A FOURTEEN-YEAR MONITORING IN A PHENOLOGICAL GARDEN, STUDY OF PLANT SPECIES, CLIMATE TRENDS AND THEIR RELATIONSHIPS IN CENTRAL ITALY

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Abstract. The plant adaptation to climate trends appears as a main research field in the recent past. In the Mediterranean environment a 14-year (1997-2010) phenological study was realized inside a phenological garden to investigate the climate/ plant relationships. The average phenological data thus obtained provide a mean model of development for the different species in relation to the 14-year period of observation (1997-2010). Meteorological recordings showed June and July as the months with the highest temperature increases during the central period of the study period. The phenological data showed a double-trend behaviour during the historical series considering the first two growth phases (V3, V5) that was not seen for the later phases (V7, V8). Moreover, different leaf presence periods on the tree were calculated for some of the plant species, and commonly the clearest trends were seen for V3 to V7 with a decreasing period length from 1997 to 2002-2003 and a successive quite constant behaviour. The lowest correlations between annual vegetative phases and temperature variations were manifested above all by two species (*Sambucus nigra* L. and *Robinia pseudoacacia* L.) for which the first leaf development phases appeared probably influenced by photoperiod.

Keywords: *central Italy, climatic trend, phenological garden, phenology*

Introduction

Phenological events of the vegetation, such as bud breaking, flowering and leaf colouring, are closely associated with lower atmospheric conditions as the seasons change (Zhao and Schwartz, 2003). Phenology in its present meaning is the study of events that lead to the manifestation of phenomena that are associated with the functioning of plant organs or of the plant as a whole. Detecting the growing season variability of terrestrial vegetation is crucial for the identification of responses of ecosystems to recent climate change over seasonal and inter-annual time scales (Chen et al., 2005). The great advantage of phenological observations is that they are particularly suitable to illustrate and communicate the ongoing impact of climate change.

In temperate zones, the reproductive cycle of plants is largely controlled by temperature and day length, while at lower latitudes, rainfall and evapo-transpiration also need to be taken into account (Menzel et al., 2001; Estrella et al., 2007; Orlandi et al., 2009, 2013; Bonofiglio et al., 2009). Given these relationships, a significant increase in global temperature should also be visible along series of long-term phenological observations. Indeed, changes in plant phenology are considered to be the most sensitive and observable indicators of plant responses to climate change, as has been demonstrated by a large number of studies (Fornaciari et al., 1998, 2000; Chmielewski and Rötzer, 2001; Chmielewski et al., 2004; Estrella et al., 2006). Further studies have linked inter-annual variability of plant phenology to large-scale weather features, such as the North Atlantic Oscillation and the El Nino Southern Oscillation (Beaubien and Freeland, 2000; Menzel et al., 2005; Avolio et al., 2008).

In climatology and ecology, phenology and syn-phenology approaches are used to evaluate the degree of climate change that has occurred, and also to consider the potential consequences on living organisms in general and in particular on the vegetable kingdom with phyto-phenology (Kramer et al., 2000; Mutke et al., 2003; Orlandi et al., 2005a, b). Numerous studies using phenological records have documented phenological changes throughout the northern hemisphere (including Europe), and they have supported the same general conclusion: the growing season is beginning earlier (Cook et al., 2005). Such an altered pattern of seasonal progress might also influence, for example, the food chain, animal migration, and cross-breeding between populations (Walther et al., 2002).

Another important application of the phenological models is the evaluation of the spatial-distribution-pattern of a species under the hypothesis of future climate change. Phenological studies can interpret the reproductive success of a plant population each year, and the growth and survival probability of individual plants, along with their fitness under particular climate conditions (Cleland et al., 2007).

The present study was carried out in a phenological garden that is located near Perugia, in central Italy, and which contains indicator species that are common to all international phenological gardens (IPGs) (Orlandi et al., 2007). This phenological garden also contains indicator species that are common to the Italian phenological gardens and that are representative of this geographical area. The aims of the present study were therefore to determine and analyse the mean development trends of the plant species considered, and to show the plant adaptability to the Mediterranean environment, over a 14-year period (1997-2010). In addition, plant phenology was used as a tool to investigate the climate/ plant relationships.

Materials and methods

The phenological garden is located near to the city of Perugia, in central Italy (coordinates, 42°60' N, 12°18' E; altitude, 265 m a.s.l), and it is one of the few Italian sites that is a member of the European network founded in 1957 by F. Schnelle and E. Volkert, within the Phenology Study Group of the International Society of Biometeorology. The plant species in the phenological garden were obtained from mother plants that were received from the German Weather Service, the European coordinator for the distribution of IPG clones. The National Working Group for Phenological Gardens selected the species that were to be adopted as indicator species from those proposed by the IPG. As all of these species are typically from northern European climates, which are characterised by cold winters, mild summers and abundant rainfall, the group selected species that would adapt easily to the Mediterranean climate.

