

Influence of meteorological conditions on male flower phenology of *Cupressus sempervirens* and correlation with pollen production in Florence

Tommaso Torrigiani Malaspina · Lorenzo Cecchi ·
Marco Morabito · Marzia Onorari ·
Maria Paola Domeneghetti · Simone Orlandini

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Abstract A description of the phenological development of the male flower of *Cupressus sempervirens* in Florence was performed on the basis of a one-year field monitoring of ten cypress populations in different climatic conditions; daily mean temperature was registered in each population and Cupressaceae pollen concentration in the atmosphere of Florence was monitored. Several methodologies were applied on the aerobiological data in order to identify the main pollen season (MPS) of Cupressaceae in Florence. The method that identified the MPS as 75% of the total annual pollen (MPS75) showed the best correlation with the phenological phase, which corresponded to the dispersal period of cypress. A clear relationship among male cypress phenology, daily mean temperature and Cupressaceae airborne pollen was shown. A phenological model able to simulate male cypress development was finally realized and validated on the basis of 6 years of aerobiological data. The model can predict the starting and ending date of the MPS75 of Cupressaceae in Florence and can be used fruitfully by the allergic population that can profit by the possibility of beginning an antiallergic therapy several days before the first symptoms and ending it when the pollen concentration in the air is low.

Keywords Cypress phenology · Airborne pollen · Phenological model · Pollinosis

Introduction

Cupressus sempervirens, belonging to Cupressaceae family, is an evergreen resinous tree that releases an enormous amount of anemophilous pollen (Belmonte et al. 1999). For this reason, it has been recognized to be responsible for a large part of the total annual amount of airborne pollen in several Mediterranean areas. In the city of Cordoba, southern Spain, Cupressaceae pollen represents at least 30% of the total pollen count during the winter season (Ruiz de Calvijo et al. 1988), whereas in Italy and Albania it reaches 20–40% of the annual pollen rain (Mandrioli et al. 2000; Priftanji et al. 2000).

Moreover, in the last few decades, Cupressaceae pollen has been identified as a source of increasing pollinosis in the Mediterranean countries such as France (Panzani et al. 1986), Spain (Subiza et al. 1995) and Italy (Caiaffa et al. 1993; Papa et al. 2000). It is also responsible for winter pollinosis in a period of the year when no other allergenic plants are flowering (Caramiello et al. 1991; D'Amato e Liccardi 1994).

In recent years, phenological, biometeorological and aerobiological investigations on allergenic plants have gained greater importance because of the increasing prevalence of allergic diseases worldwide. Many of these studies were performed with the aim of building a phenological model that is able to forecast the start, duration and severity of the pollen season at the local level (Rodriguez-Rajo et al. 2004); this is important to plan the start of antiallergic treatment before the beginning of pollination in order to optimize its effectiveness (Laaidi 2001).

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T. Torrigiani Malaspina (✉) · L. Cecchi ·
M. Morabito · S. Orlandini
Interdepartmental Centre of Bioclimatology,
University of Florence, Florence, Italy
e-mail: tommaso.torrigiani@unifi.it

M. Onorari · M. P. Domeneghetti
Articolazione Funzionale di Aerobiologia,
ARPAT, Pistoia, Italy

Moreover, the forecast of cypress pollen season is particularly important for the allergic population, as the period of pollination may last for more than a month because of the gradual mechanism of microsporophyllous maturation (from the bottom to the top of the flower) and, furthermore, because pollination shows a high variability year by year, depending on the meteorological factors (Hidalgo et al. 2003). Calleja and Farrera (2003) showed that the dates of maximum pollination may differ by up to 29 days and precocity in pollination seems to run parallel to the ongoing global warming.

