

PHENOLOGICAL TIMING OF SOME ARBOREAL SPECIES IN A PHENOLOGICAL GARDEN IN PERUGIA (ITALY)

STUDIO DELLE FASI FENOLOGICHE DI ALCUNE SPECIE ARBOREE IN UN GIARDINO FENOLOGICO A PERUGIA (ITALIA)

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Abstract

Phenology is the manifestation of the phenomena associated with the life cycle of plant. It depends on the plant's internal and external factors, as for example, climate.

Considering the fact that phenology is one of the best tools for analysing the consequences of climatic change in the ecosystems, this study investigates how the climatic trend of a ten year period (1997-2006) influenced the reproductive phase manifestation of eight plant species, both typical ones and those adapted to the Mediterranean environment, situated in the phenological garden near Perugia, Italy. The TMax amounts appear to be closer to the flowering dates in comparison to the other meteorological variables for all the species investigated, indicating that for the reproductive development the forcing temperatures are represented above all by the higher temperature that works as an environmental timer.

The subdivision of the historical series in two sub-periods of five years evidenced a phenomenon of delay of the four reproductive phases in almost all vegetative species in the recent years. The average delay is more considerable for the two species of *Salix*, especially for the R3 phase, as a consequence a flower development slowdown.

Meteorological data showed that currently the first months of the year are characterized by a tendency for colder temperatures; afterwards, as the seasons progress, the tendency inverts, to a warming trend in summer.

Key words: Climate, Plant relationship, Phenological garden, Phenology.

Riassunto

La fenologia in generale e la fitofenologia in particolare si interessano della manifestazione dei fenomeni associati con il ciclo vitale della pianta. Queste diverse manifestazioni dipendono dai fattori interni ed esterni alla pianta, come per esempio il clima. Considerando che la fenologia è uno dei migliori strumenti per analizzare le conseguenze dei cambiamenti climatici in ecosistemi, questo lavoro studia come l'andamento climatico durante il periodo decennale (dal 1997 al 2006) ha influenzato la manifestazione della fase riproduttiva di 8 specie di piante, tipiche o adattate all'ambiente Mediterraneo, situate nel giardino fenologico vicino Perugia, nel centro Italia.

Per tutte le specie investigate, le sommatorie realizzate con le temperature massime risultano individuare con maggiore precisione le date di fioritura, rispetto ad altre variabili meteorologiche, indicando che per lo sviluppo riproduttivo le temperature forzanti primaverili sono influenzate maggiormente dai valori più alti di temperatura i quali determinano in ultima analisi la calibrazione dell' "orologio biologico".

*La suddivisione della serie storica in due periodi di 5 anni ha evidenziato il fenomeno di ritardo di 4 fasi riproduttive in quasi tutte specie vegetative negli anni recenti. Il ritardo medio è più rilevante per due specie di *Salix*, specialmente per la fase R3, come conseguenza del ritardo dello sviluppo del fiore.*

Dati meteorologici hanno mostrato che i primi mesi dell'anno sono caratterizzati da temperature più fredde; dopodichè, man mano che la stagione avanza, la tendenza si inverte, e nell'estate si registrano temperature più calde.

Parole chiave: Clima, Giardino fenologico, fenofasi, fioritura

Introduction

One of the oldest definitions of phenology was given by Lieth in 1974 who described it as "the art of observing life cycle phases or activities of plant and animals in their temporal occurrence throughout the year". In its present meaning, plant phenology is the study of events which contribute to the manifestation of phenomena associated with the functioning of some plant organs, or of

a plant as a whole. Factors such as photoperiod, air and soil temperature, solar illumination and snow cover influence phenology of plants (Reed *et al.* 1994, Bailey and Harrington 2006). Among the observable phenological phenomena are leaf unfolding, flowering, leaf fall and other cyclic phenomena. Using phenology we can observe and register the different stages of plant devel-

opment and the specific phenological scales allow us to code the observations obtaining objective results (Marletto *et al.* 1992).

The essential element of phenological observation is the time data, since the observation includes a phenomenon description and its collocation in time.

There are numerous fields where phenological studies can be applied. In agriculture, the study of species (phenology and biorhythms) in relation to climate is carried out for productive and forecasting purposes, for the analysis of mechanisms of seed and bud dormancy, of growth rhythms and also for studying the plant/climate, plant/topology and plant/substrate relationships. The study of plant community complexes (sym-phenology and sym-biorhythms) has been applied in meadow, pasture, forest and water resource management programs. In the medical-health field, the phenological studies of allergenic (or potentially allergenic) species allow us to monitor their development and pollination. Using phenological data, maps of pollen and spore flows were created; when integrated with meteorological models, they were used for the elaboration of regional phenological calendars (Fornaciari *et al.* 1998, Galán *et al.* 2001). In ecology and climatology, both phenology and sym-phenology are used to determine the degree of climatic changes and their potential consequences (Kramer *et al.* 2000, Linkosalo *et al.* 2000, Mutke *et al.* 2003, Orlandi *et al.* 2005a).

A new application of phenology deals with prediction of tree species' distribution using a process-based model. In this case, the processes such as plant survival and reproduction are studied from the phenological point of view (Chuine and Beaubien 2001).

Phenological stages are the result of two different kinds of factors: internal and external ones. Internal factors are biorhythms; i.e., the rhythms regulated by genetic factors of the species. External factors are environmental and climatic ones; probably, the same ones which caused plant adaptations now fixed in its genome. Climatic and astronomical factors, repeating regularly over the long-term directly and indirectly cause the manifestation of phenological stages.

