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Seasonal variation in diurnal atmospheric grass pollen concentration profiles

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Abstract. In this study, the diurnal atmospheric grass pollen concentration profile within the Danish city of Aarhus was shown to change in a systematic manner as the pollen season progressed. Although diurnal grass pollen profiles can differ greatly from day-to-day, it is common practice to establish the time of day when peak concentrations are most likely to occur using seasonally averaged diurnal profiles. Atmospheric pollen loads are highly dependent upon emissions, and different species of grass are known to flower and emit pollen at different times of the day and during different periods of the pollen season. Pollen concentrations are also influenced by meteorological factors – directly through those parameters that govern pollen dispersion and transport, and indirectly through the weather-driven flowering process. We found that three different profiles dominated the grass pollen season in Aarhus – a twin peak profile during the early season, a single evening profile during the middle of the season, and a single midday peak during the late season. Whilst this variation could not be explained by meteorological factors, no inconsistencies were found with the theory that it was driven by a succession of different grass species with different diurnal flowering patterns dominating atmospheric pollen loads as the season progressed. The potential for exposure was found to be significantly greater during the late-season period than during either the early- or mid-season periods.

1 Introduction

Grass pollen is recognised as one of the principle causes of pollen allergy in Europe, with national sensitisation rates of up to 26 % reported for the region (Bousquet et al., 2007). Atmospheric grass pollen concentrations typically fluctuate over the course of a 24 h period, and diurnal patterns can differ greatly from day-to-day. The factors responsible for these differences are, however, not well understood. Pollen forecasts usually attempt to predict daily average concentrations, with this information disseminated to allergy sufferers in order that they might better manage their symptoms. The elicitation of allergy symptoms is dependent on an individual's recent exposure history (Connell, 1969), whilst exposure is directly related to the amount of time spent outdoors (Kailin, 1964; Mitakakis et al., 2000) and the ambient concentration at that time (Riediker et al., 2000). The time of day that pollen concentrations peak may thus be of greater importance to the allergy sufferer than the daily average concentrations typically made available to the public (Käpylä, 1981). A better understanding of what drives variation in diurnal grass pollen profiles may lead to improved advice on how allergy sufferers may best avoid exposure, and would furthermore help to improve the accuracy of pollen dispersion models (Viner et al., 2010).

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It is common practice in aerobiology to produce average diurnal pollen concentration curves based on entire pollen seasons in order to establish typical patterns of variation and times of peaks in concentration. These profiles have been found for grass pollen to vary with location, with single evening peaks (Emberlin and Norris-Hill, 1991; Mullins et al., 1986; Yang et al., 2003), single morning peaks (Galán et al., 1989, 1991; Trigo et al., 1997), two-peak profiles (Rantio-Lehtimäki et al., 1991; Kosisky et al., 2010), and invariant profiles (Gassmann et al., 2002) reported in the UK and Taiwan, Spain, Finland and the USA, and Argentina respectively. Intra-seasonal variation in diurnal profiles has, however, received scant attention in existing published literature. Mullins et al. (1986) compared profiles averaged over two different months (June and July) but found no discrepancies between the two, whilst Norris-Hill (1999) noted four different profiles for the four quarters of the season, and sought to relate these to rainfall patterns.

Pollen concentrations are influenced both by pollen emission and by the meteorological parameters that determine dispersion, transport and deposition (Galán et al., 1995). Different grass species release their pollen at different times of season (León-Ruiz et al., 2011) and at different times of day (Emecz, 1962). Diurnal flowering patterns are furthermore known to change in accordance with meteorological factors in a species-specific manner (Subba Reddi et al., 1988). The changing character of the diurnal grass pollen profile may thus be driven by the weather, the flowering patterns of local grasses, or both.

The objective of this study was to investigate and explain seasonal variation in the diurnal atmospheric grass pollen profile. This was achieved in the following manner:

1. Systematic changes in the diurnal grass pollen concentration profile were shown to occur in the Danish city of Aarhus as the pollen season progressed, with profiles for the early, middle and late periods of the season showing different statistical properties.

2. The potential for exposure during these three periods was estimated and compared in order to test whether the different profiles have the potential to lead to significant differences in exposure.

3. The hypothesis that the seasonal variation was driven by meteorological factors was tested against the alternative hypothesis that it related to a progression of different grass species dominating pollen emissions as the season developed.

4. An inventory of the principle grass species likely to be common in Aarhus was compiled together with available information on their flowering cycles, in order to support the interpretation of results.

2 Materials and methods

Medial time stamps are reported for both pollen concentration and meteorological data, as is the convention in aerobiology, with all time stamps given in Central European Time (UTC +1).

2.1 Site description and data provenance

The study was conducted using data from Aarhus, Denmark's second largest city. Aarhus lies on the east coast of Jutland, the peninsula that constitutes the western part of Denmark, and has a population of around 250 000 (Statistics Denmark, 2012). Aarhus is a green city in which numerous parks and unmanaged[1] natural areas are found. The surrounding countryside consists largely of arable land, with common crops including seedling grasses, permanent grass and rye, all of which are potential sources of grass pollen (Skjøth et al., 2013).

For the years 2009–2011, three temporary pollen monitoring stations were operational in Aarhus during the grass pollen season. The three monitoring stations were situated within 8 km of one another (Fig. 1). Each consisted of a Burkard Seven Day Recording Volumetric Spore Trap (Hirst, 1952) installed at roof level, 15–20 m above ground level. The Central Aarhus monitoring station was situated in the centre of the city on the roof of Aarhus Municipality Department of Nature and Environment, and was surrounded by a regularly managed lawn. The TV-2 monitoring station lay in the northern outskirts of the city on the roof of the TV-2 Østjylland TV station, close to open countryside and less than 100 m from an unmanaged grass field. The Rundhøjskolen monitoring station was situated on top of a school building in the southern suburbs of the city.

