

Climate change: consequences on the pollination of grasses in Perugia (Central Italy). A 33-year-long study

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Abstract Many works carried out in the last decades have shown that the pollen season for taxa flowering in winter and spring, in temperate regions, has tended to be earlier, probably due to the continuous rise in temperature. The mean annual temperature in Perugia, Central Italy, was about 0.5 °C higher in the last three decades compared with that registered from 1952 to 1981. The increase of temperature took place mainly in winter and spring, while no significant variation was recorded during the summer and autumn. This scenario shows variations in the timing and behavior of flowering of many spontaneous plants such as grasses, whose phenology is strongly influenced by air temperature. This work reports fluctuations in the airborne grass pollen presence in Perugia over a 33-year period (1982–2014), in order to study the influence of the warming registered in recent years on the behavior of pollen release of this taxon. The grass pollen season in Perugia typically lasts from the beginning of May to late July. The start dates showed a marked trend to an earlier beginning of the season (−0.4 day/year), as well as a strong correlation with the average temperatures of March and April. The peak is reached around 30th May, but the annual pollen index (API) is following a decreasing trend. The correlation between starting dates and spring temperatures could be interesting for the constitution of a forecasting model capable of predicting the presence of airborne grass pollen, helping to plan therapies for allergic people.

Keywords Climate change · Pollination · Grasses · Poaceae · Italy

Introduction

The prevalence of allergic diseases worldwide is strongly increasing in both developed and developing countries, with about 30–40 % of the world population now being affected by one or more allergic conditions (Pawankar et al. 2011). Most of these are elicited by airborne particles and in particular pollen. Among allergenic pollen-producing species, grasses (Poaceae) are a major issue worldwide, sensitizing about 80 % of allergic patients (D’Amato et al. 2007; Jochner et al. 2012). Pollen from Poaceae causes allergic responses in 35 % of the European population (D’Amato et al. 2007; Weger et al. 2013). In Italy, allergic reactions to grass pollen represent more than 50 % of all pollinosis, which affects about 10 million people, especially children and adolescents (Brighetti et al. 2014). Tripodi et al. (2012) found that 87.5 % of pediatric patients were affected from rhinoconjunctivitis and about 90 % had a positive skin reaction to *Phleum pratense* pollen extract.

The grass family is the most numerous in the world, comprising more than 600 genera and over 10,000 species, of which more than 400 can be found in Europe (D’Amato et al. 1991). They are herbaceous, wind pollinated, ubiquitous plants, adapted to a wide range of habitats, both spontaneous and cultivated. About 20 species, belonging to five sub-families (Pooideae, Panicoideae, Chloridoideae, Arundinoideae, and Bambusoideae), represent the most frequent cause of grass pollinosis (Hrabina 2007), and their risk factor is usually connected with an exceptionally abundant pollen production and widespread distribution, also in urban areas. (D’Amato 2011). Most temperate grasses have a dual induction requirement for

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flowering, a primary induction which is brought about by low temperature (vernalization) and/or short days (SD), and a secondary induction which requires a transition to long days and is enhanced by moderately high temperatures (Heide 1994; Dahl et al. 2013). Depending on the region, large differences are observed in the dates of first appearance of airborne grass pollen grains, as well as in the duration of the pollen season and concentrations (Frenguelli 2002; Beggs 2004; Chłopek 2007; Kasprzyk and Walanus 2010; Myszkowska 2010; Puc 2011). Resulting from the different flowering time of the numerous species of the family, the grass pollen season lasts longer than other taxa, especially in the southern part of Europe, where it covers ca. 6 months (April–September) (Weeke and Spieksma 1991). Furthermore, grasses are a stenopalynous taxon, that is, their pollen grains exhibit a very high intra-family similarity (Perveen 2006). This makes it impossible to distinguish them by microscope observation, and thus, it is not simple to outline pollen seasons for the single species.

The pollen production and flowering time are under genetic and physiological control, but environmental conditions—especially climate—can have a strong influence on them (Pacini 2000; García de León et al. 2015). Many studies have been carried out which correlate different meteorological parameters with Poaceae pollination time and intensity (Galán et al. 1995; Iglesias et al. 1998; Sánchez-Mesa et al. 2003; Dahl et al. 2013). For example, water availability and high temperatures prior to flowering seem to have a positive effect on the anthesis and quantity of pollen produced (Sánchez-Mesa et al. 2005; Green et al. 2004). Obviously, meteorological parameters such as rainfall, relative humidity, wind velocity, and direction also have a strong influence on pollination (Puc and Puc 2004; Sánchez-Mesa et al. 2005; Jato et al. 2009). Smith et al. (2009) have shown that large-scale air mass phenomena like the North Atlantic Oscillation (NAO) may also affect the amounts of airborne grass pollen concentration, even if this influence varies spatially across Europe.

