

# Phenological investigations of different winter-deciduous species growing under Mediterranean conditions

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**Abstract** – Phenological stages are the result of biorhythms and environmental factors, these last are probably the same ones that caused, during evolution, adjustments of the species to different climate. The present study was carried out in a Phenological Garden located in central Italy (Perugia, Umbria Region) which contains indicator species, common to all International Phenological Gardens. The aim of this study was to determine and analyse the average trends of development of eight plant species and their phenological adjustment to the Mediterranean environment, over a nine-year period (1997–2005). The results of the statistical analyses show a strong relationship between the temperature trends and vegetative seasonal evolutions interpreted by phenological data for all the species considered. Moreover, it was demonstrated that the plants studied may approach or close completely the timing gaps eventually created during the first phenological phases, adjusting thus the beginning of subsequent phenophases.

phenology / garden / climate / trends / Mediterranean

**Résumé** – **Recherches sur la phénologie de différentes espèces décidues sous climat méditerranéen.** Les stades phénologiques résultent des biorhythmes et des facteurs environnementaux qui sont probablement ceux là même qui ont provoqué les changements d'aires de répartition des espèces pendant leur évolution, en réponse aux changements climatiques. La présente étude a été réalisée dans un Jardin phénologique situé dans le centre de l'Italie (Perugia, Ombrie) où l'on trouve des espèces indicatrices communes à tous les Jardins phénologiques internationaux. Le but de cette étude a été de déterminer et d'analyser les tendances moyennes de développement de huit espèces de plantes et leur ajustement phénologique à l'environnement méditerranéen, dans une période de neuf ans (1997–2005). Les résultats des analyses statistiques montrent une forte corrélation entre les tendances des températures et le développement végétatif saisonnier, pour toutes les espèces étudiées. On a également démontré que les plantes étudiées peuvent réduire ou éliminer les décalages temporels entre les premières phases phénologiques, en ajustant le début des phénophases suivantes.

phénologie / jardin / climat méditerranéen / tendances

## 1. INTRODUCTION

In the first 1970s, Lieth defined Phenology as the study of the timing of recurring biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species [18]. During the 1980s other researchers interpreted phenology as the study of the seasonal timing of life cycle events of organisms [30]. Moreover, in the 1990s it was considered that factors influencing phenology vary by species, but include photoperiod, soil moisture and temperature, air temperature, solar illumination and snow cover [31].

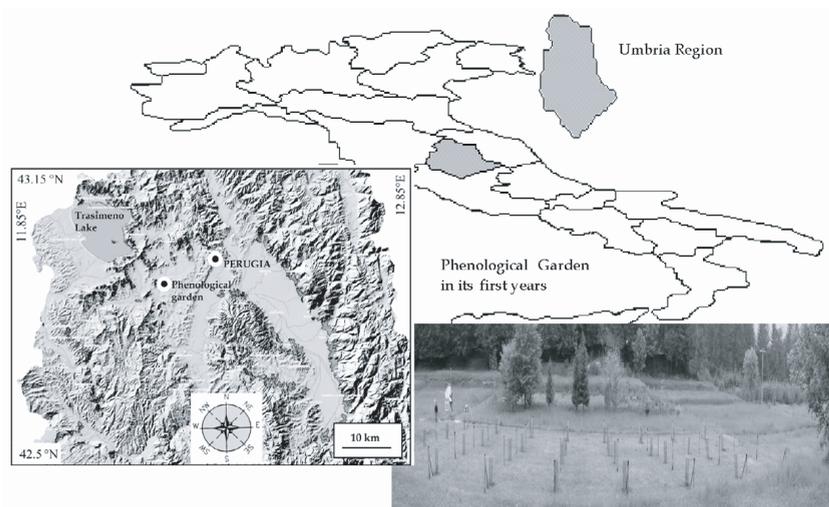
The observed phenomena (the phenological stages) include flowering, the leaf unfolding, the leaf fall and any other observable cyclic phenomenon. Phenological stages are the result of internal factors which are biorhythms; i.e., the rhythms regulated by the genetic constitution of the species, and external factors which are environmental and particularly climatic ones. The long-term repetitive cycles of climatic and astronomical factors are the direct and indirect exogenous causes of the biorhythms; while meteorological conditions may induce

some temporary limited phenological adjustments, which may evolve or not in adaptation and exaptation phenomena in relation to the typical plasticity of the plant species [1].

The study of the phenology of plant communities (syn-phenology and syn-biorhythms) has been applied in land, pasture, forest and water resource management programs [8, 37]. In climatology and ecology, phenology and syn-phenology are used to determine the degree of climatic changes that have occurred and to predict the potential consequences [15, 18, 23, 24]. In particular, several studies were conducted to investigate the phenological behaviour of various species in different Mediterranean climate conditions, which sometimes can be characterized by rather high natural variability due to the presence of important limiting factors such as very cold winters (chilling phenomena) or dry summers causing a water stress [2, 6, 7, 16, 22].

However, in general large Mediterranean areas are characterized by moderate climate with a relatively small range of temperatures between the winter lows and the summer highs. The daily range of temperatures during the summer is wide, except along the immediate coasts. The winter temperatures rarely reach the level of freezing, although in some years chilling phenomena may occur in high altitude and

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**Figure 1.** Phenological Garden located near Perugia, in the Umbria Region (central Italy).

internal “continental” areas and may severely damage evergreen shrubs and trees of different species, both cultivated and wild. In the summer, the temperatures are warm, but do not reach the high temperatures of inland desert areas. In the Mediterranean area (i.e., Spain, southern France, Italy, southern Croatia, Montenegro, Macedonia, Albania, Greece and northern Africa), the summer months usually are hot and dry; almost all rainfall in this area occurs in the winter, the mean annual temperatures for several locations can go down the 10 °C in winter and over 30 °C during summer [3].

Several studies were carried out concerning the coupling of winter-deciduous species’ seasonal evolution to the Mediterranean climate and possible utilization of these species as bio-indicators in climate change investigations [10, 17, 20, 25, 26, 34]. Generally, all organisms may be considered “potential” bio-indicators, when they are correctly inserted in the ecosystem because plants can highlight the alterations caused by different factors; a response to any kind of disturbance must thus be interpreted and evaluated because it summarizes the synergic action of all environmental components. Therefore, current climate changes can influence, more or less seriously, the vegetative-reproductive cycle of a plant species [21].

The aim of this study was to determine and analyse the average development trends of some winter-deciduous species and to evidence the ones more phenologically adjusted to the Mediterranean environment, over a nine-year period (1997–2005). In addition, the phenological study was used as a tool to investigate the climate/plant relationships and, in particular, to monitor current climatic changes with the expectation that the future utilization of long-term database in large study areas could be useful in the prediction of future climatic scenario in the Mediterranean area.

