

FACTORS INVOLVED IN THE PHENOLOGICAL MECHANISM OF *ALNUS* FLOWERING IN CENTRAL EUROPE

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Abstract: The objectives of this paper are to ascertain the main factors involved in the phenological mechanism of alder flowering in Central Europe by understanding the influence of the main meteorological parameters, the North Atlantic Oscillation (NAO) effect and the study of the Chill and Heat requirements to overcome dormancy. Airborne pollen (1995–2007) was collected in Poznań (Poland) by means a volumetric spore trap. Temperatures for February, and January and February averages of the NAO are generally key factors affecting the timing of the alder pollen seasons. Chilling accumulation (which started in Poznań at the beginning of November, while the end took place during the month of January) of 985 CH with a threshold temperature of -0.25°C, followed by 118 GDD°C with a threshold temperature of 0.5°C, were necessary to overcome dormancy and produce the onset of flowering. The calculated dormancy requirements, mean temperatures of the four decades of the year, and January and February average NAO index recorded during the period before flowering, were used to construct linear and multiple regression models in order to forecast the start date of the alder pollen seasons. Its accuracy was tested using data from 2007, and the difference between the predicted and observed dates ranged from 3–7 days.

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INTRODUCTION

In the temperate climatic zone of the Northern Hemisphere, trees belonging to the order Fagales, which include members of the Betulaceae and Fagaceae families, are important sources of allergenic pollen [9, 31, 41]. The major allergens of pollen from trees belonging to the Fagales order are structurally and immunochemically similar and therefore have a degree of cross reactivity [31, 36, 39]. This study focuses on pollen grains from a member of the Betulaceae family, *Alnus* (alder) [2]. Exposure to the pollen of *Alnus*, which is the one of the earliest Betulaceae to flower, can effectively extend the pollen season of *Betula*,

another member of the Betulaceae family and an important aeroallergen [11, 42]. Individuals can be sensitised to *Betula* pollen before the season begins and lower birch pollen concentrations may elicit stronger symptoms in those sensitive to birch pollen [7, 11]. *Alnus* pollen grains are also important pollinosis agents, but studying spatial and temporal variations in *Alnus* pollen concentrations is also essential for evaluating the ecological conservation of Central European woodland. Possible changes in the distribution of the Fagaceae family due to hotter early spring temperatures in recent years [8, 11] are of great economic importance to the timber and agricultural industries [4, 18].

Climatic parameters that are subject to variations, such as temperature, photoperiod or precipitation [16], induce physiological adaptations during the life cycles of arboreal plants, especially in tree species that flower at the beginning of spring. Thus, the growth of woody plants in winter slows down and they enter into a physiological state called “dormancy” in which cell water is prevented from freezing [46]. Dormancy is commonly separated into a “rest period”, which is a period of low temperatures when the buds remain dormant due to growth-arresting physiological conditions, followed by a “quiescent period” of unfavourable environmental conditions with relatively warm temperatures [5, 14, 46]. Knowing the amount of chilling and heat required by plants to overcome dormancy enables us to determine the onset of flowering [14, 15, 24, 37]. Since the presence of pollen in the air is the result of flowering, aerobiological data has been used in recent years as phenological information in order to calculate chilling and heat requirements [15, 18, 25, 38]. This paper aims to develop models to quantify the dormancy period, its duration and the consequent heat requirement needed to trigger flowering in early winter-flowering trees in Poznań, Central Europe. Classical Chilling Hour models do not separate dormancy into rest and quiescent periods [5]. In this study, a sequential model predicting the number of days of dormancy, based on the accumulation of Chill Hours during rest [3] and Growth Degree Days °C during quiescence [14] is described.

The influence of the North Atlantic Oscillation (NAO) on ecological processes was also evaluated. Temperature is described as the most important factor affecting phenological phases in temperate ecosystems [1, 35]. Plant phenology in this region is also influenced, via temperature, by year-to-year variations in the NAO [10, 45]. The NAO is a mode of interannual variability in atmospheric circulation associated with changes in the surface westerlies across the North Atlantic and into Europe [22, 32]. A lengthening of the active growing season in Europe has been related to increases in winter and spring temperatures, which may in turn be associated with strongly positive indices of the NAO [30, 35]. The NAO is more likely to affect ecological mechanisms in winter, although the link between winter indices of the NAO and climatic conditions may persist through to summer [35].