Each of the last three decades has been successively warmer than any preceding decade since 1850. The period from 1983 to 2012 was very likely the warmest 30-year period of the last 800 years in the Northern Hemisphere (IPCC 2014). Recent analysis has shown significant changes in Europe, including Mediterranean areas, and it is very likely that this trend will persist throughout the twenty-first century (IPCC 2013, 2014). Time series data on the pollen content of the air show that these changes have clearly influenced the intensity of pollination in some taxa, especially woody plants, but also weeds and grasses (Frenguelli et al. 2014a, b). Poaceae in particular are predicted to increase their pollen production considerably (Emberlin et al. 1994; García-Mozo et al. 2011). Moreover, temporal fluctuations have also been noted in flowering onset: many studies carried out in recent decades

have shown that the pollen season for taxa flowering in winter and spring in temperate regions, such as many grasses, tend to become earlier and trends in pollination generally show significant variations, probably due to the continuous rise of the average air temperature (Frenguelli et al. 2014a, b).

This work reports fluctuations in the airborne grass pollen presence in Perugia (Central Italy), where airborne grass pollen is being monitored continuously since 1982. The pollination behavior of this taxon has been studied in order to understand if it can be considered as a bio-indicator of climate change. Moreover, the effects of diverse meteorological parameters on Poaceae pollination have been dissected, with the aim of collecting useful information for the improvement of predictive models, which represent an important contribution to primary prevention strategies for susceptible individuals. Each climatic area is characterized by particular pollination patterns and weather conditions, leading to such models being widely studied across different regions (Sánchez-Mesa et al. 2002; Smith and Emberlin 2006; Rodríguez-Rajo et al. 2010; Piotrowska 2012; Brighetti et al. 2014). Similar studies have also been carried out in other taxa (Rodríguez-Rajo et al. 2003; Kasprzyk 2009; Sabariego et al. 2012; Oteros et al. 2013).

Materials and methods

This study was conducted using aerobiological data of Poaceae, compiled over 33 years (1982–2014) of monitoring in Perugia (Central Italy). The city lies on a hill (493 m above sea level) and overlooks the middle valley of the river Tevere, between the Lake Trasimeno and the Tyrrhenian versant of the Marche-Umbria Apennines. Its climate can be classified as semi-continental (Rivas-Martínez et al. 2011), with an average temperature of 13.4 °C and average rainfall of 780.5 mm/year in the studied period. Meteorological data were obtained from the station placed in the experimental fields of the Department of Agricultural, Food and Environmental Sciences in Papiano, about 15 km from the pollen trap.

Grass pollen data were recorded by means of a 7-day volumetric Hirst-type (Hirst 1952) pollen trap (Lanzoni®, Bologna), located in Perugia on a rooftop at 20 m above ground level. The sampler was calibrated to aspire an air flow of 10 L/min, designed to mimic the human respiration process. The trapping surface consisted of a Melinex stripe coated with 2 % silicon solution, and was mounted on a rotating drum dragged by a clockwork mechanism at the speed of 2 mm/h. The Melinex stripe was substituted every 7 days, cut into parts—each corresponding to a day of monitoring—and fixed on a glass slide with gelatine and fuchsin. The preparations were analyzed by optical microscope, and airborne pollen concentrations were expressed as a daily number of pollen grains per cubic meter of air.

The most common and widespread spontaneous and forage grasses present in Perugia area are *Festuca arundinacea* (tall fescue), *Dactylis glomerata* (orchard grass), *Poa* spp. (meadow grass and bluegrass), *Lolium perenne* (perennial ryegrass), *Holcus lanatus* (Yorkshire fog), *Bromus* spp. (bromes), *Avena fatua* (common wild oat), *Hordeum murinum* (wall barley), *Alopecurus myosuroides* (black grass), *Cynodon dactylon* (Bermuda grass), *Setaria* spp. (foxtail), and *Digitaria* spp. (crabgrass) (Frenguelli and Tedeschini 2009).

The main parameters describing the pollination (start, end, duration, peak day, annual pollen sum = annual pollen index (API)) were analyzed. Moreover, the behavior of pollination curves by two shape statistical parameters, kurtosis and skewness, was checked.

We tested different criteria to describe the start and end of the pollen season (cumulative, threshold, and percentage methods, Jato et al. 2006). In this paper, we applied the 5 % threshold method (Nilsson and Persson 1981), which gave the smallest standard deviation in the start dates. According to this criterion, the pollen season starts on the date when the sum of pollen grains from the beginning of the year reaches or exceeds 5 % of the total annual pollen concentration, and ends when the 95 % of this total is reached or exceeded.

The shape of the pollination curve and its variations through the years were investigated by two parameters: the skewness, which is a measure of the asymmetry of the probability of the distribution of a real-valued random variable, and the kurtosis, which is a measure of its “peakedness.” In detail, skewness indicates the amount and direction of the curve skew (departure from horizontal symmetry), and kurtosis tells how tall and sharp the central peak is.