## 2. MATERIALS AND METHODS

### 2.1. Study species and sites

The management of Phenological Garden, apparently, is not very complicated, considering the fact that indicator plants should be left

growing in a natural way as long as possible. Following the standard procedures for planting and managing species in phenological gardens, study plants were watered and fertilized during the adaptation period after planting (the first 2 or 3 years), while pruning and antiparasitic treatments were applied continuously [33].

The Perugia Phenological Garden contains some indicator species common to all International Phenological Gardens (IPG) [32]. It is located at the distance of 25 km from Perugia (the regional capital of the Region of Umbria, in central Italy) in an area of Mediterranean climate with a subcontinental influence.

The garden’s total area is 1.9 ha and it has the following geographic coordinates: 43° 00′ 40″ North latitude and 12° 14′ 52″ East (Greenwich) longitude. The area is exposed to South/South-East and partially protected from the cold winds coming from North. However, since the indicator species are located in the highest and the most open site of the area, they are subject to the variations of wind direction. The ground, being on a slope with a gradient of about 12°, presents the difference in altitude of 10 m, from 270 m a.s.l. to 260 m a.s.l. (Fig. 1).

Meteorological data were recorded in the station of the Italian Central Ecological Office located in Marsciano (Perugia area) near to the Phenological Garden (about 50 m), at the altitude of 211 m a.s.l. with coordinates of 43° 00′ 15″ North latitude and 12° 18′ 00″ East longitude.

The mean annual temperature and total annual rainfall recorded during the 9-year period evidenced values of about 13 °C and 650 mm.

The plant species of the Phenological Garden were obtained from mother plants received from the German Weather Service, the European coordinator for the distribution of IPG clones. The National Working Group for Phenological Gardens selected the plants that were adopted as indicator species from the species proposed by the IPG. Since all the species are typically from northern European climates which are characterised by cold winters, mild summers and abundant rainfall, the group of selected species would adapt to the Mediterranean climate with the only exception of the *Salix* species that may have some problems due to the Mediterranean summer droughts.

Moreover the Phenological Garden contains indicator species that are common only to the Italian Phenological Gardens and that are representative of the geographical area where the garden is located.

The winter-deciduous indicator species examined were:

(1) *Cornus sanguinea* L.

Family: Cornaceae; common names: dogberry, dogwood.

It flowers in the period of April–June and fructifies in August–September. This plant is present in all Europe (except for the extreme north) and in western Asia. It is distributed in the entire national territory, from sea level to 1200 m.

(2) *Crataegus monogyna* Jacq.

Family: Rosaceae; common names: hawthorn, thornbush.

This is a spiny bush or a small tree. It flowers in the late spring (May to early June) and fructifies in summer. It is distributed in the entire national territory, both on plain and in hill areas.

(3) *Corylus avellana* L.

Family: Corylaceae; common name: hazel.

This is a deciduous shrub or a small tree. It flowers in January–March and fructifies in August–September. It is present in the entire national territory, in Europe, western Asia and northern Africa.

(4) *Ligustrum vulgare* L.

Family: Oleaceae; common name: privet.

This is a deciduous shrub or a small tree, up to 2–3 m tall. The plant flowers in May–June and fructifies in September. It is widely present in Europe (western, central and southern), extends on north up to southern Scandinavia and in western Asia. It is distributed in the entire national territory, except for the islands.

(5) *Robinia pseudoacacia* L.

Family: Fabaceae; common name: black locust, acacia.

This is a deciduous tree up to 25 m high. It flowers in the period of May–July and fructifies in summer. It is native to the central America, but has been widely planted and naturalized in Europe and Asia. It is present in the entire national territory and is considered an invasive species in some areas.

(6) *Salix acutifolia* Willd.

Family: Salicaceae; common name: violet willow, sharp-leaf willow.

This is a deciduous shrub or a small tree up to 10 m high. It flowers in the period of February–April, before the bud burst, and fructifies in May–June. It is mainly diffused in central and northern Europe and does not grow spontaneously in Italy.

(7) *Salix smithiana* Willd.

Family: Salicaceae; common name: Smith's willow.

This is a deciduous shrub or a small tree up to 9 m high. It flowers in the period of February–April, before the bud burst, and fructifies in May–June. It is mainly diffused in Europe, and does not grow spontaneously in Italy.

(8) *Sambucus nigra* L.

Family: Caprifoliaceae; common name: elderberry.

This is a deciduous shrub up to 8 m high. It flowers in the period of April–July and fructifies in September. It is diffused in Europe, including Britain. In Italy it is present in the entire national territory, from sea level to 1800 m.

The information about the cited plant species were obtained from different Flora guides [29, 35].

## 2.2. Plant sampling

The phenological sampling frequency was weekly (52 samples/year) and was carried out according to some basic criteria using phenological keys described by various authors [4, 34] and on

the basis of the experience of the International Phenological Gardens [32]. In particular, for the vegetative cycle the following phenological phases were considered [27]:

(V1) bud dormancy; (V2) swollen bud next to the opening; (V3) swollen bud and bud burst, with folded leaves; (V4) bud just opened and young open leaves; (V5) young open leaves; (V6) young and adult leaves; (V7) adult leaves; (V8) beginning of autumn leaf colouring; (V9) leaves mostly coloured; (V10) beginning of leaf withering; (V11) leaves mostly withered; (V12) beginning of leaf fall; (V13) leaves mostly fallen.

In the Perugia Phenological Garden five plants for each species were planted in 1994. The phenological survey of these plants started after three years from the date of planting. From 1997 the observations were conducted on three individuals for each species for obtaining a mean interpretation of the phenological phases, considering the possible random variability even in genetically similar plants. The mean date for the onset of the various phenophases was obtained by taking the mathematical average of the dates when it appeared in each individual plant (phenoid). Some vegetative phases, however, may not be represented in all the phenoids, so the mean values are calculated only in the plants in which these phases are shown. Generally, the phenological observations were carried out on the same three phenoids as indicated by the Phenological Garden protocols. However, during 2001 one plant of *S. acutifolia* had some problems; so it was substituted by the one of two remaining plants of the same age present in the garden and this new plant has been monitored since 2002.

## 2.3. Calculations and statistical analyses

The average of the starting date of every phenophase was calculated considering the three phenoids of all the study species. These averages provide a mean model of development in relationship to the species and to the year of observation. Using yearly development dates, the mean values of the phenological data were computed for the different species in relationship to the nine years studied (1997–2005) in order to obtain the mean development trends in the study area. For a general view of the annual behaviours of the studied species and their progressive vegetative developments, plots of the seasonal evolution were obtained.