Uniform materials and methods were used at the three monitoring stations. Pollen data were collected and processed in accordance with the guidelines of the British Aerobiology Federation (1994). Samples were collected on Melinex tape coated with silicone fluid using standard seven-day drums. Weekly sample traces were divided into seven 24 h sections and mounted on microscope slides using a stain-bearing gelatine mountant. For each daily slide, bihourly concentration data were obtained by counting the number of pollen grains deposited along 12 transverse transects at 640 times magnification (equating to 9.75 % of each daily slide) under a light microscope, with counts converted into concentrations in grains m^{-3}.

Three-hour averaged wind speed, wind direction, surface air temperature, dewpoint temperature and precipitation data were obtained from the Flyveplads Kirstinesminde weather station (WMO Station ID 06074), situated just north of Aarhus (Fig. 1), courtesy of the UK Meteorological Office (2012). Three-hour averaged saturated and actual vapour

[1]Management is defined as cutting on a regular basis such that flowering does not occur.

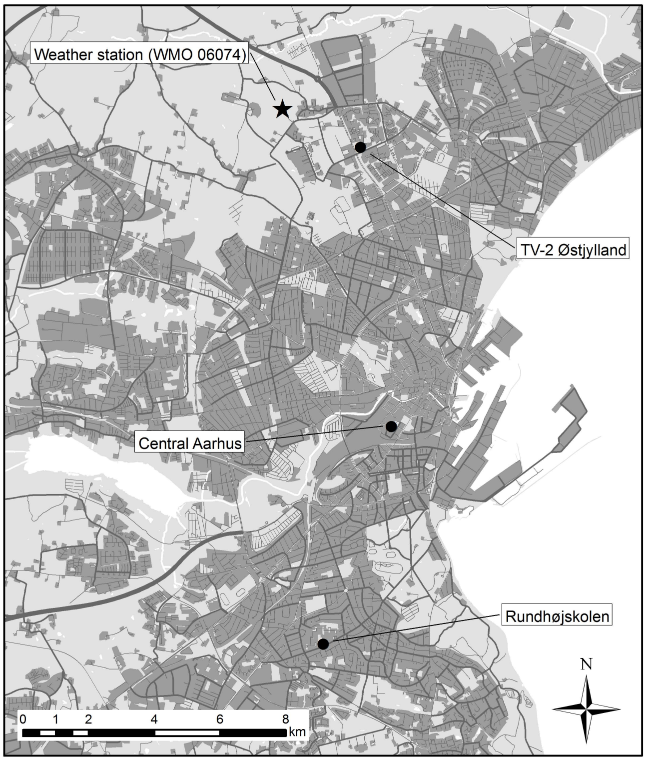

Fig. 1. Map of Aarhus showing the locations of the three temporary pollen monitoring stations and the weather station.

pressures were calculated from ambient and dewpoint temperatures respectively using Eq. (3) of Henderson-Sellers (1984). Vapour pressure deficit (VPD) was then computed using method 1A of Howell and Dusek (1995).

The daily dynamics of population activity were modelled using the time–activity diurnal curve for the population of the USA presented by Klepeis et al. (2001). The proportion of the population outdoors was determined for hours of the day corresponding to pollen data as the sum of the "residence-outdoors", "near vehicle (outdoors)" and "other outdoors" activity categories.

2.2 Data reduction and processing

2.2.1 Pollen data

The Aarhus grass pollen season ran from 20 May to 29 July in 2009, from 6 June to 8 August in 2010, and from 21 May to 27 July in 2011. Here we define the start (end) of the season as the first (last) day that a daily average of ≥ 10 grains m^{-3} was recorded at one of the three monitoring stations.

For each monitoring station and each year, the grass pollen concentration time series was divided into 24 h daily "profiles" (midnight–midnight). All daily profiles with a corresponding daily average concentration of < 20 grains m^{-3} were discarded, on the basis that at low concentrations the resolution of data becomes poor[2]. All profiles that coincided with precipitation were also discarded, since rain removes pollen grains from the air with great efficiency (McDonald, 1962) and may thus strongly influence profile shape. For each of the remaining 157 profiles (48, 54 and 55 for the Central Aarhus, Rundhøjskolen and TV-2 stations respectively, relating to 69 different calendar days), peaks in the diurnal pollen curve were identified according to the following criteria:

1. Each peak was required to have a minimum[3] bi-hourly concentration of ≥ 50 grains m^{-3}. Profiles where concentrations failed to exceed this threshold were considered to have no peak.

2. Overall maximum bi-hourly concentrations satisfying (1) were designated primary peaks.

3. Local bi-hourly concentration maxima occurring ≥ 6 h before or after a primary peak were designated secondary peaks, provided that the trough between the two was at least 50 grains m^{-3} deep.

4. Two candidate peaks of equal magnitude ≤ 4 h apart were considered a single peak and given an intermediary time stamp: for example, a peak bi-hourly concentration of 100 grains m^{-3} occurring at both 17:00 and 19:00 was defined as a single peak at 18:00.

5. Two candidate peaks of equal magnitude occurring six or more hours apart were considered separate peaks.

6. Apparent peaks close to midnight that were associated with an actual peak occurring on the preceding evening were rejected (e.g. a peak at 01:00 was rejected if a greater concentration occurred at 23:00 on the previous evening).