Recent studies have pointed out the importance of improving aerobiological investigations by using phenological monitoring: it is well demonstrated that the aerobiological spectrum reflects the composition of local vegetation, but it also strongly depends on a multitude of factors such as meteorological conditions, pollen production and dispersal capacity, plant number and the localization of the pollen trap (Keynan et al. 1989). Consequently, it cannot be assumed that airborne pollen is equivalent to phenology, and phenological observations are needed to provide more accurate information on the airborne spectrum (Hidalgo et al. 2003). Phenological observations are also very useful in choosing the best methodology to calculate the start and the end of the pollen season in a given area, on the basis of aerobiological data (Jato et al. 2006).

Several aerobiological and phenological investigations on *C. sempervirens* have been carried out in the Mediterranean area (Hidalgo et al. 1999, 2003; Galan et al. 1998a, 1998b), but no phenological model has been created so far. The Cupressus genera is not indigenous to Italy, but *C. sempervirens* represents a very important and dominant species in Tuscany, Central Italy, and especially in the Florence area. It was introduced by the Etruscans and Romans and nowadays its abundance is due both to plentiful reforestations all around the city and successive naturalization, and to ornamental use in parks and graveyards. Nevertheless, phenological and aerobiological investigations on *C. sempervirens* in Tuscany are not found in literature. Several other species of the Cupressaceae family are present in the provincial district of Florence (*Juniperus* spp., *Taxus Baccata*, *Cupressus arizonica*), but their presence is small. They are used ornamentally in gardens and parks in the urban area.

The first aim of this study was to investigate the relationship between meteorological variables and the male cypress flower's development, and between male cypress flowering and the atmospheric concentration of airborne cypress pollen, in order to provide an exhaustive knowledge of phenological and aerobiological trends of cypress in Florence. The overall aim was to set a phenological model able to simulate the development of male cypress

flower, in order to predict the start and end of the cypress main pollen season on the basis of meteorological conditions, and in particular on the daily mean temperature.

Materials and methods

This investigation was performed in the provincial district of Florence, Tuscany region, Central Italy. Florence ($\lambda = 11^\circ 11'E$; $\Phi = 43^\circ 47'N$) is 50 m above sea level (asl) in a closed valley bottom at the foot of the Apennines and extends along the plain in a SE-NW direction. It is crossed by the Arno river and is surrounded by hills on the south and mountains on the north, which rise to almost 1,000 m. The city has a climate, which can be defined as Mediterranean semi-continental, with cold winters and hot summers. The coldest month is January, with an average temperature of about 6°C. The warmest months are July and August, with an average temperature of 24°C. The period under study was autumn 2004 to early spring 2005.

Phenological observation

Weekly visits to 60 individuals, selected from ten populations of cypress located in the provincial district of Florence were carried out during the period January 1–April 30, 2005 in order to monitor the phenological development of male flower in each population. Five populations were placed on the extra-urban hills around Florence; the other five were selected in the urban area, close to the Arno river valley. The populations were displaced from a minimum of 49 m asl to a maximum of 370 m asl. (Table 1).

Table 1 Cypress populations under study

Population	Altitude (m)	Code	Latitude (m)	Longitude (m)	Esp (°)
Urban populations					
Isolotto	49	U1	4849520	677964	0
Anconella	53	U2	4848333	685135	0
Fabbricotti	84	U3	4851556	681538	130
Poggio	118	U4	4846621	681063	300
San Miniato	135	U5	4847800	682368	0
Extra-urban populations					
Compiobbi	145	EX1	4850058	689652	200
Belmonte	192	EX2	4844009	686431	0
M. Ceceri	249	EX3	4852896	684459	200
Frati	295	EX4	4852910	684432	320
Villamagna	370	EX5	4847976	6913683	22

