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Published version:

DOI:10.1007/s10453-016-9432-8

Novara, Cristina; Falzoi, Simone; La Morgia, Valentina; Spanna, Federico; Siniscalco, Consolata. Modelling the pollen season start in Corylus avellana and Alnus glutinosa. AEROBIOLOGIA. 32 (3) pp: 555-569.
DOI: 10.1007/s10453-016-9432-8

Modelling the pollen season start in Corylus avellana and Alnus glutinosa

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Received: 23 July 2015 / Accepted: 16 February 2016 / Published online: 27 February 2016_ Springer Science+Business Media Dordrecht 2016

Abstract Hazel (Corylus avellana L.) and black alder (Alnus glutinosa (L.) Gaertn.) are important sources of airborne pollen and represent an allergen threat during the flowering period. Researches on airborne pollen concentrations in both species are useful in allergology, as well as for fruit production for hazel. The aims of the present study were: (1) to investigate the relationships between environmental conditions and the airborne pollen concentration of hazel and black alder during the flowering period by correlation and multiple regression analysis and (2) to predict the pollen season start (PSS) by using a sequential model, in order to obtain a helpful tool in allergology and hazel cultivation. In this study, the applied method defines the pollen season as the period in which 90 % of the total season's catch occurred, using a data set of 18 years (1996–2014). The relationships between daily meteorological parameters (temperature, humidity, rainfall and wind speed) during the 14-day period that precedes the PSS and the PSS of hazel and black alder (day of the year) were investigated. The results showed that mean temperature and the number of rainy days before the PSS are the main factors influencing PSS for both taxa. Moreover, the chilling and heat needed to break dormancy were estimated in order to predict the PSS of both species. Different years and different thresholds of temperature and chill days were used to calibrate and validate the model.

Keywords Black alder _ Chilling units _ Hazel _ Pollen season start _ Sequential models

1 Introduction

Hazel (Corylus avellana L.) and black alder (Alnus glutinosa (L.) Gaertn.) are important sources of airborne pollen and represent an allergen threat during the flowering period. In particular, hazel and alder act as primers of allergic sensitization to late springflowering Betulaceae, and other pollen allergens and the clinical symptoms become more marked during the birch-pollen season (Emberlin et al. 1997; D'Amato et al. 2007; Smith et al. 2007). Pollen forecasts are helpful tools for allergy patients to plan pollen avoidance strategies and the medicine intake that reduces the symptoms (Pauling et al. 2014). Moreover, while the analysis on the reproductive biology of both hazel and black alder plays an important role in allergology, researches on hazel are also conducted for their importance in fruit production.

In Italy, the pollen season of C. avellana typically lasts from the middle of December to the end of March or early April, although interannual variation of pollen season start (PSS), length of pollen period emission and amount occurs (Mehlenbacher 1991; Frenguelli et al. 1997; Frenguelli and Bricchi 1998; Emberlin et al. 2007).

The peak flowering period for male and female flowers of hazel may not overlap on the same cultivar (Mehlenbacher 1991) and, generally, male catkins precede the occurrence of female flowers. When the catkins elongate, pollen is shed and dispersed by the wind and reaches female flowers.

The presence of pollen in the air can be used as a proxy of male flowering (Frenguelli et al. 1997), being a proxy a metric that can be used to infer information about a phenomenon without measuring the phenomenon itself (Luedeling et al. 2009a, b). When the spore trap starts to record high pollen concentrations in the air, the catkins are elongating and releasing pollen. During this phase, temperature, rainfall and atmospheric humidity play an important role because they could alter the flowering times and affect the airborne pollen concentration.

Several researches take into account the relationship between environmental factors, mainly temperature, and the start and trend of the pollen season (Mandrioli et al. 1998; Frenguelli et al. 1991, 1992, 1993; Frenguelli and Bricchi 1998; Gala´n et al. 2001; Emberlin et al. 2007; Linkosalo et al. 2008; Garcı´a- Mozo et al. 2009).

Temperature-based models are commonly used to predict the PSS or bud-burst. Spring warming models only take into account the influence of forcing temperatures (Cannell and Smith 1983; Siniscalco et al. 2014), while other models consider forcing and chilling (Chuine et al. 1999; Emberlin et al. 2007). Following three different approaches, these can be divided into parallel, sequential and alternating models. While spring warming models show very good performance in the great majority of middle springflowering and summer-flowering species, sequential and alternated models usually perform better in the winter-flowering and early spring-flowering ones (Siniscalco et al. 2014).

In this study, we only used a model based on the sequential approach. These models are based on the fact that temperate tree species require a period of rest and a period of quiescence (Cannell and Smith 1983; Lang et al. 1987). The first one is connected with physiological conditions, while, during the second one, the buds or flowers remain dormant until environmental conditions become favourable (Chuine et al. 1999; Siniscalco et al. 2014). Several models consider the need of accumulating chilling units to break rest followed by a period of forcing temperature to overcome quiescence. The transition from chilling to forcing is precisely determined by sequential models. Moreover, sequential models specifically describe the relationship between flowering start and temperature. Most winter-flowering trees, including hazel and black alder (Gonza´les-Parrado et al. 2006), have to survive periods of adverse climatic conditions, entering a period of dormancy; the onset of catkins growth requires a period of low temperatures followed by another of relatively warm temperatures. In some European sites, a trend towards earlier PSS dates has been observed and attributed to the climate change (Garcı´a-Mozo et al. 2002; Rodrı´guez-Rajo et al. 2003). Different cultivars of hazel require different amount of chilling hours to break their dormancy, as referred to in the literature (Mehlenbacher 1991). Male flowers end their dormancy after receiving from 100 to 860 h of chilling temperatures, between 0 and 7 _C, and female flowers need 290–1550 h of chilling temperatures, depending on genotype (Mehlenbacher 1991). Hazel requires a short period of forcing (Cˇ repinsˇek et al. 2012) that is different among cultivars: flowering time varies according to climate and, in particular, depends mainly on temperature (Frenguelli et al. 1991, 1992, 1993).

Black alder flowering usually starts at the end of January or at the beginning of February (Jato et al. 2000). In temperate regions, trees enter a dormant period during autumn and the start of chilling takes place at temperatures below 9.1 _C (Frenguelli et al. 1991). The chilling accumulation period starts in late October or the first fortnight of November and lasts until December or January (Frenguelli et al. 1991, 1993; Gonza´les-Parrado et al. 2006). Once chilling requirements is satisfied, heat accumulation starts. In general, the heat requirement is satisfied in a short period of time (Gonza´les-Parrado et al. 2006).

The processes involved in bud or flowers development are not well understood until now, and more physiological studies are needed to identify the biochemical responses to chilling and forcing temperatures (Lang et al. 1987). Although each species has a specific chilling requirement, this can also change from site to site and this is probably due to the adaptation of tree populations to the specific environmental conditions of sites (Luedeling et al. 2009b). In spite of this variability, knowledge of the relationships between temperature and flowering phenology in a site can be useful to predict the PSS and development for allergenic purposes, and to predict and help the choice of new areas outside its native range when the cultivation of a species has to be widened.