In this manner each profile was characterised in terms of the time at which peaks occurred, with each profile featuring 0, 1 or 2 peaks. These peak-time profiles were then grouped by site and year, and each group arranged in chronological order. Three characteristic profile types were observed to dominate at different points during the grass pollen season, meaning that the season could be divided into three distinct periods:

[2]Profiles with daily average concentrations < 20 grains m^{-3} generally failed to show a well-defined diurnal pattern, with the random nature of sample collection apparently dominating. An alternative (and relaxed) criterion of a daily maximum bi-hourly concentration > 50 grains m^{-3} was considered, but was ultimately rejected as it increased the number of poorly defined profiles. It should be noted that profiles were rejected purely based on data resolution – some sensitive allergy sufferers have been reported to experience symptoms at daily average concentrations as low as 1 grain m^{-3} (Hyde, 1972).

[3]The value 50 grains m^{-3} was chosen because this is generally considered to be the average concentration above which all individuals sensitised to grass pollen experience symptoms (Galán et al., 1995).

Period 1: The early season, characterised by a twin morning and evening peak profile.

Period 2: The middle of the season, characterised by a single evening peak profile.

Period 3: The late season, characterised by a single late morning/early afternoon peak profile.

Dates of transition between periods were determined for each monitoring station and each year as the point where the dominating diurnal profile switched from the character of one period to the character of another, and each profile was thus assigned to period 1, 2 or 3.

For each of the three periods, the peak-time distributions of data collected at the three monitoring stations were then compared using the Anderson–Darling two-sample goodness-of-fit test with adjustment for ties (Trujillo-Ortiz et al., 2007). Results were considered significant at the 95 % level, and are summarised in Table 1. For periods 1 and 3 no differences were found between the three stations. For Period 2 the distribution of TV-2 data was found to differ significantly from those of the Central Aarhus and Rundhøjskolen stations; however inspection of the data showed that distributions were very similar except that the highly dominant modal peak time for the TV-2 station was 17:00, whilst for the Central Aarhus and Rundhøjskolen stations it was 19:00. It was therefore considered appropriate to pool data collected at the different monitoring stations for each of the three seasonal periods. Using these pooled data, the Anderson–Darling test was then used to test for differences between the peak-time distributions of the three periods.

Table 1. Anderson–Darling test (with adjustment for ties) for differences in peak-time distribution between the three monitoring stations for each seasonal period. D is the Anderson–Darling rank statistic and p the associated probability.

		Central/ Rundhøjskolen	Central/ TV-2	Rundhøjskolen/ TV-2
Period 1	p	0.650	0.147	0.435
	D	0.476	1.604	0.832
Period 2	p	0.592	0.004[b]	0.030[a]
	D	0.562	4.535	2.855
Period 3	p	0.783	0.663	0.606
	D	0.234	0.443	0.540

[a] Indicates a significant difference at the 95 % level.
[b] Indicates a significant difference at the 99 % level.

2.2.2 Population exposure

In order to neutralise magnitude-related differences and isolate the qualitative shape of each diurnal profile, pollen concentration data were standardised by dividing each bi-hourly value by its respective daily maxima. A proxy for population exposure was then calculated for every other hour of the day by multiplying the standardised concentration by the proportional outdoor population. For each day, bi-hourly standardised population exposure values were summed to give a measure of the daily total standardised population exposure, and the Wilcoxon rank sum test (normal approximation applied) used to test for differences in daily total standardised population exposure between the three periods.

2.2.3 Meteorological data

Wind speed, temperature and VPD data were selected for days corresponding to the pollen dataset, and divided into three groups corresponding to the three periods of the pollen season defined above. Data for days where periods overlapped between the different monitoring stations were omitted. Data from one further day were omitted due to an incomplete record. Average diurnal profiles were plotted for each variable and each period, and differences in diurnal profile shape were tested for by grouping data by time of day. The Wilcoxon rank sum test was then applied to each temporal group. Results were considered significant at the 95 % level.

2.2.4 Grass species inventory

The inventory of grass species was composed from species listed by Frederiksen et al. (2006) as "common" or "very common" along roads and railways and in parks – the habitats where unmanaged grasses are likely to be found in Aarhus according to Skjøth et al. (2013) – and species found to be common in Copenhagen by Hald (2011). It seems likely that species that are abundant in Copenhagen will also be well represented in other large Danish cities. Data on the pollen productivity and flowering behaviour of constituent species were gathered from available existing literature and added to the inventory.

3 Results

3.1 Pollen data

The availability of pollen data and the way they were distributed between the three seasonal periods can be seen in Fig. 2. The maximum period overlap between stations within a single year was 4 days (the transition from Period 2 to 3 at the Rundhøjskolen and TV-2 stations during 2009). The transition from Period 1 to 2 occurred later in 2010 than in 2009 or 2011; the transition from Period 2 to 3 also occurred later in 2010 than in 2009, but cannot be precisely located for 2011 due to the sparsity of data. Period duration cannot in general be precisely stated due to the volume of missing data, but was typically in the order of 1–2 weeks and appears to have been briefer during 2010 than during 2009 or 2011.

