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ORIGINAL PAPER

Evidences of olive pollination date variations in relation to spring temperature trends

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Abstract Airborne pollen concentration patterns reflect flowering phenology of a given species, and it may be a sensitive regional indicator in climate change studies. This paper presents the relationship between a strategic biological event, such as olive flowering, and the air temperature trend, registered over a large scale (1982–2007) in the Umbria region. The aim of the study was to determine relationships between phenological behaviour (flowering) of olive trees and the air temperature trend (1982–2007) in the Umbria region. The phenological data on flowering phase were registered indirectly through an aerobiological monitoring technique. The obtained results showed a strong relationship between phenology and thermal trend. This characteristic was confirmed from results of correlations between temperature (mean temperature from 1st March) and flowering dates, especially that of full flowering ($r = -0.9297$). Moreover, the results showed an advance trend of 6, 8 and 10 days, respectively of start, full and end of flowering dates. The advance of the recorded flowering time in this period is to ascribe mainly to the increase of mean temperature and above all to that registered in months of May and June.

Keywords *Olea europaea* · Pollen · Phenology · Climate change · Italy

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1 Introduction

The consequences of global climate change are evident from the observation of different climatic and biological manifestations which are directly or indirectly connected with the increase of the greenhouse effect (Bernstein et al. 2007). Many studies have confirmed the relationship between climatic trend and various phenological phases of different plant and animal species (Sparks et al. 2000; Beaubien and Freeland 2000; Jaagus and Ahas 2000; Teranishi et al. 2000; Menzel et al. 2001; Peñuelas et al. 2002; Chmielewski and Rötzer 2002; Orlandi et al. 2005a, b). Therefore, it can be supposed that a hypothetical future climate change will determine modifications in a spatial distribution of the most sensitive species and a temporal shift of the onset of different phenophases (Higgins and Richardson 1999; Schwartz and Reiter 2000; Schwartz and Chen 2002; Neilson et al. 2005).

Because of the strong relationship between plant development and seasonal temperature trend, the effect of a significant increase of mean global temperature might be traceable in the long phenological series in many areas of the planet (Schwartz et al. 2006; Fitter and Fitter 2002; Donnelly et al. 2006; Williams and Abberton 2004; Menzel et al. 2001; Beaubien and Freeland 2000; Peñuelas et al. 2002; Stefanescu et al. 2003). Therefore, phenological manifestations (plant awakening, flowering, etc.) may be used as observable and sensitive indicators of alterations in the biosphere as a response to climate changes (Peñuelas and Filella 2001). In the Mediterranean basin, olive trees (*Olea europaea* L.) are one of the most widespread arboreal cultivated species which are perfectly adapted to that mild climate. The flowering of this species is most suitable for the study of potential climatic changes, in particular, for the "spring warming" effects, because of its physiological characteristics: late spring flowering, insensitivity to photoperiod (Hackett and Hartmann 1964) and high requirement of biothermic units necessary for development of the inflorescences (Chuine et al. 1998). The olive flowering date can be used as a direct, simple and sensitive biological indicator of climatic warming in the Mediterranean area (Osborne et al. 2000). Moreover, olive pollen is considered as one of the most important causes of respiratory allergic disease in the Mediterranean region (D'Amato et al. 2007). In southern Italy, the frequency of positivity to *Olea* pollen allergens among all skin prick test-positive patients is 13.49% in adults and 8.33% in children (D'Amato and Spieksma 1990). Data for olive flowering were obtained indirectly through monitoring pollen in the atmosphere using volumetric pollen samplers (Orlandi et al. 2005b). The data obtained allowed us to determine the dates of the beginning, maximum and end of flowering, which is an important source of information about the flowering of anemophilous species (Fornaciari et al. 2000; Jato et al. 2000; Galán et al. 2001; Ribeiro et al. 2005; Estrella et al. 2006).

The aim of this study was to determine in the area of central Italy:

(1) the flowering and climatic trends registered during the study period (1982–2007);
(2) the type of relationship between the phenological behaviour (flowering) of the olive and the temperature trend in the period before the date of flowering;
(3) possible influences of temperature trend on the olive flowering process.