The tree indicator species that were examined were those suggested by the IPG network:

1) *Cornus sanguinea* L. Common name: dogberry, dogwood;
2) *Corylus avellana* L. Common name: hazel;
3) *Ligustrum vulgare* L. Common name: privet;

4) *Robinia pseudoacacia* L. Common names: robinia, acacia;
5) Common IPG species, such as *Salix acutifolia* Willd. Common name: willow;
6) *Sambucus nigra* L. Common name: elder.

The phenological sampling was carried out according to the basic phenological criteria (e.g. every phenological stage should interpret a distinct biological event, and the data must be objective so that they can be compared with those of other studies), using phenological keys that have been described in various previous studies (e.g. Chmielewski and Rötzer, 2001). In particular, for the vegetative cycle, the following phenological phases were considered: V3, bud break and leaf unfolding; V5, young unfolded leaf; V7, adult leaves; and V8, beginning of leaf colouring.

The observations were conducted on three individuals for each plant species, to limit the random variability that can be seen even in genetically similar plants. The mean date for the onset of each phenophase was mathematically calculated considering together the three plants (phenoids) of the same species. The average dates thus obtained provided a mean model of the plant development in relation to the species and the year of observation. The mean values of the phenological data were computed for the different species over the 14-year period of observation (1997-2010), to obtain the mean development in the study area.

For each plant species, the differences between the phenological data of year x+1 and year x were calculated to determine any delay or advancement of the phases over the study years. Moreover, these differences were parameterised considering the positive values always as 1, and the negative values always as -1 (not considering the entity of variation), for an unbiased comparison of the behaviours of the different species across the different phenological periods.

Afterwards, the yearly variations (-1, +1, or 0 if the dates were the same) were summarised, to calculate the overall trend line for the identification of the phenological behaviour throughout the investigation period. Linear trend lines were constructed on the basis of the yearly variations, to interpret the 14-year tendency of each species for each vegetative phase. This methodology was utilized in place of a ‘canonical’ one, to reveal any consecutive variations year on year.

Moreover, to determine the relationships between spring and summer temperatures and the vegetative plants development, the daily temperature values were elaborated calculating growing degree days (GDDs). For the calculation of the GDDs, a single sine method was used. This method evaluates the hourly temperature trends according to the means of the single sine function (Zalom et al., 1983). Moreover, the GDD formulae were calculated using 7 °C as the temperature threshold for vegetative development of all of the plant species considered.

To interpret the climate variations over the 14-year study period, the yearly totals of the daily GDDs were evaluated and also compared year by year every 4 weeks from 1 January to the end of August, with the construction of polynomial trend lines.

The same parameterisation for the phenological data was carried out from a climate point of view. The different temperatures were interpreted in terms of annual increases (GDD total in the year x+1 higher that in the year x) and decreases. These differences were parameterised, and the parameters obtained were summarised, to show the potential increasing or decreasing trends over the study period.

Moreover, the yearly whole periods of leaf presence on the tree were considered for the evaluation of any potential variations that were mainly caused by the erratic appearance dates from the first leaves with photosynthesis activity (V3) to the leaf withering and loss of assimilation (V8): the leaf assimilation period. These periods were evaluated on the basis of their annual duration over the study period, with the calculation of the weeks between the different vegetative development phases: i.e. weeks from V3 to V8, from V5 to V8, from V3 to V7, and from V5 to V7.

Results and Discussion

The temperature trends of the study area were analysed through the GDD calculations, and these trends over the 14-year period were interpreted through non-linear regression analysis. *Table 1* shows the coefficients of the second degree polynomial trend lines, to reveal the main trends in the GDDs over the first 8 months of each of the study years. The main characteristics of these curves are shown by the evaluation of the sign of the 'a' coefficient (parabolic convexity) and by its value. All of the curves corresponding to the moving GDDs had negative 'a' coefficients, with the maximum in the central years of the study, and an increasing parabolic curvature, as testified by the Δa parameter. The months that showed the highest temperature increases during the central period of the study years were those of June and July. In particular, the highest GDDs were those recorded during 2003.

Table 1. *Coefficients of the second degree polynomial trend lines (ax2 + bx + c = 0) of average GDD amounts calculated every 5 weeks from January to July.*

Week	Coefficient a	Δa	Coefficient b	Coefficient c	R^2
5	-0.38		6.6	48.5	0.23
10	-0.66	-0.28	11.0	95.5	0.25
15	-1.43	-0.77	24.2	164.3	0.39
20	-2.38	-0.95	38.3	413.6	0.41
25	-3.72	-1.34	58.6	860.0	0.42
30	-4.72	-1.00	77.6	1369.5	0.37

The annual parameterised phenological delays or advancements are shown for all the plant species (*Fig. 1*). The annual variations related to the first two phenological phases (V3, V5) for all these overall totals, showed a delay tendency (highest values) from the first study years (2000) to the mean period (2003-2005), and then an advance tendency (lowest values) during the following 5-year period (2006-2010). On the other hand, the V7 and V8 phases appeared to follow yearly advancements to 2004-2005, and then year by year these phases showed progressive delay.