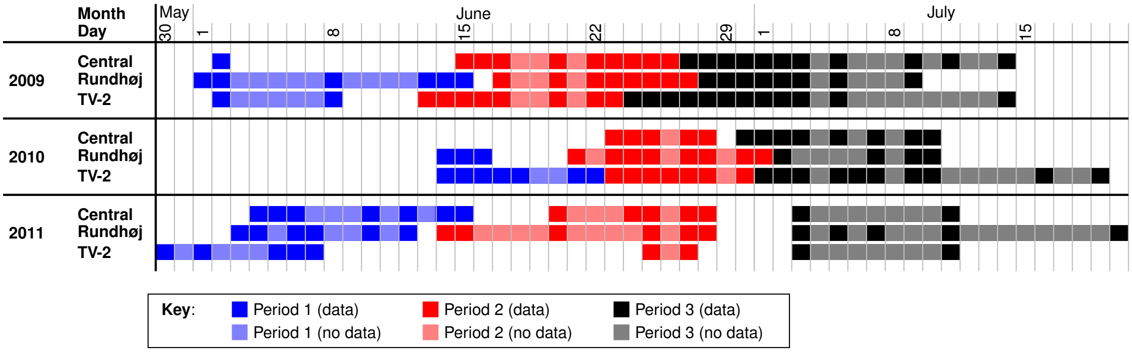

Fig. 2. Assignment of data to the three seasonal periods for the Central Aarhus (Central), Rundhøjskolen (Rundhøj) and TV-2 monitoring stations for each year. Contributing dates (i.e. dry days with daily average concentrations ≥ 20 grains m^{-3}) are in bold, and non-contributing dates for which period affiliation can be projected are faded.

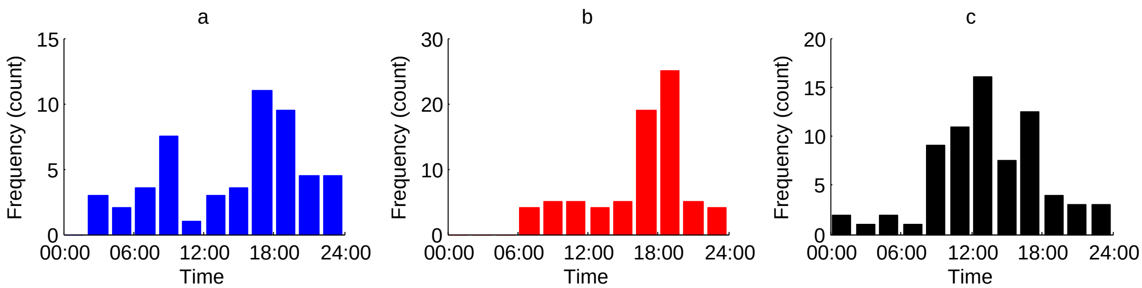

Fig. 3. Peak-time distributions for **(a)** Period 1 ($n = 37$), **(b)** Period 2 ($n = 58$) and **(c)** Period 3 ($n = 62$), where n is number of constituent profiles. Where peaks straddled two time slots, each bi-hourly bin was assigned a value of 0.5.

The peak-time distributions of the pooled data are presented in Fig. 3. Period 1 shows a bimodal tendency, with morning peaks common at 09:00 and evening peaks common at 17:00 and later. Period 2 shows a uni-modal distribution, with the majority of peaks occurring at 17:00–19:00 and otherwise background peak levels between 07:00 and 23:00. Period 3 also shows a uni-modal distribution, but with peaks common between 09:00 and 17:00 and attaining a maximum frequency at 13:00. According to the Anderson–Darling test, the peak-time distributions for the three periods differ significantly from one another at the 95 % level (periods 1 and 2: $D = 2.51$, $p = 0.048$; periods 1 and 3: $D = 3.84$, $p = 0.010$; periods 2 and 3: $D = 11.51$, $p < 0.001$).

3.2 Population exposure

Average standardised population exposure diurnal profiles are presented in Fig. 4 for the three periods, together with average standardised pollen concentration profiles. The standardised population exposure profiles of periods 1 and 2 are reasonably similar, both showing essentially only a single evening peak at 17:00. Period 3, however, shows a different profile, peaking around midday. Average concentration profiles agree qualitatively with the peak distributions of Fig. 3.

The daily total standardised population exposure mean (range) was 0.31 (0.09–0.55) for Period 1, 0.35 (0.17–0.70) for Period 2 and 0.40 (0.20–0.59) for Period 3. According to the Wilcoxon rank sum test, daily total standardised population exposure did not differ significantly between periods 1 and 2 ($W = 1535$, $p = 0.1733$), but did differ significantly

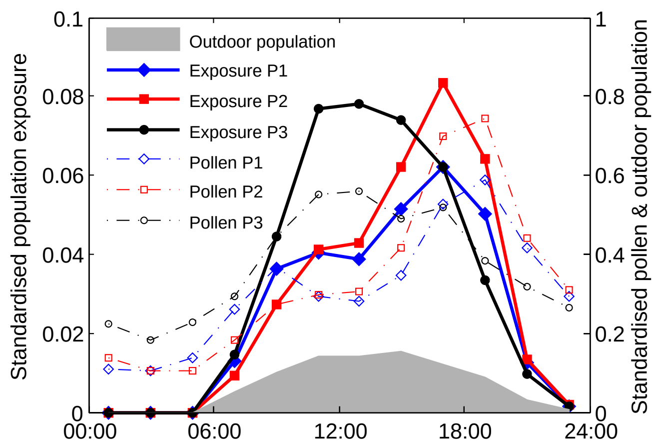

Fig. 4. Mean diurnal standardised population exposure (solid lines) and standardised pollen concentration (dotted lines) profiles for Period 1 (P1), Period 2 (P2) and Period 3 (P3). The outdoor population profile (solid), derived from the results of Klepeis et al. (2001), is also shown.

between periods 1 and 3 ($W = 1282$, $p < 0.001$) and between periods 2 and 3 ($W = 3070$, $p = 0.0061$).