The relationship between meteorological data collected in the 33 years of the study and the grass pollen season was investigated using correlation and regression analysis. All the statistics were performed using Microsoft Excel and R software.

The temperature or heat units a plant needs to accumulate until flowering can be expressed as growing degree-days or GDD°C (Baskerville and Emin 1969). This index was used to express heat requirements of grasses, after deducting different

base temperatures (5, 6, and 7 °C). For any calendar day, the number of GDD°C for that day is the average of the minimum and maximum temperature in the same 24-h period minus the base temperature. The equation for the calculation is as follows:

$$\text{GDD}^{\circ}\text{C} = [(T^{\circ}\text{max} - T^{\circ}\text{min})/2] - T^{\circ}\text{base} \text{ (Baskerville and Emin 1969).}$$

Results

Trend of monthly and annual temperatures

The average annual temperature in Perugia has increased over the 33 years of this study, revealing a clear and significant linear trend of approximately 0.04 °C/year ($R^2 = 0.367$, $p < 0.001$ —Fig. 1). In the previous three decades (from 1952 to 1981), the average annual temperature was 12.9 °C while in the 1982–2014 period, it was 13.4 °C (+0.5 °C).

The coldest year was 1991 with a mean temperature of 12.3 °C, and the hottest was 2014 with 15.2 °C. There has been a significant warming above all in spring and slightly in winter, whereas summer and autumn have not undergone significant changes (Table 1). The months which have undergone the highest mean temperature increase are March, April, and June with increases of about 1–2 °C during these three decades (Table 1). November temperature follows a positive trend as well (Table 1) but this is unlikely to influence the flowering of grasses. Also if the maximum temperature is considered, the highest increase is registered above all in March, April ($p < 0.05$), and June ($p < 0.01$), while the minimum temperature did not show any distinctive pattern (data not shown).

Analysis of pollen seasons

Variation and trend of annual pollen index

The mean value of annual pollen index (API) is 3767, with a highest total of 12,170 in the year 1985 and a lowest annual total of 903 in the year 2009.

Fig. 1 Annual mean temperature over the studied period (1982–2014) and its increasing trend ($y = 0.0369x + 12.804$, $R^2 = 0.3674$)

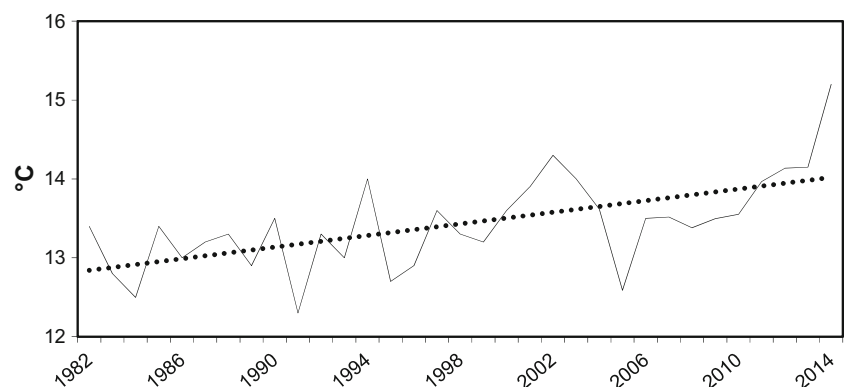


Table 1 Monthly average temperatures and statistics of the regression for each month (1982–2014). Significant trends are evidenced

	$T_{av.} (1982-2014)$	Slope ($^{\circ}\text{C}/\text{year}$)	R^2	p value
January	4.1	0.04	0.073	n.s.
February	5.1	0.04	0.044	n.s.
March	8.4	0.06	0.128	*
April	11.9	0.06	0.278	**
May	16.6	0.02	0.012	n.s.
June	20.7	0.05	0.157	*
July	23.6	-0.001	0.0001	n.s.
August	23.4	0.03	0.060	n.s.
September	18.9	0.01	0.003	n.s.
October	14.3	0.02	0.050	n.s.
November	8.9	0.06	0.154	*
December	5.2	0.03	0.035	n.s.

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

There is a significant decreasing trend in the annual sum of pollen recorded over the study period (Fig. 2).