The objective of this paper is to ascertain the main factors involved in the phenological mechanism of alder flowering in Poznań by understanding the influence of the main meteorological parameters, the North Atlantic Oscillation (NAO) effect and the study of the Chill and Heat requirements to overcome dormancy. Finally, linear regression equations based in these results were produced in order to select an accurate model to predict the start date of the alder pollen season.

MATERIAL AND METHODS

Pollen monitoring. The Allergic Diseases Diagnostic Centre at the University of Medical Sciences and the Laboratory of Aeropalynology at Adam Mickiewicz University collected the pollen data for Poznań. Daily average *Alnus* pollen counts (1995–2007) were collected in Poznań by Burkard volumetric spore traps of the Hirst design [21]. In 1995 and 1996 the trap in Poznań was situated in an old district of the city. However, from 1997–2007 the trap was sited on the roof of a thirteen story university students’ dormitory (Eskulap), approximately 1 km south-west of the city centre [8]. *Alnus* pollen dataset from Poznań was spliced together to make single datasets running from 1995–2007. Two different counting methods were employed. From 1995–1999 pollen data were collected following the methods outlined by Stach [43] where pollen grains were counted along twelve latitudinal transects. From 2000–2007 this method was changed and pollen grains were counted along four longitudinal transects, which were divided into 2 mm (1 hourly) intervals.

In the neighbourhood around the pollen-monitoring site in Poznań, there are small parks, gardens, patches of grass and ruderal vegetation, and some trees. In Poland, there are three species of alder: *Alnus glutinosa*, *A. incana* and *A. viridis* (rare). Flowering usually begins at the end of February or the beginning of March, and lasts until April [27, 47, 48]. *Alnus incana* typically flowers first, followed by *A. glutinosa* about two weeks later. However, in years when spring warming occurs rapidly both *A. glutinosa* and *A. incana* can flower simultaneously, which may affect the length of the *Alnus* pollen season [26, 28].

Climate and meteorological data. Poland has a temperate continental climate with cold winters and warm summers. However, as well as having a continental climate, Poznań is located in an area of western circulation and, as a result, winds from the west and southwest predominate. Mean January and July temperatures in Poznań are -1.4°C and 19.2°C, respectively, and mean annual precipitation is approximately 528 mm [8, 43].

Meteorological data for Poznań were recorded at the Institute of Meteorology and Water Management site at Ławica Airport (52°25′N 16°49′E), situated approximately 4.25 km west of the pollen-monitoring site at Eskulap. Data for the NAO were obtained from the NAO index, calculated from Gibraltar and Reykjavik, which is maintained by P. D. Jones at the University of East Anglia, UK [10, 34].

Analysis of pollen seasons. It was decided to examine retrospective methods that define the season as the period in which 90% [33], 95% [19] and 98% [11] of the total seasons’ catch had been recorded. The method used for defining the start of the *Alnus* pollen season was selected after examining the results of Spearman’s rank correlation tests between different start dates of the alder pollen

seasons, and temperature data from the preceding months. Start dates calculated by using the pollen season period described by Nilsson & Persson [33], which includes 90% of total annual pollen (whereby the season starts when 5% of the total catch is achieved and ends when 95% is reached), generally showed the strongest relationship with meteorological variables. As a result, it was decided to use this method when describing the characteristics of the *Alnus* pollen season. It should be noted that this technique is often used for selecting the method for calculating the start of the pollen season of early flowering trees [24, 26, 38].

Chilling and Heat requirement. Chill requirements were calculated following the method of Aron [3], which is based on the accumulation of Chilling Hours (CH) [3, 24, 40]:

$$CH = 801 + 0.2523 B + 7.57 B^2 \times 10^{-4} - 6.51 B^4 \times 10^{-10} - 11.44 T_{\min} - 3.32 T_{\max}$$

CH = number of Chilling Hours during period
 $B = 24 D (\text{Threshold} - T_{\min}) / (T_{\max} - T_{\min})$

Where T_{\min} and T_{\max} are the average minimum and maximum temperatures recording during the period, respectively, and D is the length of the study period in days.

