which traps the particles through impaction.

>>Figure 1

Copenhagen is the capital of Denmark. It is situated on the eastern coast of Zealand island (55º40’N, 12º34’E), 20 m a.s.l. The city is in located on low lying flat ground near the coast and subject to low pressure systems from the Atlantic resulting in unstable conditions throughout the year. The area is mainly urban with agricultural surroundings and biogeographically located in the northern part of the continental region with little distance to the Atlantic and Boreal regions. The annual diurnal mean temperature is 8°C and the annual precipitation is 613 mm with rainfall fairly evenly distributed throughout the year. Weather data is obtained from near the pollen trap, including hourly measurements of temperature, wind speed and direction. Daily precipitation is from the nearby synoptic meteorological site at Kastrup airport (USAF-ID 061800), obtained from the data set Global Summary of the Day exchanged by World Meteorological Organisation. The pollen trap is situated 15 m above ground level on the roof of the Danish Meteorological Institute (55°43 N, 12°34 E). The Copenhagen pollen monitoring station is part of the permanent Danish pollen monitoring network, and is typically in continuous operation from January to October.

The typical grass pollen season in Denmark is from end of May till end of August, peaking at the end of June, with an average annual pollen integral of 2200 grains * day/m^3, varying from 588 to 3222 (1985-2009). Peak daily pollen concentration occurred in 2004 with 320 pollen grains /m^3(Sommer and Rasmussen, 2011).

The city of Cordoba is placed in the south of Iberian Peninsula (37°50'N, 4°45'W), 123 m a.s.l. The area has a Mediterranean climate with some continental features. The annual mean temperature is 17.8 °C and the annual average precipitation is 621 mm, with hot dry summers. The nearby area is urban with agricultural surroundings (pasture and crops under rotation), olive plantations as well as shrub and/or herbaceous vegetation. Biogeographically Cordoba is located in the southern parts of the Mediterranean region. Weather data, including hourly measurements of temperature, precipitation, wind speed and direction, were provided by the central service for research support of the University of Cordoba (SCAI), based on readings taken at Rabanales Campus, located around 10 km north-east of the pollen sampler site.

The trap in Cordoba city is located on the roof of the Educational Sciences Faculty, at 15 m above ground level. The typical grass pollen season starts in April and ends in July. The peak concentration is recorded during May. Annual pollen integral varies from 1000 to 10000 pollen grains * day/m^3, and daily peak concentrations vary from less than 100 to more than 800 pollen grains /m^3. Pollen concentrations were obtained using a standard protocol published by the Spanish Aerobiology Network (REA) (Galán et al., 2007). Cordoba has a special location with a valley-mountain breeze known to affect pollen concentrations (Hernandez-Ceballos et al., 2013; 2014), where winds are towards the mountains in the morning and from the mountains in the evening.Both locations follow the minimal requirements of the European Aeroallergen Network (EAN) for pollen monitoring (Galán et al., 2014).

For this study, we included data from 2008 to 2011 for Cordoba and from 2001 to 2010 for Copenhagen. Days with less than 20 grass pollen grains /m^3were excluded from the analysis in the same way as in Peel et al. (2014) due to the large uncertainty in the daily pattern at very low concentrations. Days with rain were also excluded due to the efficiency of precipitation on removal of pollen from the atmosphere (McDonald, 1962), and the resulting effect on the profile.

Pollen data from Cordoba is counted for every hour. To reduce statistical error, as the orifice of the sampler is 2 mm wide and the drum runs by 2 mm per hour, we have re-calculated data into two-hours concentrations. Time stamps have been corrected corresponding to official Danish and Spanish time (UTC+ 1 hour during autumn and winter and UTC + 2 hours during spring and summer).

All the statistical analysis are performed by using the SPSS 15.0® Software Package and R Software (R Core Team, 2014). The pollen concentrations were standardized to eliminate the effect of the magnitude. The formula for standardizing two-hours grass pollen concentrations is presented in (1), where Z_i is the standardized two-hours value, xi is the real two-hours value, $\overline{X}$ is the daily mean of the two-hours pollen concentrations and SD is the daily standard deviation of the two-hours pollen concentration (Oteros et al., 2013).