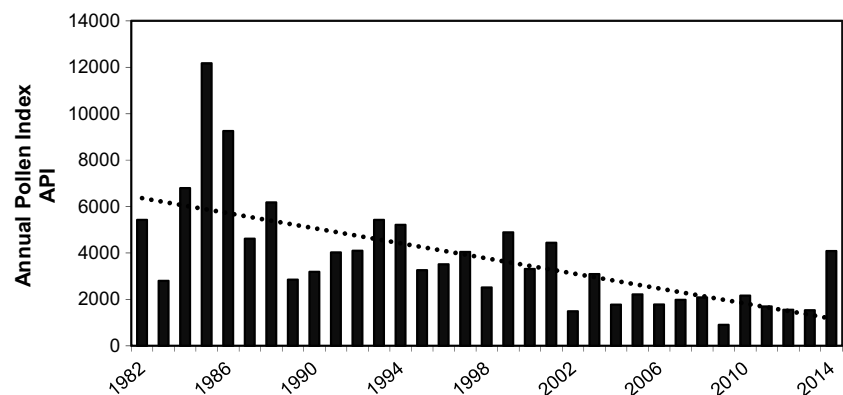
The slope of this regression is -162.15 ($p < 0.001$, $R^2 = 0.44$): this means that there is a marked negative trend, with a year-to-year decrease of the annual total of about 162 pollen grains.

Seasonal course of daily concentration

After totaling and averaging the data from the 33-year period, an overall seasonal course can be constructed which is shown in Fig. 3. Clearly, the main peak is at the end of May. The average maximum daily pollen concentration is 212 pollen grains/ m^3 , with the highest maximum of 647 pollen grains/ m^3 in the season of 1985 and the lowest in 2009 with 31 pollen grains/ m^3 . The maximum pollen concentration decreases over time with a slope of about -6.06 ($p < 0.05$).

On average, the season's maximal daily pollen occurrence was on 30th May; the earliest seasonal peak was on 21st May in 1989, and the latest was on 7th July in 2013.

Fig. 2 Annual total sums (API) of daily airborne grass pollen concentrations in 33 individual years (1982–2014) at Perugia, Central Italy. The regression line indicates the trend calculated on the 33-pollen index values sequence ($y = -162.15x + 6523.9$, $R^2 = 0.4438$)



Moreover, the start date is negatively correlated with the temperature of May ($r = -0.664$, $p < 0.001$, Fig. 4). There is no significant trend in peak dates over time, indicating that there have not been significant variations of this date during the last decades (Fig. 5).

Season starting dates, ending dates, and length

The average starting date is the 2nd May: the latest start was registered in 1984 on 17th May, the earliest in 2009 on 15th April (Fig. 6). The start of the grass pollen season was associated, since the beginning of the 1980s, with a strong advance (-0.4091 days/year, $p < 0.001$, $R^2 = 0.32$). The end dates fluctuate between 24th June (2009) and 23rd August (1984), without a statistically significant change over the studied period.

Likewise, the length of the pollen seasons is highly variable, oscillating between 61 and 131 days, but it does not follow a significant trend over the whole data set (Fig. 6).

Skewness and kurtosis

The average pollination curve approximates the shape of a normal distribution (which would have had both indices equal to 0) showing low values of kurtosis (3.8) and skewness (2.1). However, the analysis of each yearly distribution showed significant differences as underlined by kurtosis and skewness values trends (calculated on each yearly sequence of daily average airborne pollen concentrations), which are shown in Fig. 7a, b.

Despite their high variability, all the values of skewness and kurtosis for the 33 studied grass pollen seasons are >0 , which means their distributions are generally “more peaked” than a Gaussian curve and skewed to the right. From the graphics, we can observe that the two indices are strongly variable in the studied period, but their general trend is significantly increasing and that means the pollination curve is becoming more “peaked” and right skewed through the years.

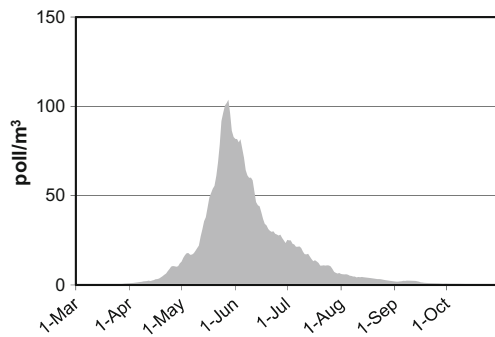


Fig. 3 Overall seasonal course of the airborne grass pollen concentration, expressed as sequence of mean daily concentrations (pollen grains/cubic meter of air) calculated from 33 years of observation

Pollination and meteorological data

Air temperature and starting dates

The influence of air temperature on the starting dates has been investigated by determining the correlations between the starting dates and the mean air temperatures during a number of different periods preceding the average starting date (Table 2).

The relationship with the March–April average temperature is the highest of all the tested ones, with a correlation coefficient of $r = -0.629$ ($p < 0.001$) (Fig. 8).