Figures 2 to *5* show the plant species that underwent significant delay or advancement trends over the study period according to the polynomial trend lines (i.e. showed R2 values ≥0.4). Only three plant species had significant trends for their phase V3 variations over the 14-year period (*Fig. 2*). In particular, the parameterised annual variations for Corylus, Robinia and Ligustrum showed a tendency towards delay (although in the last few years, Robinia showed a tendency to phenophase advancement).

In the following two Figures, again, four plant species (Cornus, Corylus, Robinia and Sambucus) showed significant phenological trends for their vegetative V5 and V7 phases (*Figs. 3, 4*). These phases showed the clearest general trends, with a tendency to advancement considering the 'young unfolded leaf' phase (V5), and on the other hand, a tendency to delay for the ‘adult leaves’ phase (V7). In particular, the analysis of phase V5 in Figure 3 shows that the clearest advancements were recorded from 2005-2006, with the phase V7 delays generally apparent from about the same period (*Fig. 4*).

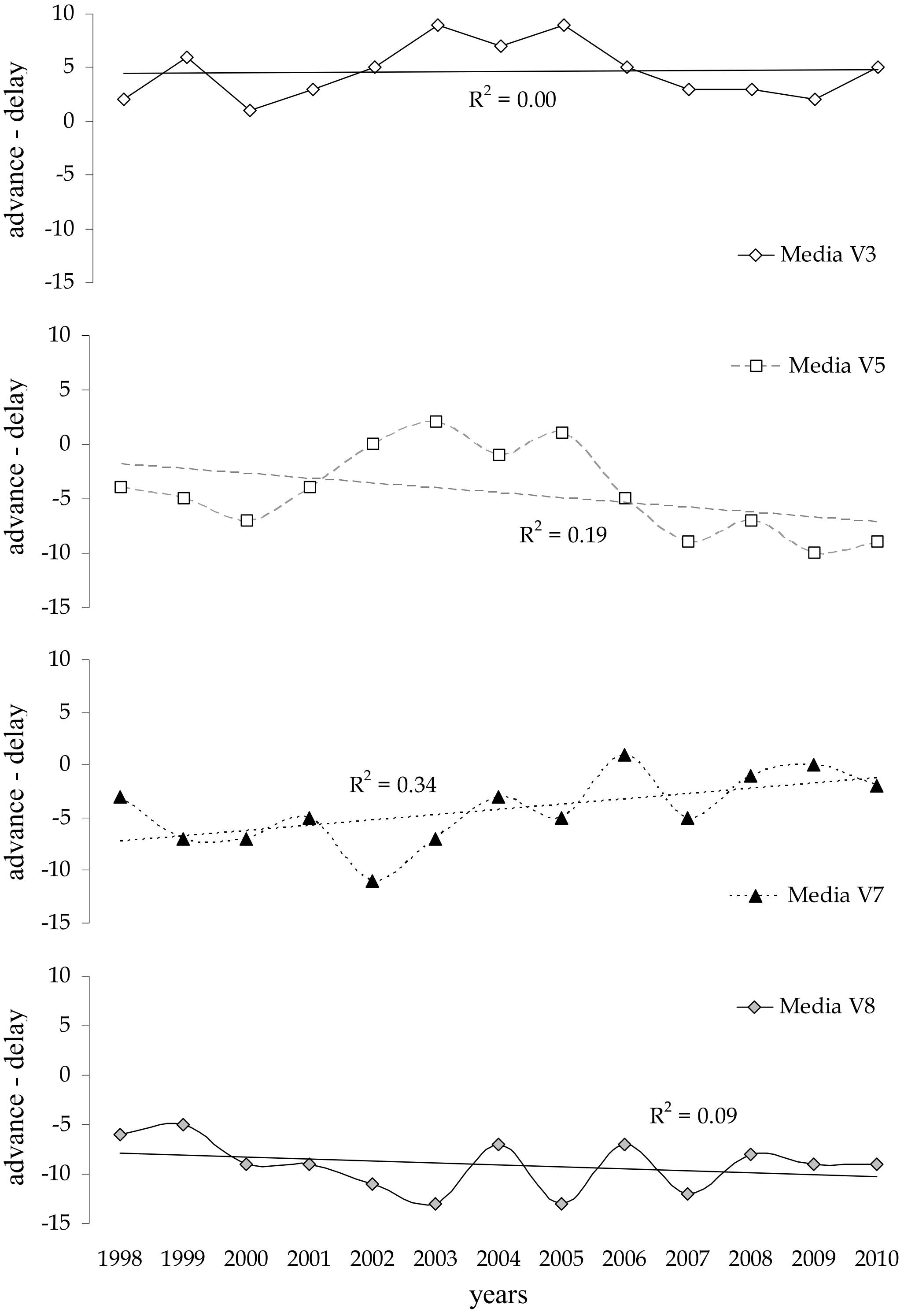

Figure 1. *Trends of normalised date delay or advancement for each of the four phenological phases, considering all of the plant species together.*