3.3 Meteorological data

Median diurnal profiles for the three periods are presented in Fig. 5, together with the results of the Wilcoxon rank sum test. No significant differences were found between the three periods for wind speed or between periods 1 and 2 for temperature or VPD, with the exception of temperature between 22:00 and 01:00, when Period 2 values tended to be higher. Temperatures during Period 3 were found to be significantly higher than those during periods 1 and 2 at all times of day. VPD was found to be significantly higher during Period 3 than Period 2 between 07:00 and 13:00, whilst no significant differences were found between periods 1 and 3.

Figure 6 shows time of peak plotted against concurrent wind direction. Winds appear to be dominated by two sectors, south-east and west; however within both sectors, peaks are seen to occur at all times of day with the exception of Period 1, where all morning peaks occur under winds from the west. This, however, appears to reflect the fact that almost all peaks during Period 1 were accompanied by westerly winds. Figure 7 shows time of peak plotted against the number of days since rain. There is no apparent relationship between the two.

3.4 Grass species inventory

Table 2 lists the 18 grass species likely to be present in Aarhus. Data on diurnal flowering behaviour was found in existing published literature for 12 of these species. Amongst these are seven species that have been reported to flower at around the time of the early peak during Period 1 (*Alopecurus pratensis*, *Dactylis glomerata*), around the time of the evening peak during periods 1 and 2 (*Arrhenatherum elatius*, according to Jones (1952)), or during the middle of the day when peaks commonly occur during Period 3 (*Festuca arundinacea*, *Lolium perenne*). There are also species that have been reported to flower twice per day at times coinciding more or less with the two peaks of Period 1 (*Anthoxanthum odoratum*, *Holcus lanatus*).

Pollen productivity estimates were found for nine of the species in Table 2, with the number of pollen grains produced per inflorescence ranging from 0.1×10^6 (*Poa annua*) to 11.7×10^6 (*Festuca arundinacea*). Of those species whose flowering times coincided with concentration peak times, three (*Dactylis glomerata*, *Festuca arundinacea*, *Lolium perenne*) were reported to be relatively productive with estimated yields of at least 2.3×10^6 grains per inflorescence, one (*Holcus lanatus*) had received conflicting productivity estimates, two (*Alopecurus pratensis*, *Arrhenatherum elatius*) had unknown productive capacity, and only one species was reported to be relatively unproductive (*Anthoxanthum odoratum*).

4 Discussion

4.1 Drivers of diurnal grass pollen concentration variation

In this study, we have shown that the diurnal pollen concentration profile for the Danish city of Aarhus varies in a systematic manner as the pollen season progresses. Using statistical methods, different diurnal patterns were shown to dominate during different periods of the season: twin morning and evening peaks characterised the early part of the season, a single evening peak the middle of the season, and a single midday peak the late season. That diurnal grass pollen profiles vary from day-to-day is well known (Käpylä, 1981). It has also previously been demonstrated that the grass pollen season can be divided into several periods with different characteristic properties. In their 7-day ahead grass pollen forecast model for the UK, Smith and Emberlin (2005) used different parametrisations for the pre-peak, peak and post-peak periods of the grass pollen season, whilst Sánchez Mesa et al. (2003) obtained greater correlation between meteorological parameters and the daily average grass pollen concentration by isolating the pre-peak period from the remainder of the season. However, as far as the authors are aware, the systematic variation found during this study has not previously been shown to occur.

Atmospheric pollen concentrations are determined by two sets of variables – those that mediate pollen release into the atmosphere, and those that mediate its dispersal from source to receptor (Galán et al., 1995). Pollen emission is regulated by biological and meteorological factors, which restrict it to a limited range of weather conditions and a specific portion of the day (Raynor et al., 1970). For plants that flower during turbulent weather, which can be crudely approximated as the hours of daylight, we would expect flowering and increased atmospheric concentrations typically to coincide. This is especially true for taxa with smooth pollen grains that are relatively easily removed from the anther (Subba Reddi and Reddi, 1985), or relatively large pollen grains whose residence time in the atmosphere is limited (Skjøth et al., 2013). For Poaceae pollen, which is both smooth and relatively large, the timing of peaks in atmospheric concentration can thus be expected in general to follow patterns of local emission.

Pollen primarily enters the atmosphere directly from the anthers following flowering. For grasses, flowering intensity generally follows regular diurnal cycles that differ from species to species, and furthermore vary with changing weather conditions (Emecz, 1962; Jones, 1952; Subba Reddi et al., 1988). Different species of grass flower at different points during the pollen season (León-Ruiz et al., 2011), meaning that as the season progresses, different subsets of the local grass flora are likely to be contributing to the atmospheric pollen load. The systematic variation observed in this study could therefore potentially be driven by two different