Heat units

Considering that in Perugia the vernalization process (needed for grasses to break dormancy and be able to flower) begins during November and is fulfilled at the end of February (Frenguelli et al. 2014b), the GDD $^{\circ}\text{C}$ were calculated from the beginning of March to flowering. The results indicated an average value of 205 GDD $^{\circ}\text{C}$ with $T^{\circ}\text{base} = 7^{\circ}\text{C}$, 268 with

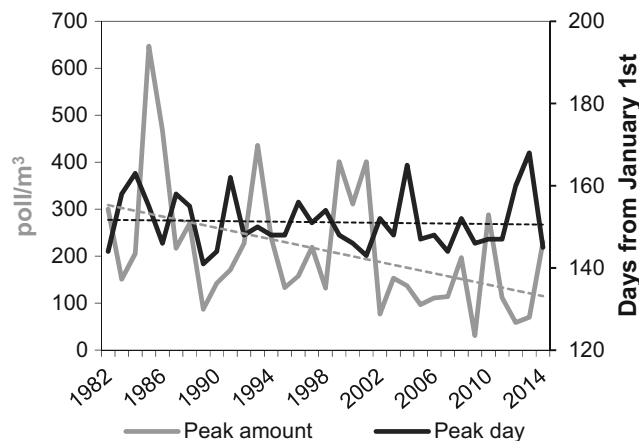


Fig. 4 The figure shows how the maximum daily concentration of the year is following a decreasing trend ($y = -6.0582x + 314.9$, $R^2 = 0.1853$), while the day of the year in which it occurs has not changed significantly from 1982 until 2014 ($y = -0.0354x + 151.72$, $R^2 = 0.0025$)

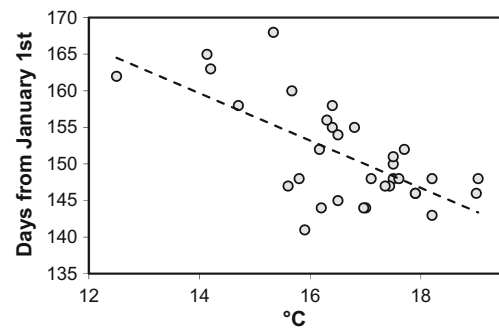


Fig. 5 Negative correlation ($r = -0.664$, $p < 0.001$) between the date of peak and mean temperature of May

$T^{\circ}\text{base} = 6^{\circ}\text{C}$, and 330 with $T^{\circ}\text{base} = 5^{\circ}\text{C}$. The GDD $^{\circ}\text{C}$ calculated by subtracting the base temperature of 7°C resulted in the lowest standard deviation and so were chosen to carry on the statistical analysis (Table 3). On average, the value of 205 GDD $_{7^{\circ}\text{C}}$ occurs between 1st and 2nd May and these dates correspond perfectly to the average onset date of the principal period of grass pollination recorded during 33 years of testing. Our data also shows that the threshold is being reached earlier through the years (Fig. 9). In the 1982–2000 period, the threshold of 205 GDD $_{7^{\circ}\text{C}}$ occurred on average between 6th and 7th May, while in the 2001–2014 years, being a warmer period, the value was reached between 27th and 28th April, about 10 days before.

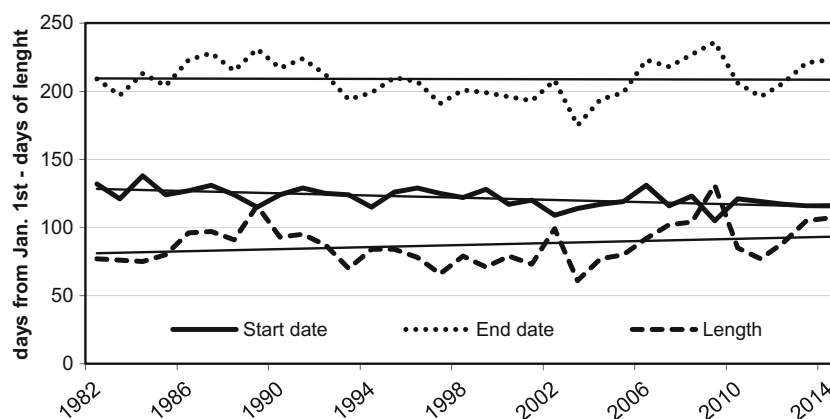
Rainfall

Unlike temperatures, rainfall has not changed significantly in the studied area and period. Even if sub-periods of the year are analyzed separately (pre-pollen season rainfall, spring rainfall), no trend is detectable (data not shown). The average yearly rainfall is 780.5 mm. The rainiest year was 2014 with 1145 mm, while the driest was 1985 with 503.2 mm (Figs. 4 and 10a). The period of the year in which precipitations mostly affect the airborne grass pollen is May. Total rainfall of May is negatively correlated with the sum of daily average pollen concentrations for the same month ($r = -0.4$, $p < 0.05$). Moreover, the higher the rainfall in May, the later the seasonal peak occurs (Figs. 4 and 10b).