There were only two plant species that showed particular trends for the ‘beginning of leaf colouring’ of phase V8 (*Fig. 5*; Corylus and Cornus) and their behaviours were different: Corylus showed clear advancement of this final vegetative phase over the study years, while Cornus showed a delay (*Fig. 5*).

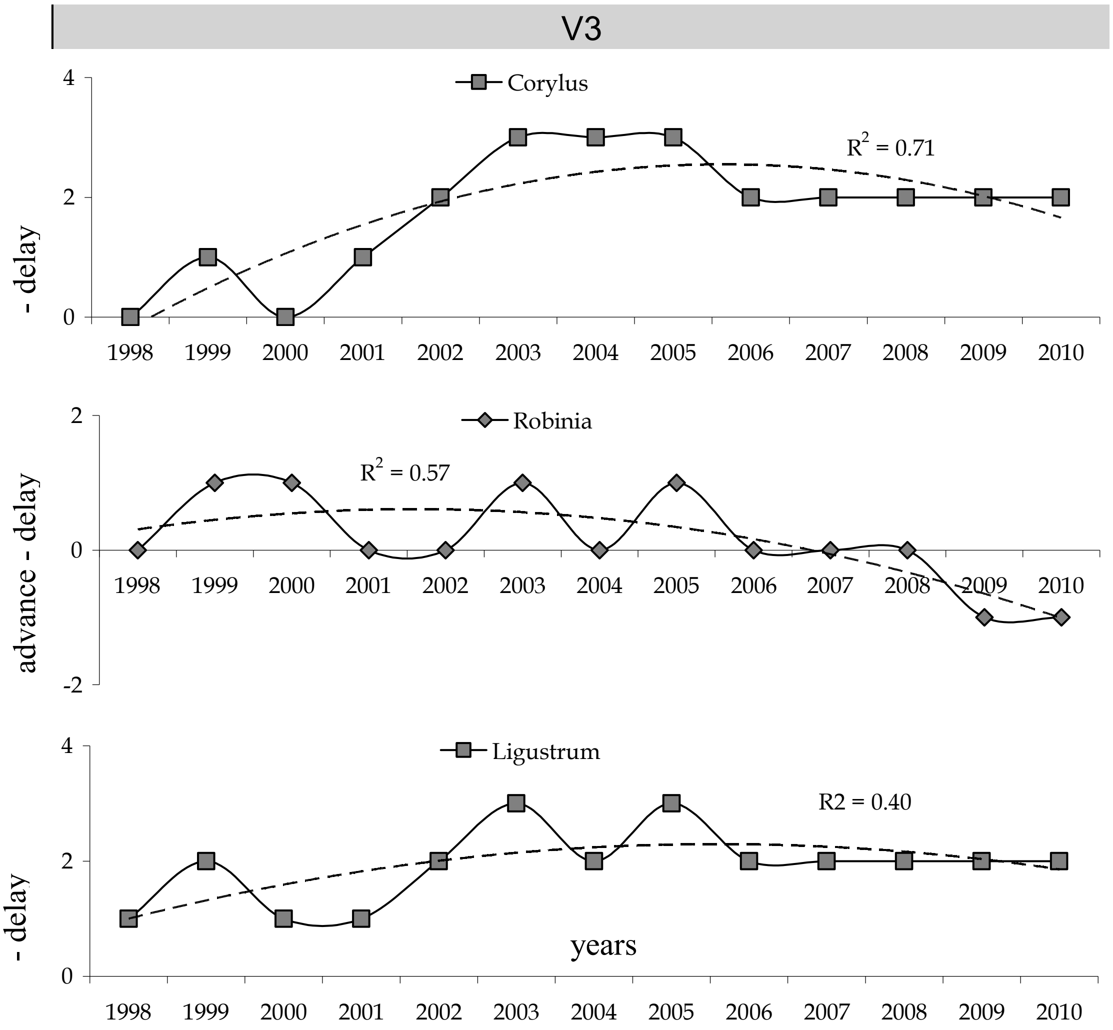

Figure 2. *Species showing significant trends of normalised date delay or advancement, considering the V3, bud break and leaf unfolding, phase.*