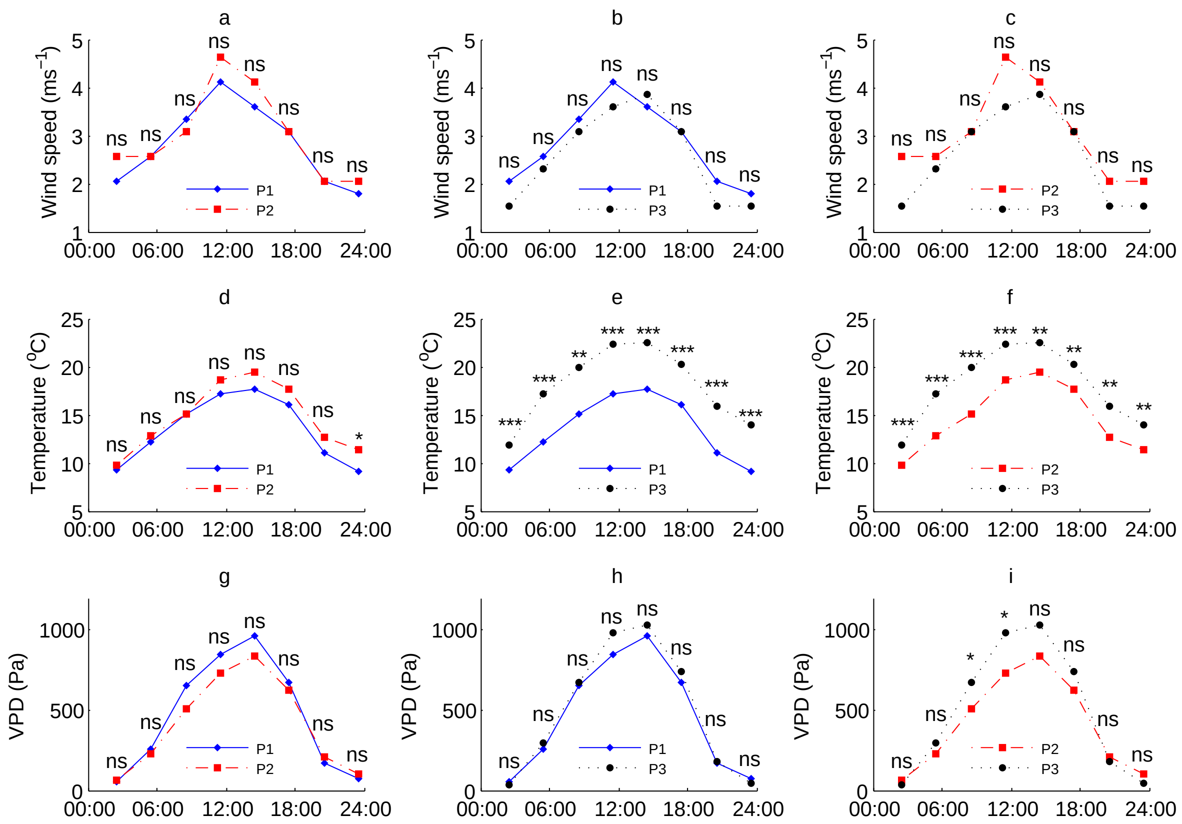

Fig. 5. Pairwise median diurnal profiles for wind speed **(a–c)**, temperature **(d–f)** and VPD **(g–i)** for Period 1 (P1, $n = 16$), Period 2 (P2, $n = 17$) and Period 3 (P3, $n = 24$). Results of the two-tailed Wilcoxon rank sum test are indicated for each three-hour weather data averaging period, with "ns" indicating no significant difference and "⋆", "⋆⋆" and "⋆⋆⋆" indicating significant differences at the 95, 99 and 99.9 % levels respectively.

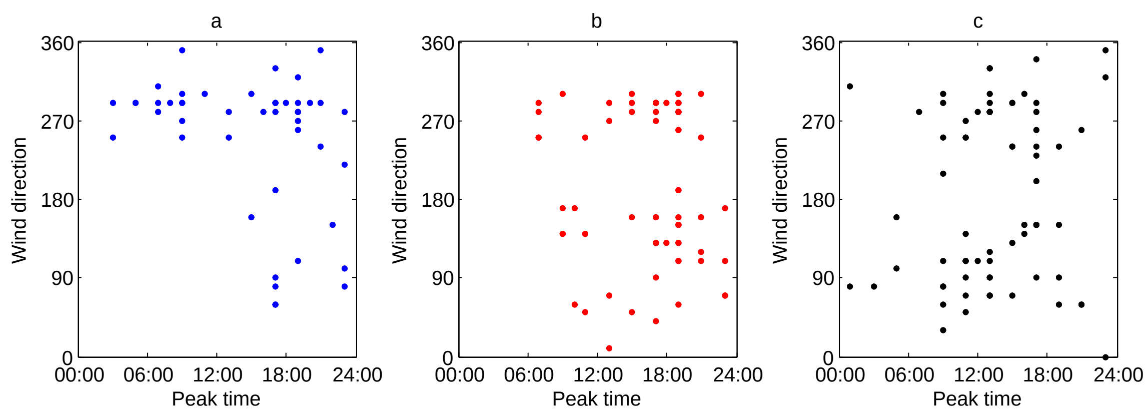

Fig. 6. Scatter plots showing time of the peak against concurrent wind direction for **(a)** Period 1, $n = 37$; **(b)** Period 2, $n = 58$; and **(c)** Period 3, $n = 62$.

factors: a difference between the weather conditions in the three periods of the season, or a succession of different grass species dominating pollen emission as the season develops.

4.2 Do meteorological factors explain the three periods ?

Flowering amongst grasses is in general dependent on a species-specific temperature threshold being exceeded (Emecz, 1962). It is possible that higher temperatures could

Table 2. Grass species that are likely to be present in and around Aarhus. Constituent species are listed by [a] Frederiksen et al. (2006) as "common" or "very common" along roads and railways and in parks, and/or were found by [b] Hald (2011) to be common in the city of Copenhagen. Details of productivity (grains/inflorescence[c,e] or spike[d] – note that an inflorescence consists of one or more spikes (Guinther, 2013)), time of flowering (range gives period of flowering & hour denotes time of peak flowering, unless otherwise indicated), minimum temperature threshold (°C) that must be exceeded in order for flowering to occur (square brackets indicate temperature that induces maximum liberation), and minimum light intensity (i – foot candles) and duration (d – hours) necessary to initiate flowering. Information is derived from the following sources: [c] Prieto-Baena et al. (2003), [d] Smart et al. (1979), [e] Aboulaich et al. (2009), [f] Emecz (1962), [g] Ogden et al. (1969), [h] Jones (1952), [i] Beddows (1931), [j] Smart and Knox (1979), [k] Evans (1916), and [l] Clark (1911). * Also known as *Agropyron repens*.