Daily airborne pollen concentrations of hazel and black alder during flowering are affected by temperature, precipitation, humidity, sunlight hours and wind speed. The strength of correlations between these meteorological factors and airborne pollen concentrations is different during the pre-peak and post-peak periods of the pollen season (Puc and Kasprzyk 2013), where the ‘‘peak’’ is the day with the highest pollen concentration in the air.

The aims of the present study are: (1) to investigate the relationships between environmental conditions and the PSS of two early flowering species (hazel and black alder) by a multiple regression method and (2) to test a method to predict the PSS in order to obtain a helpful tool in allergology and hazel cultivation. This method is based on a model proposed by Cesaraccio et al. (2004, 2005) to predict the bud-burst in orchard crops and deciduous forest species. Moreover, estimating the chilling requirements of hazel and the amount of winter chill at a given location is a central topic in agricultural research, ever since the cultivation ranges of this species have expanded in the last 20 years beyond its native range, also in the southern hemisphere (Luedeling et al. 2009a, b).

2 Materials and methods

2.1 Study area and climate conditions

Turin (45_04041ooN and 7_40o33ooE) is located in northern Italy and is surrounded by the western Alpine arch (Fig. 1). It is a 1 million inhabitants city, characterized by more than 70.000 ornamental trees planted along the city avenues and in the urban green areas.

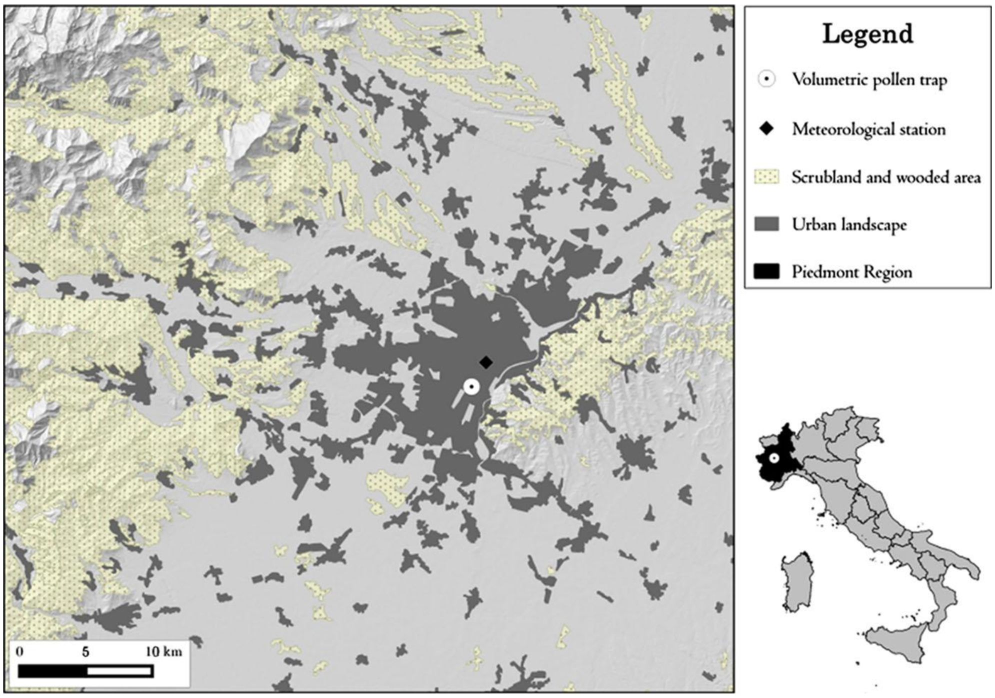


Fig. 1 Location of the volumetric pollen trap and meteorological station used in this study within the city of Turin. Dotted areas represent the possible sources of pollen of wild hazel and black alder

Turin is located at the border of the temperate and Mediterranean climates. Winters are moderately cold but dry, and summers are mild in the hills and quite hot in the plain. Rain falls mostly during spring and autumn. The average yearly amount of precipitation between 1983 and 2014 was 848.7 mm, but ranged from the wet 2010 (1327 mm) to the semiarid 2001 (438 mm). Mean annual temperature is 13.81 _C. Winds in the area are mainly blowing from the Alps to the city with a dominant west–east direction and are generally characterized by low speeds in all seasons. The mean yearly wind speed in the analysed period was 5.2 km/h, with very few days showing speeds higher then 8–10 km/h registered in each year.

2.2 Meteorological data

Meteorological data for the sampling area throughout different years were obtained at the meteorological station network of the Piedmont Regional Agency for the Protection of the Environment (ARPA Piemonte 2015). The variables considered were minimum, maximum and mean temperature (_C), minimum, maximum and mean humidity (%), rainfall (mm) and wind speed values (m/s) measured on daily basis.

2.3 Pollen monitoring

The aerobiological monitoring was carried out continuously in Turin, in the period 1983–2014, using a 7-day Hirst spore trap, VPPS 2000 Lanzoni, placed in the centre of the city at 12 m above the ground level and absorbing 10 L/min (Hirst 1952). The location of the spore trap was not changed in the monitoring period. The standard method of collection described in Mandrioli (1990) was used, and daily average pollen concentrations were expressed as grains/m^3 of air. Among the different taxa for which the daily pollen concentration was recorded, this study focused on two species: A. glutinosa (L.) Gaertn. and C. avellana L.

These two species are commonly present in the surrounding area of the city of Turin and produce an important fraction of the total amount of pollen monitored.

In the Turin area, A. glutinosa and A. viridis spread their pollen. It should be noted that, although their pollen grains cannot be easily discerned from one another morphologically, their pollen seasons in the study area do not overlap. The pollen season of A. glutinosa starts, in fact, typically around the middle of February and finishes around the end of March, while the pollen season of A. viridis starts around the second half of May, since this species is located in the mountain area. So, in this study, the period considered includes only the pollen produced by A. glutinosa.

2.4 Time series filtering, PSS, SPI computation and relationships with meteorological parameters

The study period starts in 1996 and ends in 2014. A preliminary analysis of the yearly pollen concentration time series of the two different taxa from 1986 to 2014 was performed in order to ascertain possible problems in the data. This preliminary analysis showed that in some of the years the pollen record showed long ''nodata'' periods within the typical pollen season due to incorrect spore trap functioning. These years were removed from the data set, and it was decided to consider the period from 1998 to 2000 and from 2002 to 2014 for C. avellana, and the period from 1996 to 2006, from 2008 to 2011 and from 2013 to 2014 for A. glutinosa. The number of removed years is different for the two species; in particular, it has been taken into account 16 years for hazel and 17 years for black alder.

Several methods have been proposed for defining the PSS and its end. In this study, adopting an approach already used by Emberlin et al. (2007), we defined the pollen season as the period in which 90 % (Nilsson and Persson 1981), or 95 % (Goldberg et al. 1988) or 98 % (Emberlin et al. 1993) of the total season's catch occurred. The main peak appearance date (MPA) was calculated for each year as the day in which the maximum value of daily pollen concentration is reached (Moriondo et al. 2001).