Following the calculation of the parameterised annual variations of these phenological phases, the annual phenological data for the different plant species were analysed for statistical correlations between these biological trends and the temperatures (GDDs). *Table 2* gives the correlation data for each of the four vegetative phenological phases for the plant species that showed significant phenological trends. The highest correspondence was characterised by positive signs that showed particular relationships between phenological delay–GDD increase, and phenological advancement–GDD decrease, above all for the V7 (mean R^2, 0.86) and V8 (mean R^2, 0.75) phases, with the lowest correlations seen for V5 (mean R^2, 0.43). In this sense, high correlation values can be interpreted as a strong relationship between vegetative development and temperature. These show a strong dependence on the temperature variations of all the vegetative phases, but particularly of adult leaves and the start of leaf colouring. The lower relationship of the initial vegetative phases would probably be related to other variables, such as the photoperiod (V3) or the water availability (V5) (Olmsted, 1951; Kramer, 1994, 2000).

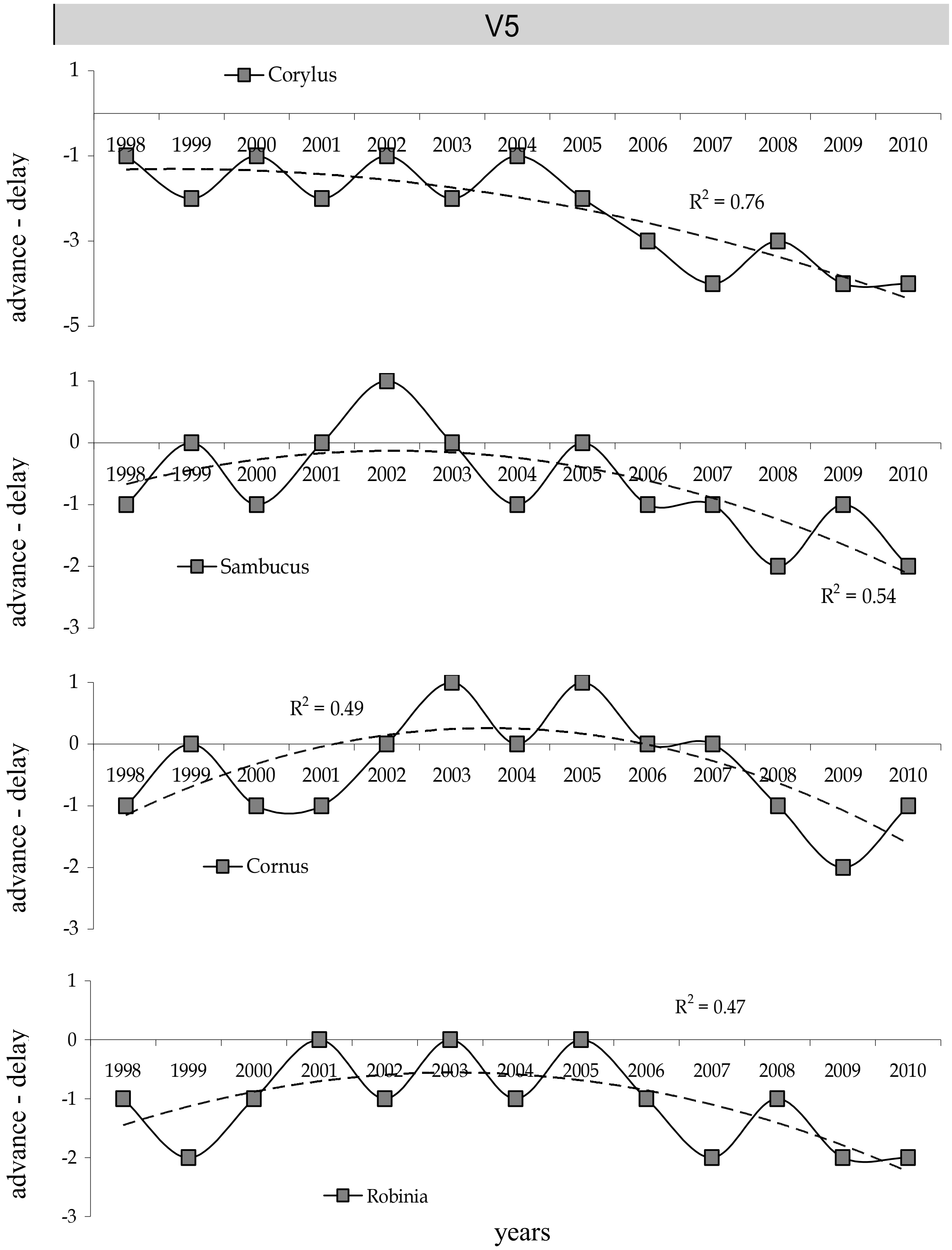

Figure 3. Species showing significant trends of normalised date delay or advancement, considering the V5, young unfolded leaf, phase.