Chilling was deemed to have commenced when daily mean temperatures were $\leq 12.5^\circ\text{C}$ for two or more consecutive days. The value of 12.5°C was chosen to establish the start of the chilling period because this is the temperature below which winter flowering woody plants are considered to begin to satisfy their chilling requirements [14, 24, 37]. It is very difficult to predict the exact moment when the adverse period finishes. In our study, the end of chilling is marked as the first day when the daily mean temperature reached the minimum values and started to follow a positive trend [38], and the moment in which is registered the inflexion point of a polynomial second degree trend that followed temperatures from October of the preceding year to April of the same year as pollination. To calculate and quantify the most precise chilling requirements, a set of threshold temperatures from -3 – 8°C in steps of 0.25°C were assessed

The Heat requirements were obtained from the sum of the daily maximum and mean temperatures after deducting different base temperatures (from 0 – 8°C in steps of 0.25°C) and expressed as Growth Degree Days (GDD $^\circ\text{C}$) [15, 24]. GDD $^\circ\text{C}$ were calculated from the day after the chilling period each year to the start of the pollen season. Only temperatures above or equal to 0°C were used to calculate cumulative sums, negative temperatures were considered as 0°C [17, 25, 38].

Statistical analysis. Spearman's rank correlation tests were used to explore the relationships between dependent variables (start dates of the *Alnus* pollen seasons) and different independent variables (meteorological data) recorded in Poznań from 1995–2006. The results of correlation analysis were then used to produce simple and multiple

Table 1. Significant correlations between start dates of *Alnus* pollen seasons (1995–2006) and temperature and rainfall data recorded from the previous period to flowering. **Correlation significant at 0.01 level; *Correlation significant at 0.05 level.

		Start 5%	Start 2.5%	Start 1%
Average temperature	February	-0.888**	ns	ns
	10-day mean temperature: day 31–40	-0.893**	-0.791**	-0.748**
	10-day mean temperature: day 41–50	-0.687*	ns	ns
Rain	December	-0.680*	-0.792**	-0.771**
	10-day mean rain: day 31–40	-0.649*	-0.712*	-0.736**

regression models predicting the start of the pollen alder seasons. The regression models were constructed using data from 1995–2006, and its accuracy tested using data from 2007. The independent variables considered were mean monthly and 10-day-mean daily average temperature and daily rainfall data from September (the previous year) to March (in the year of pollination), and averages of the NAO from December–March (from the winter preceding pollination).

RESULTS

Start dates of the *Alnus* pollen season from 1995–2006, defined by using the 5%, 2.5% and 1% methods, were entered into correlation analysis with monthly and 10-day-mean temperature and rainfall data from October (the previous year) to February (in the year of pollination) (Tab. 1). The behaviour of the alder flowering followed a similar pattern during the years studied with a difference of 60–70 days in the start dates between years: varied from 2 February in 2002 to the 7 April in 1996. In recent years there has been a gradual delay (not significant) in the onset of the pollen seasons (Fig. 1). A non significant trend was also witnessed with the end and duration of the pollen seasons.

The results of correlation analysis between start dates of the *Alnus* pollen seasons in Poznań and meteorological data recorded during the previous period show that February temperatures (particularly the mean temperatures of its first decade) are generally the most important factor affecting the timing of the pollen seasons (Tab. 1). The significant negative relationships between start dates and temperature suggest that high temperatures at this time will produce earlier starts of the pollen season. Significant negative correlations also exist between the start dates of alder pollen seasons and rainfall of December and rainfall of the first decade of February.