An attempt was made to determine the nine-year meteorological trends for the study area. The cumulative values of meteorological variables were calculated from 1 January to five different dates corresponding to the 10th, 20th, 30th, 40th and 50th weeks of the year and linear trend lines were constructed.

These dates correspond to the regular intervals of temperature accumulation and therefore, subdivide the entire annual cycle in five homogeneous sub-periods. Also, they define temperature summations for each study area in relationship to the important climatic periods such as: last winter, including chilling phenomenon (until the 10th week); spring, including forcing phenomenon (20th tweek); summer, considering principal heat waves (30th week); autumn, considering total summer period and seasonal water stress (40th week); first winter, considering dormancy induction (50th week) [9].

To summarize the phenological data variability, an analysis of each vegetative phase was realized during the entire period of nine years. Coefficients of Variation (CV) were calculated according to the standard formula (Standard Deviation/Mean) and tabulated, based on the yearly mean values for each species. This evaluation gives us

**Table I.** Results of the Pearson correlation analysis (all the coefficients have a *P*-value lower or equal to 0.001).

Species	Phases	Tmin	Tmax	Rain	Sun. dur.	Species	Phases	Tmin	Tmax	Rain	Sun. dur.
<i>Cornus sanguinea</i> L.	2	-0.04	0.98	0.50	0.89	<i>Corylus avellana</i> L.	2	0.23	0.96	0.24	0.89
	3	0.12	0.96	0.42	0.76		3	0.23	0.95	0.33	0.68
	4	0.10	0.89	0.23	0.63		4	0.60	0.88	0.59	0.59
	5	0.76	0.97	0.66	0.89		5	0.90	0.98	0.58	0.94
	6	0.97	0.99	0.69	0.96		6	0.97	0.99	0.55	0.97
	7	0.98	0.99	0.75	0.97		7	0.97	0.99	0.63	0.97
	8	0.93	0.95	0.30	0.93		8	0.95	0.96	0.28	0.96
	9	0.89	0.93	-0.16	0.67		9	0.95	0.96	0.34	0.92
	10	0.51	0.77	-0.38	0.52		10	0.91	0.93	0.38	0.87
	11	0.80	0.95	-0.79	0.54		11	0.94	0.93	0.18	0.85
	12	0.70	0.87	-0.77	0.74		12	0.97	0.93	0.31	0.86
	13	0.68	0.82	-0.69	0.75		13	0.93	0.85	0.26	0.61
	<i>Crataegus monogyna</i> Jacq.	2	0.11	0.93	0.21		0.82	<i>Ligustrum vulgare</i> L.	2	-0.12	0.97
3		0.14	0.84	-0.16	0.17	3	0.17		0.98	0.46	0.89
4		0.68	0.93	0.68	0.57	4	0.35		0.95	0.35	0.56
5		0.92	0.98	0.65	0.91	5	0.70		0.96	0.40	0.88
6		0.98	0.99	0.66	0.98	6	0.98		1.00	0.69	0.98
7		0.97	0.99	0.46	0.99	7	0.98		0.99	0.72	0.99
8		0.98	0.98	0.54	0.98	8	0.94		0.95	0.13	0.96
9		0.96	0.94	0.66	0.95	9	0.79		0.89	-0.18	0.50
10		0.96	0.91	0.57	0.92	10	0.39		0.68	0.26	0.41
11		0.97	0.92	0.49	0.90	11	0.24		0.63	0.25	0.56
12		0.96	0.89	0.32	0.86	12	0.41		0.68	0.11	0.69
13		0.91	0.85	0.41	0.81						
<i>Salix acutifolia</i> Willd.		2	-0.02	0.85	0.50	0.65	<i>Salix smithiana</i> Willd.		2	-0.07	0.94
	3	0.03	0.90	0.25	0.41	3		0.13	0.92	0.39	0.57
	4	0.16	0.81	0.18	0.31	4		0.56	0.96	0.61	0.71
	5	0.74	0.96	0.27	0.88	5		0.58	0.96	0.56	0.73
	6	0.98	0.99	0.74	0.98	6		0.96	0.99	0.60	0.97
	7	0.98	1.00	0.65	0.98	7		0.98	1.00	0.63	0.98
	8	0.89	0.92	0.06	0.88	8		0.95	0.98	0.55	0.97
	9	0.61	0.69	-0.13	0.62	9		0.87	0.87	-0.03	0.94
	10	0.58	0.60	-0.05	0.61	10		0.85	0.83	0.12	0.91
	11	0.70	0.62	0.09	0.65	11		0.87	0.82	0.06	0.95
	12	0.75	0.68	0.08	0.76	12		0.92	0.86	-0.03	0.96
	13	0.71	0.60	0.24	0.64	13		0.72	0.73	-0.11	0.91
	<i>Robinia pseudoacacia</i> L.	2	0.04	0.87	-0.03	0.31		<i>Sambucus nigra</i> L.	2	0.56	0.97
3		0.15	0.87	0.13	0.32	3	0.07		0.84	-0.26	0.69
4		0.66	0.96	0.56	0.81	4	0.66		0.96	0.52	0.87
5		0.96	0.99	0.44	0.97	5	0.83		0.97	0.58	0.89
6		0.98	0.99	0.58	0.99	6	0.94		0.98	0.70	0.90
7		0.97	0.99	0.18	0.97	7	0.96		0.98	0.47	0.95
8		0.91	0.93	0.29	0.92	8	0.94		0.97	0.15	0.92
9		0.93	0.92	0.27	0.82	9	0.90		0.93	0.26	0.89
10		0.94	0.93	0.37	0.84	10	0.90		0.90	0.45	0.80
11		0.95	0.92	0.41	0.79	11	0.87		0.87	0.47	0.70
12		0.94	0.90	0.40	0.72	12	0.84		0.84	0.38	0.58
13		0.63	0.58	0.30	0.39	13	0.80		0.79	0.50	0.31

indirectly the homogeneity degree of all the phenophases for each species during their annual vegetative growth.