Monitoring was performed by a specialized operator and it consisted in measuring the size and identifying the pheno-phase of eight randomly selected male flowers of each plant. Previous experience on this genus shows that this methodology is a reliable means of describing the phenology (Hidalgo et al. 2003). Five phenological phases were considered, as described by Hidalgo et al. (2003), based on dimension, color, stage of microsporogenesis and blossoming behavior of androecium. The phenophases identified are the following: Phenophase 1 (PHP1) — bud differentiation, male flower size $1.2 \text{ mm} \pm 0.3$, divided into two sub-phenophases; Phenophase 2 (PHP2) — immature flower, male flower size $3.0 \text{ mm} \pm 0.5$, divided into three sub-phenophases; Phenophase 3 (PHP3) — near to flowering, male flower size $4.9 \text{ mm} \pm 0.5$; Phenophase 4 (PHP4) — full flowering, male flower size $7.0 \text{ mm} \pm 1.0$; Phenophase 5 (PHP5) — senescent period, male flower size $7.0 \text{ mm} \pm 1.5$. The starting date of each phase corresponded to the day when 50% of the flowers monitored showed the given phase.

Aerobiological monitoring

Cupressaceae daily pollen concentration was collected during the period 1994–2005; 2005 data were used to correlate phenological and aerobiological data and to build the model. Data from the period 1994–2004 were used to validate the model; as aerobiological data of several years resulted in being incomplete, only data from the years 1994, 1995, 1998, 2000, 2002 and 2004 were used to validate the model. The air was sampled by an instrument (VPPS 2000 provided by Lanzoni srl, via Michelino 93b Bologna, Italy) that applies the principle initially proposed by Hirst (Hirst 1952) and recommended by the International Biological Program (Benninghoff and Edmonds 1972). The instrument was located on the flat roof of a building situated in the city of Florence 20 m ($\lambda = 11^\circ 15' \text{E}$; $\Phi = 43^\circ 48' \text{N}$) above ground level and far away from walls and protection. The sampler was regulated to handle airflows of 10 l/min and it was at all times oriented to the wind by a wind vane. Pollen grains impacted on a cylindrical drum covered with a plastic strip coated with a uniform layer of adhesive solution (Mandrioli et Puppi 1978). Every week, the strip was removed and cut into seven equal segments corresponding to the 7 days of the week. The samples were examined using optical microscopes. The pollen grains were generally counted not on the whole $14 \times 48 \text{ mm}$ sampled area, but on a fraction of the entire deposit of particles. The slides were examined on parallel (longer side of the slide) horizontal sweeps using the continuous sweep technique. The minimum number of horizontal sweeps is the one that assures a total observed area not less than 18% of the sampled area

(statistical count). The results were expressed as the number of daily pollen grains per cubic meter of air. Data were provided by 'Articolazione Funzionale di Aerobiologia-ARPAT, Pistoia'.

The aerobiological data were used to calculate the main pollen season (MPS); on the basis of previous studies carried on by several authors (Nilsson et Persson 1981; Andersen 1991; Emberlin et al. 1993; Spieksma et al. 1998; Giner et al. 2002) in different environments and for different species, different methodologies were adopted: the MPS was considered as the period with 75, 80, 90 and 95% of the total amount of pollen during the season. The total amount of pollen during the season was calculated as a sum of the number of daily pollen grains per cubic meter of air.

These calculated MPS were compared with the full flower period (PHP4) that resulted from phenological monitoring. The method that identified the best correlation between MPS and PHP4 was adopted to identify the start and the end of the MPS to be used to build the model and to analyze its performance.

Meteorological measurements: in order to correlate phenological data with meteorological conditions, hourly temperature was recorded close to each cypress population under study. Temperature was obtained by HOBO H8 Pro Temperature and Relative Humidity data loggers provided by 'Onset Computer Corporation', 470 MacArthur Boulevard Bourne, MA, USA.

In order to analyze the phenological model performances, hourly meteorological data of air temperature ($^\circ \text{C}$) in Florence, for the period 1993–2004, were obtained from the urban weather station located at the center of Florence (Coordinate Gauss-Boaga x : 1681423 y : 4849368) and managed by the Regional Office for Environmental Protection in Tuscany. Mean daily temperatures were calculated for each year.