Therefore, phenology is considered as one of the best ways to analyze climate and its shifts which cause consequences in ecosystems (Peñuelas and Filella 2001, Walther *et al.* 2002). During the last 100 years the available climatic data indicate that the climate has warmed (Peñuelas *et al.* 2002), the temperature increased by about 0.6 °C (Walther *et al.* 2002) particularly since the 1970's (Hansen *et al.* 1999, IPCC, 2001). Therefore, phenology became an important tool to indicate biological response to climate warming (Sherry *et al.* 2007). Global warming may cause several types of changes in species such as the density of the species, genetic frequencies and also changes in the timing of the events (Parmesan and Yohe 2003, Root *et al.* 2003).

In the Northern hemisphere, the influence of global warming on plant phenology has been studied. This influence has been seen in an earlier flowering and a longer period of active growth (Cleland *et al.* 2006). The main factors which contribute to phenological shifts are air temperature (Bradley *et al.* 1999, Beaubien and Free-

land 2000, Sparks *et al.* 2000, Chmielewski *et al.* 2003) and day length, especially in temperate zones (Menzel 2002).

The study of the impact of an eventual climate change on reproductive stages is important because flowering and fruiting determine consequences in future plant generations influencing evolutionary processes (Sherry *et al.* 2007). Over the long-term, these consequences, other than producing a biological response, may also be studied from the economic point of view, especially for yield of crop species (Parmesan and Yohe 2003).

The aim of this study was to analyse the average trends of reproductive development of some vegetative species, typical ones or adapted to the Mediterranean environment, over a ten year period (1997-2006). The study was carried out in the phenological garden situated near Perugia, central Italy. The use of climatic variables, which show yearly temperature accumulation, allowed us to examine for some species the correspondence between climate and reproductive development.

Materials and Methods

Meteorological data

The phenological garden of Perugia (central Italy), situated at 43° 10' North, 12° 42' East, has a sub-continental climate with minimum temperature that can be around the zero in winter (december, january) and maximum temperature that can reach values of 35°-40° during summer (july, august). The necessary meteorological information on a continuous basis was provided daily by the local station of the National Agro-Meteorological Network, situated a short distance from the garden. The temperature data were taken into consideration for investigating how the trends of the reproductive cycles of different species in the years can be related to climatic changes. In particular, the principal meteorological trends were interpreted by the use of daily values of minimum temperature in °C (Tmin), maximum temperature in °C (TMax), precipitation in mm (Rain) and absolute heliophany in minutes (EDT). For each variable, the amounts of daily values from 1 January to the 10th, 20th and the 30th weeks of the year were calculated. These periods represent time intervals during which the reproductive phenophases of the studied species were recorded.

The indicator species

The selection of indicator species, among the species proposed by the IPG, was carried out by the National Working Group for Phenological Gardens. Eight species were chosen to study: *Cornus sanguinea* L.; *Crataegus monogyna* Jacq.; *Corylus avellana* L.; *Ligustrum vulgare* L.; *Robinia pseudoacacia* L.; *Salix acutifolia* Willd.; *Salix smithiana* Willd., *Sambucus nigra* L.

Some species easily adaptable to the Mediterranean climate were chosen, like those of the genus *Salix*, this permitted us to connect with the International Phenological Network.

Sampling was carried out according to the methods and criteria of the National Working Group for Phenological Gardens, which allowed us to register how many plants

