

Phenological surveys of allergenic species in the neighbourhood of Bologna (Italy)

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Abstract

The results of phenological observations in a geomorphologically differentiated area are shown. Phenological surveys were conducted on flowering and pollen emission of some common and widely diffused Graminaceae species, using a 7-step key. By means of multiple regression analysis altitude, latitude, slope and exposure were identified as significant environmental factors in giving rise to the pollination: in particular with respect to altitude the phenological pattern showed a negative gradient below 100 m of altitude and a positive one above.

1. Introduction

Phenology of allergenic species is an important tool to understand the pollen sources activities in order to investigate their relationship with aerobiological surveys (Puppi Branzi and Zanotti, 1992; Bricchi et al., 1992; Mandrioli et al., 1993). In Italy phenological pattern of some allergenic species has been already investigated by the Phenological Working Group of Italian Association of Aerobiology (AIA) (Gruppo di Lavoro di Fenologia dell'Associazione Italiana di Aerobiologia, 1998); nevertheless the present knowledge on this subject is still incomplete.

In this paper we present the results of phenological observations conducted in 1997 in the neighbourhood of Bologna, on some Graminaceae species, notoriously involved in rhinitis and asthma. This study is a part of a wider one that has the aim to obtain phenological maps by observation of herbaceous and woody spontaneous species. The observed species were spontaneous ones, very common and widely spread in the region.

2. Materials and methods

The Graminaceae observed are the spring flowering species: *Alopecurus myosuroides*, *Anthoxanthum odoratum*, *Brachypodium pinnatum*, *Bromus erectus*, *Bromus hordeaceus*, *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis*, *Poa trivialis*.

The investigated area (Figure 1, about 700 sq. km) lies to the East of Bologna, in northern Italy, between 44°20' and 44°36' N latitude and between 11°28' and 11°42' E longitude. We chose a set of more than 50 sampling stations sited at various altitudes between 10 (near Mezzolara) and 500 m a.s.l (near Frassineto). Some of them were located in the widely cultivated plain (Po Plain) and some in the hills, with a much more natural landscape: plain and hills are divided by Emilia road. Every station consisted of an area as much as possible homogeneous as regards the topographic and vegetational features. The observations were conducted using the following phenological key in 7 steps:

1. 000 absence of visible inflorescences

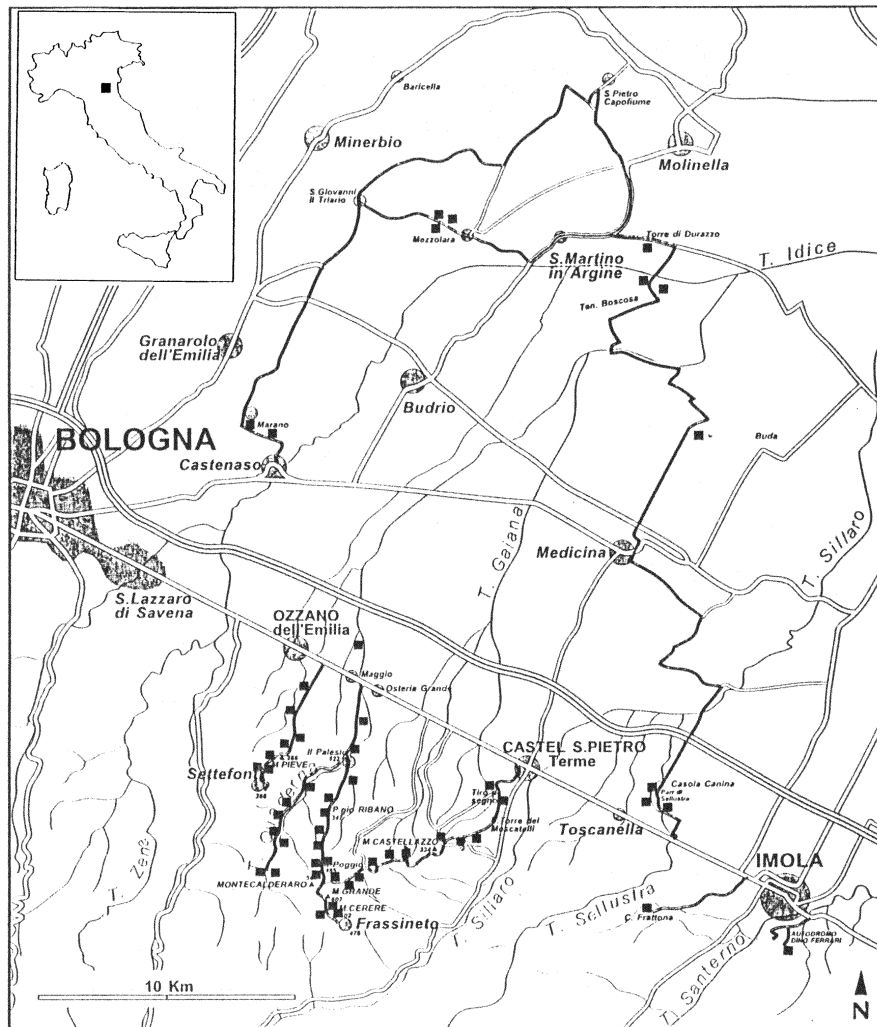


Figure 1. The investigated area: phenological stations are marked with small squares.

2. +00 inflorescences partially or fully emerged:
stamens not extruded
3. ++0 stamens partially extruded
4. +++ full flowering: all stamens extruded, full
pollen emission
5. 0++ part of stamens withered
6. 00+ all stamens withered
7. 000 beginning of the fructification

Beside every step the symbols of Marcello's key (Marcello, 1935) are reported.

According to this key the flowering period occurs from step 2 to step 6 and the pollination period, that is pollen emission time, occurs from step 3 to step 5. The sampling period extended for three

months, from March to May, and the phenological data were collected every week. In each station, a number of different plants (20 to 25) for each species were recorded. The collected data were processed applying statistical methods (SPSS statistical package) which were linear and multiple regression analysis. By means of linear regression analysis the best estimates of the beginning and of the end of the pollination period were obtained by interpolation. These interpolations make possible to draw phenological calendars, a graphic way to show the pollen emission period. The phenological calendars may be referred to a single station with different species, or to a single species, with different stations indicated. So we can point out