The phenological model was set up with the method of the degree day accumulation that consists of cumulatively summing the daily average temperatures above a thermal threshold from a determined date until each phenophase. Different thermal thresholds, above which the daily temperature can be considered effective, were tested, from 0 to 10°C . The model was applied for each threshold and for each population, and then the mean μ and the standard deviation σ of the sum of the temperatures were calculated. The more appropriate threshold was the one that minimized the ratio σ/μ , which corresponds to the coefficient of variation for all the populations.

In order to validate the model, 6 years of aerobiological and meteorological data from the period 1994–2004 were utilized. For each year considered to validate the model, the observed MPS was calculated from aerobiological data with the methods of 75%, and the phenological development of male cypress flower was simulated on the basis of

meteorological data. On the basis of this simulation, the starting date and ending date of PHP4 were calculated (predicted MPS). Starting and ending date of observed and predicted MPS were confronted and statistically correlated, and the errors (differences in days between predicted and observed MPS) were evaluated applying several methodologies. In particular, mean absolute error, root mean square error, mean bias error and standard deviation were calculated.

Results

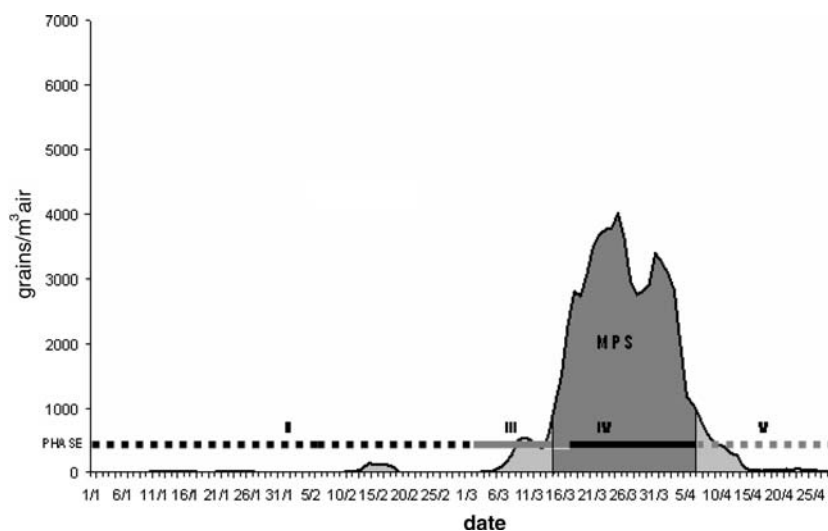
Phenological monitoring

PHP3, PHP4 and PHP5 were followed and described from the beginning to the end (see Table 2). The shortest period was PHP3, lasting from 10 to 17 days, with an average

Table 2 Starting date and duration of the phenophases monitored

Population	Starting date of phase			Length of phase (days)	
	3	4	5	3	4
U1	28 Feb	12 Mar	29 Mar	14	16
U2	26 Feb	14 Mar	6 Apr	16	24
U3	7 Mar	17 Mar	2 Apr	10	16
U4	6 Mar	17 Mar	30 Mar	11	13
U5	10 Mar	22 Mar	6 Apr	12	14
EX1	8 Mar	20 Mar	3 Apr	11	15
EX2	8 Mar	21 Mar	3 Apr	13	13
EX3	23 Feb	11 Mar	29 Mar	17	18
EX4	7 Mar	20 Mar	11 Apr	13	22
EX5	9 Mar	20 Mar	4 Apr	11	15
Average				13	18

Fig. 1 Trend of pollen concentration and phenophases duration. The peak of pollen concentration that precedes MPS is due to the flowering of *C. arizonica*



value of 13 days. PHP4, the most important period for allergological applications because it coincides with the dispersal period, persisted for an average of 18 days with a minimum of 13 days and a maximum of 24. There is a clear relationship between the duration of the dispersal period and the altitude of the population; in general, those with a length of PHP4 above 20 days stand above 250 m asl, and all urban populations showed a length of PHP4 less than 17 days.