2 Materials and methods

2.1 Study area

The Umbria region is situated in the central part of the Italian peninsula between 42°26′N and 43°31′N latitude (Fig. 1). The climate of this region is of the Mediterranean type, slightly mitigated because of its distance from the sea. The other characteristic is the presence of the Apennine Mountains in the north-eastern part of the region, which constitutes a barrier to the cold air currents coming from north-east, especially during winter. The mean annual temperature in the study period (1982–2007) was 13.6°C, when the mean value of the coldest month (January) was 4.3°C and that of the hottest month (July) was 27°C. The average annual rainfall was ≈900 mm, concentrated in the autumn-winter period regarding both the amount and the number of rainfall days. The total area of the region is 8,456 km^2 and 3.2% of it is cultivated in olives, especially in the hill zones between 170 and 500 m a.s.l. altitude. The main olive cultivars (Frantoio, Leccino, Moraiolo and Dolce Agogia) are autochthonous and represent 90% of the olive population of the entire area. They are characterized by the synchronism of the flowering period, in particular regarding full flowering (Pannelli et al. 2000).

2.2 Climatic data

The meteorological variable used in the study is the temperature (maximum, minimum and average), registered from 1982 to 2007 at the S. Egidio meteorological station (43°05′N latitude, 12°29′E longitude) distant ≈7 km from the pollen sampling station. The data analysis was useful to determine the thermal characteristics of the study area over the last 26 years. In particular, to better analyze the temperature trend in the first 6 months of the year, the GDD (Growing degree days) relative to every month were calculated.

The GDD for each month were calculated by the method of simple mean value, using the threshold temperature (T_b) equal to 0°C:

$$GDD = \Sigma[(T_{max} + T_{min})/2] - T_b$$

where, T_{max} is the maximum daily temperature and T_{min} is the minimum daily temperature. The 0°C