$$Z_i = \frac{x_i - \overline{X}}{SD} \qquad (1)$$

"Clustering" is the generic name of a big variety of procedures used for grouping a set of objects into relatively homogeneous groups. The standardized two-hours values are analysed using hierarchical cluster analysis (HCA). Hierarchical clustering analysis was performed using Ward's method, in which the distance of each element, in our case between each day, to the centroid of the cluster to which it belongs was evaluated. The mean vector of all standardized two-hours pollen concentrations was calculated, determining the multivariate centroid for each cluster. The squared Euclidean distances between each element and the centroid (mean vector) of all clusters were then calculated and expressed as a distances matrix. The Euclidean distance (ED) is defined as the sum of the differences between the values of the attributes of each compared pair of entities:

$$ED = \sqrt{\sum_{i=1}^{n} (p_i - q_i)^2}$$

Where *p* and *q* are the values of the pollen concentration at the same hour (i) of different days in the study.

Finally, distances for all elements are combined. This method starts defining n number of clusters, where n = number of study cases. The algorithm tries to minimize the total within-cluster variance after merging clusters. The algorithm proceeds iteratively and at each stage joins the two most similar clusters, continuing until there is just a single cluster. For every step of the iteration an optimal pair of clusters to merge needs to be found. For disjoint clusters (X,Y,Z), the implementation of the Ward's minimum variance method is mathematically expressed as:

$$D(X \cup Y, Z) = \frac{n_x + n_z}{n_x + n_y + n_z} D(X, Z) + \frac{n_y + n_z}{n_x + n_y + n_z} D(Y, Z) - \frac{n_z}{n_x + n_y + n_z} D(X, Y)$$

where nx, ny and nz are the size of the clusters.

The number of natural daily pollen patterns in every city was examined graphically by a dendogram and the Elbow plot (Appendix 1). The Elbow method calculates the total intra-cluster variance according to the number of clusters.

After determined k, the optimal number of clusters. “K-means” conglomerate method was applied for generating clusters. Patterns in grass pollen were generated on the basis of similar two-hours standardized pollen data. From various types of cluster analysis available, this is deemed to the most appropriate, in that it provides a more flexible approach and does not assume any specific distribution of variables. Appendix 1 shows the result of k-means analysis by a “clustplot or Bivariate Cluster Plot”, which is a representation of the cases and the k clusters in a 2D space ordered according to the two principal components of the data. The clustplot was with the R package “cluster” (Maechler et al., 2017).

The relationship between pollen profiles and daily weather parameters was carried out using an average-comparison method. Tested daily weather parameters were: temperature, humidity, global radiation, wind speed and wind direction. Wind direction was available for Cordoba as the hourly percentage of wind source from each octant and for Copenhagen as the predominant wind direction within 30 minutes. In the case of Cordoba, we selected the most common wind direction every hour as the prevalent direction with the aim of getting degrees’ units. We calculated one value of predominant wind direction per day in degrees (0º-360º) by the circular average of the wind directions.

Aerobiological data are often non-normally distributed, which was verified using the Saphiro-Wilk test. Variances and homogeneity was tested by Fligner-Killeen Test (Conover et al., 1981). Due to the non-normality and the presence of outliers (tested by plotting boxplots), a Robust Anova analysis is applied for analysing the correlation between patterns in pollen concentrations and weather parameters. Significant differences in weather variables between clusters were examined applying posthoc Tukey test to analyse in which clusters they are present. The analysis is performed using “WRS2” package of R (Mair et al., 2015; R Core Team, 2015).

The effect of wind direction on the clustering pattern was studied by circular statistics (Sadyś et al., 2015, Borycka and Kasprzyk, 2014, Maya-Manzano et al., 2017). The circular average of the prevalent wind direction was calculated for the days associated with each cluster. Circular statistics is performed by using "Circular" package of R (Agostinelli and Lund, 2013; R Core Team, 2015). Differences in wind direction between clusters were analysed by applying circular ANOVA.

Results

A total of 259 days of data for Copenhagen and 184 days for Cordoba met the above listed criteria of no rain and pollen concentrations above 20 pollen grains / m^3. Three well defined diurnal profiles were observed in both locations by the above described method. Days with high distance to cluster center were not included in further analysis, since those days do not have a well-defined hourly pattern. For Copenhagen, this condition applied to 69 days, and for Cordoba, 60 days. Appendix 1 shows the distribution of all the cases clustered by their dissimilarities in the HC dendrogram (A,B) and the Elbow plot (C,D), the graphical evaluation in both cases suggest that the dataset can be clustered in three well defined groups (K=3) for each city. Appendix 1 (E,F) shows the distribution of the cases in a two-dimensional space conformed by the component 1 and component 2 of the k-means analysis, the cases are separated in three groups according to the diurnal pollen profiles.