Moreover, a correspondence analysis (CA) and a detrended correspondence analysis (DCA) were carried out to compare the phenological matrix (phenological dates) with the environmental matrix of Tmin, Tmax, Rain and Sunshine duration (heliophany) data. The data used in these analyses were the mean values calculated in the period 1997–2005 for every species. In consideration of the results con-

ducted in the DCA chart a Pearson correlation analysis was carried out to establish the effective numeric interactions between meteorological variables and phenophases. This type of analysis considered the progressive dates (in weeks) of the 13 phenological phases and the daily values of the principal meteorological variables as minimum and maximum temperatures (°C), rain (mm) and sunshine duration (min), calculated since 1 January to the dates of each phase for every plant species analysed during the nine years (9 samples). The

daily summation values are usually utilized to interpret the potential relationships between the accumulation of thermal degrees (thermal amounts) and the vegetative development of the plant species and to forecast the different growth phases [19]. Also, a multiple regression analysis was used to determine in a mathematical form the relationship degrees between meteorological variable amounts and the vegetative development of the species. The meteorological data were used as the independent variables, while the vegetative development dates (in weeks) were used as the dependent variable. To verify the possible use of the data to predict vegetative phases in our context, a simulation of the 2005 dates was made (“in sample” reconstruction) to test the two climatic and biological trends.

The CA was carried out with the use of the MVSP software (MultiVariate Statistical Package) applying an algorithm in which the solution for each axis is calculated separately. It was done using the reciprocal averaging method described by Hill [13]. In consideration of the present results, the Pearson correlation analyses between the meteorological variables and the phenological dates are reported (Tab. I). The correlation and the regression analyses were carried out using the S-Plus statistical software; in particular, the default *P*-value utilized in the correlation analyses was equal to 0.001 value.

The one-way ANOVA analysis (calculated with the use of the S-Plus statistical package) between fitted and real phenophase dates was realized to evidence the significance level of the predictions.

### 3. RESULTS

Generally, for the different species considered in this study the phenological phases corresponding to the beginning of growing season (phases V4-V5) occurred from the 13th to the 15th week (Fig. 2). These results are in agreement with those reported in previous studies [1] conducted in similar latitudes, in which such phenomena occurred at the end of March or the beginning of April. Moreover, the phenological phases that correspond to the end of the growing season (phases V8-V9) occurred in the period around the 40th week (the end of October), in response to the characteristics of the studied area. Linear trend lines were added to the charts of each species with the relative  $R^2$  values. Even if in almost all the cases the vegetative seasonal development is more than proportional until the young leaves phase (V6), while in the second part of vegetative growth the increase is less than proportional, yet the linear trend lines appear to interpret very well the essential phenological trends ( $R^2$  between 0.93 and 0.97). In two cases (*C. avellana* and *S. nigra*) the development from phase V2 to phase V6 is realized according to the perfect linear trend, and then from phase V7 to the end of the vegetative growth the development proceeds as for the other species (less than proportional).

The trend of CV is sufficiently similar for the different species: in the first phenological phases (V2; V3) the values are the highest, while generally in the two successive phases they become lower than 0.2. In phase V6 the values have the last increase and then become definitively lower in the successive phases. In three cases (*C. monogyna*, *S. acutifolia*, *R. pseudoacacia*) the phenological phases are quite homogeneous in terms of date registration during the nine years, showing CV values always lower than 0.2. In particular, in these

species high values in the first phases are missing and the higher CV are presented by the phases V5-V6.

In Figure 3, the linear trend lines from 1997 to 2005 for all the phenological phases are shown (part A). Moreover, in the same figure the mathematical angular coefficients of the linear trend lines for the different phases are reported for each species to show the slope of the phenophases' timing expressed in weeks per year (part B). In linear functions ( $y = mx + b$ ) the angular coefficients (slopes) are represented by the coefficient of  $x$ , therefore, the  $m$  is the slope. Generally, the slope is commonly used to describe the measurement of the steepness, incline, or grade of a straight line, a higher slope value indicates a steeper incline.

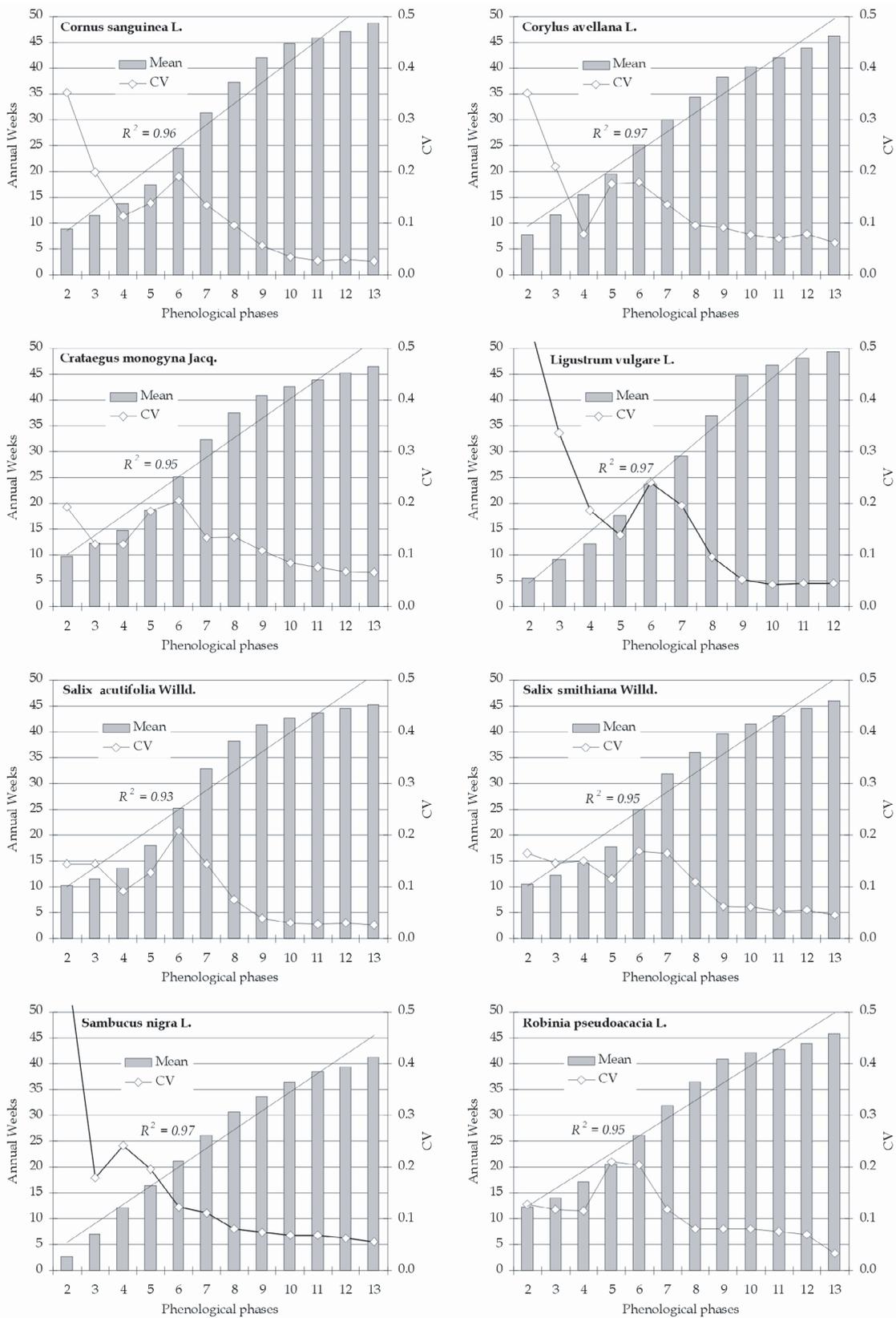
In the upper part of the Figure 3 where a mean interpretation of the phenomena is possible, considering contemporary all the species, it can be noted that for the first vegetative phases (V2-V3-V4) linear trend lines have positive angular coefficients (rising trends), while for the successive phases (V5-V6-V7-V8, evidenced in the Fig. 3 part B) the angular coefficients are negative. The linear trend lines for the last phases (V9-V10-V11-V12-V13), calculated using the mean values with all the species, evidenced angular coefficients near zero, with practically constant trends in the nine years. A positive angular coefficient is linked to the growing linear trend line and hence to the delay of phenological dates from 1997 to 2005.