Aerobiological monitoring and determination of the MPS

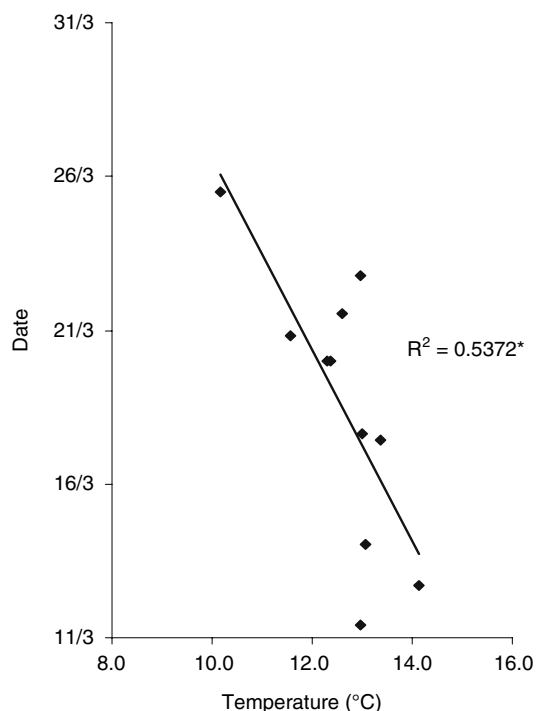
Cupressaceae pollen concentration in 2005 in Florence and cypress phenophases duration are shown in Fig. 1. Total annual count in this year reached 72,991 grains/m³ of air with a maximum daily value of 5,858. Table 3 shows the starting and ending date of the PHP4, and the starting and ending date of the MPS calculated with different methodologies on the year of the study. Comparing PHP4 with the four MPS, it emerges that the method for finding MPS, which best correlated with PHP4 is the one that considers MPS as the period that includes 75% of the total annual count (MPS75). In the following analysis of this study, only MPS75 will be considered.

Meteorological conditions–male cypress phenology relationship

Statistical analysis pointed out the relationship between daily mean temperature and male cypress phenology (Figs. 2, 3). In particular, the correlation between daily mean temperature and PHP4 is significant at the 0.05 level, and between daily mean temperature and PHP5 at the 0.01 level. These results show that the development of the male

Table 3 Starting date and ending date of the PHP4, starting date and ending date of the MPS calculated with different methodologies in 2005, and error in days

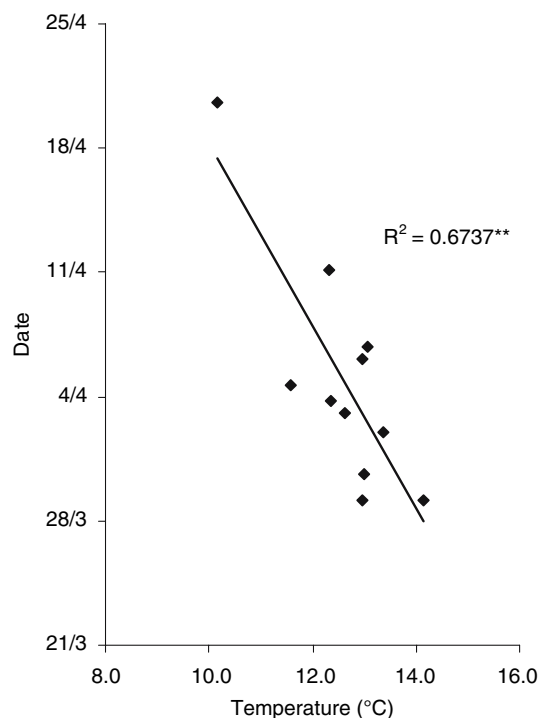
Main flower period (PHP4)		Main pollen season calculated with different methods							
		95%		90%		80%		75%	
Start	End	Start	End	Start	End	Start	End	Start	End
19/3	6/4	8/3	11/4	10/3	9/4	15/3	7/4	16/3	6/4
Error (days)		11	5	9	3	4	1	3	0

**Fig. 2** Starting date of phenological phase 4 vs. daily mean temperature. * = Correlation is significant at the 0.05 level

cypress flower strongly depends on meteorological conditions and in particular on daily mean temperature; the modeling of this relationship can be used to simulate the development of male cypress flower in order to forecast the start and duration of PHP4, and consequently of the MPS75.