Pollen collected during the main pollen period was expressed as the seasonal pollen index (SPI = daily pollen concentration from the beginning to the end of pollen season calculated with the three methods; the SPI is dimensionless) (Moriondo et al. 2001).

The relationships between meteorological variables and the day of the year (DOY) of PSS of C. avellana and A. glutinosa were investigated using correlation and regression analysis. Values of the meteorological parameters, specifically mean temperature, rainfall (with 1-, 3-, 5-, 10-mm thresholds), wind speed and mean humidity, were calculated for the 14-day period preceding PSS. The 14-day period was adopted to highlight the mechanical effect of rainfall on the catkins and early dispersed pollen. Correlation analysis was performed for each threshold (90, 95 and 98 %) adopted to calculate the PSS dates. Each threshold for each taxon was preventively tested to assess the departure from normal distribution and thus to identify the most appropriate test (Pearson's r or Spearman's rho). The results obtained by correlation analysis were used to identify the threshold (90, 95 or 98 %) with which we obtained the higher correlation with the DOY of PPS. So, only one threshold was used for the subsequent regression analysis. For the latter, as DOY showed a continuous and normally distributed response variable, it was related to the meteorological covariates by linear regression. The regression analysis procedure involved three main steps. First, a multicollinearity analysis was performed among meteorological variables in order to remove possible issues of redundancy in subsequent analysis. Second, a global model involving only uncorrelated variables was fitted to the data, and third a backward model selection procedure was performed to finally identify the minimal adequate model to describe the data. Lastly, the analysis of standardized coefficients was performed to determine the most important variable. Correlation and regression analyses were performed with exploratory and descriptive purposes in order to highlight the influence of meteorological parameters on PSS.

All analyses were performed using R software (R Core Team 2014), version 3.1.1.

2.5 The applied model

The model proposed by Cesaraccio et al. (2004; 2005) to predict the bud-burst is a sequential model called Chill days (C_D) based on the concept of chill days (C_d) to break rest and accumulation of anti-chill days (C_a) to overcoming quiescence. Negative C_d values are accumulated until they reach a value called chilling requirement (C_R). The chilling requirement is met on the day when the C_d B C_R, which corresponds to breaking rest. On the following day, the model begins to add anti-chill days on each day starting at C_R, until C_R ? C_a C 0 at the predicted bud-burst. The chill days and anti-chill days both depend on the selection of a temperature threshold (T_C) and C_R, so these parameters are iterated to find the combination that best predicts the bud-burst dates (Cesaraccio et al. 2004, 2005).

There are five possible cases for calculating C_d and C_a that relate the maximum (T_x) and minimum (T_n) temperature to the threshold temperature T_C and 0 _C. In Table 1 are reported the equations for each of the five cases.

The DOY_{Sept1} of the PSS start date was calculated in the applied model from the 1st of September of the previous year for both taxa in order to simplify the computations.

Table 1 Chill days (C_d) and anti-chill days (C_a) equations for the five cases

Case	Temperature cases	Chill days	Anti-chill days
1	$0 \le T_C \le T_n \le T_x$	$C_d = 0$	$C_a = T_M - T_C$
2	$0 \le T_n \le T_C \le T_x$	$C_d = -\left[(T_M - T_n) - \frac{(T_x-T_C)^2}{2(T_x-T_n)}\right]$	$C_a = \frac{(T_x-T_C)^2}{2(T_x-T_n)}$
3	$0 \le T_n \le T_x \le T_C$	$C_d = -(T_M - T_n)$	$C_a = 0$
4	$T_n < 0 \le T_x \le T_C$	$C_d = -\left[\frac{T_x^2}{2(T_x-T_n)}\right]$	$C_a = 0$
5	$T_n < 0 < T_C < T_x$	$C_d = -\frac{T_x^2}{2(T_x-T_n)} - \frac{(T_x-T_C)^2}{2(T_x-T_n)}$	$C_a = \frac{(T_x-T_C)^2}{2(T_x-T_n)}$

Different cases depend on relationship between maximum (T_x), minimum (T_n) and mean (T_M) daily temperature, the threshold temperature T_C and 0 °C. (Table 2 in Cesaraccio et al. 2005)

The PSS of hazel and black alder was determined, applying the model which calculated the best values for the chill threshold (T_C) and the chilling requirement (C_R) by iteration to minimize the value of the root-mean-square error (RMSE) between predicted and observed data of PSS. Typically, the values for C_R vary between -1 and -100 chill days for both species, while T_C values vary from 4 to 8 _C for A. glutinosa and from 3 to 6 _C for C. avellana. All equations have been developed using software R (R Core Team 2014), version 3.1.1.

2.6 Test statistics

In order to evaluate the accuracy of Chill days and the agreement between observed and estimated data of PPS of C. avellana and A. glutinosa, four indices were taken into account. In particular, the mean absolute error (MAE; Shaeffer 1980, from 0 to ??, optimum 0), the relative root-mean-square error (RRMSE; Jørgensen et al. 1986; from 0 to ??, optimum 0), the efficiency index (EF, Nash and Sutcliffe 1970; from -? to 1, optimum 1) and the coefficient of residual mass index (CRM; Loague and Green 1991; from -? to ??, optimum 0) were used to validate the model proposed by Cesaraccio et al. (2004, 2005) both in the calibration phase and in the validation phase.

Both the MAE and the RRMSE measure how close the predictions are to the observed data and can range from 0 (best) and ??. The EF identifies inefficient models, as negative values indicate that the mean of all measures is a better predictor than the model used. The CRM indicates the tendency of the model to overestimate (if negative) or underestimate (if positive) the observed data.

3 Results

3.1 Time series filtering, PSS, SPI computation and relationships with meteorological parameters

The PSS of A. glutinosa and C. avellana was calculated using the 90 % threshold, showed the highest number of correlations and the strongest relationship with meteorological variables (Table 3), and therefore it was decided to take into account this threshold for subsequent analyses. The use of the 98 % method had the advantage of including the maximum number of pollen counts in the analysis (Emberlin et al. 2007), but in this case, it showed the lowest correlation with the meteorological variables for both taxa.

The PSS for black alder showed a range of 36 days. The PSS date varies from 1st February in 2008 to 9 March in 2005 (Table 2). For hazel, there is a range of 47 days in the start dates, which varies from 8 January in 2013 to 24 February in 2010 (Table 2). It was not possible to highlight an early flowering trend during the studied period.

The length of pollen season of black alder and hazel in Piedmont varied over the years from a minimum of 20 days for alder in 2010 and 22 days for hazel in 2002 to a maximum of 115 days for alder in 2000 and 99 days for hazel in 2013 (Table 2).