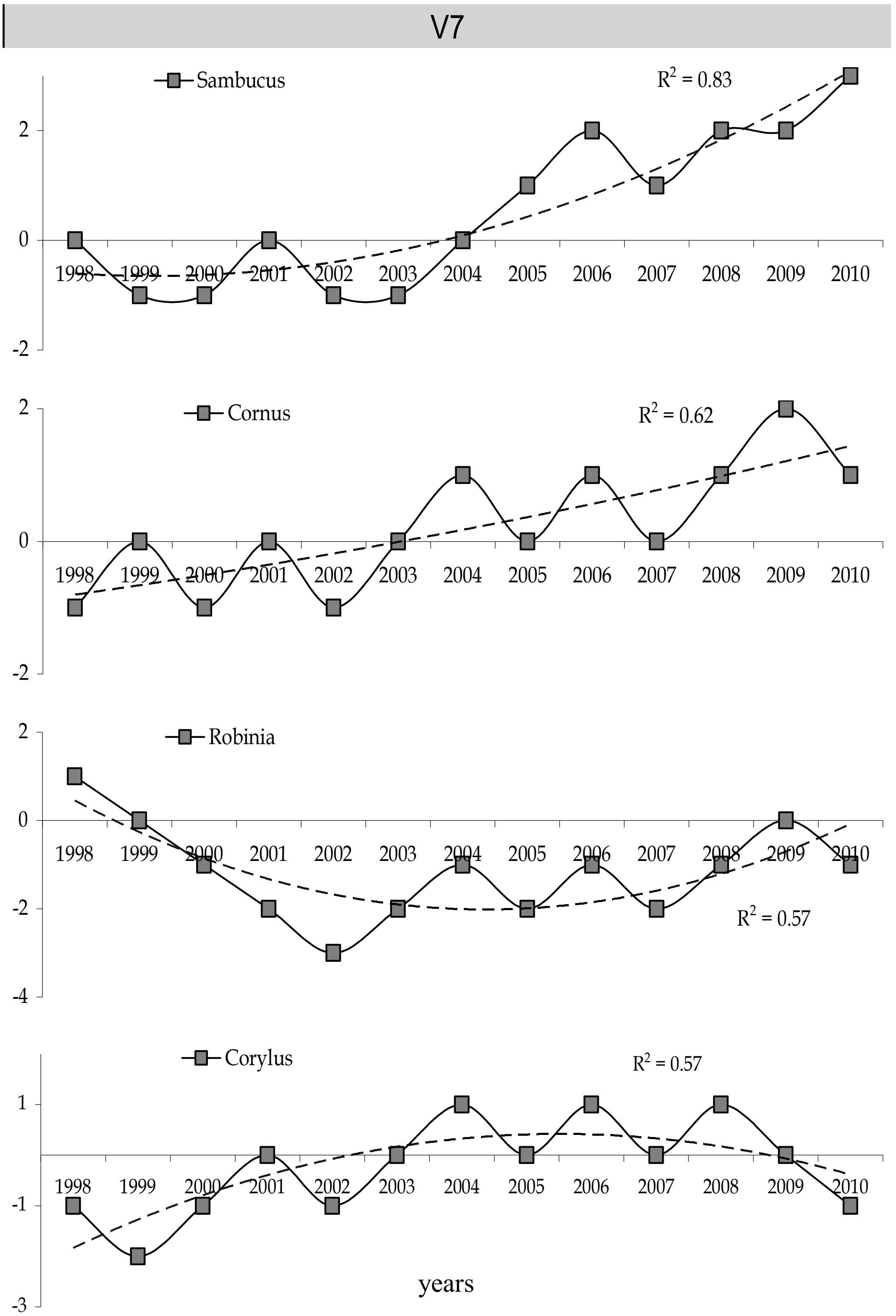

Figure 4. *Species showing significant trends of normalised date delay or advancement, considering the V7, adult leaves, phase.*