In the lower part of Figure 3, the angular coefficients reported for all the different species evidenced positive values for phases V2, V3 and V4, while for phase V5 only the *Salix smithiana* showed a positive value and all the other species a negative one. Phases V6 and V7 confirmed the presence of negative coefficients and consequently of negative linear trends (advance of dates from 1997 to 2005), phase V8 evidenced negative values but near zero only for *S. nigra* and *R. pseudoacacia*. In the last phases (from V9) only the *C. monogyna* and *S. smithiana* species showed negative angular coefficients, while the others were positive or almost zero.

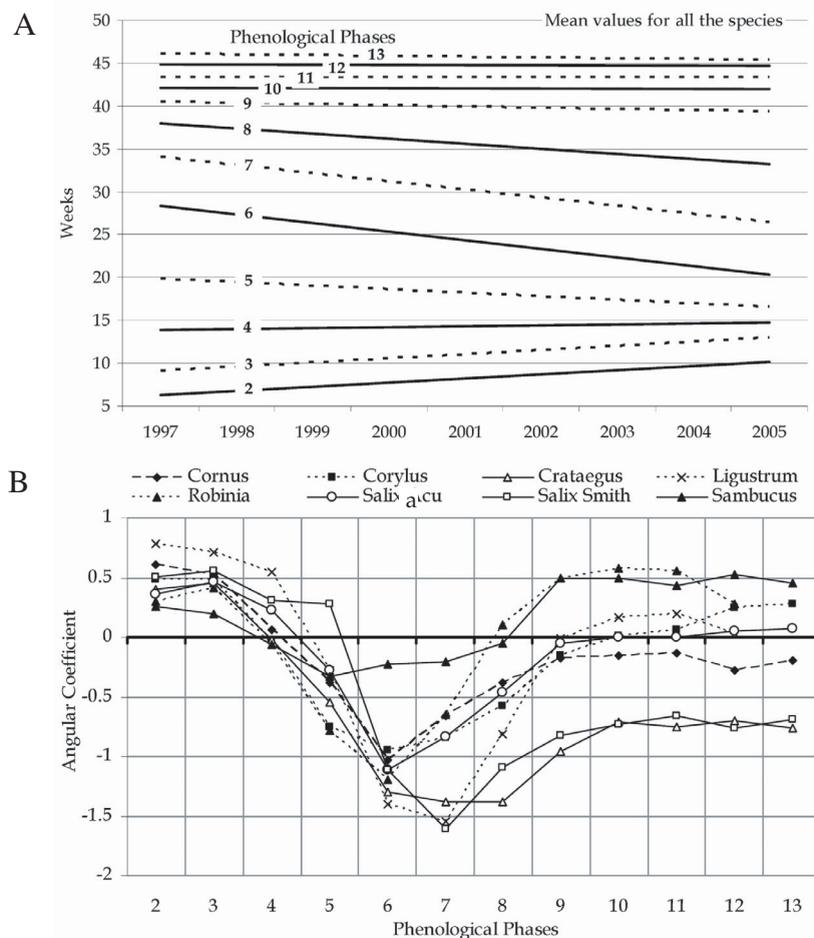
A meteorological analysis was conducted with the summations of daily temperature, rain and sunshine duration data to evidence possible trends in the nine years of the study from January to five conventional annual dates (Fig. 4). The minimum temperature amounts showed a negative angular coefficient with a progressive reduction until zero, corresponding to the phenomenon of marked temperature reduction in the first months of the last years (2002-2005) associated to the temperature homogeneity of the central and final part of the year during the historical series. The maximum temperatures confirmed the trends shown by the minimum ones with negative angular coefficients in the two first stages (the 10th and the 20th week) and successive positive values from the 30th week.

The rain amounts showed a small reduction in the first stages, while in the 40th and 50th weeks the daily summations increased in the last years of study. In particular, in the last two years (2004–2005) very high precipitations were recorded during the last months of the year.

The summations of the daily values of sunshine duration evidenced declining values in the historical series (1997–2005) for all the stages, but with lower values for the last weeks of the year, probably related to the increase of rain.



**Figure 2.** Graphs of the mean dates calculated over 9-year period of the beginning of each phenophase (bars) with linear trend lines and their  $R^2$ . The coefficients of variation (CV) of each phenophase (lines stand) were calculated on the plant sample size ( $n = 3$ ).