Forecasting model

Tables 4 and 5 show the mean (μ), the standard deviation (σ) and the coefficient of variation (σ/μ) of the sums of temperature needed to reach respectively the starting date and the ending date of the MPS for the considered thresholds. The coefficient of variation was minimal for the threshold of 0°C, resulting respectively in 0.0552 for the

**Fig. 3** Starting date of phenological phase 5 vs. daily mean temperature. ** = Correlation is significant at the 0.01 level

starting date and 0.0515 for the ending date. The sum of temperature request resulted respectively in 1386.2°C and 1576.9°C.

Validation of the model

Table 6 shows the predicted and observed starting and ending dates of the MPS in the years considered to validate the model. The model's forecasting of the start of the pollen season resulted in errors ranging from 1 to 6 days, whereas its forecasting of the end date resulted in errors ranging from 0 to 9 days. Figures 4 and 5 show the result of the linear regression of observed versus predicted starting date and ending date of the MPS. Both correlations are significant ($P < 0.05$).

Discussion

Aerobiological monitoring and determination of the main pollen season

It is well known that the methodology adopted to determine the MPS may influence the results in aerobiological investigations (Jato et al. 2006). In the case of this study, for example, choosing one of the most commonly used methods in literature, such as 95, 98% or others that in-

Table 4 The method of summing temperature: GDD calculated with several thresholds needed to predict the starting date of the pollen season

Population	Sum of mean temperature (°C) above the thresholds (°C) of					
	0	1	2	3	5	10
U1	1502.3	1334.7	1181.7	1033.5	777.3	340.7
U2	1300.9	1131.9	993.9	865.5	634.1	261.4
U3	1434.7	1264	1112.0	968.2	721.0	297.6
U4	1434.8	1264.6	1113.6	969.3	721.0	295.6
U5	1458.3	1284.9	1130.9	984.8	734.0	297.0
EX1	1367.1	1193.3	1050.3	912.4	670.0	261.9
EX2	1397.3	1226.2	1077.2	935.5	693.4	270.4
EX3	1360.3	1202.4	1058.4	919.1	684.2	287.5
EX4	1362.1	1187.1	1048.1	913.6	674.0	269.8
EX5	1243.7	1081.9	945.9	818.9	600.1	222.6
Mean μ	1386.2	1217.1	1071.2	932.1	690.9	280.5
Standard deviation σ	76.5	74.7	68.4	61.4	51.0	31.2
Coef. of variation σ/μ	0.0552	0.0614	0.0638	0.0659	0.0738	0.1111

Table 5 The method of summing temperature: GDD calculated with several thresholds needed to predict the ending date of pollen season

Population	Sum of mean temperature (°C) above the thresholds (°C) of					
	0	1	2	3	5	10
U1	1714.8	1531.2	1362.2	1198.0	909.8	393.2
U2	1567.6	1376.6	1216.6	1066.2	790.8	308.2
U3	1628.3	1442.6	1275.6	1116.8	839.6	341.4
U4	1589.0	1406.7	1243.7	1087.5	815.2	329.7
U5	1632.1	1444.7	1276.7	1116.6	837.8	330.9
EX1	1526.4	1339.5	1183.5	1032.6	764.2	291.1
EX2	1543.3	1360.2	1199.2	1045.5	779.4	296.4
EX3	1569.1	1394.2	1233.2	1076.8	807.9	326.4
EX4	1595.7	1399.7	1239.7	1084.2	802.7	300.3
EX5	1402.5	1226.6	1076.6	935.6	688.8	242.6
Mean μ	1576.9	1392.2	1230.7	1076.0	803.6	316.0
Standard deviation σ	81.3	79.1	73.7	67.5	57.1	39.2
Coefficient of variation σ/μ	0.0515	0.0568	0.0599	0.0627	0.0711	0.1240