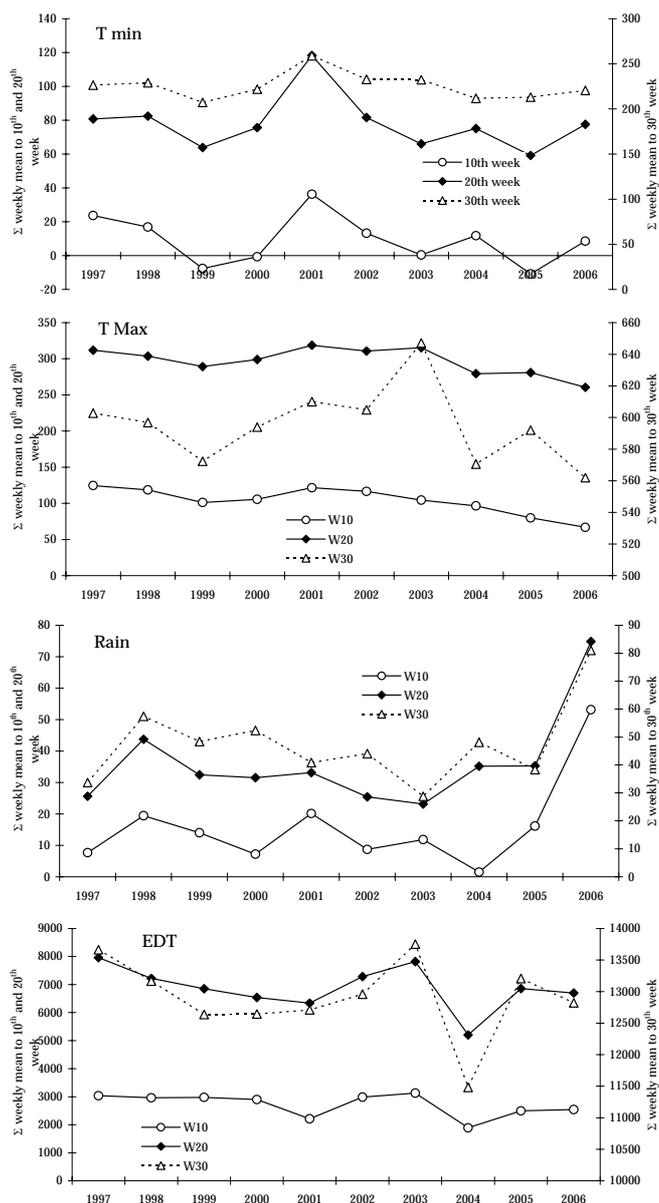


Fig. 1 - Tmin, TMax, Rain and EDT weekly amounts from 1 January to the 10th, 20th and the 30th weeks of the year expressed by polynomial trend lines of the 2nd degree.

Fig. 1 – *Sommatorie settimanali di Tmin, Tmax, Pioggia e EDT dal 1° gennaio alla 10°-20°-30° settimana dell'anno espresse con curve di tendenza polinomiale (2° grado).*

of the same species show the same phenophase. Because of the intrinsic variability of the vegetative organisms due to biotic factors, it is necessary to conduct the observations on multiple different individuals of the same species to be able to create a representative sample of all possible variation within each species. For this reason, in the phenological garden the observations and monitoring were carried out on three individuals for each species (Phenoids) during the whole year. To obtain the best results for mean trends of species, the average dates of the beginning of different phenological phases were calculated considering the three phenoids. The observations were codified by means of phenological keys, which describe synthetically the various phases of phenological

events, such as flowering, fructification and leaf unfolding.

The National Working Group for Phenological Gardens has codified 14 vegetative phenological phases (V1-V14) and 12 reproductive phases (R1-R12). This study analyzes the phases of reproductive development, from the phase of formation of flower buds or male aments (R3) until the phase of complete withering of flowers and aments (R6). R03: during this phase there are contemporary swollen buds, close to blooming, and open flowers, and also male aments which, in part, produce pollen. R04: represents full blooming with open flowers ready for pollination and male aments that produce pollen. R05: a withering begins; flowers and aments have almost completed their flowering. R06: complete withering, the flowering has terminated and the plant has only withered flowers and aments. We considered only four phases of the 12 of the reproductive cycle in order to show the part of the cycle that is related to the development of new flowers. The average dates of phenophase fulfilment, calculated on three phenoids, represent a mean model of development for the species and for that year. On the basis of these annual models, the series of phenological data for various species related to the nine years of research (1997-2006) were produced, and the results for mean development trends and the duration of different phenological phases in the study area were obtained.

Climate – plant relationships

On the basis of daily meteorological data, according with the timing of weekly phenological observations, the 7-day and monthly means of Tmin, TMax, Rain and EDT for every year of the historical series were calculated in order to investigate the principal relationships between meteorological variables and the four reproductive phenophase dates with the use of the inferential statistical technique.

In particular, multiple regression analyses were carried out among the full flowering dates (R4 phase) and the meteorological variables' amounts calculated from 1 January to the R4 dates in order to determine the real relationships and the dependence degree, considering the biological variable as the dependent variable.

We used Growing Degree Day (GDD) and Growing Degree Hour (GDH) which define the amount of heat necessary for the successive development of vegetative organisms, to condense the climatic information into a few parameters that relate phenology and temperature.

The GDH were calculated applying the method proposed by Anderson *et al.* (1986), while for GDD the method of Baskerville & Emin (1969) based on the maximum and minimum temperatures was applied. In GDD mathematical formulas 12 threshold temperatures from 4 to 15 °C were used (Orlandi *et al.* 2005b).

The weekly accumulations both of GDD and of GDH, beginning from 1 January (as the initial date of accumulation) until the dates of appearance of the reproductive phases examined (R3-R6) for each species were calculated. To study the relationships between temperatures and the vegetative species development, we used a correlation analysis of the annual values of reproductive

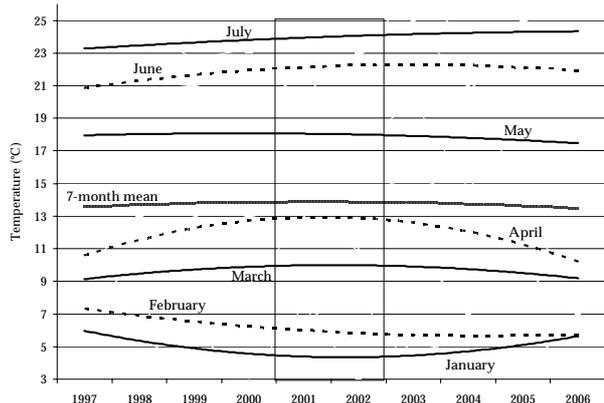


Fig. 2 - Monthly means of average temperature from January to July expressed by polynomial trend lines.

Fig. 2 - Medie mensili della temperatura media da gennaio a luglio espresse da curve di tendenza polinomiali.

development during the four phases examined and the corresponding temperature accumulations (GDD-GDH). The best correlations between GDD accumulations, calculated with the different threshold temperatures, and the dates of the onset of four reproductive phases indicate for each phase the best threshold appropriate to start considering the temperature accumulation.