In order to evaluate the effect that changes in the NAO could induce in the alder flowering, start dates of the

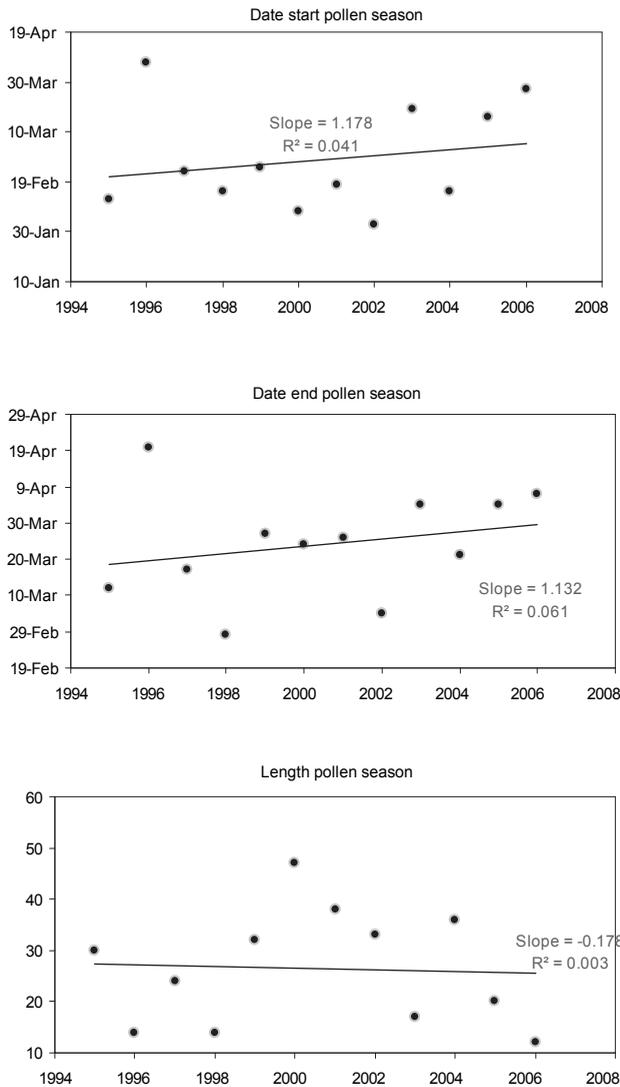


Figure 1. Dates of the start, final, and length of the *Alnus* pollen season calculated by using the 90% method [33] and its trends.

Table 2. Spearman's correlations between start dates of *Alnus* pollen season defined using the 5% method and winter averages of the NAO. **Correlation significant at 0.01 level; *Correlation significant at 0.05 level.

	R ²
NAO December–January–February average	-0.669*
NAO January–February average	-0.756**

alder pollen season were also entered into Spearman's rank correlation tests with winter averages of the NAO during the period before the pollen season (Tab. 2). Significant negative correlations were found between start dates of the alder pollen seasons and December–February indices of the NAO. The highest correlation coefficient was found between the NAO average of the months of January and February.

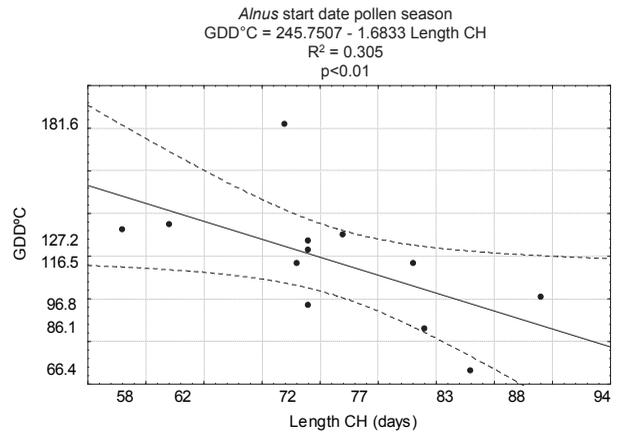


Figure 2. Model to predict the number of heat requirements (GDD°C) to overcome dormancy from length of the chilling period of *Alnus* and its regression lines.