Figure 2 represents the average and the 95% confidence intervals (CI) of each of the three pre-defined clusters for Cordoba. Great variation is seen between clusters in the time of peak pollen concentrations. Cluster 1 is the most frequent pattern, with 40 % of the cases, showing the typical afternoon peak. Cluster 2 represents 33 % of the days included, and shows an early morning peak, with substantial concentrations before daylight starts (around 7 in Cordoba). Cluster 3 represents 27 % of the days included and has a two-peak pattern with morning and evening peaks. Clusters 2 and 3 are closer between then than to cluster 1, both groups of days shows a peak in the morning, buth with the difference of pollen concentrations during the night.

>>Figure 2

Figure 3 shows the average and the 95%-CI of the three pre-defined clusters for Copenhagen. Cluster 1 is the most frequent pattern, with 57% of cases, showing peaks recorded during the early evening. Cluster 2 represents 13% of cases, and consists of days with peak concentrations during the night. Cluster 3 represents 30% of cases, and shows a midday-afternoon double peak.

>>Figure 3

By applying comparison of mean methods, we found relationships between pollen patterns and meteorological variables (Table 1). A total of 166 days for Copenhagen and 86 days for Cordoba were included. Differences were seen between the days of the year in which most of the cases of each patterns are observed in Cordoba, however not significant. Cluster 1 has the highest fraction of observations from early in the season, while Cluster 3 has the main fraction of observations during the late pollen season (Appendix 2). This fact could be related to the association of the flowering features of different species to different patterns, but also could be a masking factor for the differences caused by meteorology. Global radiation is significantly lower in cluster 1, this is probably the consequence of cluster 1 happening more frequently during the earlier part of the season.

>> Table 1

By comparing pollen patterns with weather parameters in Copenhagen we only found a significant difference for wind directions. The main wind direction was from West in Cluster 1 and from South-West in Clusters 2 and 3.

>>Table 2

Discussion

It is known that the pollen load varies across the duration of a day, and that methods for predicting the time of the day where maximum peaks are reached have still not been developed (Bogawski and Smith, 2016). Due to pollen grains being biological particles with an important impact on human health, the study of diurnal profiles of pollen is very useful to prevent high exposures (e.g. Sommer et al., 2009). For this reason, several papers have focused on hourly pollen information and the parameters mainly influencing this variation, finding great diversity in the daily rhythms of pollination (Beddows, 1931; Jones, 1952; Kasprzyk, 2006; Muñoz Rodríguez et al., 2010; Peel et al., 2014; Pérez-Badía et al., 2011; Puc, 2012; Rojo et al., 2015).

The variation in diurnal pollen patterns is especially clear in the case of multi-species pollen types such as Poaceae, and the time of maximum concentration is difficult to predict as an average that only shows one most frequently found pattern without accounting for other possible patterns. Alba et al. (2000) found also that there is not a single diurnal pattern even for pollen measurements originating from a single species (*Olea europaea* L.). They postulated that limiting the visualizing of the average behaviour of airborne pollen (through the average diurnal pattern) limits the analysis of the diurnal pollen pattern, resulting in the understanding to be incomplete. They found that 54% of the observed days fitted a single dispersal pattern, on the remaining days (46%) the pollen dispersal was highly irregular. In our study we found three possible patterns where approximately 70% of the studied days without rain and a daily pollen concentration above 20 pollen grains m-3 could be fitted. For 27% of days for Copenhagen, and 32% of days for Cordoba the pattern showed a high distance to cluster center, i.e. a pattern not fitting any of the three clusters.

Many factors are involved in the variation of the diurnal pattern of pollen concentrations. In the case of Poaceae, differences in pollination features of the different species can have an important influence. Several papers report considerable variation in the pattern, linking this to species flowering at different time, peak occurring mostly in the morning or in the afternoon (Kapyla, 1981; Peel et al., 2014). It could therefore be important to determine this by a dedicated phenological study focusing on the species that contribute to the majority of airborne grass pollen concentrations in order to determine the time of the day at which they liberate the pollen, and potential differences in the effect of meteorology on the different species. This can also be expected to be site-specific, and transferable uniform patterns may not be possible, however uniform methods may be developed.