Results

Climate trends

Principal meteorological variable trends were interpreted by the use of summations of Tmin, Tmax, Rain and EDT from 1 January to the 10th, 20th and the 30th weeks of the year (Fig. 1a). The maximum value was reached in 2001, after which the temperature amount values recorded until the 20th-30th weeks decreased. The trends of temperature amounts calculated until the 10th week appear as decreasing.

The maximum temperature trends (Fig. 1b) interpreted by the chart confirmed a reaching of the maximum values in the first years of 2000, followed by a marked temperature decrease for the three reference dates. Moreover, rain showed an opposite tendency compared to temperature, since the chart evidenced the minimum value in 2001 and increasing values in 2005-2006.

Finally, the solar radiation trend (Fig. 1d) decreased annually; particularly, the chart constructed with the amounts recorded until the 20th week reached the minimum values in 2003.

We also studied the reproductive period for all the species from the meteorological point of view using monthly means from January to July (Fig. 2). The different months show three different trends. The trend of the first two months (Jan.-Feb.) is generally decreasing, even if in the last three years January had increasing values. The trends of March, April and May, on the other hand, reached their maximum value in 2001-2002 and in April the constructed curve is at the top of a

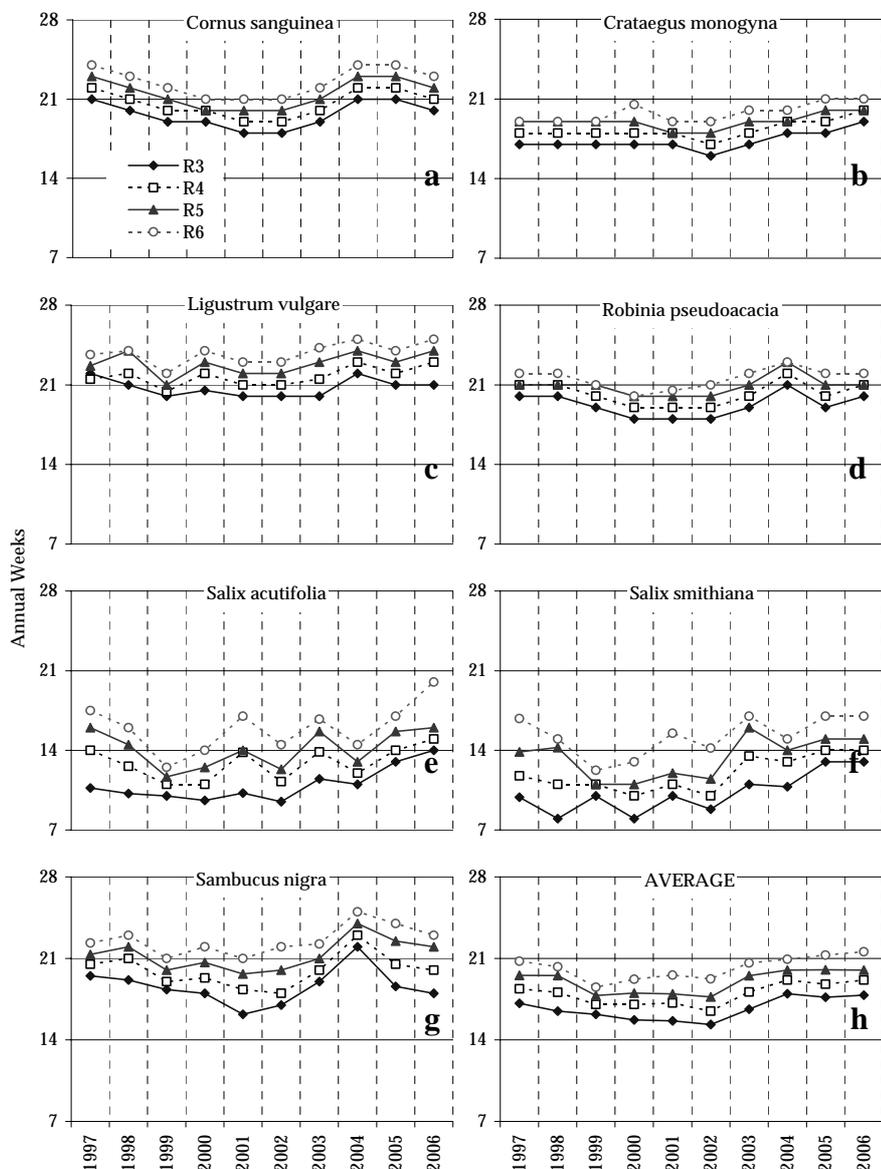


Fig. 3 - Trends of the four reproductive phases analysed (R3-R6) of the historical series studied (1997-2006).

Fig. 3 - Andamenti delle quattro fenofasi riproduttive (R3-R6) della serie storica considerata (1997-2006).