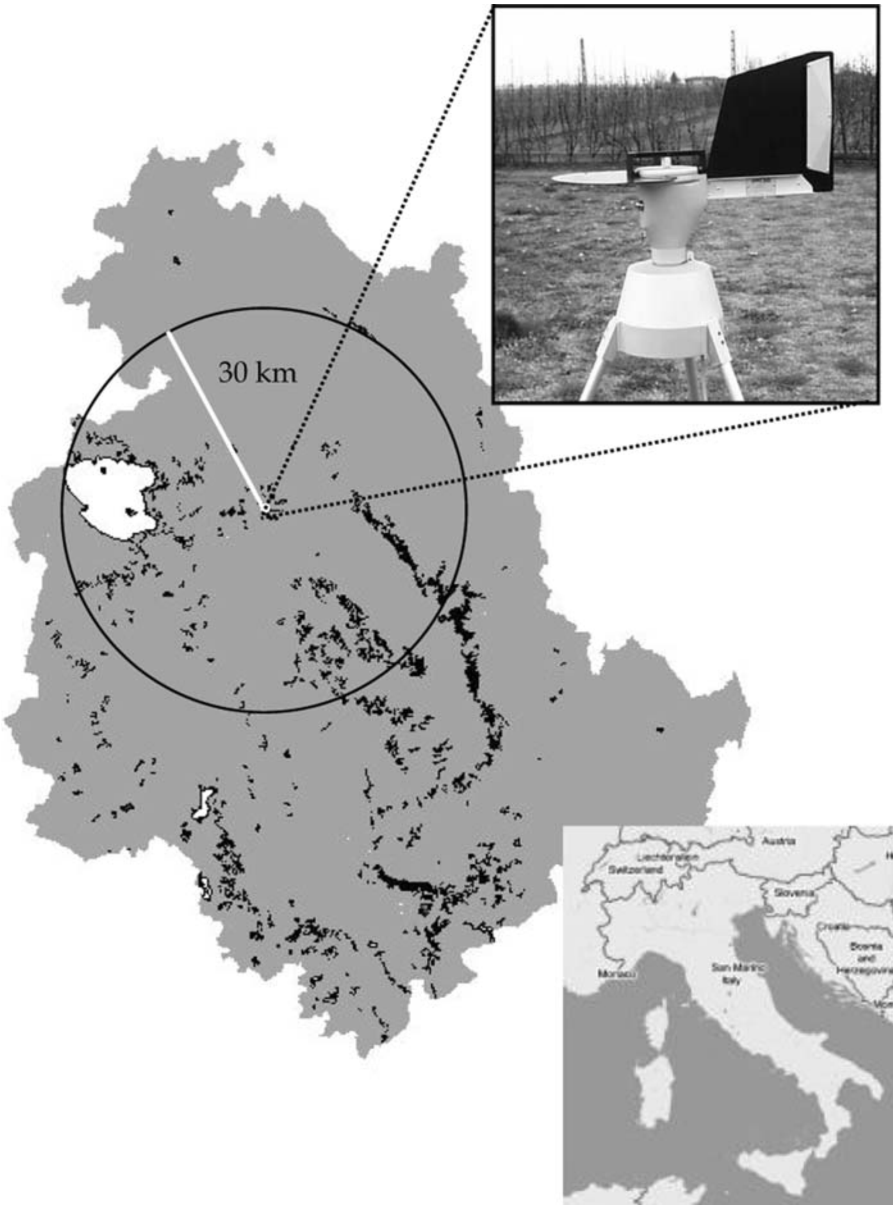


Fig. 1 The Umbria region and the pollen monitoring area

threshold temperature was not derived from a previous statistical analysis but to be consistent with the use of the mean temperature calcilated in pre-flowering periods, for the successive correlation and linear regression analyses. The temperature summation represents a synthetic and useful indicator to describe the temperature trend of a certain area. The calculation of the monthly temperature summations allows us to evidence the months with a particularly marked (increasing or decreasing) tendency in the temperature trend. The GDD were calculated during the first 6 months of the year to determine the real existence of the positive temperature trend "spring warming" during the study period (1982–2007).

2.3 Pollen monitoring technique and the analysis of pollen seasons

The pollen sampling activity was carried out with the use of the pollen volumetric samplers of the Hirst type (Hirst 1952). The model utilized was the VPPS 2000 Lanzoni model, in function from 1982 at the

Agriculture Faculty of the University of Perugia (43°06′N latitude, 12°24′E longitude, 400 m a.s.l. altitude). The instrument has an aspiring force of 10 litres per minute, that is 0.6 m^3 of air each hour. The determination of the daily pollen concentrations permits the definition of the pollination curve, which graphically represents the flowering trend in the year (Fornaciari et al. 2002; Orlandi et al. 2003). Hence, the daily pollen concentrations represent a precious source of information on flowering, because they reflect the reaction of the tree population around the monitoring station and not of single trees, as occurs during direct phenological observations (Osborne et al. 2000). The other important element is the capacity of the volumetric method to capture aero-transported pollen at considerable distances from the releasing site ≈30–50 km, depending on orography of the observed area (Osborne et al. 2000; Chuine et al. 2000; Galán et al. 2004). The mean pollination period (MPP) was calculated preventively, in order to determine the relationship between the meteorological parameters and the olive flowering. In this case we used the criterion described by Galan in 2000 which considers as the start date of flowering the date when the pollen concentration reached 1% of the total annual pollen concentration of a species (IP). By subtracting 1% of the total pollen from the initial and the final parts of the pollination curve, respectively, the dates of the start of flowering (SF) and the end of flowering (EF) can be determined. At the same time, the full flowering date (FF) corresponds to the day with the maximum daily pollen concentration. These dates are the most representative phenological dates of the entire olive tree population and are not influenced by the possible presence of micro-climates which could induce local olive plants to emit pollen particularly in advance in comparison to the mean date.

2.4 Determination of the relationships between the flowering dates and the air temperature

Phenological development in nature is strictly correlated to the atmospheric conditions (Jaagus and Ahas 2000; Richardson et al. 1974). To examine the relationship between climate and a determined phenological event, the correlation between the values of meteorological parameters registered during the specific period and the starting dates of phenological manifestation were calculated (Jaagus and Ahas 2000; Ahas and Aasa 2006; Emberlin et al. 2007; Bonofiglio et al. 2008). In our study, to determine the pre-flowering period that greatly influences the olive flowering date, the mean temperature values (calculated in 7 determined pre-flowering periods) were correlated to the flowering dates expressed in days from January 1st (SF and FF). The mean temperature values, used for the correlation, were calculated starting from 7 different dates: January 1 (1 Ja), January 15 (15 Ja), February 1 (1 Fe), February 15 (15 Fe), March 1 (1 Ma), March 15 (15 Ma) and April 1 (1 Apr), while the SF and FF dates for determination of different periods were calculated using the mean value of the start and full flowering dates obtained during the 26 years of the study.

The relationship between the meteorological variable (mean temperature) and the biological one (dates of flowering) has been studied by linear regression analysis and by establishing Pearson's correlation coefficients, reflecting the extent of correlation. The importance of the correlation coefficients was judged with error probability levels of 1%. The NCSS 2007 (Statistical Software) has been used for all statistical analysis. From a statistical analysis of the mean temperature and the flowering dates, it was possible to determine the period which greatly influences the date of flowering, degree and the type of relationship between the two variables.