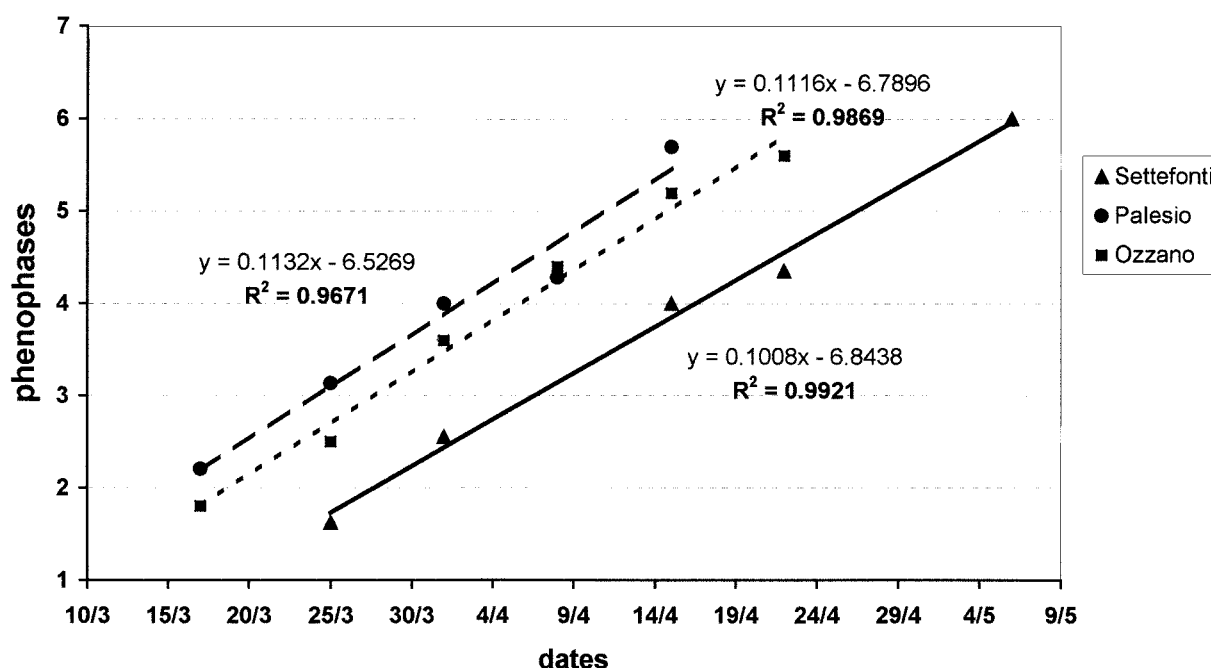


Figure 2. Flowering of *Anthoxanthum odoratum* in three stations. Dots represent average phenophases in the different dates. Regression lines, regression equations and their significances are shown.

the earliest and the latest species in each station and compare the phenological behaviour of a species in different places.

The second method of data analysis was based on the multiple regression analysis, that allowed to compute the correlation among the dates of pollination beginning (phase 3), chosen as dependent variable, and several environmental factors, as independent variables: altitude, exposure (expressed as incident solar energy in April, according the method used in Puppi Branzi and Zanotti, 1989), slope, latitude and longitude.

The aims of this analysis were:

- to find correlations between the phenological behaviour of all species and topographic factors. This analysis is useful in phenological mapping, because it gives a spatial interpolation model (the multiple regression equation) for unknown points. The effects of the environmental significant factors are calculated by the regression coefficients.
- to estimate the differences in phenological pattern among stations in order to distinguish them in earlier and later ones and to obtain the “phenological station delays” as regression coefficients
- to estimate the differences in phenological pattern among the species and to distinguish them in

earlier and later ones by means of the “phenological species delay” (as regression coefficients) as regards to a reference species (*Anthoxanthum odoratum*).

Results

3.1 Linear regression analysis

In Figure 2, as an example, the experimental points and the regression lines of the flowering of *Anthoxanthum odoratum* in three different stations are reported. All the regressions are significant and the same may be stated for all the considered species. Therefore phenological calendars obtained both for species and station are reliable.

In the phenological calendar of the plain station “La Boschosa”, the difference between the earliest species (*Alopecurus myosuroides*) and the latest one (*Lolium perenne*) at the beginning of pollination was of about 45 days. *Alopecurus myosuroides* had a very long pollen emission period (26 days), *Poa pratensis* a very short one (about one week) (Figure 3). In the crest hilly station “Settefonti”, the difference between the earliest species (*Alopecurus myosuroides*) and the