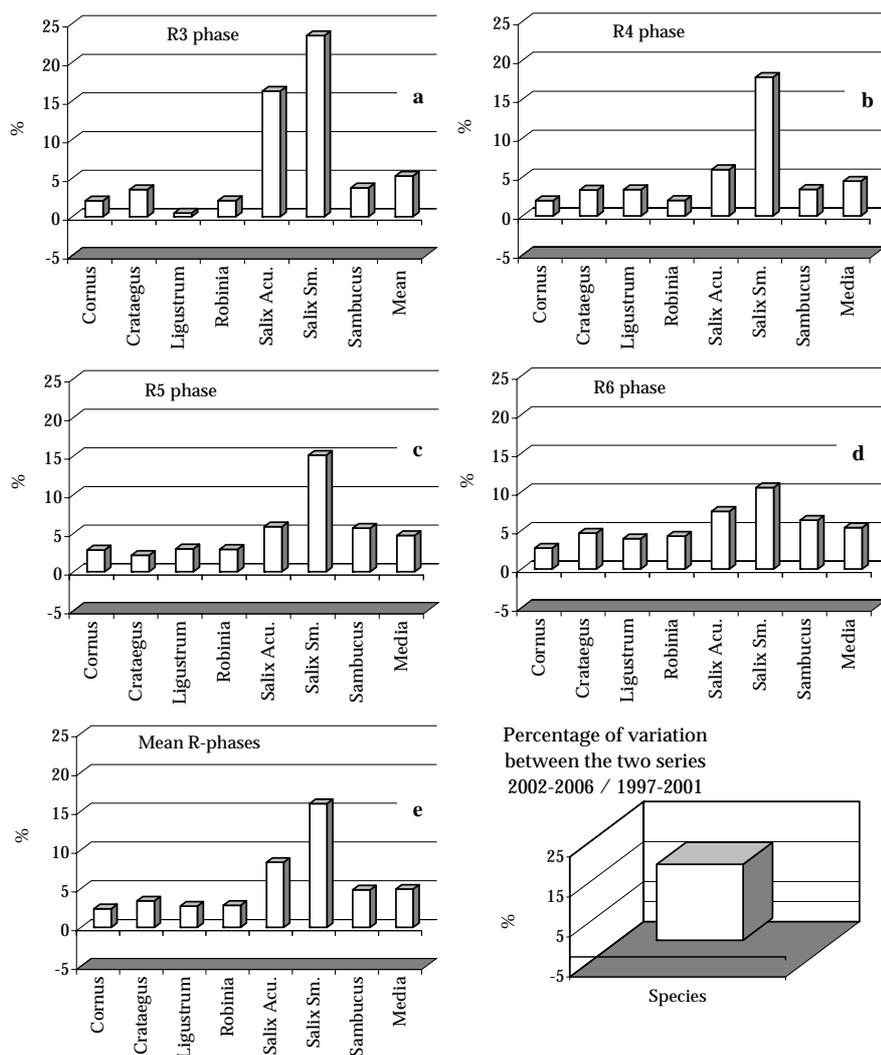


Fig. 4 - Percentage variation between the average values calculated in two sub-periods (2002-2006 / 1997-2001) for all the reproductive phases (R3-R6) for every species.

Fig. 4 – Variazione percentuale tra le medie calcolate in due sotto periodi (2002-2006 / 1997-2001) per tutte le fasi riproduttive (R3-R6) e per tutte le specie.

parabolic one. The June and July months showed increasing trends, especially due to the July values which increased year by year.

Indicator species results

Fig. 3 show the relation of each species to the trends of four reproductive phases of the historical series studied (1997-2006). Phenological analysis allowed us to compare the duration of the reproductive season, which goes from the beginning of flowers' opening and aments' maturation (R3) until the complete withering (R6) and also permitted us to examine the trends of each reproductive phase.

In *Cornus sanguinea*, Fig. 3a shows that the reproductive period is rather short and lasts three weeks on the average. The trends of each phase are very similar. During 1997-2001 there was an advancing trend (Fig 3h) which was reversed in 2002-2006. Also, *Crataegus monogyna* (Fig 3b) had a rather short reproductive season: in all

years, the reproductive phases were completed within three weeks. In the years studied, there were no considerable differences between the dates when the phases occurred. However, from 2002 until the end of the historical series, there was a trend to delay the dates for about two weeks.

The species *Ligustrum vulgare* (Fig 3c) is the latest blooming: the R3 phase did not occur until the 22nd week. All the years of the historical series had a rather short reproductive period, except for 2003, when it lasted four weeks. The periods of manifestation of phenological phases in this species did not show any particular trend, even during the last years of the series.

The species *Robinia pseudoacacia* (Fig 3d) in the first years had a rather concentrated reproductive period of about two weeks, with a tendency for a delay from 2001. Starting in 2002, we note a tendency for delay in reproductive cycle timing.

Two species of *Salix* (Fig 3 e and f) have a particular development. First of all, they are generally characterized by a quite long reproductive period, even if in 1999 they had a shortened timing of the cycle. For *Salix smithiana*, flowering starts very early, on the average 8-9 weeks before the other species. In the years observed, the

development of the phases was quite heterogeneous, though in the period from 2002-2006 a delay in the beginning of phases (rising trend on the graph) can be seen. For *Sambucus nigra*, (Fig 3g) the data evidenced a homogeneous flowering period in the first years, while starting from 2002 a considerable delay in the manifestation of all the phases of our interest (with a discordant data for 2005) was registered.

Fig. 3h, made on the basis of average values of reproductive phases for all the species considered, reflects the tendency for delay of flowering in the last years of our study. It is clearly evidenced that from 2002 the reproductive phases occurred with some delay (more or less significant), compared to the previous period. In order to show the differences in development begun in 2002, graphs for every species were made comparing the average values of the phenological phases calculated in two sub-periods: 1997-2001 and 2002-2006 (Fig. 4). This figure shows the percentage variation between the aver-

Tab. 1 - The variation coefficients of meteorological variable amounts to the four reproductive phenophases (R3-4-5-6) calculated in the period 1997-2006.