Species	Productivity	Time of flowering	Temp	Light i/d
Agrostis capillaris[a]	–	–	–	–/–
Agrostis stolonifera[b]	2 426 609[c]; 777 058[e]	–	–	–/–
Alopecurus pratensis[a]	–	07:52[f]; 06:00–11:00[i]	11[f]	–/10[f]
Anthoxanthum odoratum[a]	621 363[e]	05:00–10:00 and 17:00[i]	–	–/–
Arrhenatherum elatius[a,b]	–	15:00–19:00[h]; 05:00–11:00 and 18:00–19:00[i]	–	–/–
Bromus hordeaceus[a]	245 176[c]; 407 489[e]	06:00[i]	12[i]	–/–
Dactylis glomerata[a,b]	7 971 347[c]; 3 700 000[d];	06:25–08:35[f] (peak); 04:00–10:00[h];	15.5[f]	1600/8[f]
	3 419 469[e]	04:00–10:30[i]		
Elytrigia repens[a,b,*]	–	14:00–18:00[h]; 16:30[i]	23[i]	–/–
Festuca arundinacea[a]	11 697 131[c]	15:08[f]; 06:00[i]	17[f][14[i]]	3600/5[f]
Festuca brevipila[a]	–	–	–	–/–
Festuca pratensis[a]	–	10:00[f]; 06:00[i]	15[f][14[i]]	1200/2[f]
Festuca rubra[a,b]	–	06:00; 09:45–14:30 (peak 12:00–13:00)[i]	–	–/–
Holcus lanatus[a,b]	875 715[c]; 4 500 000[d]	Typically ∼ 06:00–07:00 and ∼ 18:00–19:00 though other patterns also reported[i]	–	–/–
Lolium perenne[a,b]	2 300 000[d]	11:45–15:20[f] (peak); 09:00–12:00[i] 12:00–14:00 & 20:00–22:00[j] (peak)	14–17[f]	2000–5200/1.5–3[f]
Phleum pratense[a,b]	–	05:48–08:54[f] (peak); mostly 06:00–08:00[g]; ∼ 03:00[l]; 02:00–04:00[k] (peak); 04:00–09:00[h] 4:30–10:00[i]	16–17[f]	2200–3000/10[f]
Poa annua[b]	115 511[c]; 142 911[e]	04:30[i]	11[i]	–/–
Poa pratensis[a,b]	–	03:00–08:00[h]	–	–/–
Poa trivialis[b]	2 088 492[e]	–	–	–/–

lead to thresholds being exceeded earlier in the day, bringing the time of flowering forward. Temperatures tended to be higher during Period 3 than during periods 1 or 2 at all times of day; however the earliest peaks occurred during Period 1. Temperatures also showed a tendency to be higher during Period 2 than during period 1 between 22:00 and 01:00. We would expect that if this had any effect, it would lead to earlier peaks during Period 2 that during Period 1; however, the opposite is in fact seen.

Anther dehiscence, the process during which the anthers split open to release pollen, occurs following dehydration (Stanley and Linskens, 1974, p. 24). VPD may be considered a proxy for the drying power of the air, and greater VPD earlier in the day may thus lead to earlier drying, emission and concentration peaks. The shift from an evening to a midday peak as Period 2 transitions into Period 3 is indeed accompanied by a significant increase in VPD between 07:00 and 13:00; however the VPD values that accompanied the earliest

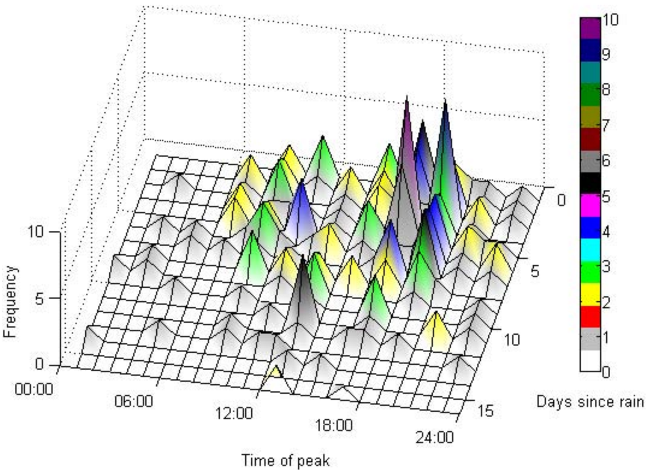

Fig. 7. Frequency map of peak time against the number of days since ≥ 1 mm rain.

peak, which occurred during Period 1, did not differ significantly from those of periods 2 or 3 at any time of day.

Horizontal transport is dependent on wind direction (Stull, 1988, p. 3–5), and if major sources are not found in all compass directions about a monitoring station, it is possible that concentration peaks are the result of wind directions that carry pollen from source to monitoring station. Although all morning peaks were recorded under westerly winds during Period 1, morning peaks are seen to occur under winds from all directions of the compass during periods 2 and 3.

Wind speed is associated both with the primary emission of pollen from the anthers (Emecz, 1962; Lu et al., 2005) and with secondary emission through resuspension (Sánchez Mesa et al., 2003; Sehmel, 1980); however, no significant difference in wind speed was found between the three periods of the grass pollen season. Norris-Hill (1999) proposed that the timing of diurnal grass pollen peaks may be related to the time elapsed since rainfall due to the availability of pollen for resuspension. In this study, we find no relationship between the two.

In summary, we thus find no evidence in support of the theory that the systematic changes in the diurnal grass pollen concentration pattern in Aarhus are related to meteorological factors.