3 Results

3.1 Meteorological analysis

Figure 2 shows the trend of the mean annual air temperature registered from 1982 to 2007. An evident increase of the mean temperature can be seen, with the lowest value (12.2°C) in 1985 and the highest one (14.9°C) in 2007. The increase of the mean temperature was calculated as 0.06°C/year with the R-Squared value of 0.4488 ($R^2 = 0.4488$). Monthly GDD calculated for the first 6 months of each year from 1982 to 2007 evidenced an increasing trend during the months of May and June (Fig. 3). In analyzing the details of Fig. 3 the temperature in January and February did not show any significant increase. For these 2 months, the R-Squared (R^2) and the regression coefficients (slope) values were lower

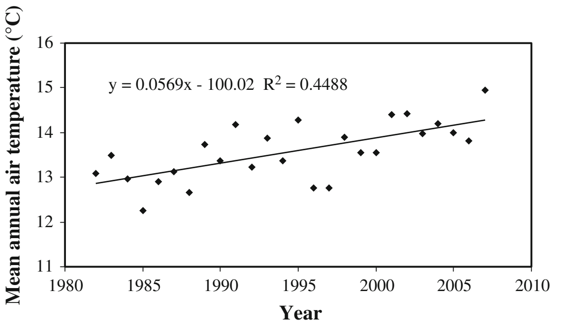


Fig. 2 Trend of the mean annual air temperature registered from 1982 to 2007 at the Perugia meteorological station

than 0.06 and 1.44, respectively. Different trends were observed for May ($R^2 = 0.3136$ and slope = 4.13) and June ($R^2 = 0.5625$ and slope = 5.67). The months of March and April showed an intermediate behaviour with respect to the other two types described above.

3.2 Start and duration of the pollen season

The pollen monitoring results demonstrated that the olive flowering in central Italy generally occurs in the period between the end of May and the entire month of June. On the basis of our observations made during the 26 study years (1982–2007), it can be noticed that the mean pollination period (MPP) was concentrated mainly in June (May 28 to June 27) with a mean duration of 30 days (Fig. 4).

It can be noted that the dates of flowering (SF, FF and EF) vary greatly through the years; in particular, the SF date with the maximum oscillation of 36 days between the date registered in 1984 (June 18) and that

Fig. 3 The GDD monthly values with the relative line trends calculated during the 26 years of the study (1982/2007). Ja (January), Fe (February), Ma (March), Ap (April), May (May), Ju (June)

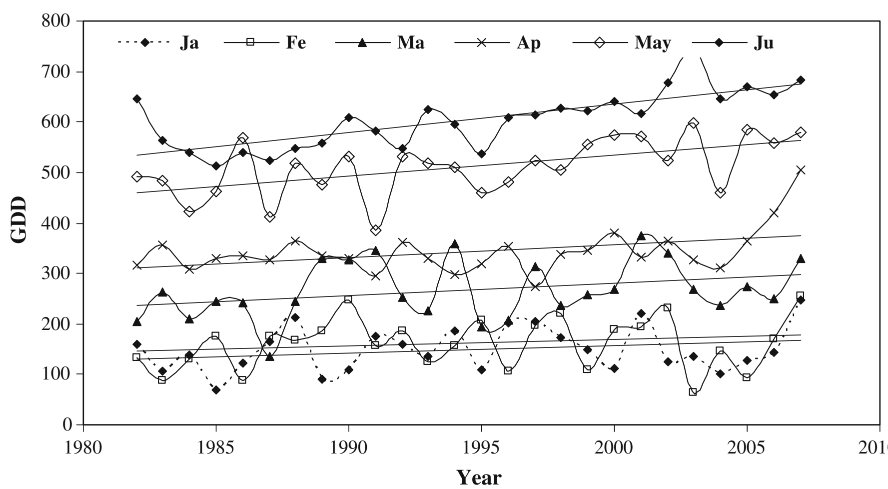
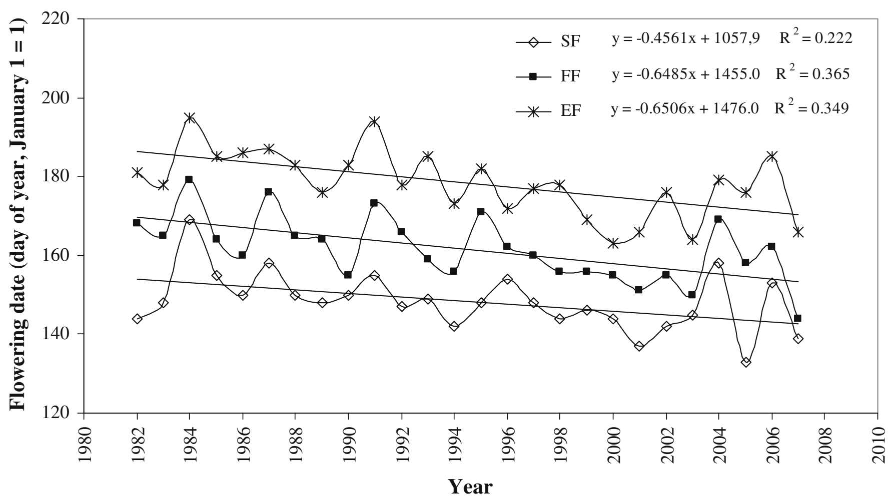