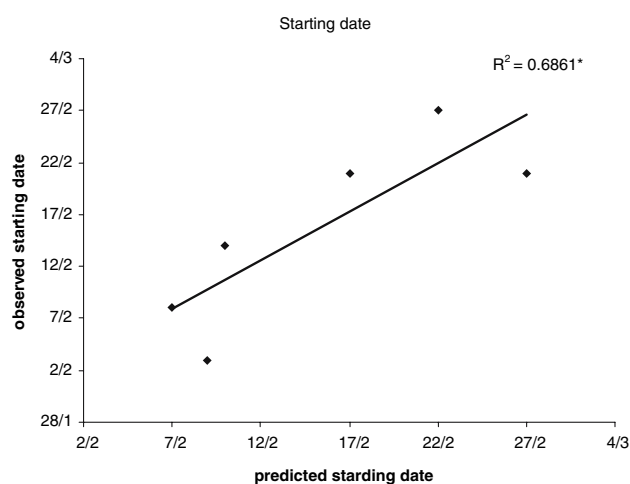
clude most of total annual pollen count, the MPS should result longer than PHP4. For example with the method of 95%, the starting date of MPS should occur 11 days before the beginning of PHP4 and end 5 days after. These differences also depend on the way the starting and ending date of PHP4 have been determined. In this study, PHP4 was considered as the average of the PHP4 of the ten populations. Choosing a different method to determinate PHP4, such as from the starting date of the earliest population to the ending date of the last population, the results will be obviously different and will be better correlated with an MPS calculated with a method that includes a greater part of the total annual count. As appears in recent publications, the limit of the pollen season should be set in accordance with the aim of the survey, and phenological observations are recommended to choose the best meth-

odology (Jato et al. 2006; Hidlago et al. 2003). As the overall aim of these investigations was to set a phenological model able to simulate the start and end of the MPS, and as MPS was defined with the method that guarantees the best correlation with PHP4, we can assume that this study complied with these recommendations.

The use of an optical microscope to identify pollen grains did not allow us to distinguish the pollen of *C. sempervirens* from the pollen of other Cupressaceae; however, we have to consider the very small presence of other Cupressaceae in Florence, with respect to *C. sempervirens*, and consequently the scanty contribution to the total annual count of Cupressaceae pollen. Moreover, the periods of pollination of the other Cupressaceae present in Florence (*T. baccata*, *C. arizonica* and *J. communis*) occur in different periods of the year with respect to the period of

Table 6 Differences between predicted and observed starting and ending date of MPS, and between start and end MPS 2005 with start and end MPS in the others years

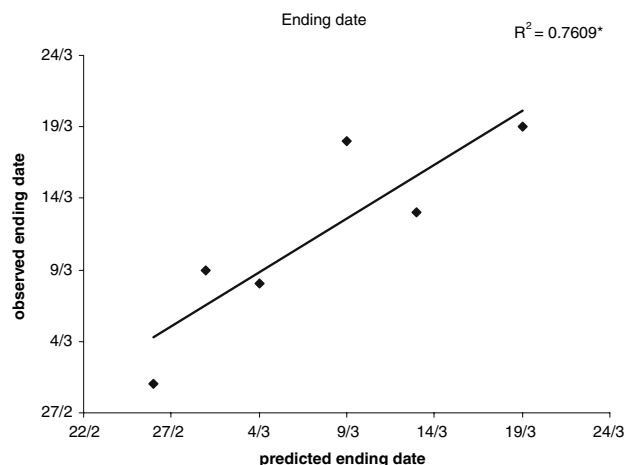
Year	Predicted		Observed		Error (days)	
	Start	End	Start	End	Start	End
1994	9 Feb	4 Mar	3 Feb	8 Mar	6	–4
1995	10 Feb	1 Mar	14 Feb	9 Mar	–4	–8
1998	7 Feb	26 Feb	8 Feb	1 Mar	–1	–4
2000	17 Feb	9 Mar	21 Feb	18 Mar	–4	–9
2002	27 Feb	13 Mar	21 Feb	13 Mar	6	0
2004	22 Feb	19 Mar	27 Feb	19 Mar	–5	0
	Mean absolute error				4.33	4.17
	Mean bias error				–0.33	–4.17
	Root mean square error				0.82	10.2
	Standard deviation				5.09	3.82