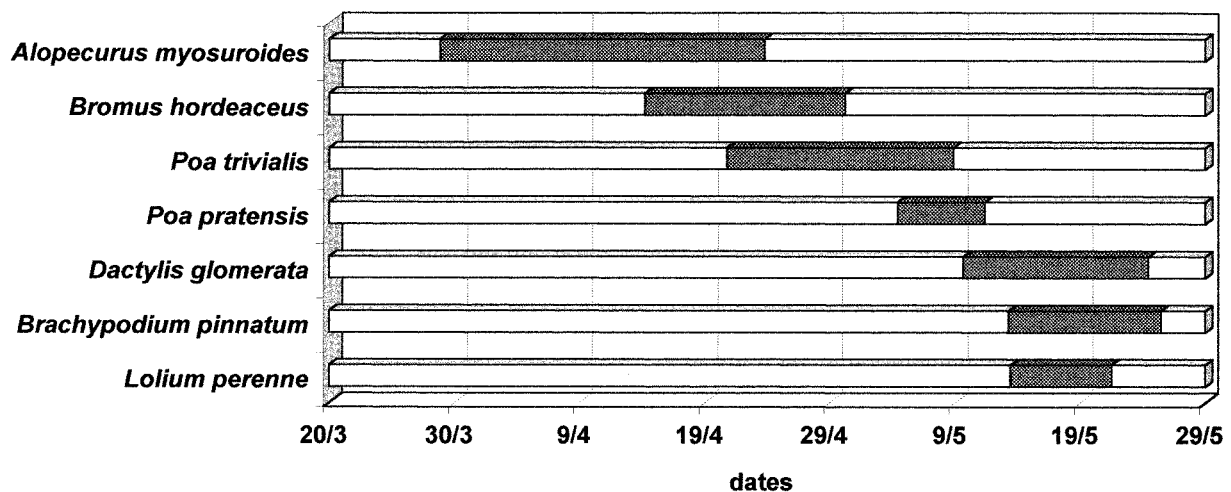


Figure 3. Phenological calendar of the plain station "La Boscosa" (44°34' N latitude, 11°39' E longitude). Hatching highlights the pollination period (phases 3 to 5).

Table 1. Multiple regression analysis results $R^2 = 0.880$; Multiple $R = 0.938$; $p < 0.01^{**}$.

Variables	B = regression coefficients	T	Sig. T
Latitude (primes)	0.28	2.344	0.020
Exposure (Mj/m ² per day)	-1.29	-5.554	0.000
Slope (degrees)	-0.10	-2.309	0.022
Altitude (hm)	1.87	4.154	0.000
<i>Alopecurus myosuroides</i>	-2.00	-0.709	0.479
<i>Bromus hordeaceus</i>	10.09	3.561	0.001
<i>Poa trivialis</i>	21.71	7.757	0.000
<i>Poa pratensis</i>	26.37	9.497	0.000
<i>Lolium perenne</i>	34.43	11.819	0.000
<i>Bromus erectus</i>	38.08	12.231	0.000
<i>Brachypodium pinnatum</i>	40.54	9.942	0.000
<i>Dactylis glomerata</i>	42.13	13.170	0.000

(*Anthoxanthum odoratum* was chosen as reference species)

Table 2. Average dates of pollination period of investigated species in a reference situation (station at 100 m height, without slope, at 44°24' N latitude, 11°30' E longitude) calculated by means of the multiple regression equation.

Species	Pollination beginning	Pollination period	Pollination end
<i>Anthoxanthum odoratum</i>	90	20	110
<i>Alopecurus myosuroides</i>	88	28	116
<i>Bromus hordeaceus</i>	100	18	118
<i>Poa trivialis</i>	112	16	128
<i>Poa pratensis</i>	116	16	132
<i>Lolium perenne</i>	124	17	141
<i>Bromus erectus</i>	128	14	142
<i>Brachypodium pinnatum</i>	130	17	147
<i>Dactylis glomerata</i>	132	15	147

Pollination beginning and pollination end dates are expressed in days from the beginning of the year. Pollination period is the difference between the two values.

latest one (*Bromus erectus*) at the beginning of pollination was of about 43 days. The pollen emission periods of *Alopecurus myosuroides* and *Poa pratensis* were of about 1 month and of 10 days respectively (Figure 4).

In Figure 5 the phenological calendar of *Bromus hordeaceus* in different stations is reported and ordered by height. This calendar refers only to flat stations. The flowering and pollen emission began later in higher stations and in the lower stations, while the earlier stations were those sited at mid altitude (between 75 and 200 m).

As regards the variability of pollination start of species (Figure 6), in the whole area we observe that

species like *Alopecurus* and *Poa trivialis* reached the beginning of pollen emission gradually in about 1 month; other species like *Bromus erectus* flowered almost simultaneously. Early species present more variability in flowering beginning (standard deviation of about 5–6 days) than later species (standard deviation of 2–4 days).