Fig. 4 Dates of the olive flowering (SF, FF and EF) and the relative trends registered at the Perugia station from 1982 to 2007. SF (start of flowering), FF (full flowering) and EF (end of flowering)



in 2005 (May 13). Also, the FF and EF dates ranged from 35 and 32 days, respectively. The wide range of flowering dates may suggest an insensitivity of the floral development to the photoperiod (Osborne et al. 2000). This result confirms the strict relationship between the olive phenology and a climatic trend, especially temperature. Moreover, the pollen monitoring results were used for analysing the tendency of the onset of flowering. The curves graphically represented in Fig. 4 evidence a tendency towards the anticipation of flowering, with the regression coefficients of the linear trends which show a negative value, in particular for the FF dates (−0.6485) and for the EF dates (−0.6506). The anticipation of the SF date is less consistent (−0.4561). The direct consequence of the different intensity of flowering anticipation (SF, FF and EF) is a decrease of the MPP, observed in the 26 study years.

The tendency to an anticipation of flowering dates is evidenced by the results of the Box plots relative to two different periods C1 (1982/1994) and C2 (1995/2007) showed in Fig. 5. In particular, in Fig. 5A the "position" results (Average, Median and Skewness) of two different box plots demonstrate that between the first period (C1) and the second one (C2) there is an advancement of the SF mean dates of about 6 days. The same behaviour was observed for the FF dates where the date advancement between the C1 and C2 periods was ≈8 days (Fig. 5B). The advancement of the EF dates between the C1 and C2 periods is even higher, with a difference of 10 days between the Average values and of 7 days between the Median ones (Fig. 5C). Also the Box plots relative to the flowering duration evidenced a reduction of the flowering period between the first (C1) and the second (C2) periods. In this case, the reduction of the Average date was ≈5 days and that of the Median date was ≈2 days (Fig. 6).

3.3 Correlation of the SF and FF dates with air temperature

The results of correlation and regression analysis between the flowering dates (SF and FF) and the mean temperature, reported in the Tables 1 and 2, put in evidence a negative correlation between the two variables. Negative correlation coefficients indicates that earlier flowering dates are related to an increase in mean temperature. The negative sign was observed in all the correlation analysis results, both for the SF and the FF dates. The analysis of the SF dates evidenced that the highest correlation values ($r = -0.6837$ and $P \leq 0.01$) were obtained using the mean temperatures calculated for the period from March 1 to the start of flowering (Table 1). In this case, the regression has a significance level of 99% ($P \leq 0.01$) and a slope ($\lambda = -3.9504$). The variation of ± 1°C of the mean temperature corresponds to the variation of ± 3.9 days of the SF mean date. The most significant results were obtained using the full flowering (FF) mean date. Also in this case, the highest correlation results ($r = -0.9297$) were obtained using the mean temperature calculated from March 1 until the FF mean date (Table 2). The results relative to that period, besides a high correlation value, showed a significance level of 99% ($P \leq 0.01$) and a slope ($\lambda = -6.3053$). Moreover, from the correlation results it is possible to determine the period which greatly influences the olive flowering date in this area. Both for the SF date and for the FF date, the period starts from March 1. This date shows the highest correlation between the meteorological variable (temperature) and the biological one (date of flowering).

3.4 Trend of IP from 1982 to 2007

Box-plots were constructed considering two periods C1 and C2 (Fig. 7). The principal results evidence a decreasing trend of the yearly concentrations of pollen grains recorded during the same two periods. The box-plots show a clear reduction of the average Ip value during C2 (3,339 pollen grains) in comparison to the average value of the precedent interval (C1 = 4,092). The difference is greater (1,339 pollens) considering the median values calculated in C1 (3,560) and C2 (2,221). Moreover, the C2 period presents a lower homogeneous distribution of values (skewness = 2.38) in comparison to C1, probably due to the "outlier" value (11,494 pollens) recorded during 1999.