Table 2 Summary of pollen season parameters for *A. glutinosa* and *C. avellana*

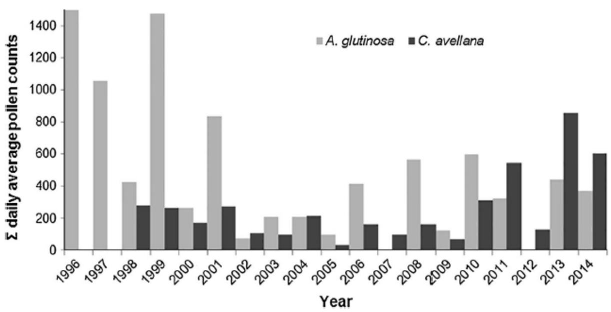
Alnus glutinosa					*Corylus avellana*				
Year	PSS (DOY)	MPA (DOY)	SPI	PS's length (days)	Year	PSS (DOY)	MPA (DOY)	SPI	PS's length (days)
1996	62	83	1380	27	1998	32	44	249	32
1997	44	50	957	102	1999	24	36	231	29
1998	43	60	383	100	2000	22	39	155	91
1999	38	54	1336	43	2002	30	41	97	22
2000	35	51	206	115	2003	37	63	88	42
2001	45	46	690	104	2004	15	37	193	89
2002	40	42	64	110	2005	32	32	31	72
2003	56	63	190	91	2006	38	59	146	54
2004	56	64	190	92	2007	18	26	88	55
2005	68	72	88	73	2008	21	61	151	40
2006	59	59	373	27	2009	40	57	64	40
2008	32	44	501	31	2010	55	61	284	32
2009	55	67	110	93	2011	36	41	488	41
2010	56	62	543	20	2012	10	89	115	81
2011	41	50	289	103	2013	8	69	784	99
2013	38	69	401	40	2014	20	67	538	55
2014	44	54	337	28					

DOY is the day from January 1. Pollen season start (PSS) was calculated using 90 % threshold. Also reported are the main peak appearance (MPA) date in DOY, the seasonal pollen index (SPI) and the duration of pollen season (PS's length)

The main peak appearance date (MPA) was calculated for each year as the day in which the maximum value of daily pollen concentrations is reached and varied from 11 February in 2002 to 23 March in 1996 for black alder and from 26 January in 2007 to 29 March in 2012 for hazel.

The duration of the main pollination period was 70 days for black alder and 54 days for hazel. The total season counts of pollen for both taxa are reported for all years (Fig. 2). The highest SPI of black alder pollen was 1380 grains in 1996, while the lowest was 64 grains in 2002. For hazel, the highest annual total sum was recorded in 2013 (784 grains) and the lowest one was recorded in 2005 (31 grains). For both species, a slight biennial pattern can be observed in the total annual sums with years characterized by high pollen concentration alternated to years with the lowest pollen sums.

Fig. 2 Total season counts of *A. glutinosa* and *C. avellana* from 1996 to 2014 in Turin. Daily average counts were calculated as pollen grains/m^3. Season pollen was calculated using 90 % threshold



3.2 Correlation and regression analysis

Table 3 summarizes the results of the correlation tests performed to assess the relationships between hazel and black alder DOY of PSS and meteorological variables. Only the variables with significant correlation values with PSS are shown.

Table 3 Results of the correlations analysis between black alder and hazel DOY of PSS and meteorological variables

Meteorological variables (14-day period before PSS)	*A. glutinosa* PSS						*C. avellana* PSS					
	90 % threshold		95 % threshold		98 % threshold		90 % threshold		95 % threshold		98 % threshold	
	r or *rho*	*p* value	*r* or *rho*	*p* value	*r* or *rho*	*p* value	*r* or *rho*	*p* value	*r* or *rho*	*p* value	*r* or *rho*	*p* value
Min temperature	–	–	–	–	–	–	–	–	–	–	−0.536	0.058
Max temperature	−0.785	<0.001	–	–	–	–	−0.530	0.062	−0.515	0.059	−0.537	0.057
Mean temperature	−0.787	<0.001	–	–	–	–	−0.591	0.033	−0.540	0.045	−0.596	0.031
Mean humidity	–	–	–	–	−0.55	0.049	–	–	–	–	–	–
Number of rainy days >1 mm	–	–	–	–	–	–	0.054	0.054	–	–	–	–
Number of rainy days >3 mm	0.573	0.032	–	–	–	–	0.643	0.017	0.510	0.061	–	–
Number of rainy days >5 mm	–	–	–	–	–	–	0.594	0.032	–	–	–	–
Number of rainy days >10 mm	–	–	0.532	0.049		–	0.527	0.064	0.599	0.023	–	–
Average wind speed	–	–	–	–	–	–	–	–	0.534	0.049	–	–

The table shows the correlation coefficients (*r* or *rho*, as appropriate according to the distribution of each variable) and the corresponding *p* value (two-tailed)

On the basis of the obtained results, we considered the 90 %threshold, which showed the most significant correlations with meteorological data. For hazel and black alder, there was a significant negative correlation between maximum and mean temperature and PSS, while the number of rainy days with different thresholds showed a positive relationship with the PSS; in particular for black alder, the correlation coefficient for the number of days with rainfall higher than 3 mm was positive. Taking into account the results of correlation analyses, multiple regression backward analysis was only performed for each species for the 90 % threshold (Tables 4, 5).
For black alder, the best model (structure 2) highlighted a significant negative effect of the mean temperature and positive effect of the occurrence of rain events ([10 mm) (Table 4), while for hazel, the best model (structure 2) included a significant negative effect of the mean temperature and positive effect for rain events ([3 mm) (Table 5).

Table 4 Results of linear regression analysis between DOY of PSS of black alder and meteorological variables during the period of 14 days preceding the PSS

PSS 90 %	Model structure 1		Model structure 2		Model structure 3	
	Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
Intercept	101.216	0.003	97.600	<0.001	71.746	<0.001
Mean temperature	−5.190	0.016	−5.352	0.002	−5.416	0.002
Max humidity	−0.336	0.189	−0.291	0.201		
Occurrence of rain events >10 mm	6.960	0.114	7.585	0.047	7.789	0.041
Wind average speed	−1.064	0.890				
R^2 adjusted	0.613		0.625		0.546	

Results (unstandardized coefficients) are shown only for the global model and the two best alternative model structures identified via backward selection

Table 5 Results of linear regression analysis between DOY of PSS of hazel and meteorological variables during the period of 14 days preceding the PSS

PSS 90 %	Model structure 1		Model structure 2		Model structure 3	
	Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
Intercept	53.967	0.201	45.828	0.077	51.772	0.001
Mean temperature	−6.330	0.060	−6.379	0.044	−5.928	0.024
Mean humidity	−0.099	0.794				
Occurrence of rain events >3 mm	12.483	0.129	11.401	0.083	8.702	0.108
Wind average speed	3.801	0.816	5.243	0.719		
R^2 adjusted	0.322		0.392		0.311	

Results (unstandardized coefficients) are shown only for the global model and the two best alternative model structures identified via backward selection

Finally, the analysis of standardized coefficient showed that the mean temperature represented the most important parameter for both taxa (mean temperature standardized coefficient for alder's best model -0.642 and for hazel's best model -0.556), highlighting a negative relationship between the PSS and the mean temperature of the previous period. Moreover, for both species, the rainfall during the period before of PSS showed a positive effect on start date (standardized coefficient for alder's best model 0.377 and for hazel's best model 0.434).