Tab. 1 - Coefficienti di variazione delle sommatorie delle variabili meteorologiche fino alle date di realizzazione delle quattro fenofasi riproduttive (R3 -4-5-6) dal 1997 al 2006.

	Phases	Tmin	Tmax	Rain	EDT
<i>Robinia pseudo-acacia</i>	R3	0,25	0,08	0,46	0,13
	R4	0,21	0,08	0,42	0,13
	R5	0,17	0,06	0,40	0,10
	R6	0,15	0,07	0,40	0,13
<i>Ligustrum vulgare</i>	R3	0,20	0,07	0,41	0,12
	R4	0,19	0,06	0,41	0,09
	R5	0,17	0,07	0,39	0,09
	R6	0,14	0,06	0,37	0,08
<i>Cornus sanguinea</i>	R3	0,32	0,16	0,45	0,20
	R4	0,28	0,15	0,41	0,19
	R5	0,27	0,15	0,39	0,19
	R6	0,25	0,15	0,38	0,18
<i>Crataegus monogyna</i>	R3	0,33	0,04	0,52	0,13
	R4	0,30	0,04	0,51	0,12
	R5	0,20	0,04	0,46	0,14
	R6	0,20	0,05	0,43	0,12
<i>Sambucus nigra</i>	R3	0,31	0,15	0,46	0,15
	R4	0,25	0,11	0,46	0,13
	R5	0,19	0,09	0,42	0,12
	R6	0,16	0,08	0,39	0,10
<i>Salix acutifolia</i>	R3	1,12	0,12	0,98	0,21
	R4	0,93	0,16	0,90	0,21
	R5	0,78	0,18	0,80	0,22
	R6	0,72	0,20	0,73	0,22
<i>Salix Smithiana</i>	R3	1,40	0,17	1,01	0,26
	R4	0,88	0,13	0,92	0,19
	R5	0,70	0,17	0,83	0,23
	R6	0,63	0,16	0,70	0,21

age values of the two sub-periods for the entire reproductive cycle (average R3, R4, R5 and R6). A positive percentage value means that a phenological delay occurred during the second sub-period (2002-2006); a negative value on the other hand can be associated with an advance. The R3 phase (Fig 4a), on the average for all the species considered, shows a delay manifesting in the second period. The most significant delay was registered for the two species of *Salix*, while for *Ligustrum vulgare* it was less considerable. On the average, for the other species, the R3 phase occurred with a one week delay, compared to the first years of monitoring. The R4 phase (Fig 4b) was delayed during 2002-2006, compared to the previous period, with the exception of *Cornus sanguinea*. The difference between values is quite low (on the average, one week), while for *Salix smithiana* the calculated delay is about two weeks. The last two phases, R5 and R6 (Fig 4c and d), occurred with an average delay of one week in the second series (except for *Cornus sanguinea*).

In particular, *Salix smithiana* is the species for which the delay was the most evident.

The mean values calculated for the four reproductive phases (Fig 4e) show a brief delay in the last study years for all the species within the 5% of variation, except for the two "northern" *Salix* species, for which the variation in the second sub-period was 10% higher than in the first one.

Statistical analysis results

In order to estimate variability or stability of temperature, precipitation and heliophany registered along with the flowering phenomenon, the variation coefficients of these meteorological variables to the dates of the four reproductive phenophases from 1997 to 2006 are reported in Tab. 1. The results demonstrate that maximum temperature generally shows less variability than the other variables considering the accumulations registered in 10 years of study. Among all the species, *Crataegus monogyna* is the most stable (0.04-0.05) together with *Ligustrum vulgare* and *Robinia pseudoacacia* (with values lower than 0.1). Heliophany, also shows low variability, in the range of 10-20% and which is characterized by a certain homogeneity among different species.

The accumulations of the minimum temperatures showed the variation coefficients within the range 0.2-0.3, except for the two *Salix* species which have definitely higher values, sometimes exceeding 1.0. Finally precipitations, essentially due to their aleatory character, register rather variable values year by year, showing a percentage higher than 40%.

Tab. 2 shows the results of multiple regression analyses. Again, the most satisfactory interpretation of the biological phenomenon was found using TMax and Rain and can be demonstrated considering both the multiple R-squared and the significance values (|t|) for each species. The higher variance is obtained for *Cornus sanguinea*, for which the two meteorological variables are responsible for more than 93% of the total variation. *Crataegus monogyna* needs only one variable (Rain) to obtain acceptable statistical results, although lower in comparison to the other species ($R^2 = 0.69$).

The dates of the four reproductive phases were correlated to the temperature accumulations calculated with GDH and GDD (Fig. 5). The statistical analysis for *Cornus sanguinea* showed that the correlation between the considered variables is very high, both for GDH and all GDD thresholds (the calculated values are within the range $0.90 < r < 0.96$). For *Crataegus monogyna*, the highest correlation for the R3 phenophase corresponds to GDD13, and is the same for the R5 phase. However, the first one is more correlated ($r=0.75$) than the second one ($r=0.56$). For the other two phases (R4 and R6) the maximum correlation corresponds to GDD11; but also in this case, the correlation coefficient is not very high for the R4 phase ($r=0.56$), and instead it is more considerable for the R6 phase ($r=0.81$). For *Ligustrum vulgare*, the R3 phase is more correlated to GDH ($r=0.59$) than to any considerable threshold of GDD. The R4, R5 and R6 phases have the highest correlation with GDD7; still, the coefficients near the maximum ones correspond to the immediately adjacent thresholds.