Discussion

The climatic trend registered in Perugia over the last three decades is in accordance with the tendencies recorded globally. The average global combined land and ocean temperature has increased about 0.85°C over the last century and is predicted to continue rising (IPCC 2013, 2014). According to the European Environmental Agency (2015) and IPCC (2013), annual average land temperature over Europe, including the Mediterranean area, is projected to further increase by more

Fig. 6 Trend of start dates, end dates, and season length during the 33 years studied (start dates solid line, $y = -0.4091x + 939.15$, $R^2 = 0.3223$; end dates dotted line, $y = -0.0301x + 269.04$, $R^2 = 0.0004$; length broken line, $y = 0.3792x - 670.51$, $R^2 = 0.0594$)



than global average temperature over the rest of this century, and this increase is expected to take place especially in spring and summer months. Temperature data from Perugia confirm these tendencies, with March, April, and June being the months which temperature has risen the most during the last 33 years. March and April in particular need to be taken into account in this study, as long as they are the months immediately preceding the period in which Poaceae begin to flower in this area.

Different authors have shown how warming can affect reproductive physiology and phenology of plants (Menzel et al. 2006, Gordo and Sanz 2009, Ziello et al. 2012). For example, it can cause an increase in pollen production (Zhang et al. 2014), which is also a consequence of increasing concentrations of atmospheric CO₂ (Ziska and Caulfield 2000, Hamaoui-Laguel et al. 2015, Ziska and Ebi 2015), and/or an advance in flowering time (Walther et al. 2002). This study shows how, in Perugia, this kind of advance has been clearly evidenced in grasses but, conversely, a decreasing API has been detected (Fig. 2), with an average value for the last years about halved in respect of the end of last century. A similar trend has been observed by Ridolo et al. (2007) in the city of Parma (Northern Italy), but is in contrast with what has been reported in other areas of the Mediterranean region (García-Mozo et al. 2011, García de León et al. 2015),

and needs to be explained taking into account different exogenous factors that could influence the quantity of grasses of different species and the pollen produced and released by each of them. In particular, we have reasons to motivate this trend with recent changes in the land use and urban green management. The reference spore trap is located in a marginal area of the urban centre, and it is reasonable to think that, in the last decades, new constructions have substituted green and fallow areas often inhabited by several common grasses. An example is the restoration, carried out in 1996, of the “medieval garden” located in close proximity of the trap, previously covered in fallow and marginal vegetation (www.comune.perugia.it). In addition, we could consider also the possible role played by an improved urban vegetation management. Recent local and national legislation requires a series of measures in the maintenance of urban green areas (e.g., grass cut before flowering/pollen dispersal, planting of low-pollen-producing species) in order to encourage the interaction between citizen and environment while promoting human health (Ministero della Salute 2010, Comune di Perugia 2015). In Italy, the API is subject to high variability between regions and seasons (Frenguelli et al. 2015). The reason for this oscillation is in the complexity of factors—not only climate—that govern flowering and pollen dispersal/settling/resuspension

Fig. 7 a, b Trends for skewness and kurtosis yearly values ($y_{kur} = 0.4445x - 872.71$, $R^2 = 0.1312$, $y_{ske} = 0.0402x - 76.884$, $R^2 = 0.1444$)

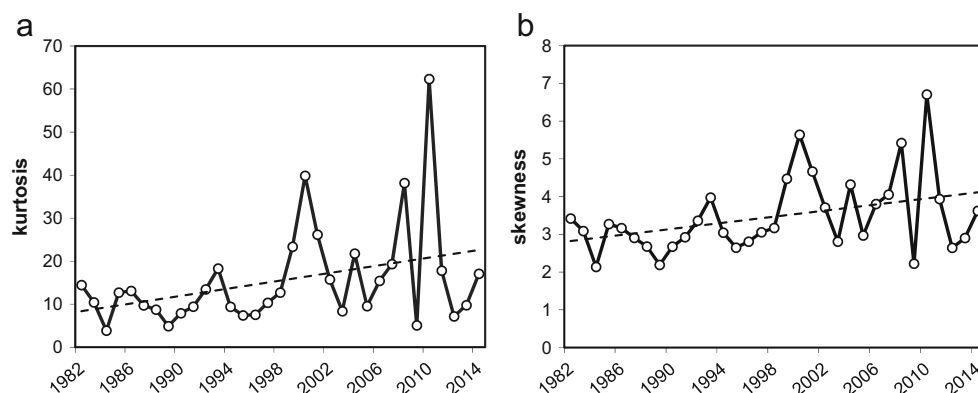


Table 2 Correlations between starting dates of grass pollen season and mean air temperatures in different preceding periods

Independent variable	Correlation coefficient (<i>r</i>)
February mean monthly temperature	−0.224 (n.s.)
March mean monthly temperature	−0.488**
April mean monthly temperature	−0.378*
February + March mean temperature	−0.420*
March + April mean temperature	−0.629***

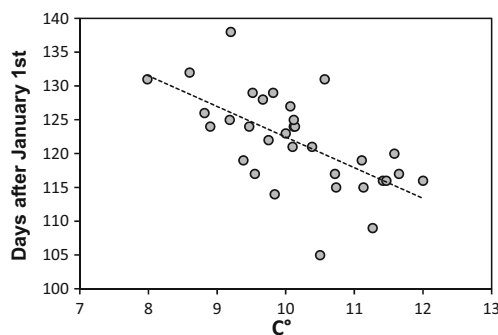
*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

***Correlation is significant at the 0.001 level

dynamics, and makes it too difficult to predict a trend for grass pollen concentrations in the future.

