Abstract: In the last few centuries phenological observations were commonly considered as useful and inexpensive ‘plant-instruments’ which respond to many meteorological and environmental factors. At first, phenological observations were carried out by volunteers interested in nature, while in the last century the first phenological networks were instituted in different countries. The present study was carried out at the 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 vegetative and reproductive development of seven plant species, adapted to the Mediterranean environment, over an eleven-year period (1997-2007). Two periods (“effective leaf assimilation” ELA and “effective flowering period” EFP) were considered to show the meteorological influence on plants development during the same periods. High correlation values were seen between ELA and rain amounts recorded at the 40th week (autumn season) which show that rain events delay the beginning of leaf colouring extending in this manner the ELA period. Though the reproductive phases demonstrated lower variability in comparison to the vegetative ones, a certain lengthening of the effective flowering period was evidenced.

Keywords: phenology, tree plants, climate

INTRODUCTION
Phenology in its present meaning is the study of the events that lead to the manifestation of phenomena associated with the functioning of some plant organs or of the plant as a whole. The observed phenomena (the phenological stages) include flowering, the appearance of leaves, leaf drop or any other observable cyclic phenomenon with their exact occurrence during the year. In temperate zones the reproductive cycle of plants is mostly controlled by temperature and day length, while at lower latitudes rainfall and evapotranspiration must also be taken into account. The timing of spring events in mid to high latitude plants such as budding, leafing and flowering is mainly regulated by temperatures after dormancy and a number of studies have found good correlation between spring phases and air temperatures (Chmielewski and Rötzer 2001; Chmielewski et al. 2004; Estrella et al. 2006; Fornaciari et al. 1998, 2000; Makrodimos et al. 2008). Thus, phenological phases may serve as proxies for spring temperatures. While the climate signal controlling spring phenology is quite well understood, autumn phenology is less clearly explained.

Several studies have linked inter annual variability in phenology to large-scale weather features such as the North Atlantic Oscillation (NAO) and El Nino-
Southern Oscillation (ENSO). Changes in plant phenology are considered to be the most sensitive and observable indicator of plant responses to climate change.

In climatology and ecology, phenology and syn-phenology are used to determine the degree of climatic changes that have occurred and to consider their potential consequences (Kramer et al. 2000; Mutke et al. 2003; Orlandi et al. 2005a, b).

In his pioneer work in 1955, Schnelle discussed the value of phenological observations and concluded that these inexpensive and useful ‘plant-instruments’ are integral instruments which respond to many meteorological and environmental factors. He concluded that the best method to analyse impacts on plants would be to ask the plants themselves. In most cases, phenological observations have been carried out by volunteers interested in nature. The great advantage of phenological observations is that they are extremely suitable to illustrate and communicate climate change impacts. Another important application of phenology models is to evaluate species distribution from the perspective of future climatic change. Phenological studies interpret the reproductive success of a plant population each year, the growth and survival probability of individuals and their fitness under particular climatic conditions (Cleland et al. 2007).

The present study was carried out at a phenological garden located near Perugia, central Italy, which contains indicator species, common to all International Phenological Gardens (Orlandi et al. 2007). These species 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 species which were adopted as indicator species from those 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 selected species that would adapt easily to the Mediterranean climate.

The phenological garden also contains indicator species that are common to the Italian Phenological Gardens and that are representative of the geographical area. Aims of this study were to determine and analyse the average development trends of the considered plant species and to evidence plant adaptability to the Mediterranean environment, over an eleven-year period (1997-2007). In addition, phenology was used as a tool to investigate the climate/plant relationships.

MATERIALS AND METHODS

The tree indicator species examined were those suggested by the International Phenological Garden Network:

1) *Cornus sanguinea* L.; Common name: dogberry, dogwood.
2) *Crataegus monogyna* Jacq., Common names: hawthorn, thornbush.
3) *Corylus avellana* L., Common name: hazel.
4) *Ligustrum vulgare* L., Common name: privet.
5) *Robinia pseudoacacia* L. Common names: robinia, acacia;
6) along with common IPG species such as *Salix acutifolia* Willd. Common name: willow;
7) *Sambucus nigra* L. Common name: elder.

The phenological sampling was carried out according to the basic criteria (every phenological stage interprets a distinct biological event, the data must be objective, so that they can be compared with those of other researchers, etc.) using phenological keys described by various authors (Chmielewski and Rötzer 2001; Spano et al. 1999).

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; V8) beginning of leaf colouring.

For the reproductive cycle, the following phenological phases were considered:

B3) swollen buds and open flowers, mature and immature aments; B4) full blooming: open buds and flowers, mature aments; B5) withering begins: open and withered flowers, mature and withered aments; B6) complete withering: withered flowers and aments.

The observations were conducted on three individuals for each plant species to limit the random variability, possible even in genetically similar plants. The mean date for the onset of each phenophase was calculated mathematically considering contemporary the three plants (phenoids) of the same species.