Tab. 2 - The results of multiple regression analyses for the species considered (Full flowering dates as dependent variable, TMax and Rain amounts from 1 January to R4 phase as independent variables).**Tab. 2** – Risultati delle analisi di regressione multipla per le specie considerate (date di piena fioritura intese come variabili dipendenti, sommatorie di Tmax e Pioggia dal 1° gennaio alla fase R4 intese come variabili indipendenti).

<i>Cornus sanguinea</i> L.					<i>Crataegus monogyna</i> Jacq.				
Coeff.:	Value	Std.Er.	t value	Pr(> t)	Coeff.:	Value	Std.Er.	t value	Pr(> t)
(Intercept)	7.9077	1.3515	5.8507	**	(Intercept)	16.9644	0.3539	47.9312	**
TMax	0.0361	0.0036	9.8650	**					
Rain	0.0368	0.0116	3.1705	*	Rain	0.0406	0.0096	4.1934	**
Residual st. error: 0.5236 on 9 degrees of freedom					Residual st. error: 0.4882 on 9 degrees of freedom				
Multiple R-Squared: 0.9335					Multiple R-Squared: 0.6873				
F-statistic: 49.1887 on 2 and 7 degrees of freedom, the p-value is 7.55E-05					F-statistic: 17.5853 on 1 and 8 degrees of freedom, the p-value is 0.0030				
<i>Ligustrum vulgare</i> L.					<i>Robinia pseudoacacia</i> L.				
Coeff.:	Value	Std.Er.	t value	Pr(> t)	Coeff.:	Value	Std.Er.	t value	Pr(> t)
(Intercept)	12.4937	2.9752	4.1992	**	(Intercept)	8.9097	2.4335	3.6612	**
TMax	0.0234	0.0088	2.6591	*	TMax	0.0329	0.0078	4.1849	**
Rain	0.0345	0.0103	3.3262	*	Rain	0.0361	0.0114	3.1468	*
Residual st. error: 0.4896 on 9 degrees of freedom					Residual st. error: 0.5344 on 9 degrees of freedom				
Multiple R-Squared: 0.7370					Multiple R-Squared: 0.7919				
F-statistic: 9.8110 on 2 and 7 degrees of freedom, the p-value is 0.0093					F-statistic: 13.3057 on 2 and 7 degrees of freedom, the p-value is 0.0041				
<i>Salix acutifolia</i> Willd.					<i>Salix smithiana</i> Willd.				
Coeff.:	Value	Std.Er.	t value	Pr(> t)	Coeff.:	Value	Std.Er.	t value	Pr(> t)
(Intercept)	5.8228	1.4428	4.0356	**	(Intercept)	1.8311	2.3010	0.7957	*
TMax	0.0417	0.0097	4.2876	**	TMax	0.0690	0.0167	4.1209	**
Rain	0.0458	0.0136	3.3576	*	Rain	0.0465	0.0176	2.6424	*
Residual st. error: 0.6866 on 9 degrees of freedom					Residual st. error: 0.8617 on 9 degrees of freedom				
Multiple R-Squared: 0.8301					Multiple R-Squared: 0.7664				
F-statistic: 17.1084 on 2 and 7 degrees of freedom, the p-value is 0.0020					F-statistic: 11.4839 on 2 and 7 degrees of freedom, the p-value is 0.0061				
<i>Sambucus nigra</i> L.									
Coeff.:	Value	Std. Er.	t value	Pr(> t)					
(Intercept)	7.8981	1.9231	4.1069	**					
TMax	0.0368	0.0064	5.7347	**					
Rain	0.0362	0.0124	2.9154	*					
Residual st. error: 0.6131 on 9 degrees of freedom									
Multiple R-Squared: 0.8595									
F-statistic: 21.4120 on 2 and 7 degrees of freedom, the p-value is 0.0010									

* p<0.05

** p<0.01

For *Robinia pseudoacacia*, the maximum correlation in all the phases was registered with GDH. The correlation coefficients for the first three phases are very considerable, exceeding 0.70, while in the R6 phase the coefficient is lower ($r=0.63$). For *Salix acutifolia*, in all the reproductive phases the maximum correlation occurs with GDH, even if the highest coefficients calculated with some GDD thresholds are quite close to them. For example, in the R3 phase the coefficient calculated with GDH is 0.65, while with GDD10 and GDD11 it is 0.63.

For *Salix smithiana*, the maximum correlation in the R3 phase occurs with GDD10 and GDD11 thresholds, while in the other phases with GDH. Nevertheless, the most significant GDD are GDD8-GDD9 for the R4 phase, GDD6-GDD7 for the R5 phase and GDD6 for the R6

phase. The behaviour of *Sambucus nigra* in regression analysis is very similar to that of *Salix smithiana*, even if the correlation coefficients are more considerable and on the average reach values close to those of *Cornus sanguinea*.

In conclusion the highest thresholds considering the GDD amounts were shown by *Sambucus nigra*, both the *Salix* and above all by the *Crataegus monogyna* while the lowest thresholds were those evidenced by *Ligustrum vulgare* considering all the reproductive phases.