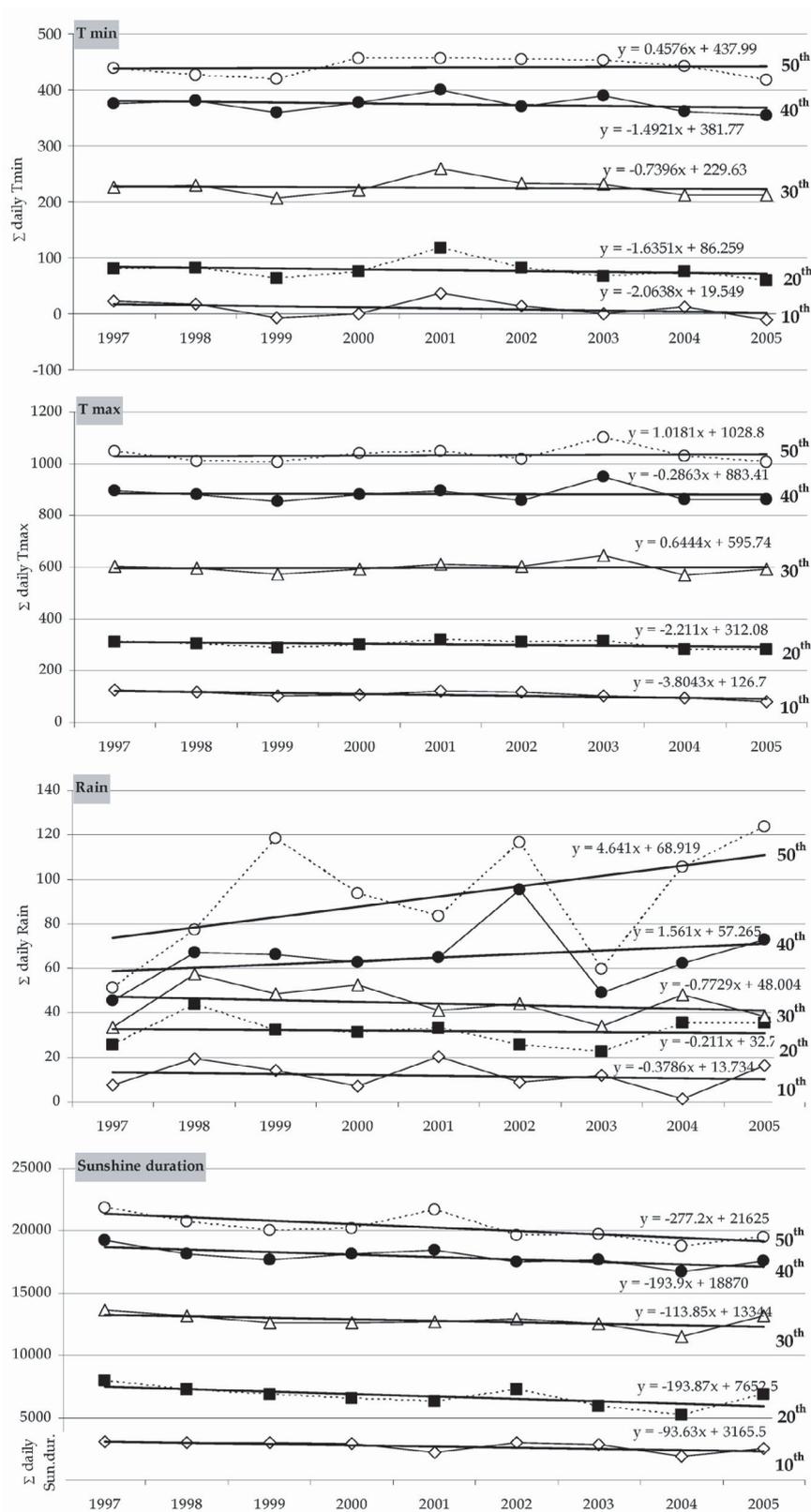


**Figure 3.** Linear trend lines from 1997 to 2005, evidenced by different type-lines, constructed by the mean values of all the species for all the phenological phases (part A) and angular coefficients of the trend lines for each species expressed as weeks/year (part B).

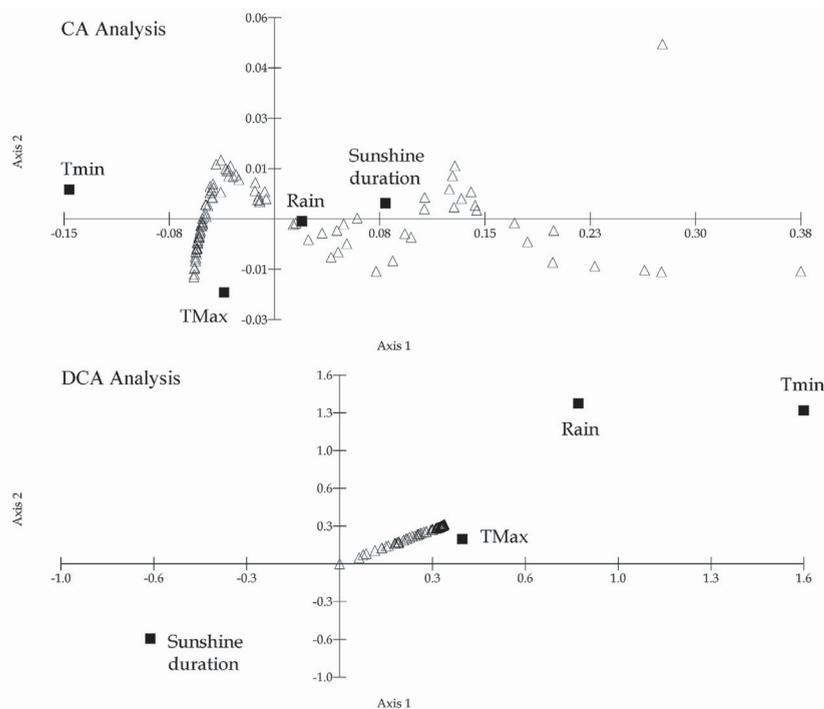
In Figure 5, the CA results demonstrate that only with a detrending investigation a linear trend can be shown by the different species and that both temperature values (principally Tmin) and precipitation have great influence in the phenophases timing while sunshine duration appear to have a secondary importance. In consideration of the present results, the Pearson correlation analyses between the meteorological variables and the phenological dates are reported (Tab. I). The most important results of this type of analyses for all the species can be shown considering the high values related to the maximum temperature for all the vegetative phases during the entire year. The minimum temperature shows high correlation values from the fourth phenological phase (V4), while in the first three phases the values are lower than 0.6. The total rainfall shows high values only for the central phases (V5-V7). On the other hand, the sunshine duration shows a correlation similar to that of Tmax, but lower for the first phases (V2-V4). The species that appeared to be the most related to the meteorological variables and for which the correlation values of at least one variable do not decrease more than 0.8 for the entire vegetative cycle are *C. avellana*, *C. monogyna*, *S. smithiana* and *S. nigra*.

Moreover, to test the relationship between meteorological variables and phenological phases, multiple regression analyses were realized for every species studied considering the historical series since 1997 to 2005. In Table II, the regression results are reported with the indication of  $R^2$ , variable coefficients and  $t$ -test. The percentage of explained variability was very high for all the species as was the significance of the predictive variables. The temperature variables (Tmin and Tmax) were the most important independent variables and were involved in the regression models for all the species, while rain was involved in the regression calculation for 4 species and sunshine duration for 5 ones. All the considered species showed very high results in terms of data interpretation with excellent significances in terms of  $R$ -square and  $P$ -value, moreover the species *C. avellana* and *S. acutifolia* evidenced the best Residual standard error values.