3.2 Multiple regression analysis

This analysis shows that, among environmental factors, latitude, altitude, slope and incident solar energy, are significantly correlated with the beginning

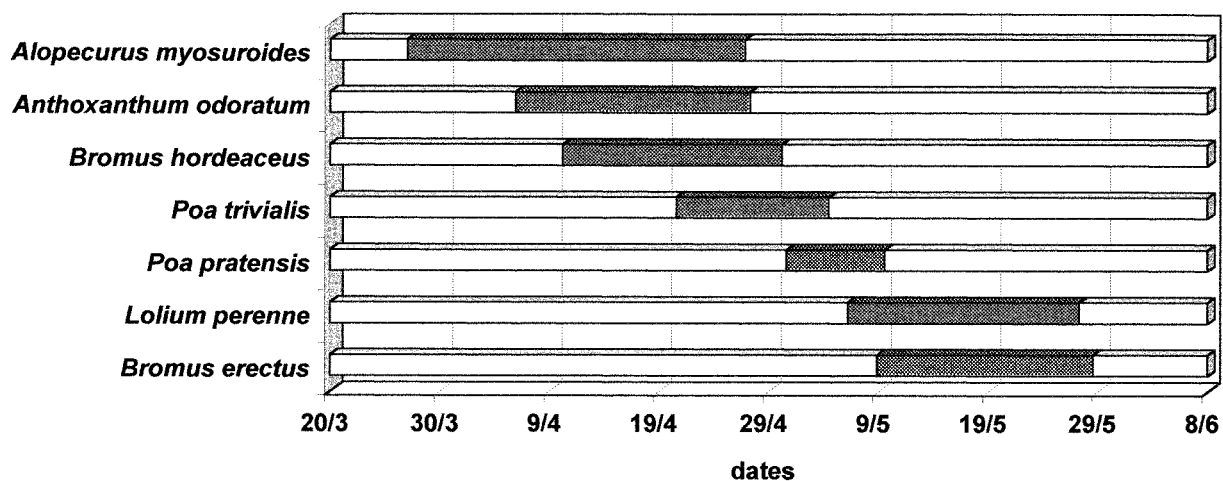


Figure 4. Phenological calendar of the hilly station "Settefonti" ($44^{\circ}24'$ N latitude, $11^{\circ}28'$ E longitude). Hatching highlights the pollination period (phases 3 to 5).