Chilling requirements to overcome dormancy were calculated following the method of Aron (1983). Chilling accumulation, started in Poznań at the beginning of November while the end took place during the month of January. The length of the chilling requirements for overcoming the dormancy period varied annually with an average of 76 days (Tab. 3). The most accurate base temperatures ranged between 0°C and -1.25°C, and the lowest coefficients of standard variation were found with a base temperature of -0.25°C and an average chilling accumulation of 985 CH (Tab. 3). Once chilling requirements were attained, heat accumulation began and lasted until the onset of flowering (Tab. 4). The lowest coefficients of standard variation were found with maximum temperatures and a base temperature of 0.5°C. Considerable year-on-year differences in heat accumulation were recorded over the study period. The heat requirement for *Alnus* ranged between 182–66 GDD°C with an average amount of 118 GDD°C and duration of 41 days as average during the studied period (Tab. 4).

A number of significant correlations exist between alder start dates, the amount of Chill and Heat units accumulated, and the length of the accumulation periods (Tab. 5). The strongest positive relationship ($p < 0.01$) was obtained between the start of the pollen season and the length of the heat period, whilst negative correlation existed between the length of the chilling period and the number of GDD required to overcome dormancy.

The calculated dormancy requirements, temperatures, and indices of the NAO recorded during the period before flowering were used to construct linear and multiple regression models in order to forecast the start date of the alder pollen seasons (Tab. 6). Taking into account the meteorological parameters with highest correlation coefficients we propose different models in order to ascertain the most accurate: (a) equations based on meteorological variables; (b) based on the most significant NAO index; (c) combining all the aforementioned parameters; (d) considering the dormancy variables with high correlation coefficient. The

Table 3. Chilling requirements calculated following Aron method [3] during the study period taking into account different threshold temperatures. (Mean=average value, SD=standard deviation, CV(%)=Coefficient of standard variation in percentage). Final date and the length of the Chill period are also shown.

	Threshold Temperature							Final date	Length (days)
	-1.25	-1	-0.75	-0.5	-0.25	0	0.25		
1995	810	788	773	770	784	812	868	14-Jan	74
1996	1244	1196	1124	1023	890	721	512	28-Dec	58
1997	1258	1258	1226	1153	1031	848	595	2-Jan	62
1998	811	818	807	788	770	758	759	14-Jan	74
1999	1026	1090	1150	1200	1233	1240	1211	13-Jan	73
2000	777	782	804	841	894	959	1030	14-Jan	74
2001	796	777	767	772	795	835	893	3-Feb	94
2002	1196	1235	1250	1230	1164	1038	840	17-Jan	77
2003	1258	1256	1225	1158	1046	882		12-Jan	72
2004	930	991	1056	1120	1176	1217	1233	24-Jan	84
2005	802	782	769	769	788	825	879	28-Jan	88
2006	1133	982	1249	1246	1243	1240	1238	23-Jan	83
Mean	1003	996	1017	1006	985	948	914	16-Jan	76
SD	203.73	203.81	212.93	201.08	186.17	190.57	245.98	10.34	10.18
CV (%)	20.30	20.46	20.94	19.99	18.91	20.10	26.90	0.03	13.37

results of regression analysis showed high adjusted-R² values ranging from 0.305–0.826. The accuracy of the models was tested using data from 2007, which was not included in the construction of the models. The difference between the predicted and observed dates ranged from 3–7 days (Tab. 6). The more precise forecast model (with the high adjusted-R² value and the more precise predicted start alder pollen season date) consider the combination of the average mean temperature during the first decade of February and the average of the January and February NAO index, forecasting the start date of the alder pollen season with a delay of 3 days. On the contrary, the equation produced to forecast the GDD°C necessary to overcome dormancy (Fig. 2), considering as estimator the length of the chilling period (during 2007 the length of the Chill period was 87 days and the equation predicted a quantity of 99 GDD°C), forecast the *Alnus* 2007 pollen season with an advance of 4 days.

DISCUSSION

Ecological processes are affected by climatic conditions, with temperature being the most important factor affecting phenological phases in temperate ecosystems [1, 35]. A number of studies addressing the effect of possible climate change on the beginning of flowering in Europe report that, in recent years, winter-flowering species have tended to delay the onset of the flowering period [23, 40] while spring-flowering species [7, 12] have tended to begin the pollen season progressively earlier. Conversely, the results of this study shows that the start of the alder pollen seasons in Poznań are gradually getting later (non-significant trend).