3.3 The applied model

All the equations have been computed in order to identify the best values for T_C and C_R to minimize the RRMSE value (Table 6) and to obtain the best prediction of PSS dates.

Table 6 Test statistics of calibration and validation of the applied model for black alder and hazel

	A. glutinosa		*C. avellana*	
	Calibration	Validation	Calibration	Validation
T_C	3.7	3.7	3.0	3.0
C_R	−65	−65	−52	−52
SD observed	9.410	9.943	12.694	11.369
SD predicted	9.236	8.873	12.044	11.900
Intercept	3.281	−18.343	−5.049	18.179
Slope	0.980	1.112	1.035	0.893
p value	0.000	0.000	0.000	0.000
RRMSE	2.402	1.492	2.272	4.599
MAE	2.067	1.262	1.953	3.517
EF	0.926	0.975	0.963	0.816
CRM	0.000	0.001	0.000	0.015
R^2	0.926	0.985	0.965	0.875

Threshold of temperature (T_C), chilling requirement (C_R), standard deviation of observed and predicted values, relative root-mean-square error (RRMSE), mean absolute error (MAE), modelling efficiency (EF) and coefficient of residual mass (CRM)

Moreover, the relationship among the amount of chilling and forcing units and the difference between observed and predicted DOY_{Sept1} has been investigated. Figure 3 shows the relation between observed and predicted DOY_{Sept1} in the calibration and validation phases for both species (Table 6).

For black alder, the years 2005, 2006, 2008, 2009, 2010, 2011, 2013, 2014 were used for the calibration of the model, and from 1996 to 2004, for validation (Fig. 3a, b). For hazel, the years from 2006 to 2014 were used for calibration and the years from 1998 to 2005 were used for validation (Fig. 3c, d). The best values for chill threshold (T_C) and chilling requirement (C_R) for black alder and hazel are shown in Table 6.

The temperature threshold (T_C) was different for the two species and varies between 3.7 _C for black alder (Fig. 4) and 3.0 _C for hazel (Fig. 5). The chilling requirement (C_R) was met on the day when the accumulation of chill days (C_d) is less than or equal to C_R and was different for the two species. In fact, black alder reached this value of C_R (-65 chill days) some days before hazel (-52 chill days). The date in which the accumulation of forcing units occurs varies from 19 December to 7 January for black alder in calibration phase and from 15 December to 2 February in the validation one (Fig. 4). The date for C. avellana varies from 15 December to 12 January (calibration) and from 11 December to 2 February (validation) (Fig. 5). The model applied is characterized by a high value of R_2 (0.926 for calibration and 0.985 for validation for A. glutinosa, and 0.965 and 0.875 for calibration and validation, respectively, for C. avellana). The EF was very close to the optimum (0.926 and 0.975 for calibration and validation, respectively, for A. glutinosa; 0.963 and 0.816 for calibration and validation, respectively, for C. avellana). The relative root-meansquare error (RRMSE) between observed and simulated onset dates was lower than 2.5 and 5 % for calibration and validation phases, respectively. The coefficient of residual mass (CRMs) was always close to 0, and significance (p value) was less than 0.05.

4 Discussion

The results obtained in this paper underline the influence of meteorological variables (mean temperature and rainfall) during the 14 days immediately preceding the male flowering and, consequently, the emission of pollen. Correlation and regression analyses have demonstrated the strong influence of temperature during the previous period of PSS, but they have also further highlighted that the temperature is not the only variable which influences the emission of pollen. In fact, the analysis of standardized regression coefficients showed the importance of the rainfall during the 14 days that precede anthesis. A prolonged period of rain could delay the opening of the anthers and cause a delay of PSS; indeed, pollen shedding is low during rainy periods. Conversely, a low relative humidity determines the opening of anthers and the release of pollen as the result from dehydration of walls of anther sacs (Kozlowski and Pallardy 2002).

Even if the period immediately before flowering plays an important role in the PSS, a longer period starting in the previous months drives the development of the reproductive structures and therefore prepares flowering and subsequently the pollen season.

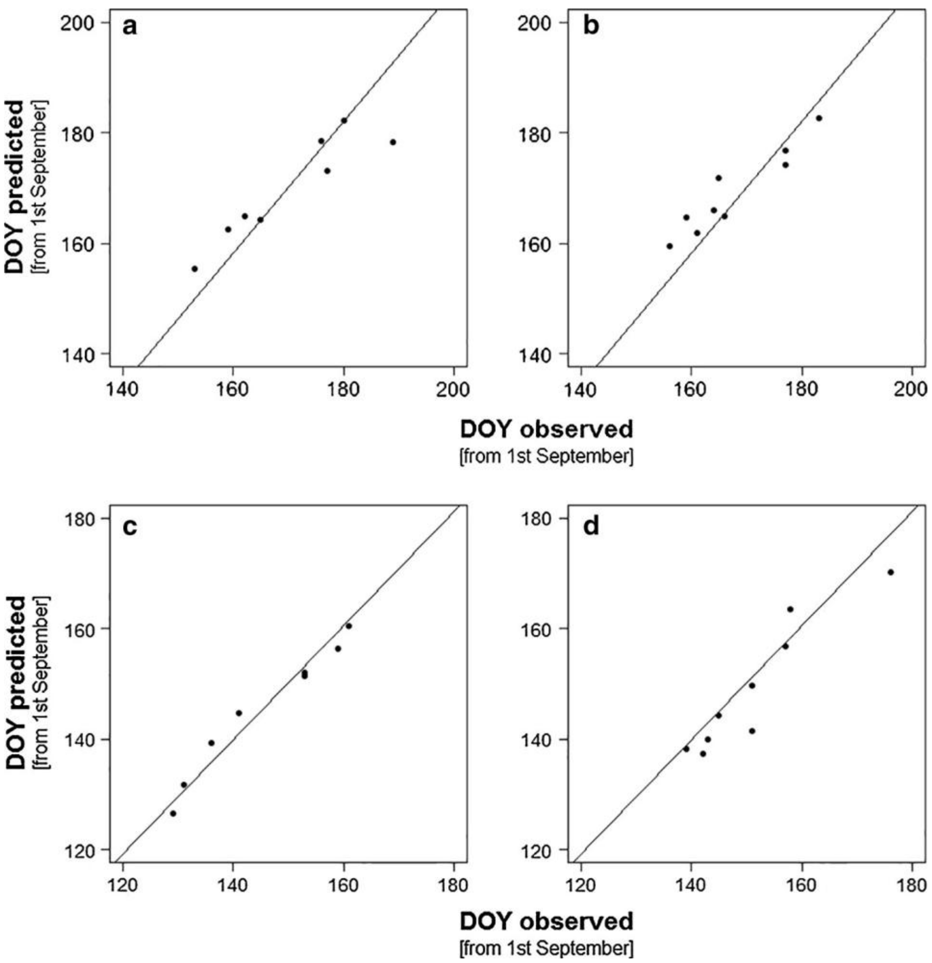


Fig. 3 Observed vs predicted day (days from 1st September) of occurrence of the PSS of A. glutinosa, in calibration (a) and validation (b) phases and in calibration (c) and validation (d) phases of C. avellana