León Ruiz et al. (2011) found that in Cordoba only four species were major contributors to the Poaceae airborne pollen curve (Dactylis glomerata, Lolium rigidum, Trisetaria panacea, Vulpia geniculata) while Kraaijeveld et al (2015) found a larger number of important species in the Netherlands. Cebrino et al. (2016) support these results and show that the majority of the pollen sources are found locally. Peel et al. (2014) found a relationship between diurnal profiles and the time of season potentially linking this to the flowering of different species. This fact could be explained by the existence of different pollination features depending on the grass species. In this study we did not see a clear difference related to time of season, and could therefore not explain the patterns as being primarily driven by the succession of flowering species.

Another factor that must be taken into account is the distance between pollen sources and the trap (Perez Badia et al., 2011), although this fact could be less relevant for Poaceae as these taxa are densely distributed everywhere, inside and around cities. Nevertheless, distance from the pollen source can be also of great importance and show large variations within short distances (Skjøth et al., 2013b). Depending on the distance from the pollen source and flowering phenology, wind direction seems to be determinant for explaining some intra-diurnal variations of pollen loads (Rojo et al., 2015). In our study the wind direction showed significant differences between clusters for Copenhagen, with Cluster 1 having more winds from West and Southwest. This is the most frequent pattern with early evening peaks. A large residential area with gardens, lawns and associated grass covered recreational areas is located approximately 0.5-1.5 km in this direction. However, whether this area is a major source of the pollen will highly depend on the cutting frequency of the lawns and meadows (Skjøth et al., 2013b).

The pollen patterns in Cordoba and Copenhagen were both affected by wind directions although not to a large degree. In Cordoba, the valley-mountain breeze (Hernandez-Ceballos et al., 2013; 2014) is dominating the wind directions for the three clusters of days, and therefore no significant differences are seen here. Differences are therefore unlikely due to differences in source areas for this site, however a separate analysis would be required to establish this. Our result along with the previous results by Norris-Hill and Emberlin (1991) suggest that the foot print area could be an important factor to take into account in further grass pollen studies. Even highly local sources could be of great importance (Skjøth et al 2013). Ideally they should focus on both the variation in the daily pattern as in our study as well as the dominating species and the associated ecosystems found within the atmospheric foot print.

Different grass species are associated with main ecosystems and geographical regions as defined by the biogeographical regions of Europe and used in the habitats directive. This is clearly illustrated in the contribution from a large number of species to the overall grass pollen integral found in Leiden, within the Atlantic biogeographical region (Kraaijeveld et al., 2015) and four the dominating species found in Cordoba (Cebrino et al., 2016). In the Poaceae family, the liberation of pollen is controlled by factors inherent to each species and occurs in a short period of hours each day but pollen grains can remain in the air where their dispersion is again affected by meteorological parameters (Myszkowska, 2014). These meteorological effects also vary during the day, e.g. as in the valley winds affecting Cordoba and the associated pollen concentrations (Hernandez-Ceballos et al., 2013; 2014). In this sense, Norris-Hill and Emberlin (1991) tried to divide days into categories taking into account temperature, humidity and wind direction, finding small differences in the time of maximum pollen concentration with temperature and wind direction.

This study was carried out in two different urban environments. Exposure to grass pollen in urban environments is particular important because some air pollutants seems to correlate with the daily patterns of pollen concentrations (Ørby et al., 2015). Puc (2012) also saw strong correlation between intra-hourly pollen concentrations and gaseous air pollutants. This is important because co-exposure of air pollutants and pollen can reduce the threshold for an allergenic response (Molfino et al., 1991; D’Amato et al., 2010). In the case of Cordoba (Spain) a previous study showed that the peaks of non-biological particles in the air throughout the day are related to activities carried out by human beings in the city occurring in the morning and late in the evenings (commercial and working hours), which are probably related with resuspension process of particles (Cariñanos et al., 1999). Many of these particles originating from traffic pollution. During these hours sensitive individuals must exercise precautions. Simoleit et al. (2015) also comment that the combination between pollution and pollen load in the air represent a special health threat for urban population as pollen are considered to be more allergenic in a polluted atmosphere (D´Amato et al, 2010; Schiavoni et al 2017). Combined with the current study indicating that a high proportion of days where pollen peaks at these times, susceptible induvial may be of increased risk and must exercise precautions. The combined effects of air pollutants and aeroallergens is an important area, in particular in the urban zone, and that there need to be a focus on short-term exposure of both air pollutants and aeroallergens.