Moreover, to test the robustness of the regression equations obtained, a reconstruction of the data for 2005 was realized. In Figure 6, the real and fitted data are shown for the different species and the residuals are graphed in the related charts. The regression results evidenced good values for almost all the species with residuals included in one week for



**Figure 4.** Meteorological variable amounts to 5 conventional dates (10th, 20th, 30th, 40th, 50th weeks) measured at the meteorological station located near the Perugia Phenological Garden at the altitude of 211 m above sea level with coordinates of 43° 00' 15" North and 12° 18' 00" East.



**Figure 5.** The Correspondence Analysis (CA) and Detrended Correspondence Analysis (DCA) results considering meteorological variables and phenological phases. Scores for the variables (■) and cases (Δ) are graphed together, the symmetric scaling was used in the CA while the sample scores were scaled to the standard deviation of the species abundance along the gradient represented by the axis in the DCA.

*C. avellana*, *C. monogyna* and *S. nigra*. The species *S. acutifolia* showed only in the first phases (V1-V5) residuals included in two weeks, while *C. sanguinea*, *L. vulgare*, *S. Smithiana* and *R. pseudoacacia* had residuals higher than two weeks. A particular behaviour was evidenced by *L. vulgare* which until the beginning of leaf colouring (phase V8) presented phenological dates well reconstructed by the regression model, while from the 9th phase this relationship was interrupted. In the Figure 6 the one-way ANOVA results between fitted and real phenophase dates were embedded in the chart of each species to evidence the level of significance of the realized predictions. In all the cases studied, the two series appear very close to each other and there are not highly significant differences between dates.

#### 4. DISCUSSION AND CONCLUSION

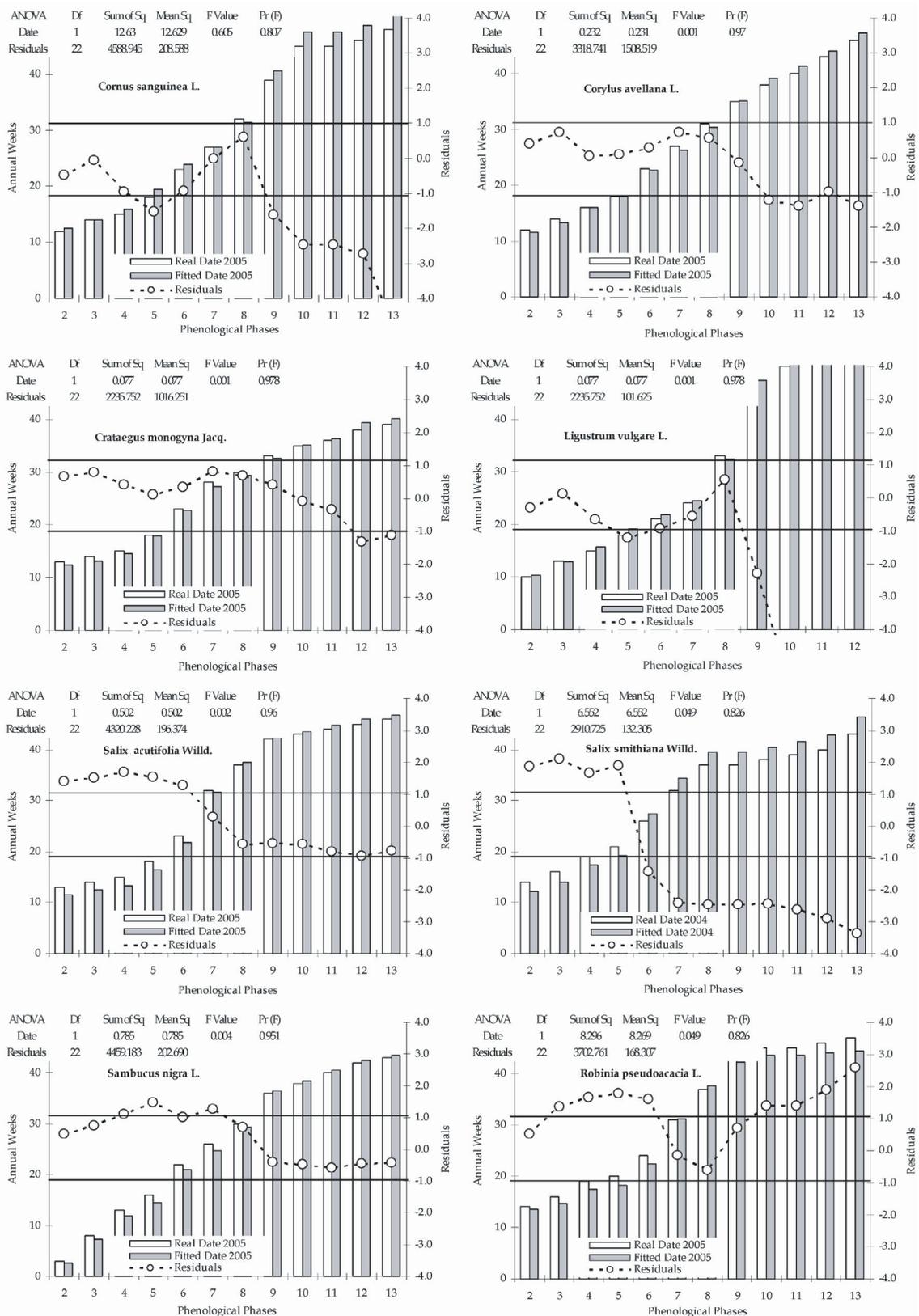
The results of the variation analysis show that the dates of the appearance of young and developing leaves, until leaf maturity (V8), were very unstable in all the deciduous species. These results suggest that once dormancy breaks in all the species (quite heterogeneous), the successive developmental phases are less variable until an ulterior period of decrease in the variability of starting dates between years that coincides with senescence (colouring and withering). Plants' hormonal changes in September–October induce the physiological changes which continue until the final phenological phase. These conclusions concur with the earlier studies in which the annual timing of leaf unfolding is to a great extent a temperature response, so the beginning of the growing season (leaf unfolding and development) should reflect the thermal regime,

while leaf withering and falling in autumn is a more complex process which is also induced by the lack of light and coldness [4].

The meteorological analysis evidenced a different behaviour of the temperatures (minimum and maximum) recorded in the first weeks of the year in comparison to those recorded from the 20th to the 25th week. In particular, a double trend phenomenon of lower winter temperatures associated with higher spring temperatures is noticed.