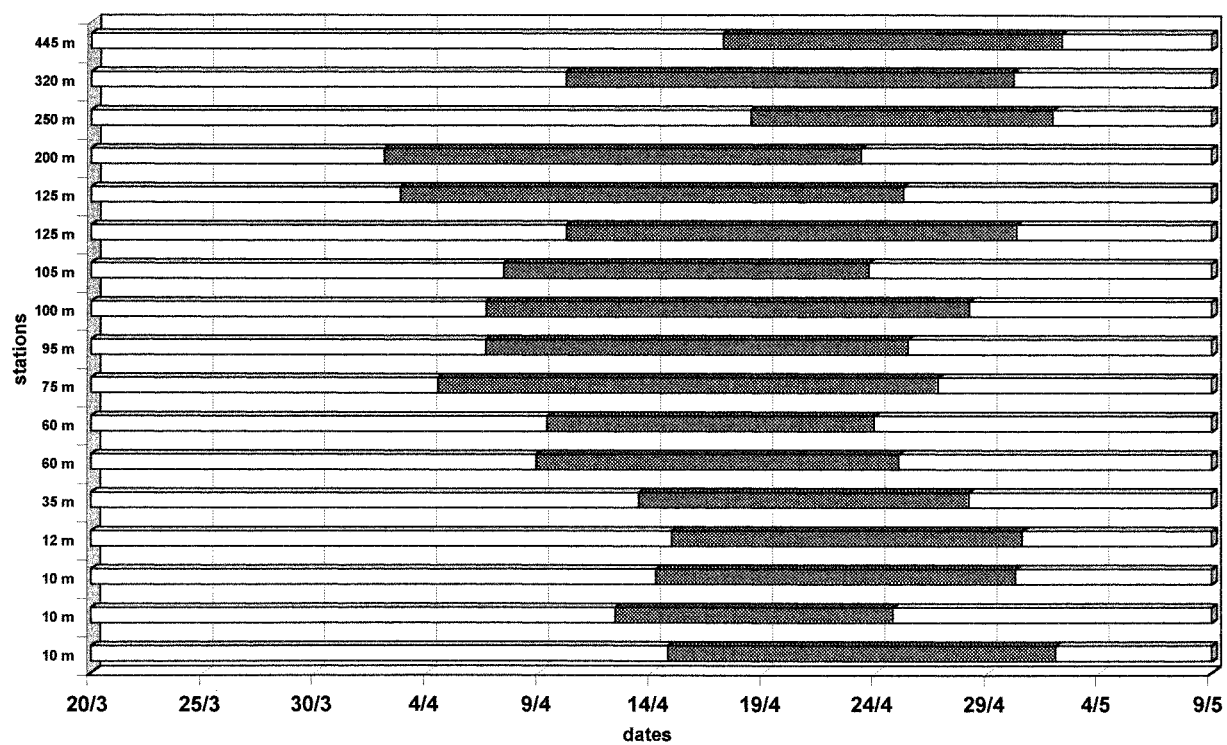


Figure 5. Phenological calendar of *Bromus hordeaceus* (in plain and crest stations) This calendar refers only to flat stations. Hatching highlights the pollination period (phases 3 to 5).

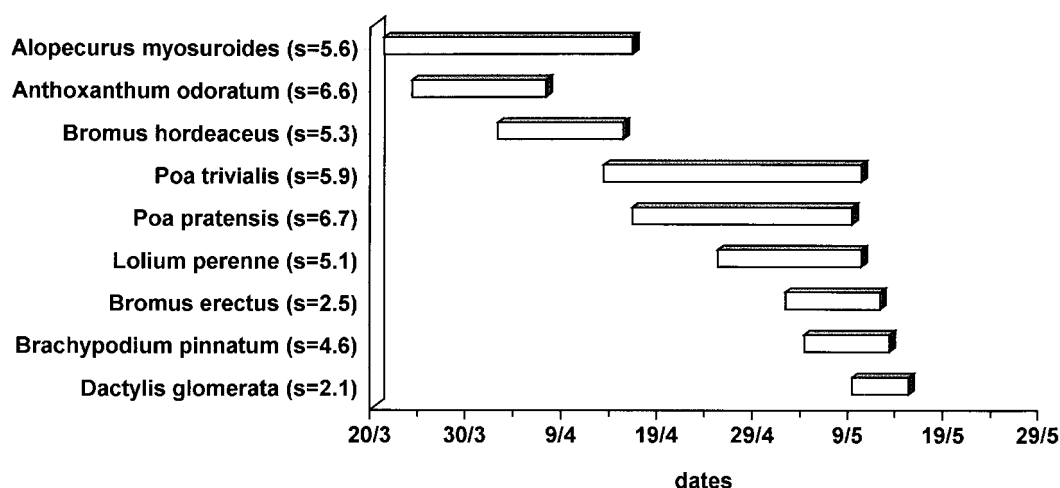


Figure 6. Variability of pollination start. Bars represent the variation interval; the standard deviations (s) are reported in brackets.

of pollen emission (expressed in days). In particular (Table 1):

- the phenological altitude gradient is 1.9 day every 100 m: this means that the pollen emission begins almost 2 days later every 100 m of height increase.
- the incident solar energy gradient is -1.3 day of phenological delay (which means 1.3 days in advance every Mj/m^2 per day). For instance: the difference in the beginning of pollen emission between southern ($\text{Mj/m}^2 = 22$) and northern exposures ($\text{Mj/m}^2 = 12$) (with the slope of 25°) at the same altitude, is about 13 days.
- the latitude gradient is 0.3 days of delay every prime of latitude.
- the slope gradient is 1 day in advance every 10 degrees of slope.

With regard to the species, the maximum difference (about 44 days) in pollination start occurred between *Alopecurus myosuroides* ($B = -2$) and *Dactylis glomerata* ($B = 42$) (as reference species *Anthoxanthum odoratum* was chosen ($B = 0$)).

By means of the multiple regression equation obtained (Table 1) we can easily calculate the beginning and the end of pollination of all the species investigated in each point of the territory. In Table 2 average pollination periods of all the investigated species (in a reference station at 100 m a.s.l.) are shown.