**Fig. 4** Observed starting date of MPS vs. predicted starting date of MPS. * = Correlation is significant at the 0.05 level

pollination of *C. sempervirens*; so pollen production of these species do not influence Cupressaceae pollen concentration in the period of aerobiological monitoring (January–May). Only *C. arizonica* flowers during the period under study and the pollen peak is clearly recognizable, occurring approximately 1 month before MPS75 (Fig. 1). Anyway, the method of 75%, adopted to determine Cupressaceae pollen season, ensures the exclusion of these pollen from the MPS and allow as to assimilate Cupressaceae MPS to *C. sempervirens* MPS.

Setting and validating the phenological model

A phenological model able to simulate male flower development for *C. sempervirens* in Florence is now available. On the basis of the methods applied, the model

**Fig. 5** Observed ending date of MPS vs. predicted ending date of MPS. * = Correlation is significant at the 0.05 level

predicts the starting and the ending dates of MPS that correspond to the day when the count of pollen reaches 12.5 and 87.5% of the total annual count, respectively.

In the 6 years of validation, the model produced errors ranging from 1 to 6 days in predicting the starting date and from 0 to 9 days in predicting the ending date. One of the sources of these errors is related to the differences between the thermal sensor used to set the model and the one used to validate it. In fact, daily mean temperature used to set the model was obtained from HOBO, whereas daily mean temperature used to validate the model was obtained from a weather station. Despite these errors, the model gives a satisfactory result, also considering that the cypress pollen season shows a high variation, and in a Mediterranean environment the starting and ending date of the cypress pollen season can differ up to 1 month each year (Hidalgo et al. 2003; Calleja et Ferrera 2003). As emerges in this study, the variability might be higher in Florence. The forecast of the start and end of the cypress pollen season on the basis of the mean starting and ending date might produce errors of 1 month or more. This fact makes the phenological model more useful in Florence than in areas with less variability.

This model can be fruitfully utilized by the allergic population, since it provides information useful to begin antiallergic therapy some days before the symptoms appear and to end it when the pollen concentration in the air is low. This is of particular importance in Florence, where cypress pollen concentration reaches several thousand grains/m³, because of the very high diffusion of this species in the surrounding area. Moreover, the impact of this pollen on the allergic population in Italy is very high and shows an increasing trend in the last few decades, as in several Mediterranean countries (Panzani et al. 1986; Papa et al. 2001). Meteorological forecasts are nowadays more and

more reliable, especially the first 72 h, so it would be possible to forecast the effective starting date at least 1 week before. Considering that symptoms of cypress pollinosis may be severe (Ariano et al. 2002), and that treatment should begin some days before (Michel and Bousquet 1997), a reliable forecast could present the allergic population with the possibility of preventing most of the symptoms. Furthermore, the ability of the model to forecast the ending date could also be useful for giving information on the duration of the pollen season and allowing patients to stop treatment in a timely manner. As a limit, it should be noted that highly sensitized subjects usually have symptoms when the pollen concentration is low, both at the start and the end of the season, needing a longer treatment period; however, they are generally a small percentage of the whole allergic population.

In conclusion, the model is able to simulate cypress phenology and gives satisfactory results in forecasting the start and the end of the cypress pollen season, representing a new tool for pollen forecasting in Florence.

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