Rain decrease in the first months of the year, although of small entity could be related to the contemporary temperature reduction and to the delay of the first vegetative development phase. The present climatic scenario induces us to imagine the presence of generally cooler winters with less precipitations (reduced number of snowfalls and consequently reduced water supplies for the spring periods) that may induce delayed vegetative growth. On the other hand, from 2002 the rain appears to increase and it is concentrated in the autumn and early winter period with the presence of temperatures higher than the mean for the period (even in this case with low probability of snowfall). This climatic scenario,

even if less known to the large public, should be placed in the global climate warming context. Indeed, we can suppose that this last general phenomenon may induce in some areas of our planet (and the Mediterranean area can be a valid candidate for that) some contrasting chain reactions which could lead to the local cooling events. Some recent theories hypothesized that abrupt climate warming, above all at the poles, could cause the glaciers to melt and the cold polar water could influence the ocean streams with successive consequences even in the Mediterranean sea, although the water



**Figure 6.** Real and Fitted phenophase dates of the different species and the residuals (for 2005) are graphed in the related charts. The one-way ANOVA results between Real and Fitted phenophase dates are embedded in the chart of each species.

**Table II.** Multiple Regression analysis considering data since 1997 to 2005.

<i>Cornus sanguinea</i> L.				<i>Robinia pseudoacacia</i> L.			
Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )	Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )
(Intercept)	1.8413	0.4559	4.0392 0.003	(Intercept)	7.6260	0.4479	17.0253 *
Tmin	-0.0734	0.0081	-9.0609 *	TMax	0.0377	0.0006	58.9902 *
TMax	0.0605	0.0041	14.6699 *				
Rain	0.1958	0.0140	14.0212 *				
Residual St. error: 0.1976 on 8 degrees of freedom				Residual St. error: 0.7066 on 10 degrees of freedom			
Multiple R-Squared: 0.9999				Multiple R-Squared: 0.9971			
<i>Corylus avellana</i> L.				<i>Salix acutifolia</i> Willd.			
Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )	Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )
(Intercept)	1.8652	0.2239	8.3304 *	(Intercept)	0.4111	0.3564	1.1535 0.282
Tmin	-0.0958	0.0099	-9.6719 *	Tmin	-0.1621	0.0081	-19.9116 *
TMax	0.1010	0.0132	7.6380 *	TMax	0.1820	0.0079	23.1352 *
Rain	0.0985	0.0161	6.1098 *	Sun. dur.	-0.0034	0.0002	-14.3408 *
Sun. dur.	-0.0012	0.0004	-3.0059 *				
Residual St. error: 0.0993 on 7 degrees of freedom				Residual St. error: 0.1207 on 8 degrees of freedom			
Multiple R-Squared: 1				Multiple R-Squared: 0.9999			
<i>Crataegus monogyna</i> Jacq.				<i>Salix smithiana</i> Willd.			
Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )	Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )
(Intercept)	1.8481	0.4592	4.0246 0.005	(Intercept)	0.5323	0.4004	1.3296 0.220
Tmin	-0.0967	0.0157	-6.1768 *	Tmin	-0.1640	0.0083	-19.8677 *
TMax	0.1027	0.0188	5.4666 *	TMax	0.1929	0.0082	23.5506 *
Rain	0.1123	0.0264	4.2627 0.003	Sun. dur.	-0.0039	0.0003	-14.5402 *
Sun. dur.	-0.0013	0.0005	-2.5217 0.039				
Residual St. error: 0.1349 on 7 degrees of freedom				Residual St. error: 0.1303 on 8 degrees of freedom			
Multiple R-Squared: 0.9999				Multiple R-Squared: 0.9999			
<i>Ligustrum vulgare</i> L.				<i>Sambucus nigra</i> L.			
Coeff.:	Value	Std.Er.	t value <i>P</i> (>  t )	Coeff.:	Value	Std. Er.	t value <i>P</i> (>  t )
(Intercept)	1.5430	0.2741	5.6290 *	(Intercept)	0.7822	0.1869	4.1848 0.003
Tmin	-0.0754	0.0056	-13.5207 *	Tmin	-0.1548	0.0102	-15.1731 *
TMax	0.0627	0.0030	21.0840 *	TMax	0.1697	0.0166	10.2193 *
Rain	0.1826	0.0135	13.4831 *	Sun. dur	-0.0029	0.0006	-4.8118 0.001
Residual St. error: 0.1929 on 7 degrees of freedom				Residual St. error: 0.1842 on 8 degrees of freedom			
Multiple R-Squared: 0.9999				Multiple R-Squared: 0.9999			

\* *P*-value ≤ 0.001.

movement here is slow due to the only one connection with the Atlantic Ocean, the Strait of Gibraltar [5, 11, 12, 14, 28, 36].

All the species investigated evidenced high relationships between biological growth and meteorological trends, moreover considering all the species, the evaluation of the incremental ratios of each phenophase showed the highest values in correspondence with the phases V5-V8 (advance of phenological dates) demonstrating that the plants studied may approach or close completely the timing gaps created during the first phenological phases, adjusting thus the beginning of subsequent phenophases.

This particular plants' capacity could be very useful in a possible future cooling climate scenario, reducing the potential phenomenon of the decoupling of species interactions. While, on the other hand, in a warming scenario the lengthening of plant growing season could alter the structure and functioning of plant communities.

The behaviour of the temperature variables may be linked to the phenological trends. The delay of the first phenological dates could be related to the lower values of the temperature summations recorded to the 30th week. On the other hand, the successive advance of the central phases (V5-V8) may be associated with the higher maximum temperatures recorded from the 30th week.

On the other hand, the reconstruction of 2005 data probably offers the best results for the species *C. avellana* and *S. nigra* due to the particular vegetative development of these species which is very similar to a linear trend until phase V11. In this case linear regression is particularly suitable to infer the real biological performance.

The behaviours of the vegetative growth of the species *C. avellana* and *S. smithiana* are substantially similar and considering their high relationships with meteorological trends during the entire year, they can be considered as bio-monitor

species in the area of study (central Italy). In particular, for the *C. avellana* the phase V2 was registered during the 6th–7th week in the first years of the series and during the 12th week in the last years. The phase V3 was registered during the 11th–12th week until 2001 and during the 13th–14th week in the last years. The *S. acutifolia* species showed the same dates for the first two phases (with only a brief delay for the phase V2 in the first years), so both the species reflected, with the first 2 steps (V2–V3), the meteorological trend recorded until the 10th week and consequently the “delay” phenomenon or the “cooling” phenomenon of the first two months of the years in the studied area. The dates related to phase V4 for the two species evidenced a constant trend, while from the phase V5 the trend was inverted. The contemporary advance of vegetative phases and the particular growth of the minimum temperature amounts suggest the presence of a “warming” phenomenon from the end of May to the end of October including the last part of spring, the entire summer period and the first part of autumn.

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