By means of another regression analysis, significant phenological differences among stations are found. The maximum phenological difference among stations (23 days), occurs between a flat station situ-

ated at about 100 m (the earliest one), and a station situated at a northern exposure at 270 m of altitude (the latest one): in this case the exposure effect on phenological delay overcomes the contribution of altitude. We notice that the relationship between altitude and phenological pattern does not seem to be strictly linear. Between 0 and 100 m we can see a negative phenological gradient corresponding to about -5 days/100 m (the earlier stations are situated at 100 m and the later at 10 m) (Figure 7). Vice versa plotting the phenological delay of stations above 100 m up to 450 m in relationship with altitude we can see a positive pattern (the earlier stations are at 100 m, the later at 450 m) (Figure 8). In this case the positive altitude gradient is of 3.7 days/100 m.

The plots point out a thermal inversion at the low altitudes and the presence of a thermal belt around 100 m of altitude, where the earliest stations are therefore located.

Discussion

The obtained significant results point out the validity of the adopted observation method, based on statistical measurements. The number of specimens for every species observed was suitable to obtain a good estimate of the average phenophases of each population. Linear regression, used to obtain phenological calendars, closely approximates the pollination trend, as results from the regression significance. These calendars allow to distinguish the earlier species from the later ones and to see the overlapping periods

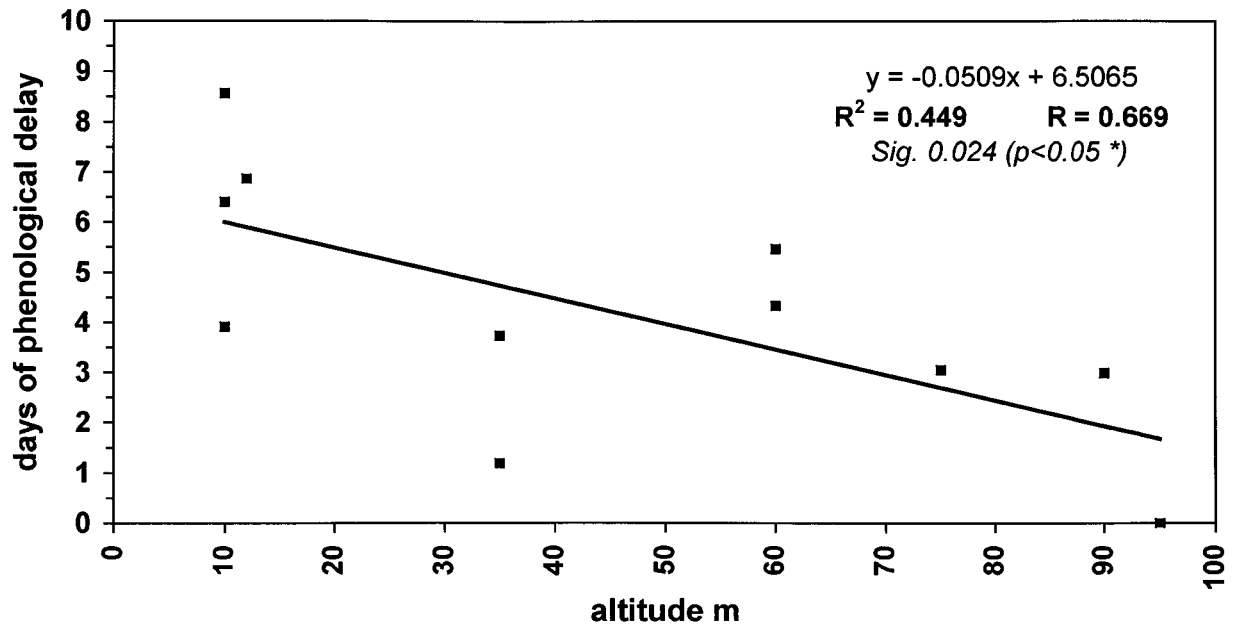


Figure 7. Plot of the beginning of the pollen emission period of the plain station (between 0 and 100 m a.s.l.). Phenological delay in relation with altitude: dots represent the different stations, located below 100 m a.s.l. (sloping stations are excluded from the plot).