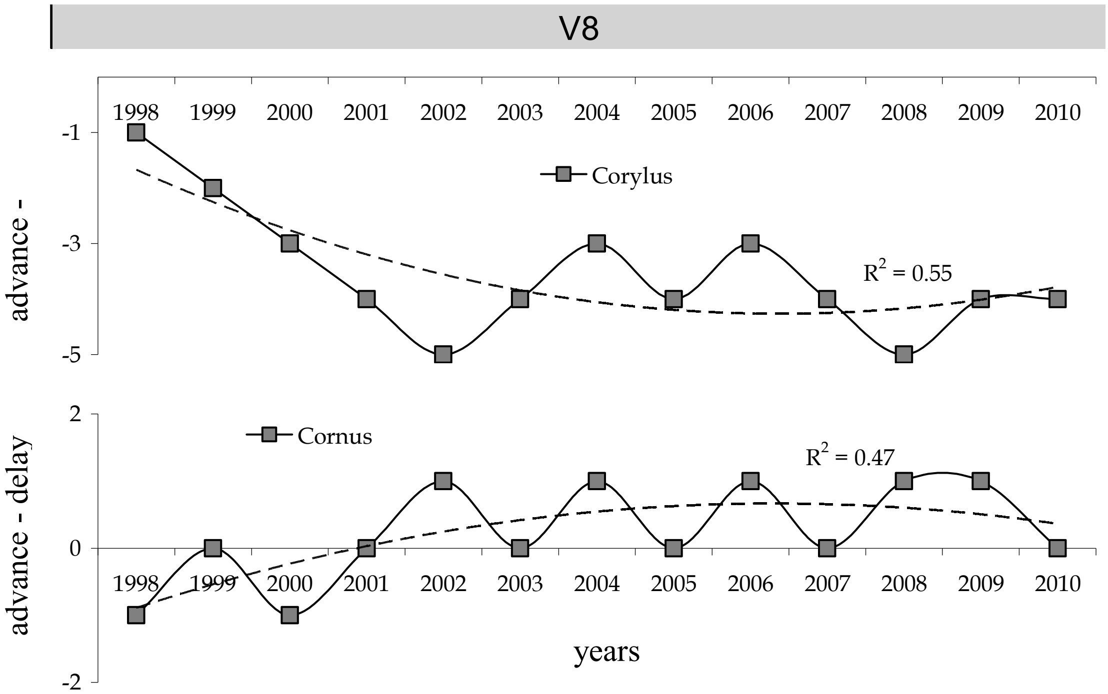

Figure 5. Species showing significant trends of normalised date delay or advancement, considering the V8, beginning of leaf colouring, phase.

Table 2. *Correlation data between the phenological dates and the GDD variations for each phenological phases for the plant species that showed significant phenological trends.*

Species	Correlation[a]	Mean correlation[a]
	V3	
Ligustrum	0.50	0.58
Corylus	0.58	
Robinia	0.65	
	V5	
Corylus	0.46	0.43
Sambucus	0.31	
Robinia	0.30	
Cornus	0.66	
	V7	
Corylus	0.82	0.86
Sambucus	0.82	
Robinia	0.87	
Cornus	0.91	
	V8	
Corylus	0.72	0.75
Cornus	0.78	

[a] *Correlation between phenological delay or advancement versus GDD increase or decrease.*

Moreover, the different periods of leaf presence on the tree were calculated for those plant species that showed significant trends across all of these four vegetative phenological phases, and thus for *Corylus*, *Robinia* and *Cornus*. The "leaf assimilation" period that manifested the clearest trend was that calculated from V3 to V7, as reported in *Figure 6*.