Although the two sites can be assumed to have differences in the composition of species, both sites had three clusters with some similarity in the daily pattern: Cluster type 1: late afternoon peaks, Cluster type 2: partly or entirely dominated by night time/early morning conditions, and Cluster type 3: a double peak. This result is partly the consequence of the method, determining the most distinctively different patterns. However, even with great differences in species composition, meteorology and dominating local wind patterns and patterns objectively analysed through statistical clustering, both sites showed a uniform peak in the afternoon or evening as the most frequent pattern. For Denmark, the evening peak was also seen as the dominating peak in the main season in the city of Aarhus (Peel et al, 2014). This indicates that the advice given for allergen-avoidance should emphasize that peak concentrations may occur at all times of day, but the most frequent peak, dominating the seasonal peak, is in the early evening.

Conclusions

Here we propose a new method based on clustering methodology and standardization of pollen concentrations to study variations of airborne pollen in two-hours periods. The different hourly-patterns recorded at southern Europe (Spain) and northern Europe (Denmark) could not directly be related with the meteorological conditions at either location.

The studies carried out in both cities show strong variation in the diurnal pattern of grass pollen in the air, with approximately 70% of days (without rain and daily pollen concentrations above 20 pollen grains m-3) fitting 3 statistically (although not significant) determined clusters of patterns, with peaks at either both morning, midday, evening or night. For both sites however, one late afternoon (Cordoba) or early evening peak (Copenhagen) is the most frequent distinctive pattern.

The peak can occur at all hours of the day, most likely depending on flowering patterns of the dominant grass species and a complex effect of meteorological parameters. In view of the results average curves are not satisfactory for describing the diurnal pattern of grass pollen as they mask the day to day variation and long term season effects.

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	sig.	C1 Mean (SD)	C2 Mean (SD)	C3 Mean (SD)
DOY	0.09	136.82 (18.58)	143.86 (17.69)	144.91 (18.39)
Temperature (ºC)	0.14	20.13 (3.64)	21.33 (3.45)	21.98 (3.1)
Humidity (%)	0.56	55.94 (11.43)	52.99 (10.22)	55.23 (7.52)
Global radiation (W/m^2)	**0.02**	286.34 (42.82)	314.00 (43.98)	314.47 (31.83)
Wind speed (m/s)	0.29	1.54 (0.48)	1.69 (0.57)	1.82 (0.6)
Wind direction (º)	0.40	351.38 (248º to 54º)	9.30 (256º to 77º)	8.20 (264º to 58º)

Table 1. Córdoba (Spain). Differences in daily environmental parameters between days defined with different hourly patterns in airborne pollen. Robust ANOVA significance. C1, Cluster 1. C2, Cluster 2. C3, Cluster 3. DOY; Day of the Year. Wind direction is calculated by circular statistics approach. Only maximum and minimum are shown for wind direction, not SD, due to the circular properties.

	sig.	C1 Mean (SD)	C2 Mean (SD)	C3 Mean (SD)
DOY	0.47	174.06 (13.15)	177.6 (15.37)	175.74 (14.61)
Temperature (ºC)	0.20	17.11 (3.09)	17.98 (2.89)	17.69 (2.69)
Humidity (%)	0.72	66.78 (9.74)	69.64 (10.18)	68.05 (7.94)
Global radiation (W/m^2)	0.19	272.64 (59.46)	246.25 (71.24)	255.12 (69.81)
Wind speed (m/s)	0.17	3.55 (0.95)	3.16 (1)	3.45 (0.98)
Wind direction (º)	**0.01**	267.34 (90º to 78º)	211.6 (35º to 333º)	221.2 (54º to 45º)

Table 2. Copenhagen (Denmark). Differences in daily environmental parameters between days defined with different hourly patterns in airborne pollen. Robust ANOVA significance. C1, Cluster 1. C2, Cluster 2. C3, Cluster 3. DOY; Day of the Year. Wind direction is calculated by circular statistics approach. Only maximum and minimum are shown for wind direction, not SD, due to the circular properties.

525 **Figure 1.** Biogeographical regions and the locations of Copenhagen and Cordoba.