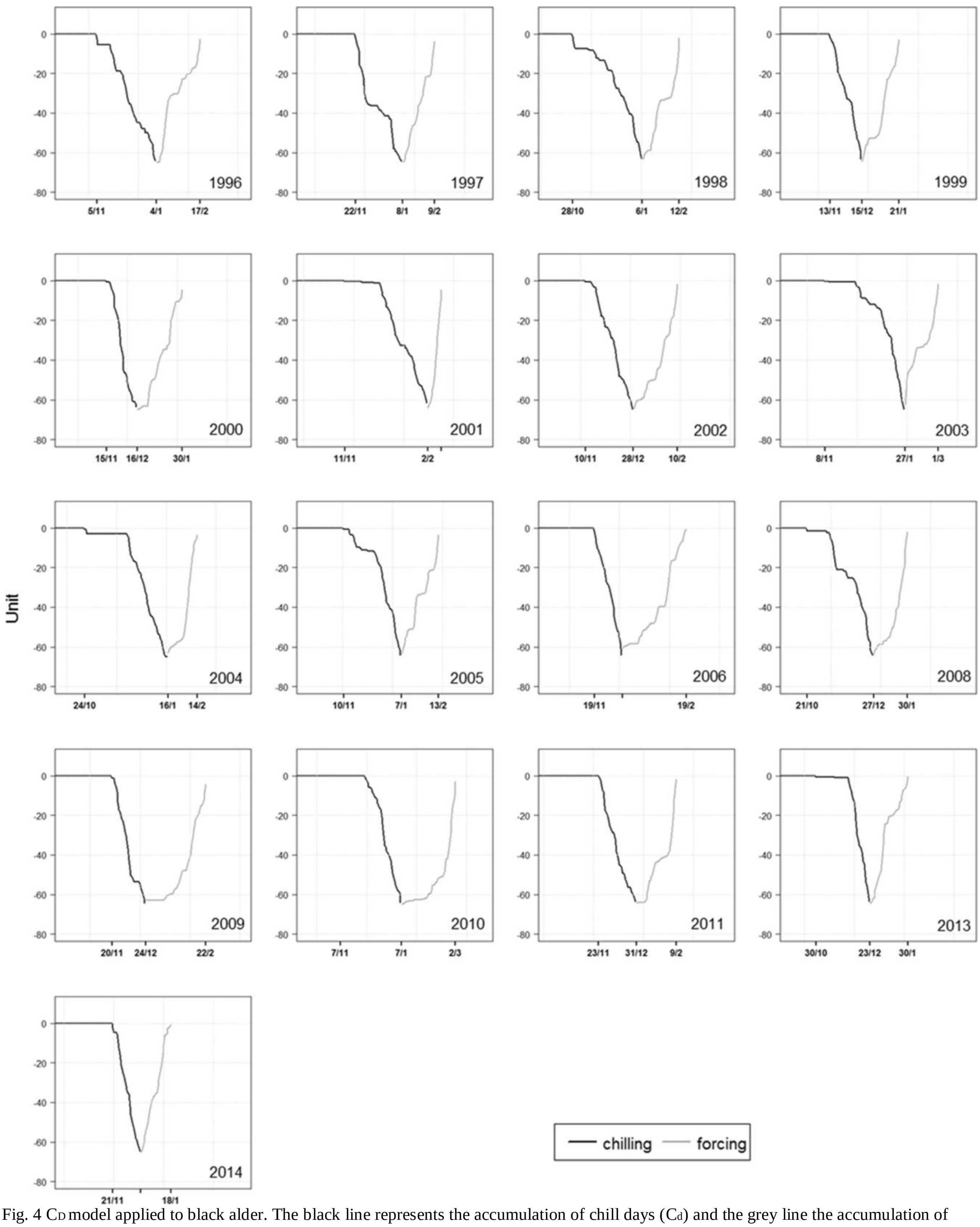


Fig. 4 C_D model applied to black alder. The black line represents the accumulation of chill days (C_d) and the grey line the accumulation of anti-chill days (C_a). When the chilling requirement (C_R) is met, the rest is broken. The quiescence is overcome when the anti-chill days curve achieves zero