4.3 Could flowering time explain the three periods?

Pollen production can vary hugely between species; indeed, Table 2 shows that pollen production amongst the species common in Aarhus may be expected to vary over at least 2 orders of magnitude. Clearly species that are both abundant and prolific pollen producers will hold greater influence over atmospheric pollen concentrations, and thus it is possible that the diurnal pattern of pollen concentration variation is determined by only a handful of species. León-Ruiz et al. (2011) identified only four locally occurring species as being likely to contribute significantly to atmospheric pollen concentrations in Córdoba, Spain. Of the species likely to be relatively abundant in Aarhus, the times of flowering of seven are expected to coincide with the times of day that peak concentrations tended to occur; of these seven species, at least three are thought to be relatively prolific pollen producers (see Table 2).

It seems probable that the period during which an individual grass species has the potential to dominate pollen emission will largely be limited to the "full flowering" phase of the flowering cycle, the period during which the central 50 % of anthers dehisce. León-Ruiz et al. (2011) found that the length of the full flowering phase varied between species, but was typically in the range 1–2 weeks, i.e. comparable with the typical lengths of the three periods identified in this study. The full flowering phase was also found to be briefer during years when flowering began late. The start of the Aarhus grass pollen season, here defined as the first day with an average concentration ≥ 10 grains m^{-3} at any of the three monitoring stations, occurred later in 2010 (6 June) than in either 2009 or 2011 (20 and 21 May respectively). This coincided with a tendency for later transition dates between the three periods, and also with apparently briefer periods.

It is well known that local-scale variation in micro-climate can cause the onset of flowering to vary by several days. The urban heat island can, for example, advance the flowering of grasses within a city compared with on the outskirts (Emberlin et al., 1993; Rodríguez-Rajo et al., 2010), whilst the onset of flowering in *Platanus* trees has been reported to differ between different areas of the same city (Alcázar et al., 2004). In the present study, the dates of transition between the three periods were found to differ between monitoring stations by up to 4 days, meaning that differences in diurnal pattern were found over distances under 5 km. The resulting overlap between periods at the different monitoring stations could be explained by small differences in the onset and culmination of the main flowering phase of specific species.

The theory that the observed systematic variation in the diurnal grass pollen concentration profile is driven by a progression of different grass species dominating grass pollen emissions as the season progresses is therefore consistent both with the characteristics of the three seasonal periods, and with the flowering behaviour of the grass species thought to have a significant local presence. No contradictory evidence has been found.

4.4 Implication of the three periods on population exposure

One way of preventing or minimising the development of allergic symptoms is to minimise exposure by pursuing allergen avoidance strategies (Custovic et al., 1998). The Danish Asthma and Allergy Association, for example, advise patients to remain indoors around midday (Astma-Allergi Danmark, 2013), the time that grass pollen concentrations are expected to peak at the Copenhagen pollen monitoring station (Sommer et al., 2006). This study, however, reveals that the time of peak pollen concentrations can vary through the season. One way of evaluating the significance of this finding from an allergy sufferer's perspective is to test whether the potential for exposure is likely to differ between the three periods.

The daily total standardised population exposure was significantly greater during Period 3 than during periods 1 and 2 (this does not imply that greater magnitude exposure is expected during period 3 – standardised population exposure is a relative value that can be used for comparing the impact of "typical" days from the three seasonal periods with similar magnitude pollen concentration peaks). That daily total standardised population exposure did not differ significantly between periods 1 and 2 likely reflects the similarity between the exposure profiles of these two periods, with the early peak during Period 1 having limited influence because a relatively small proportion of the population are expected to be outdoors at this time. The increase in exposure risk during Period 3 can be explained by the coincidence of midday peaks in pollen concentration and the proportion of the population outdoors. The clinical impact of increased exposure risk at the end of the pollen season is unclear – according to the priming effect theory, symptom intensity increases following repeated exposure (Connell, 1969), which could lead to increasing sensitivity amongst allergy sufferers as the season progresses; however, in the Netherlands similar pollen concentrations have been found to provoke more intense symptoms during the early than the late grass pollen season (de Weger et al., 2011).

We would not expect the exposure experienced by a cohort of allergy sufferers adhering to allergen avoidance advice to be represented by that of the entire population. The above result indicates that individuals within a population that continue to behave "normally" and do not moderate their behaviour would be expected on average to experience greater exposure during Period 3 than during periods 1 or 2 on days with pollen concentration peaks of equal magnitude. A dynamic avoidance strategy that takes into account this seasonal variation in the diurnal concentration pattern could help an individual to reduce their personal exposure.

It is worth noting that grass pollen concentrations measured at urban background monitoring stations do not necessarily equate to those encountered by the local population – indeed, Peel et al. (2014) found a tendency for concentrations measured at roof level monitoring stations to be greater than those recorded simultaneously at street level within the key exposure environment of the urban street canyon, implying that monitoring station data may overestimate within-canyon exposure. Monitoring station and within-canyon data were, however, also found to be fairly strongly and significantly correlated (Spearman's correlations coefficients of 0.84 and 0.65 for canyons in Aarhus and London respectively), suggesting that the variation in the diurnal pattern detected in the monitoring station data analysed in this study will be replicated at street level.

5 Conclusions

In this study it was shown that the typical diurnal grass pollen concentration profile for the city of Aarhus changes as the pollen season progresses, leading to three distinct periods of the season with different profiles. This variation most likely reflects a succession of grasses flowering and dominating pollen emission as the season progresses. The change in profile shape is expected to lead to a significant increase in standardised exposure potential during the final period of the season.

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