New data that will be recorded over the coming years will show whether the results obtained will be confirmed.

Some researchers studying alder flowering report a statistically significant association between the day on which the pollen season starts and temperature during the preceding period [13, 24, 39], 40–50 days being required with temperatures below 9.1°C for the *Alnus* flowering. As in our study in Poznań, mean temperatures at the end of January and beginning of February appear to be the more important [13, 16] as correlation coefficients during this time are higher. This relationship is illustrated by the late start dates of the alder pollen seasons in Poznań in 1996 that followed a particularly cold period from the month of December to February in 1995 (the coldest in the 1995–2006 dataset, which was -4.02°C below the mean). The models proposed in this study to forecast the start of alder pollen seasons in Poznań using these meteorological parameters present a high accuracy level.

Yearly changes in plant phenology and the timing of the start of pollen seasons in Northern Europe can be also partially explained by the NAO [10, 45]. Interestingly, the high coefficient correlations with NAO index from December to February also reflects the influence exerted in the start of the *Alnus* pollen seasons, because low temperatures and low rainfall, or high temperatures and high rainfall, are often associated with different phases of the phenomenon [44]. Furthermore, a lengthening of the active growing season in Europe has been related to increases in winter and spring temperatures, which may in turn be associated with strongly positive indices of the NAO [30, 35]. The NAO is most pronounced during winter, and as shown by the significant negative correlations found in our study,

the link between NAO and alder flowering in Central Europe is particularly important during the period starting in December or January through March [10, 22, 30, 32, 35]. Accurate models were proposed in this study to forecast the start of the alder pollen seasons in Poznań using NAO indices from the months of January to February. The use of NAO values as predictive variables to forecast the start date of the pollen season has been discussed and proposed by other authors, but not previously investigated [10].

The heat and chilling requirements that *Alnus* need to break the dormancy period that precedes the start of the flowering were also studied. As in other European areas [13, 24, 39], the Chill period generally started in the first week of November. The end of chilling occurs during the end of January in Poznań, while in Southern Europe this occurs in the first fortnight of January. The amount of chilling accumulation in Poznań was higher than in other European areas [13, 40] because in colder areas the trees need to be protected over a longer period. Also, the threshold temperature for Chilling can vary according to several factors in both the plant and the environment [6, 49]. Studies conducted in Southern Europe report a very low coefficient of standard variation for threshold temperatures of 3.5–6.5°C [20, 24, 40]. However, because of the colder temperatures experienced in Central Europe, this threshold falls and we therefore propose a base temperature of -0.25°C as the most accurate.

Otherwise, the amount of GDD°C calculated for *Alnus* in different studies in Europe shows that a similar quantity of heat units as in Poznan are needed to overcome dormancy and start flowering [13, 40]. Previous work has shown that 4.5–6°C [13, 14, 18, 25] are the threshold temperatures

Table 5. Spearman correlation values between alder start dates and the amount and length of Chill and Heat requirements. **Correlation significant at 0.01 level; *Correlation significant at 0.05 level.

		R
Pollen season	Start pollen season – CH	-0.122 ns
	Start pollen season – Length CH	-0.206 ns
	Start pollen season – GDD	0.534**
	Start pollen season – Length GDD	0.887**
Chill-Heat period	LengthCH – GDD	-0.811**
	LengthCH – LengthGDD	-0.606*
	GDD – LengthGDD	0.620*

above which growth starts to be effective. However, in our case, GDD°C calculated using lower thresholds (0.5°C) might be more representative of the heat requirement of early flowering trees in Central Europe. Similar results were obtained by Rodríguez-Rajo *et al.* [39] in studies based on *Alnus* flowering in Northwestern Spain.

As several authors have reported, the coefficients of standard variation are higher for GDD°C than for the Chill period [24, 39]. The relationship between flowering onset and both chill and heat requirements has been reported by a number of authors who have found that chilling accumulation accelerate the renewal of growth once dormancy is broken; the more chilling units accumulated, the fewer forcing units are subsequently needed for budburst [15, 24, 39]. Several researchers also suggest that for *Alnus* the shorter the period in which Rest is accumulated, the longer the required period of high temperatures [3, 29, 37]. Similar results were obtained in our study. Correlations between

Table 4. Heat requirements in Growth Degree Days°C during the study period taking into account maximum temperatures and 0–1.5°C threshold temperature (Mean = average value, SD = standard deviation, CV(%) = Coefficient of standard variation in percentage). Length (in days) of the Heat period also shown.