Discussion and Conclusions

The results of the phenological observations reported in this study show how useful it is to investigate climate changes using the biological responses of vegetative or-

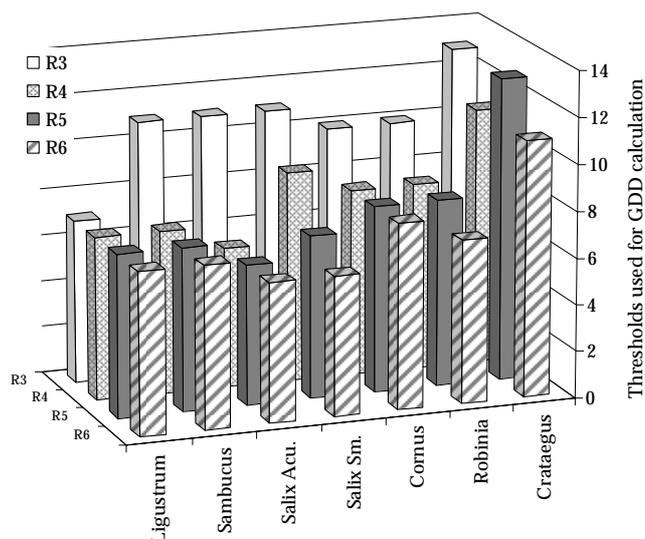


Fig. 5 - Thresholds used for GDD calculation which show the highest correlation between reproductive phases and temperature amounts.

Fig. 5 – *Temperature soglia utilizzate per il calcolo delle GDD che esprimono le maggiori correlazioni tra fasi riproduttive e sommatorie termiche.*

ganisms. In fact, vegetative development is highly influenced by temperature changes (Linkosalo *et al.* 2006), although sometimes vegetative responses, especially during the reproductive phases, can shift and not correspond with temperature due to interactions with other meteorological variables (particular values of rainfall, heliophany or biotic stress). In particular, a specific climatic discontinuity and consequently a variation of the phenological time series was discovered at the end of the 1980s, when a data set of 17 phenological phases from Germany, Austria, Switzerland and Slovenia was analysed spanning the time period from 1951 to 1998 (Scheifinger *et al.* 2002). From this point of view, the results of our observations showed that during 1999 almost all the vegetative species considered had a very short reproductive period, and the phases from R3 to R6 were very close in time. This phenomenon is most marked in the two species of *Salix*, while only the *Cornus sanguinea* species did not manifest it. However, contemporary climate analysis did not facilitate the interpretation of this manifestation, since no particular meteorological phenomena or trends were registered during the first months of the year. In particular, we did not register a considerable increase of temperature, which could explain a shortening of the development cycle during the months of April and May, when the reproductive structures are in full development. The subdivision of the historical series in the two sub-periods helped us to highlight a phenomenon of delay of the second sub-period compared to the first in the occurrence of the four reproductive phases in almost all vegetative species, as we had hypothesized. The average delay in the occurrence of the reproductive phases is more considerable for the two species of *Salix*, especially for the R3

phase, as a consequence a flower development slow-down.

The meteorological observation results showed that currently we are in a phase in which the first months of the year are characterized by a tendency for colder temperatures (as even the difference between trends of T_{min} and T_{max} recorded until the 10th week and the successive dates indicate); afterwards, as the seasons progress, the tendency inverts, and then in summer months there is a warming trend. This climate process could be supported by the hypothesis of different researchers (Schröter *et al.* 2005), which suggested that in the different regions of the world the climate is changing towards an accentuation of extreme climatic phenomena with colder winters and hotter summers.

The T_{max} amounts appear to be closer to the flowering dates in comparison to the other meteorological variables for all the species investigated, indicating that for the reproductive development the forcing temperatures are represented above all by the higher temperature that works as an environmental timer. The importance of T_{max} for the biological phenomenon determination is strengthened by the multiple regression results which demonstrate a close relationship between flowering timing and temperature summation.

The correlation analyses results between the mean phenophases' timing and the mean accumulated temperature (through GDH and GDD) evidenced a phenomenon in which the best relationship is obtained with a major temperature accumulation obtained by combining a lower threshold temperature with the successive phenophases. In fact, considering GDH, the best correlation values were obtained progressively as the reproductive season evolved, and therefore little by little more heat was accumulated. Generally, the GDD accumulations in correspondence to the highest correlation values were registered considering the decreasing thresholds (e.g. 12-10-8-7) as the season evolves (R3-R4-R5-R6). This fact suggests the importance of reaching high temperatures, especially for the initial flowering phenomenon (R3: swollen buds and open flowers) which, once initiated, would not need high heat amounts anymore, as a significant decrease of thresholds starting from the R4 phase confirmed. However, the behaviour of the *Ligustrum vulgare* and *Crataegus monogyna* species does not match this general consideration. In fact, the *Ligustrum vulgare* species seems to have a "physiological clock" which is activated by relatively low temperatures (7 °C) before the arrival of the late spring period, as the low thresholds for all the four phases showed. Also, for *Ligustrum vulgare* the low correlation values between the flowering dates and GDD suggest a scant necessity of this plant to regulate the start of flowering.

Regarding the *Crataegus monogyna* species, instead, it seems that all the flowering phases are determined by the temperature accumulations obtained using the high thresholds. Thus, the entire flowering process is optimized starting from the temperature accumulation reached in an advanced phase of the growing season.

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