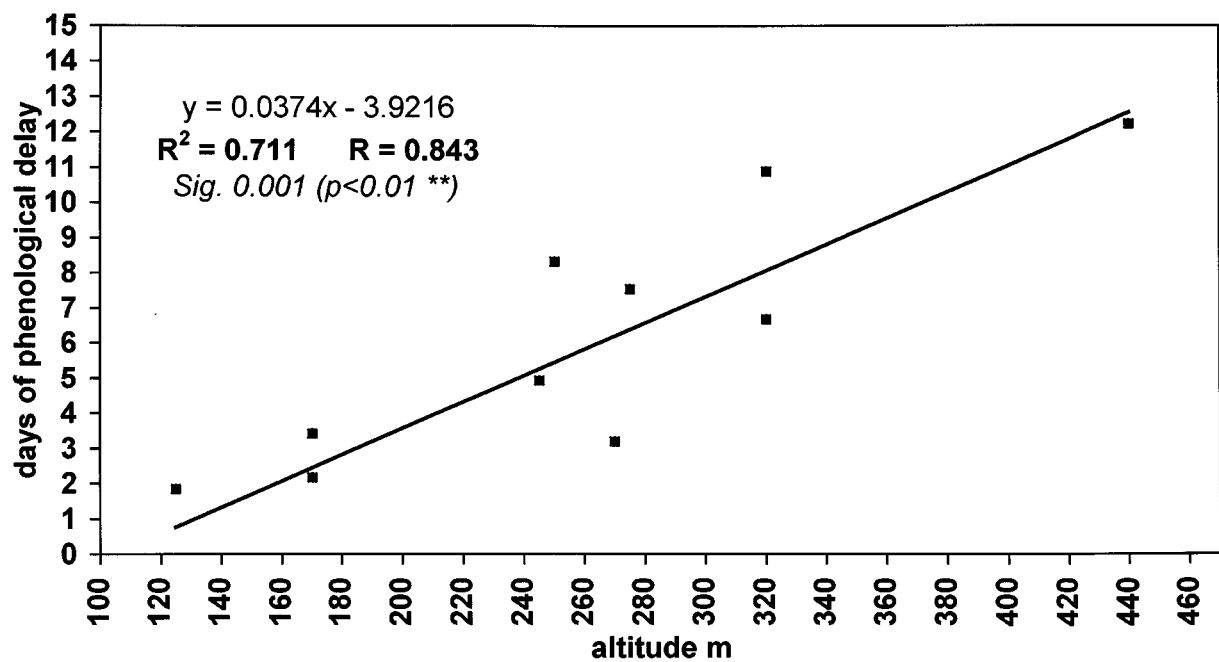


Figure 8. Plot of the beginning of pollen emission period of crest stations (between 100 and 450 m a.s.l.). Phenological delay in relation with altitude: dots represent the different stations, located above 100 m a.s.l. (sloping stations are excluded from the plot).

in the pollen emission. The examined species, all widely distributed over the territory near Bologna, and flowering from March to June, are suitable for predicting the pollination period of the spring Graminaeae. Multiple regression analysis showed the strong correlation existing among phenological events and topographic factors and may be used in achieving phenological maps. The phenological gradient of 2 days every 100 m in height is quite similar to that observed in neighbouring territories (Reno river valley) in a previous study based on six years of observation data (Puppi and Zanotti, 1989). Nevertheless the phenological pattern with height is not linear: under 100 m there is a negative gradient and over 100 m a positive one. Negative phenological gradient at a lower height is due to the climatic phenomena of thermal inversion, occurring in the Po valley in winter and spring periods. More relevant are the effects of slope and exposure. The weight of exposure, in particular, is affected by the real insolation, that varies from year to year. The multiple regression analysis is useful to order the species from a strict temporal point of view and, moreover, the method may be applied without restrictions with regard to place and time of observation, number and kinds of species: any species can be used on condition that it is observed at least in two stations.

5. Conclusions

We noted a high variability in the pollen emission period depending on species and stations. Some

species flower everywhere earlier than others. Some species flower gradually, other almost simultaneously. We have also demonstrated a correlation between the beginning of pollen emission and environmental factors such as latitude, altitude, slope and exposure. The presence of a thermal belt at 100 m of altitude is pointed out.

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