	Maximum Temperature							Length (days)
	0.00	0.25	0.50	0.75	1.00	1.25	1.50	
1995	142	134	127	120	113	105	98	29
1996	183	158	133	108	83	58	33	101
1997	161	148	135	122	109	96	83	52
1998	139	131	123	115	107	99	91	32
1999	138	127	117	106	95	84	74	25
2000	109	103	97	91	85	79	73	24
2001	108	105	101	97	93	90	86	15
2002	138	134	130	126	122	118	114	16
2003	215	198	182	165	149	132	121	67
2004	97	92	86	81	75	70	64	22
2005	90	78	66	54	42	30	23	48
2006	149	133	117	101	85	69	53	64
Mean	139	128	118	107	96	86	76	41
SD	35.89	32.00	28.96	27.06	26.56	27.52	29.75	25.95
CV (%)	25.82	24.93	24.61	25.28	27.55	32.09	39.19	62.90

Table 6. Results of simple linear regression and standard multiple regression analysis to forecast start date of *Alnus* pollen season. As independent variables: (a) meteorological factors; (b) NAO index; (c) combination of all the aforementioned parameters; (d) length of the chill period were considered. Observed and predicted start dates of *Alnus* pollen seasons produced when 2007 data were entered into, some regression models are also shown.

Model	Adj R ²	2007 observed date	2007 predicted date
a Start date = 59.6453 – (4.03907 Tmean ₃₁₋₄₀)	0.743		February 14 (-3 days)
b Start date = 67.708 – (11.065 NAO _{JanFeb})	0.546	February 17	February 24 (+7 days)
c Start date = 63.854 – (2.997 Tmean ₃₁₋₄₀) – (5.441 NAO _{JanFeb})	0.826		February 20 (+3 days)
d GDD°C = 121.498 – (0.330 * Length CH)	0.305		February 12 (-4 days)

the length of the Rest period and the date of the onset of flowering [40] shows that, in areas where the tree needs to be protected from adverse meteorological conditions in order to preserve cells from frost damage, a delay in the phenological state of anthesis has been induced. Year-on-year differences in flowering start dates within a given study area may be partly attributed to high temperatures during the Chilling period and to low temperatures during Heat accumulation [24]. Consequently, flowering may start before the end of January when temperatures are high [25], but may then be delayed until April when minimum temperatures below -15°C are recorded in December and/or early January, as in the season 1995–1996 in Poznań.

In spite of the difficulty in developing predictions for trees that flowers at the beginning of the growth season, as in this period of the year the weather is very variable, inducing very changeable starts of pollen seasons and flowering [26], the forecast *Alnus* start pollen season models proposed in our study based on the combination of meteorological factors and NAO index, or by the quantification of the heat and chill requirements – showed high accuracy. The low number of years considered in the statistical analysis could provide not entirely stable results; consequently, greater importance should be attached to those results displaying higher adjusted-R² values and greater statistical significance (p<0.01).

CONCLUSIONS

The results of this work provide valuable information, of interest from aerobiological, agronomical, ecological an allergological points of view, about the flowering of trees in the winter period in Central Europe. Temperature plays a particularly important role in the maturation of reproductive organs and is the primary factor influencing the growth and *Alnus* phenology. Although the study was conducted with 13 years of data, the results indicate that the most important effect is exerted by temperatures during January and February, and particularly during the preceding 40 days.

Chill and Heat requirements differ according to the climate of the plant habitat. In colder areas, such us Poznań, these requirements are greater because the trees need protection over a longer period. The analysis provided insight to the dormancy processes, and the effect that meteorological factors and NAO exert on *Alnus* flowering, and will

form the basis for future work to continue building predictive models, considering new data that will be recorded during the coming years.

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