4 Discussion

In many research activities the phenological phases of plant species are used as indicators which can provide further information on climate change. In our

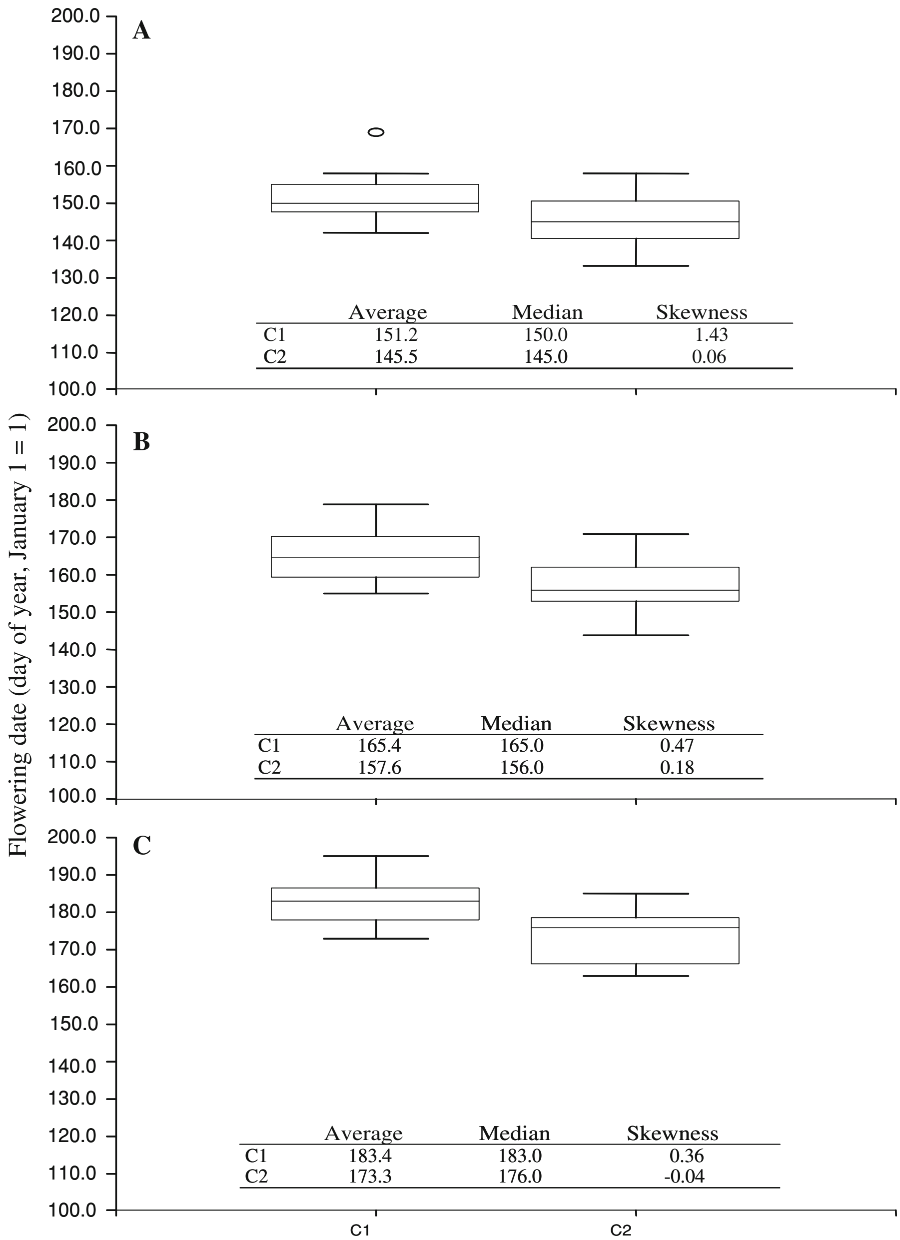


Fig. 5 *Box plots* of the flowering dates calculated for two periods: C1 (from 1982 to 1994) and C2 (from 1995 to 2007), with the relative "position" results (average, median and skewness). In **A** the SF dates, in **B** the FF dates and in **C** the EF dates are reported

study we used olive flowering as an instrument to evaluate the response of this species to the current climatic tendencies in central Italy. The climatic analyses confirmed that the temperature trend in the study area during the last 26 years corresponded to that of the planet (Alcamo et al. 2007; IPCC 2007). They evidenced an increase of the mean air temperature in the period from 1982 to 2007.