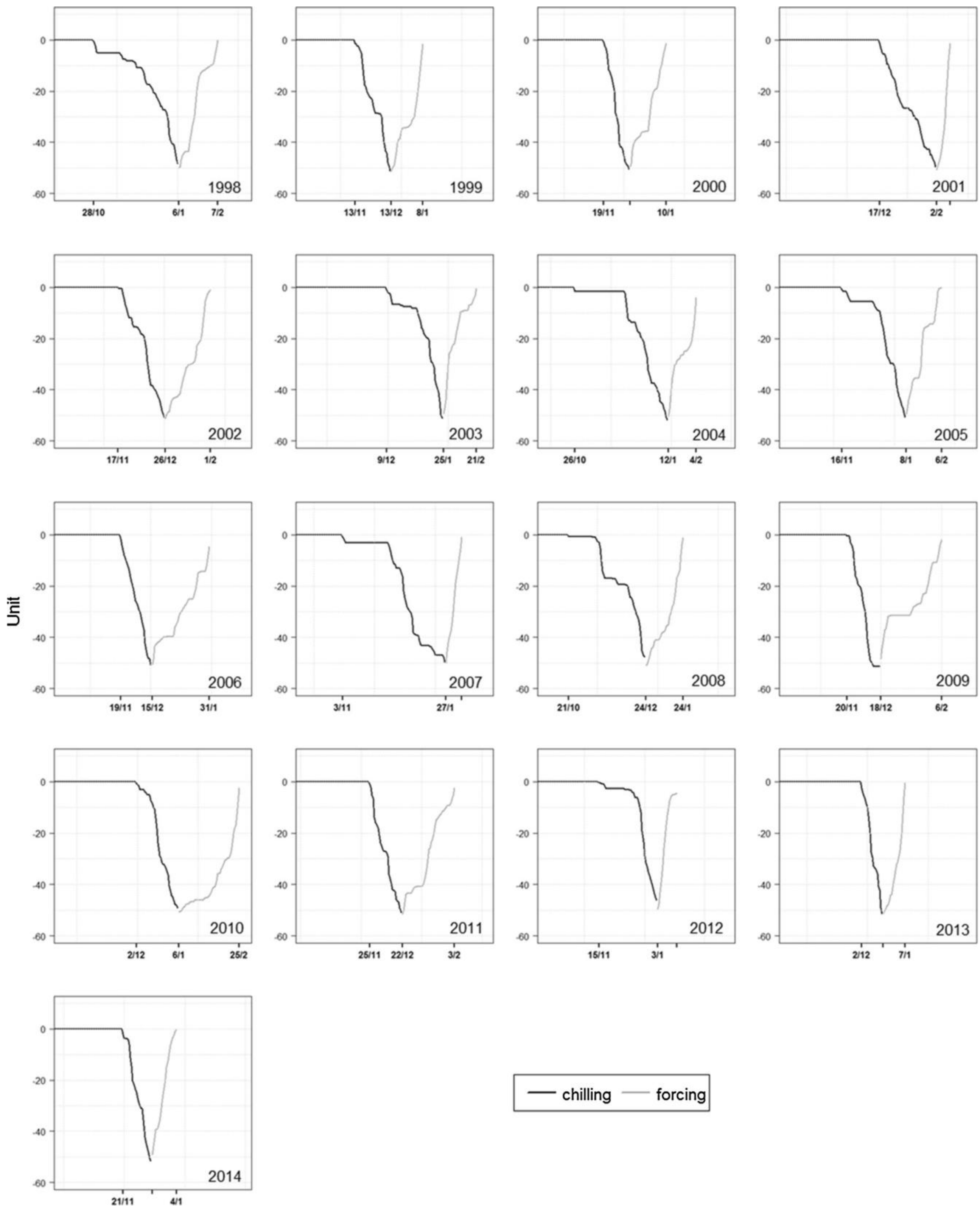


Fig. 5 C_D model applied to hazel. The black line represents the accumulation of chill days (C_d) and the grey line the accumulation of anti-chill days (C_a). When the chilling requirement (C_R) is met, the rest is broken. The quiescence is overcome when the anti-chill days curve achieves zero

The results obtained by the sequential C_D model confirmed that accumulation of chilling is fundamental for the two studied species (Mehlenbacher 1991; Gonza´les-Parrado et al. 2006). The applied model divides dormancy into a rest phase and a quiescent phase. The first phase is broken by chilling (C_d) accumulation, and the second one is overcome by temperature forcing (C_a) accumulation.

The best results were obtained with a threshold temperature of 3.7 _C and -65 chill days for A. glutinosa and 3.0 _C and -52 chill days for C. avellana. The applied model showed a high performance in predicting the date of PSS of black alder and hazel, and these results can be attributed to the model setting. In fact, the model is based on a phenological

approach, and it begins to accumulate at a certain phenological stage (e.g. harvest or leaf fall or flowering) (Cesaraccio et al. 2004). This aspect is very important because it can lead to a better interpretation of the phenomenon and an easier application in different environments.
The C_D model better matches the current theory on dormancy than the classical chill unit models, and in general, the model proved to be more accurate when used to predict bud-burst of tree species (Cesaraccio et al. 2004). Moreover, the C_D model always gives a prediction having better performance not only of the bud-burst, but also of PSS, as these results demonstrated. The C_D model predicted PSS within 5 days, although interannual variation can occur as a consequence of alternating of years characterized by high pollen concentration followed by years with low pollen sums (Mehlenbacher 1991; Frenguelli et al. 1997; Frenguelli and Bricchi 1998; Emberlin et al. 2007).
Conversely to what referred to in the literature (Garcı´a-Mozo et al. 2002; Rodrı´guez-Rajo et al. 2003), a general trend of an earlier PSS has not been registered in this paper.
The length of pollen season of black alder and hazel in this study varied over the years from a minimum of 20 days for alder in 2010 and 22 days for hazel in 2002 to a maximum of 115 days for alder in 2000 and 99 days for hazel in 2013, proving a great variability for both taxa from year to year.
The high variability of PSS from year to year and the importance of temperature (chilling and forcing during the previous months) and of mean temperatures and rainfall during the 14 days immediately before flowering should be taken into account in particular for hazel, for its importance in the fruit production. When this species is exported in new places, the climatic conditions are of primary importance in choosing the cultivation areas because the choice of cultivars may only partially overcome the environmental limits.

5 Conclusions

The present article demonstrated the influence of temperature and rainfall during the 14-day period that precedes the PSS and the applicability of the C_D model proposed by Cesaraccio et al. (2004, 2005) to predict the PSS for black alder and hazel, confirming the importance of chilling for these two early flowering species. The results showed a very high variability of the PSS, the pollen season length and the total pollen production from year to year, while an early flowering trend was not observed.
The applied model was demonstrated to be a useful tool to predict the PSS of these two allergenic species in order to support allergologists and patients. Moreover, since temperature (chilling and forcing), mean temperature and rainfall of the previous period resulted to be the factors driving the reproductive phases, a careful analysis of the climatic conditions should be performed when hazel has to be exported in a new cultivation area outside its native range.

Acknowledgments The authors would like to thank Luisella Reale for the precise and patient work of counting of the pollen grains, Enrico Corrado Borgogno Mondino for his fundamental contribution in applying the statistical model, and the Piedmont Regional Agency for the Protection of the Environment (ARPA) for the meteorological data. This research has been funded by a grant of Istituto Nazionale della Previdenza Sociale (INPS) in the context of the PhD in Biology and Applied Biotechnologies in the thesis entitled ''Influence of environmental factors on reproductive biology in hazel (C. avellana L.)''.

References

ARPA Piemonte. (2015). Banca Dati Meteorologica [Meteorological database]. http://www.regione.piemonte.it/ambiente/aria/rilev/ariaday/annali/meteorologici.

Cannell, M. G. R., & Smith, R. I. (1983). Thermal time, chill days and prediction of budburst in Picea sitchensis. Journal of Applied Ecology, 20, 951–963.

Cesaraccio, C., Spano, D., Snyder, R. L., & Duce, P. (2004). Chilling and forcing model to predict bud-burst of crop and forest species. Agricultural and Forest Meteorology, 126(1–2), 1–13.

Cesaraccio, C., Spano, D., Snyder, R. L., & Duce, P. (2005). Corrigendum to ''Chilling and forcing model to predict bud-burst of crop and forest species'' [Agricultural and Forest Meteorology, 126 (2004) 1–13]. Agricultural and Forest Meteorology, 129, 211.

Chuine, I., Cour, P.,&Rousseau, D. D. (1999). Selecting models to predict the timing of flowering of temperate trees: Implications for tree phenology modelling. Plant Cell and Environment Journal, 22, 1–13.

C ˇ repinsˇek, Z., S ˇ tampar, F., Kajfezˇ-Bogataj, L., & Solar, A. (2012). The response of Corylus avellana L. phenology to rising temperature in north-eastern Slovenia. International Journal of Biometeorology, 56, 681–694.

D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., et al. (2007). Allergenic pollen and pollen allergy in Europe. Allergy, 62, 976–990.

Emberlin, J., Mullins, J., Corden, J., Millington, W., Brooke, M., Savage, M., & Jones, S. (1997). The trend to earlier birch pollen seasons in the U.K.: A biotic response to changes in weather conditions? Grana, 36(1), 29–33.

Emberlin, J., Savage, M., & Woodman, R. (1993). Annual variation in Betula pollen season in London 1961–1990. Grana, 32, 359–363.

Emberlin, J., Smith, M., Close, R., & Adams-Groom, B. (2007). Changes in the pollen seasons of the early flowering trees Alnus spp. and Corylus spp. In Worcester, United Kingdom, 1996–2005. International Journal of Biometeorology, 51, 181–191.