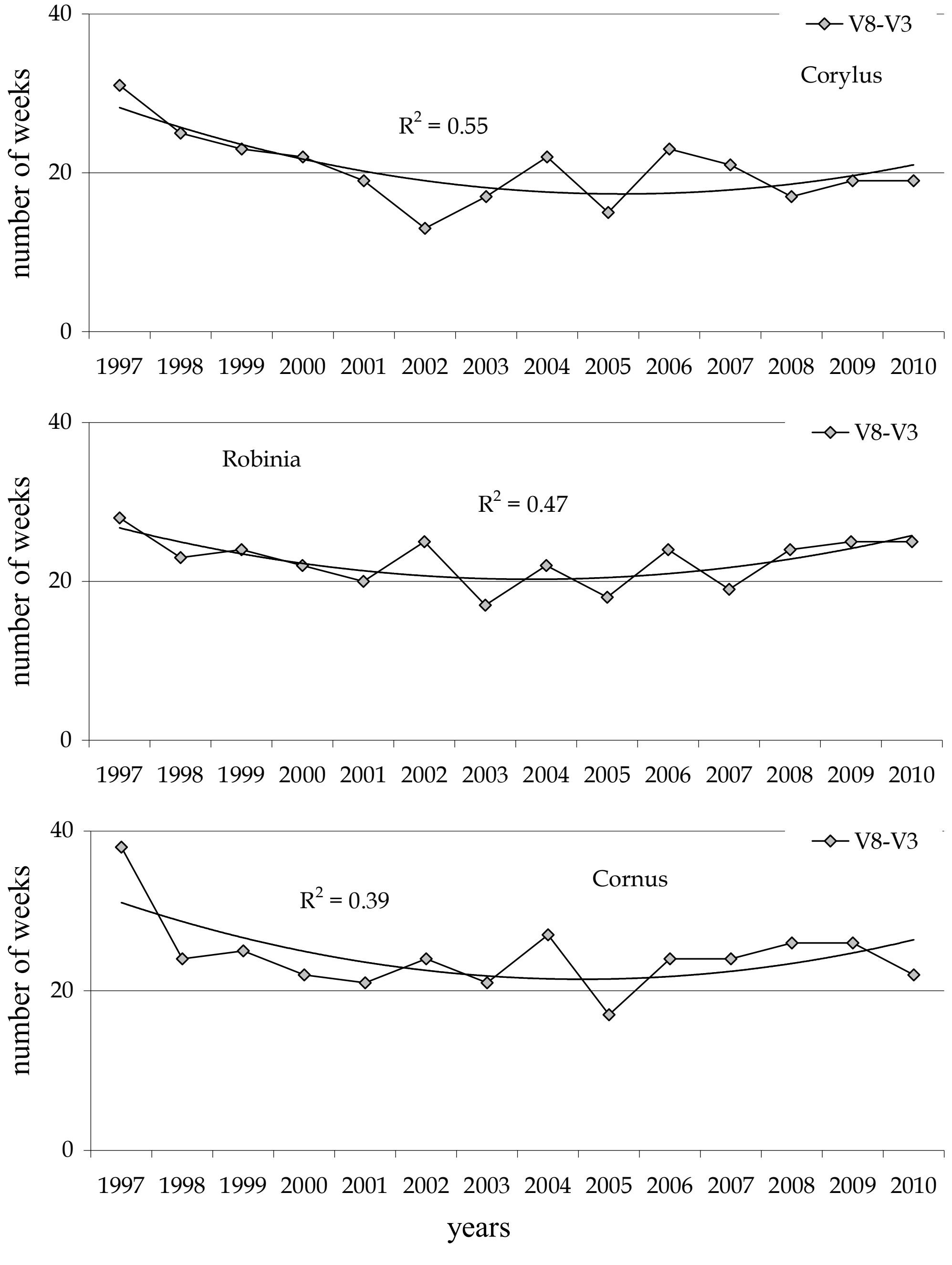

Figure 6. *Species showing significant trends of annual leaf assimilation periods (weeks from V3 to V7) over the 14-year study period.*

In physiological terms, this V3-to-V7 period represents the more important photosynthetic assimilation period for the different species, considering that the molecular chlorophyll degradation processes start from the central summer period (Farooq et al., 2009, Jaleel et al., 2009) with the leaf senescence that precedes the leaf-colouring phase (V8). Here, the V3-to-V7 leaf-assimilation periods in these three plant species considered started from about mid-March and finished at about the end of July, and overall these showed decreasing trends from 1997 to 2002-2003 for both *Corylus* and *Robinia*, and to 2000-2001 for *Cornus*. Following the decreases, these V3-to-V7 leaf-assimilation periods then showed a relatively constant phase, in which at least for *Cornus*, the last study year (2010) marked one of the lowest values, due particularly to the advancement of the V7 phase.

Conclusions

In general, the relative invariance in the start of the open-bud phase (V3) that was accompanied by the advancement of the young unfolded leaves phase (V5), which occurred particularly from 2006, led to a shortening of the leaf-opening period. In contrast, the beginning of the autumn leaf-colouring phase (V8) tended to remain constant, with the exception of the opposite trends of *Corylus* and *Cornus*, confirming that in contrast to spring events, the signal for leaf colouring in autumn is relatively ambiguous and less evident (Menzel et al., 2006). With their dependence on the meteorological features, the adult leaves show a tendency to remain on the trees longer. This will be as a consequence of the warmer season and milder temperatures favouring a delay in the successive vegetative phases, thus postponing the seasonal rest period of the plant.

At the level of the single species, it can be seen that the plants that suffered fewer phenological date variations during the study period were *Ligustrum* and *Salix*. *Corylus*, *Robinia*, *Sambucus* and *Cornus* showed the greatest variations, which are interpretable according to the phenological tendencies that depend on the variations in temperature.

These four phenological phases (V3, V5, V7, V8) showed different trends in terms of their annual delay or advancement over these 14 years of the study, with the clearest results shown in particular for the V5-to-V7 phases, while the V3 and V8 phases did not show particular trends over the study period, when all of the plant species were considered.

The lowest correlations between the annual vegetative phenological phases and the temperature variations (as GDD) were shown by *Sambucus* and *Robinia* in particular, for which the first leaf development phases appeared probably influenced by the photoperiod. Other studies have reported that the sensitivity of leaves to the photoperiod increases with aging of the expanding leaves, reaching a maximum when they are half expanded (which here corresponds to about V5, the young unfolded leaf stage) and decreasing in the later leaf development (Khudairi and Hamner, 1954; Withrow, 1959).

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