The other important result is the real independence of the olive from the photoperiod. On the basis of the consistent oscillation calculated between the dates of flowering (36 days of difference between the SF dates and 35 days–between the FF dates) it was deduced that the *Olea europaea* L. is insensitive to the photoperiod (Osborne et al. 2000). This characteristic confirms the independence of the olive

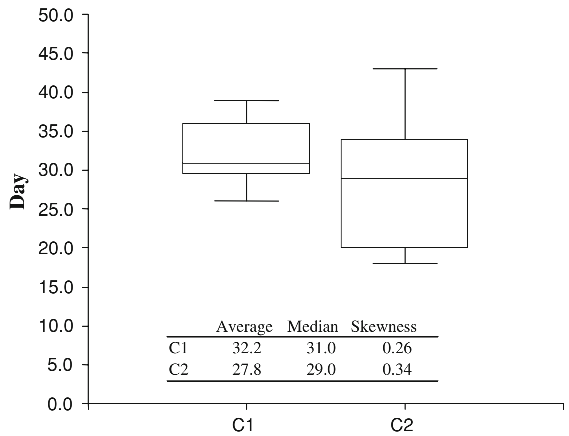


Fig. 6 *Box plots* of the flowering duration (in days) calculated for two periods: C1 (from 1982 to 1994) and C2 (from 1995 to 2007), with the relative "position" results (average, median and skewness)

Table 1 Slope of the regression line (days °C $^{-1}$) and correlation analysis results between the SF dates and the mean temperature calculated in the pre-flowering periods from 1982 to 2007

Period	Linear regression		Correlation
	Intercept	λ (Slope)	*r* (Pearson)
1 Ja–SF	188.475	−4.3173**	−0.6137**
15 Ja–SF	186.337	−3.8881**	−0.5698**
1 Fe–SF	189.236	−3.898**	−0.6164**
15 Fe–SF	191.800	−3.8886**	−0.6400**
1 Ma–SF	195.667	−3.9504**	−0.6837**
15 Ma–SF	196.376	−3.7598**	−0.6472**
1 Ap–SF	188.601	−2.9198**	−0.5746**

Slope (regression coefficients), *r* (Pearson coefficients) and *P* (significance level)

* $P \leq 0.05$, ** $P \leq 0.01$

Table 2 Slope of the regression line (days °C $^{-1}$) and correlation analysis results between the FF dates and the mean temperature calculated in the pre-flowering periods from 1982 to 2007

Period	Linear regression		Correlation
	Intercept	λ (Slope)	*r* (Pearson)
1 Ja–FF	231.558	−6.9180**	−0.8423**
15 Ja–FF	235.367	−6.9560**	−0.8521**
1 Fe–FF	235.664	−6.5232**	−0.8670**
15 Fe–FF	240.359	−6.5318**	−0.9046**
1 Ma–FF	242.839	−6.3053**	−0.9297**
15 Ma–FF	242.768	−5.9303**	−0.8735**
1 Ap–FF	237.550	−5.1560**	−0.8431**

Slope (regression coefficients), *r* (Pearson coefficients) and *P* (significance level)

* $P \leq 0.05$ ** $P \leq 0.01$

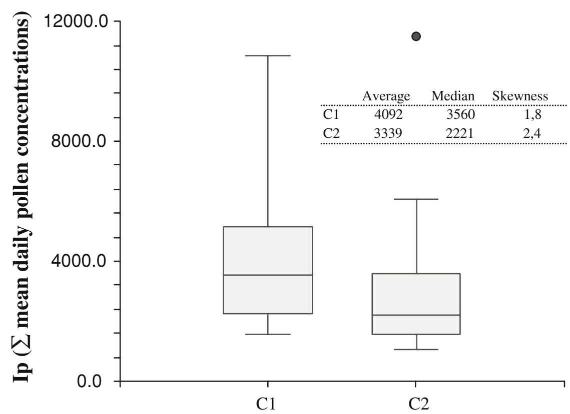


Fig. 7 *Box plots* of IP calculated for two periods: C1 and C2, with the relative "position" results (average, median and skewness)

phenological response from the day length and determines its great dependence on the temperature trend. There are also other factors influencing the velocity of plant development and consequently the time onset of different phenophases.