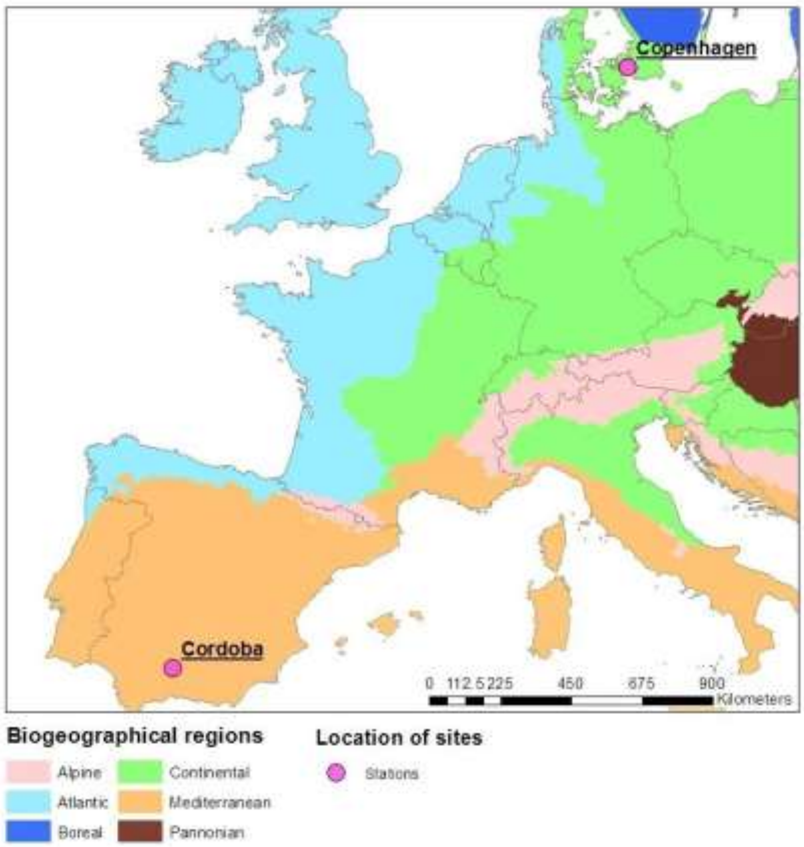

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Figure 2. Average and 95% confidence intervals (dashed lines) for each cluster of profiles of grass pollen concentrations in Córdoba, Spain. Cases: Cluster 1: 40%, Cluster 2: 33%, Cluster 3: 27%.