The taxon Poaceae is an amalgam of different species with variable ecology, mating systems, and heat requirements; thus, the annual pollination curve can have more than one peak (Gonzalez Minero et al. 1998). In Perugia, like in other sites characterized by yearly average temperature oscillation, every year has its own characteristic course, with variable periods of high and low airborne grass pollen concentrations, sometimes with a well-defined peak, sometimes with a lower and less distinct one. Nevertheless, the averaged pollination curve for grasses (1982–2014) has only one peak (Fig. 7), due to the fact that the species which contribute the most to the airborne pollen load in this area flower almost simultaneously and their pollen emission patterns overlap. We decided to study the evolution of the shape of the curve representing the pollen season using the indices of skewness (symmetry) and kurtosis (“peakedness”). The increasing trend we observed for both of them can be interpreted as the pollination curve becoming more “peaked” and right skewed through the years. Moreover, we observed how the season start tends towards a marked anticipation in time (Fig. 6), while dates of occurrence for peak and end are practically constant, and the length of the season is only slightly lengthening (not significantly). This tendency could also be linked with the rising temperatures

**Fig. 8** Correlation between mean air temperature in the March–April period and the starting dates of the grass pollen season ($r = 0.629$, $p < 0.001$)

and can be explained with an allocation of plant resources into an intense flowering in order to conclude the reproductive phase before the beginning of summer drought, which is ubiquitous in the Mediterranean area (Dahl et al. 2013). Primack (1985) stated that when flowering is synchronous in a population/region, the pollen curve is often positively skewed and the peak of pollen concentration will probably follow in a

Table 3 Heat requirements for the start of the grass pollen season using three different base temperatures (5, 6, and 7 °C)

Year	GDD°C until start day		
	Base temperature		
	5 °C	6 °C	7 °C
1982	326	253	180
1983	321	259	197
1984	414	352	258
1985	346	281	216
1986	390	322	254
1987	274	202	130
1988	310	246	182
1989	340	284	228
1990	353	288	223
1991	333	263	193
1992	358	293	228
1993	277	213	148
1994	304	248	192
1995	293	226	159
1996	374	305	236
1997	304	238	172
1998	306	243	180
1999	379	310	241
2000	307	250	193
2001	404	343	282
2002	285	235	185
2003	237	182	127
2004	242	185	128
2005	262	202	142
2006	459	387	315
2007	342	285	228
2008	321	258	195
2009	219	173	127
2010	340	278	216
2011	353	295	236
2012	367	310	253
2013	352	295	238
2014	387	330	273
SD	53.1	50.9	47.9
Mean	330	268	205
CV%	16.1	19.0	23.4

SD standard deviation, CV% coefficient of variation—percentage

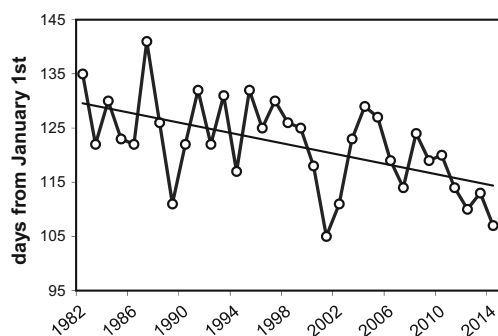


Fig. 9 Trend of the heat accumulation days needed for the grasses to reach the average threshold of 205 GDD_{7°} ($y = -0.4746x + 1070.2$, $R^2 = 0.3007$)

fairly short time after the flowering onset, and this case is recognizable in our study. Obviously, there is a certain number of grass species (most of them belonging to the Chloridoideae sub-family, such as *Cynodon* spp. and *Digitaria* spp.) which flower at the end of summer, but observing the shape of the curve, it is clear that their contribution in quantity of emitted pollen/land coverage in this area is not so relevant. However, noting the extreme differences of the indices of skewness and kurtosis also from 1 year to the following, the gradual change in the shape of the pollination curve can be presumed but needs to be further investigated, especially over a longer period.

The parameters describing the seasonal pollen course were correlated with temperature data, to investigate possible linkages. The most significant information was a strong correlation between the starting date and the cumulative average temperature of March and April (Table 3). Considering that in the area of the study, the mean onset date is the 2nd May, these 2 months are the ones that immediately precede flowering and also those which have undergone the strongest increase in temperatures in the last 33 years. Thus, a further anticipation of flowering is predictable to take place in the future, together with the continuous warming and the shift from temperate to Mediterranean climate (IPCC 2013).