Frenguelli, G., & Bricchi, E. (1998). The use of pheno-climatic model for forecasting the pollination of some arboreal taxa. Aerobiologia, 14, 39–44.

Frenguelli, G., Bricchi, E., Romano, B., Mincigrucci, G., Ferranti, F., & Antognozzi, E. (1992). The role of air temperature in determining dormancy release and flowering of Corylus avellana L. Aerobiologia, 8, 415–418.

Frenguelli, G., Ferranti, F., Romano, B., Bricchi, E., Mincigrucci, G., & Fornaciari, M. (1993). Temperature influence on differentiation and release of hazel pollen. Allergie et Immunologie, 25(4), 147–149.

Frenguelli, G., Ferranti, F., Tedeschini, E., & Andreutti, R. (1997). Volume changes in the pollen grain of Corylus avellana L. (Corylaceae) during development. Grana, 36(5), 289–292.

Frenguelli, G., Spieksma, F. T. H. M., Bricchi, E., Romano, B., Mincigrucci, G., Nikkels, A. H., et al. (1991). The influence of air temperature on the starting dates of the pollen season of Alnus and Populus. Grana, 30(1), 196–200.

Gala´n, C., Carin˜anos, P., Garcı´a-Mozo, H., Alcazar, P., & Dominguez Vilches, E. (2001). Model for forecasting Olea europaea L. airborne pollen in South-west Andalusia, Spain. International Journal of Biometeorology, 45, 59–63.

Garcı´a-Mozo, H., Gala´n, C., Belmonte, J., Bermejo, D., Candau, P., Dı´az de la Guardia, C., et al. (2009). Predicting the start and peak dates of the Poaceae pollen season in Spain using process-based models. Agricultural and Forest Meteorology, 149, 256–262.

Garcı´a-Mozo, H., Gala´n, C., Belmonte, J., Dı`az de la Guardia, C., Ferna`ndez, D., et al. (2002). Modelling start of oak pollen season in different climatic zones in Spain. Agricultural and Forest Meteorology, 110, 247–257.

Goldberg, C., Buch, H., Moseholm, L., & Weeke, E. V. (1988). Airborne pollen records in Denmark, 1977–1986. Grana, 27, 209–217.

Gonza´les-Parrado, Z., Fuertes-Rodrı´guez, C. R., Vega-Maray, A. M., Valencia-Barrera, R. M., Rodrı´guez-Rajo, F. J., & Ferna´ndez-Gonza´les, D. (2006). Chilling and heat requirements for the prediction on the beginning of the pollen season of Alnus glutinosa (L.) Geartner in Ponferrada (Leo´n, Spain). Aerobiologia, 22, 47–53.

Hirst, J. (1952). An automatic volumetric spore-trap. The Annals of Applied Biology, 36, 257–265.

Jato, V., Frenguelli, G., Rodrı´guez-Rajo, F. J., & Aira, M. J. (2000). Temperature requirements of Alnus pollen in Spain and Italy (1994–1998). Grana, 39, 240–245.

Jørgensen, S. E., Kamp-Nielsen, L., Christensen, T., Windolf- Nielsen, J., & Westergaard, B. (1986). Validation of a prognosis based upon an eutrophication model. Ecological Modelling, 32, 165–182.

Kozlowski, T. T., & Pallardy, S. G. (2002). Acclimation and adaptive responses of woody plants to environmental stresses. The Botanical Review, 68(2), 270–334.

Lang, G. A., Early, J. D., Martin, G. C., & Darnell, R. L. (1987). Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. HortScience, 22(3), 371–377.

Linkosalo, T., Lappalainen, H. K., & Hari, P. (2008). A comparison of phenological models of leaf bud burst and flowering of boreal trees using independent observations. Tree Physiology, 28(12), 1873–1882.

Loague, K., & Green, R. E. (1991). Statistical and graphical methods for evaluating solute transport models: Overview and application. Journal of Contaminant Hydrology, 7(1), 51–73.

Luedeling, E., Zhang,M., Luedeling, V.,&Girvetz, E.H. (2009a). Sensitivity of winter chill models for fruit and nut trees to clime changes expected in California’s Central Valley. Agriculture, Ecosystem and Environment, 133, 23–31.

Luedeling, E., Zhang, M., McGranahan, G., & Leslie, C. (2009b). Validation of winter chill models using historic records of walnut phenology. Agricultural and Forest Meteorology, 149, 1854–1864.

Mandrioli, P. (1990). The Italian aeroallergen network. Aerobiologia, 6, 2–59.

Mandrioli, P., Comtois, P., Dominguez Vilches, E., Galan Soldevilla, C., Isard, S., & Syzdek, L. (1998). Sampling; principles and techniques. In P. Mandrioli, P. Comtois, & V. Levizzani (Eds.),Methods in aerobiology.Bologna: Pitagora. Mehlenbacher, S. A. (1991). Chilling requirement of hazel cultivars. Scientia Horticulturae, 47, 271–282.

Moriondo, M., Orlandini, S., De Nuntiis, P., & Mandrioli, P. (2001). Effect of agrometeorological parameters on the phenology of pollen emission and production of olive trees (Olea europea L.). Aerobiologia, 7, 225–232.

Nash, J. E., & Sutcliffe, J. V. (1970). River flow forecasting through conceptual models part I. A discussion of principles. Journal of Hydrology, 10, 282–290.

Nilsson, S., & Persson, S. (1981). Tree pollen spectra in the Stockholm region (Sweden) 1973–1980. Grana, 20, 179–182.

Pauling, A., Gehrig, R., & Clot, B. (2014). Toward optimized temperature sum parameterizations for forecasting the start of the pollen season. Aerobiologia, 30, 45–57.

Puc, M., & Kasprzyk, I. (2013). The patterns of Corylus and Alnus pollen seasons and pollination periods in two Polish cities located in different climatic regions. Aerobiologia, 29, 495–511.

R Core Team. (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/. Accessed July 2015.

Rodrı´guez-Rajo, F. J., Frenguelli, G., & Jato, V. (2003). Effect of air temperature on forecasting the start of the Betula pollen season at two contrasting sites in the South of Europe (1995–2001). International Journal of Biometeorology, 47, 117–125.

Shaeffer, D. L. (1980). A model evaluation methodology applicable to environmental assessment models. Ecological Modelling, 8, 275–295.

Siniscalco, C., Caramiello, R., Migliavacca, M., Busetto, L., Mercalli, L., Colombo, R., & Richardson, A. D. (2014). Models to predict the start of the airborne pollen season. International Journal of Biometeorology, 59(7), 837–848.

Smith, M., Emberlin, J., Stach, A., Czarnecka-Operacz, M., Jenerowicz, D., & Silny, W. (2007). Regional importance of Alnus pollen as an aeroallergen: a comparative study of Alnus pollen counts from Worcester (UK) and Poznan´ (Poland). Annals of Agricultural and Environmental Medicine, 14, 123–128.