The results of many studies showed that temperature can explain a great part of plant behaviour relative to different phenological phases in many plant species (Menzel et al. 2001; Jaagus and Ahas 2000; Zhao and Schwartz 2003; Emberlin et al. 2007). The results of this study demonstrate that climate change effects, such as the tendency to an increase of the mean air temperature, influence the dates of the olive flowering.

Table 2 shows that to the variation of ±1°C of the mean air temperature in the pre-flowering period corresponded an oscillation of the FF date by ±6.3 days. Similar results were obtained by Osborne et al. (2000), showing an advance of the full flowering date of the olive in the Mediterranean area by 8.5 days per 1°C. The less evident result was calculated for the SF date with a potential variation of ±3.9 days. The anticipation of the flowering dates (SF and FF), even if not homogeneous, is significant.

Several studies were carried out on the relationship between flowering dates of several vegetative species and temperature recorded during pre-flowering period. The olive species in comparison to other studied species has a similar behaviour.

In particular, the results obtained by Fitter and Fitter (2002) on 385 species showed an advance of 4.5 days per decade. Only few species of the entire database (3% of the total) showed a different behaviour (flowering delay). Williams and Abberton (2004) determined that flowering date of *Trifolium repens* L. advanced approximately of 7.5 days per decade since 1978. Estrella et al. (2007) showed that mean temperature recorded during March–May interval caused an advance in 78 different phenological events of about 4 days°C^{-1}.

The other important result is the determination of the pre-flowering period which greatly influences the period of flowering. Tables 1 and 2 show that from March 1 the best correlation values between the temperature and the flowering dates were obtained. This means that starting from March 1 the variations of the air temperature significantly influence the period of olive flowering. Using the date of March 1, the high correlation values were calculated both for the SF dates (−0.6837) and for the FF dates (−0.9297).

The tendency for the anticipation of the flowering dates registered from 1982 to 2007 corresponds to the increase of spring temperatures in the period before flowering. The warming hypothesis was confirmed by the calculation of GDD relative to the first 6 months of the year, putting in evidence that the temperature increase generally occurred in May and June. The threshold temperature of 0°C utilised for the GDD calculation was considered in order to evaluate in which month during the pre-flowering period, from 1982 to 2007, that the highest temperature increase was recorder. These GDD were considered by a climatic point of view and not to determine the specific temperature requirements of *Olea* species in the area of investigation. During the 26-year study period the months with the increasing trend of the mean air temperature were: March, April, May and June. From those, particularly the months of May (for start flowering) and May–June (for full flowering) with their important temperature increase influenced the development of the olive flowering in central Italy, determining its evident advancement. Other studies showed, through GDD calculation with different threshold temperatures, thet temperature increase during pre-flowering induces an advance of flowering date in *O. Europaea* (Rodríguez-Rajo et al. 2004; Galán et al. 2005; Orlandi et al. 2005b). Furthermore, the authors have shown that threshold temperature is related to bio-geographical characteristics (a higher altitude results in a lower threshold temperature) (Galán et al. 2008; García-Mozo et al. 2009). Moreover, the different intensity of the advancement of the SF, FF and EF dates determined a contraction of the average pollination duration of about 5 days. The reduction of the olive pollen in the atmosphere presents problems in the agronomic area and benefits in the environmental (human health) one. In the first case, we might see a reduction of the pollination period with marked consequences on production. The principal phenomenon that could be induced is related to the reduction of the effective pollination period (Bini 1984). Other factors, such as genetic characteristics of the cultivars or agronomic techniques may also determine a reduction of the effective pollination period, but the main influence is due to the average temperature increase.

In the second case, the reduction of days and intensity of the presence of olive pollen in the atmosphere could have positive effects for people suffering from allergy from this type of pollen; especially in Mediterranean area where the species *Olea europaea* L. is widespread.

5 Conclusions

In conclusion, we can affirm that air temperature influences the olive flowering date in a decisive way. During the years of our study (1982–2007) we registered an anticipation of the flowering period (FF dates of about 8 days), which was due mostly to an increase of the mean temperature during the months of March, April, May and June. Therefore, climatic change, especially relative to the first 6 months of the year, may provoke significant changes in the olive flowering period, duration and intensity. Moreover, if in the twenty first century climate continues to change according to the IPCC forecasts in various SRES scenarios (Special Report on Emissions Scenarios), then the olive's biological response will be more consistent with environmental and agronomic consequences which ought to be evaluated. Future prospects thus seem to justify this type of study in order to verify, with the use of data from other regions, the possibility to use aerobiological data to document the olive's reaction to climate change.

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