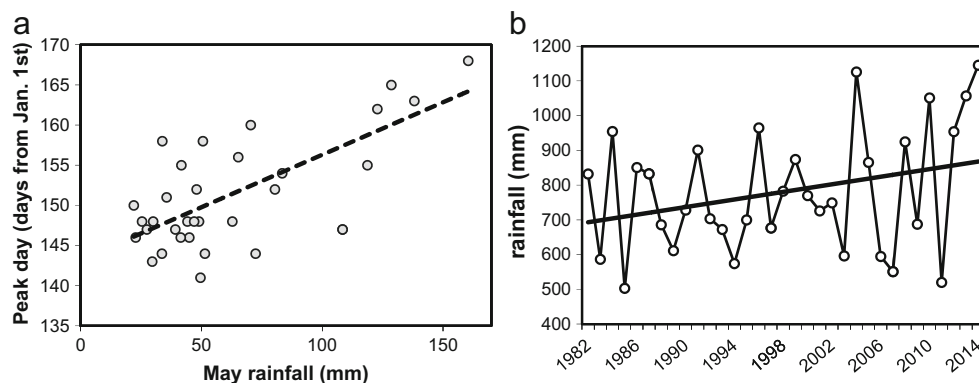
A way to determine the relation between the timing of phenological process and climate is the temperature sum

method, expressed as growing degree-days or GDD°C. Once the vernalization is fulfilled, the calendar time at which the first flower appears and the rate at which each flower develops is determined by the amount of thermal (heat) energy available for biological activity. The temperature when spring grasses initiate development, or the base temperature, is in our environment, about 7 °C. Our analysis has revealed that the average threshold of heat accumulation in this area corresponds to 205 GDD_{7°}, and its achievement, and consequent flowering, tends to be earlier over time, due to the rise of spring temperatures (Fig. 9). The climate of May is therefore linked with the peak of the grass pollen season. As shown in Fig. 4, the warmer this month is, the earlier the peak occurs. Conversely, it is delayed by high values of rainfall in the same period, which also lowers the amount of maximum daily concentration. Indeed, rainfall can alter the shape of a year's pollen curve and can influence peak characteristics, as reported in other works (Norris-Hill and Emberlin 1993, Sánchez-Mesa et al. 2005, Kasprzyk and Walanus 2010). Currently, in the area of this study, it does not interfere with the beginning or end dates nor with the length of the season, in contrast with what Antépara et al. (1995) observed. The lack of a clear trend in this sense is justified by a different sensitivity of the grass species to rainfall and, in general, water availability (Reddi et al. 1988).

Conclusions

In recent decades, there has been evidence of a significant increase of air temperature in Central Italy, such as in many other regions of the Northern hemisphere, with consequent changes of the airborne presence of anemophilous pollen. The changes recorded regarded, above all, the timing in which the pollination occurred (Frenguelli 2002) but involved also the pattern of emission and quantity of pollen released. The temperature increase in spring is believed to be the main factor responsible for the modifications noticed. In particular, the anticipated flowering of grasses observed in Perugia seems

Fig. 10 **a** Pattern of rainfall during the studied period ($y = 5.4624x + 687.62$, $R^2 = 0.0901$). **b** The date in which the peak occurs is positively correlated with the rainfall of May ($r = 0.696$, $p < 0.001$)



to be linked with the increase of the air temperature in March and April recorded in recent decades (Table 1). These trends support the theory that the timing of grass pollination is greatly dependent on temperatures, especially those to which the plants are exposed during the months preceding flowering. So, in Central Italy, the grass pollen season, and in particular its beginning, could be taken into consideration as a bio-indicator of the climatic changes. Moreover, the correlation between starting dates and spring temperature could be an interesting hint for the construction of a forecast model capable of predicting the presence of airborne grass pollen, helping to plan therapies for allergic people. On the other hand, the stability detected in the date of peaks could also provide valuable information for patients, since it means that the warning days for maximum pollen emission only vary slightly from year to year.

Conversely, in the same studied period, both the maximum daily concentration (peak) values and the API exhibited clear trends towards lower values. This result may be due to a number of factors, including the urbanization of the area around the monitoring station with a decrease in grassland, a different land use, and better management of weeds in the city, rather than variations in the weather conditions. In Northern Italy, a similar trend (which is actually quite in contrast with those observed elsewhere in Europe) has already been linked to a decrease in the incidence of rhinoconjunctivitis (Ridolo et al. 2007). There is therefore reason to believe that, in the Perugia urban area, a minor exposure to Poaceae pollen, coupled and helped with appropriate prediction models for the pollen season start, could lead to a reduced number of sensitized patients through the years.

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