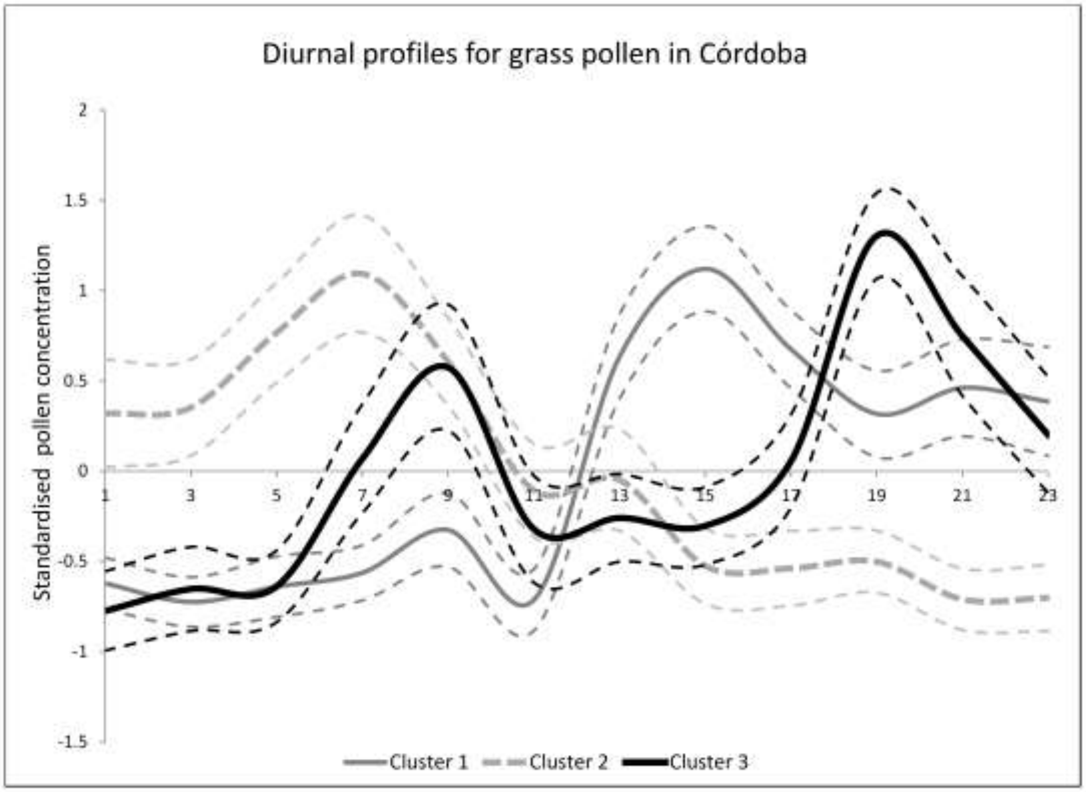
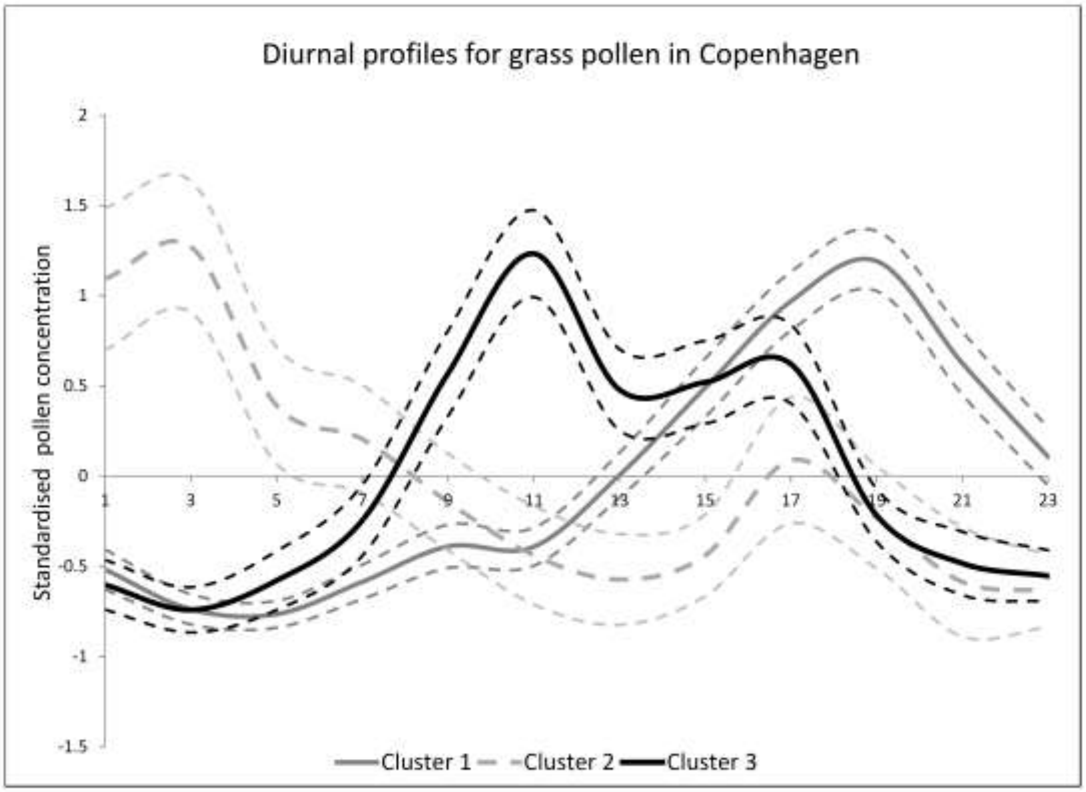
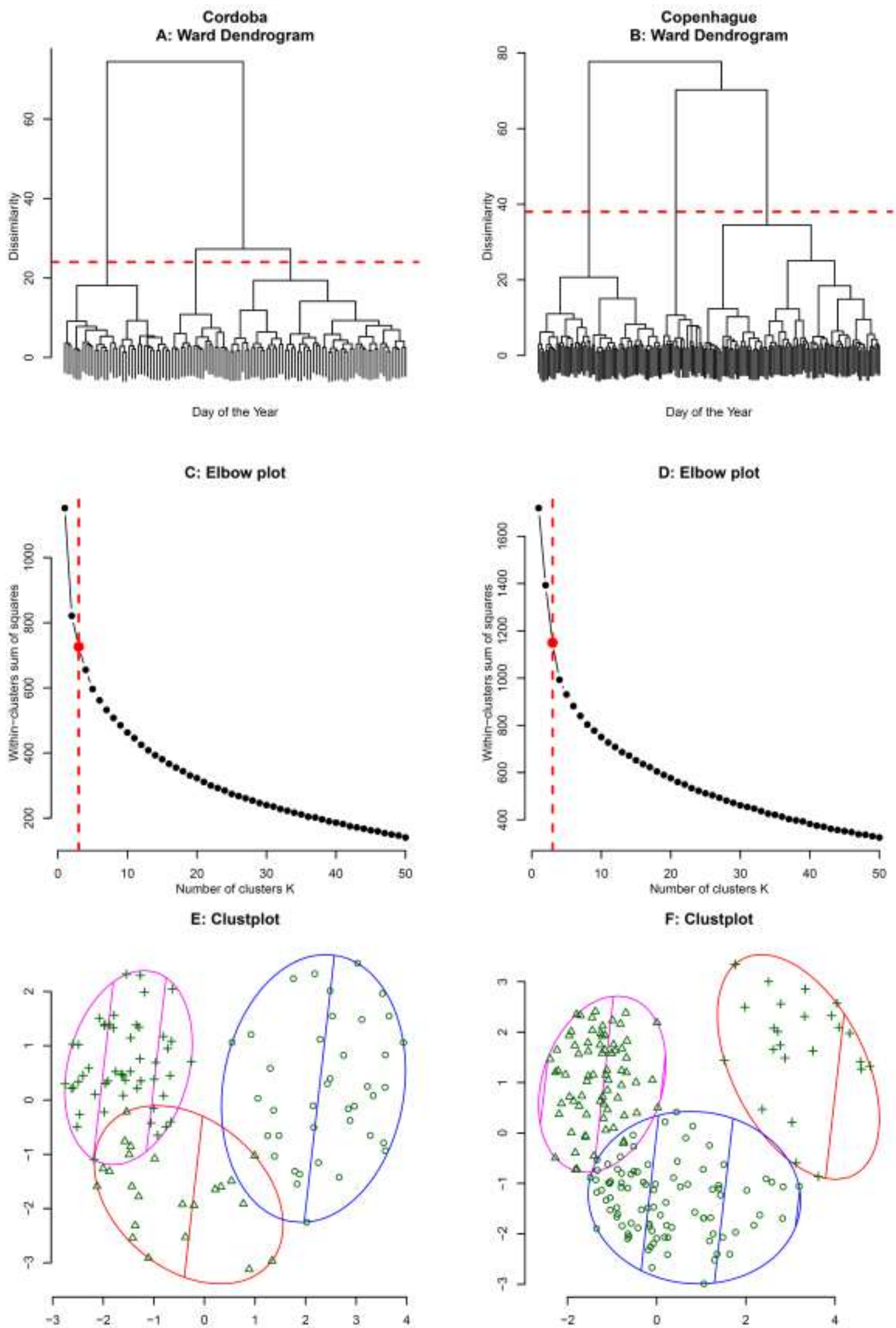


Figure 3. Average and 95% confidence intervals (dashed lines) for each cluster of profiles of grass pollen concentrations in Copenhagen, Denmark. Cases: Cluster 1: 57%, Cluster 2: 13%, Cluster 3: 30%.



Appendix 1. Hierarchical Clustering Ward dendrogram of the study cases in Cordoba location (A) and Copenhaguen (B). Elbow plot with the total sum of squares showing the explained variability in the study cases depending on the numer of clusters (K) in Cordoba(C) and Copenhagen (D). Clusterplot of the principal components (x axis: Component 1, y axis: Component 2) of the k-means analysis (k=3) in Cordoba location (E) and Copenhaguen (F).


Cordoba
A: Ward Dendrogram
Copenhague
B: Ward Dendrogram
Dissimilarity
Day of the Year
C: Elbow plot
D: Elbow plot
Within-clusters sum of squares
Number of clusters K
E: Clustplot
F: Clustplot

Appendix 2. Distribution of the days of the year in the study cases according to the cluster in Copenhagen and Cordoba.