The average dates thus obtained provide a mean model of development in relationship to the species and to the year of observation. The mean values of the phenological data were computed for the different species in relationship to the eleven-year period of observation (1997-2007) in order to obtain the mean developments in the study area. Moreover, by the meteorological point of view, the minimum, maximum temperatures and rain amounts were calculated every 10 weeks of the year (10th, 20th,
Once the dates of each phenological phase were determined, we examined the daily values of the temperature units expressed in GDD (Growing Degree Days) in order to determine the relationship between spring and summer temperature trends and the plants’ vegetative and reproductive development. For the calculation of GDD, two different methods were applied. The first method (Single triangle) uses the minimum and the maximum temperature of the n day and the minimum temperature of the n+1 day. The other method evaluated the hourly temperature trend by means of the Single Sine function (Zalom et al., 1983).
We then compared, for each species, the variability of yearly GDD summations obtained considering the same GDD method, start-end dates and the same threshold. This comparison was made using the calculation of the root mean square error (RMSE), which is considered the best instrument for the variation analysis of the distributions relative to the GDD summations (Ashcroft et al. 1977). In this phase of our research, the RMSE calculation allowed us to determine which thresholds minimized the variability among yearly GDD summations. The best threshold temperature is the one above which the heat useful for the formation of vegetative and reproductive structures is accumulated. Moreover, a regression analysis was conducted considering contemporarily all the studied species to validate the results obtained with RMSE evaluation.

For the GDD calculations the daily minimum and maximum temperatures were used, provided by the local meteorological station of the National Agrometeorological Network, situated near the phenological garden (inside the farm of the Agraria Faculty - Perugia University) at 211 meters above sea level with coordinates of 43° 00' North and 12° 18' East. In particular the phenological garden was realized during 1994 in that area considering the nearness of the meteorological station which furnishes daily values of all the principal meteorological variables.

We also considered the entire period when the leaves are in full activity, which is from the appearance of the first leaves with photosynthesis activity (V3) until the leaves wither and do not assimilate any more (V8). The present period was named as “Effective Leaf Assimilation” (ELA).

Moreover, we considered the duration of the effective flowering period, from the moment when the reproductive structures are ready for the fecundation process until their withering and death. This period was named the “Effective Flowering Period” (EFP). The influences of principal meteorological variable amounts, calculated to the moment of vegetative (V5-V8) and reproductive (R3-R6) phases, on ELA and EFP have been considered through Pearson correlation analyses.

**RESULTS AND DISCUSSION**

To better understand the extent of variations observed during the study period (1997-2007), a
complete picture of all the meteorological trends can be very useful. The charts (Figure 1) represent the thermal accumulations of the maximum and minimum temperatures and the rainfall summations. The values were calculated on a 10 week basis, until reaching the 50th week of the year (practically the central period of December).

Examining the graphs of the maximum and the minimum temperatures it can be noted that at the 50th week, the central years (2001-2003) are the warmest (highest temperature amounts) during the study period.

On the whole, the rainfall amounts at 20th and 30th weeks represent an opposite behaviour with regard to the temperature. Indeed, in the central study years (2001-2003) lowest amounts were recorded above all during spring and summer periods.

Examining the latter behaviour together with the temperature summations it can be affirmed clearly that the warmest and the driest year was 2003.

Figure 2 represents the mean dates of vegetative and reproductive phases for each examined species, calculated during the 11-year study period. The mean dates were calculated considering the exact days of phenological phases realization year by year, even if they were presented as weekly data. The charts show that the Cornus species reaches in 13 weeks the adult leaf phase, and it reaches rather quickly the autumn leaf colouring phase. The Corylus species shows similar behaviour, but it presents leaf colouring earlier. The Crataegus species reaches the adult leaf phase and the successive phase about two weeks later than Corylus.

The Ligustrum species shows the longest vegetative cycle: it starts with opening buds in April before the 10th week and it reaches the leaf colouring phase in the 37th week, with a mean time from the V3 phase to the V8 phase of about 200 days. The Robinia species has the colouring phase in the same week as the Ligustrum, though it needs about 23 weeks (160 days) to reach it. This plant has the shortest vegetative cycle; it reaches the V3 phase only on the 14th week, sprouting young leaves very quickly. It then has a very uniform development of the vegetative cycle, slightly slowing down during the passage to the adult leaf phase. The Salix species needs about 16 weeks to pass from the V5 phase to the V7 phase, after which it reaches the V8 phase in only 5 weeks. Among the examined species, it is the last to reach the adult leaf and colouring phases. The Sambucus species is the first one to show young leaves on the 7th week and it is the earliest to reach the colouring phase on the 32nd week.
Sambucus anticipates the other plants in all the vegetative phases, completing its biological cycle in about 25 weeks.

Regarding the reproductive phases, we can note a great similarity among the various plant behaviours, with a maximum duration of about 8 weeks.

Considering the examined plants individually, it can be noted that Corylus is the first one to differentiate its reproductive organs, in this case the aments, which are well developed from the 4th week. It is the only plant which emits the reproductive organs first and the vegetative ones successively. The full flowering and the beginning of withering occur in a few days, while complete withering takes place after five weeks from the precedent phase. Among all the examined species, Corylus has the longest reproductive cycle, with a duration of about 8 weeks.

The second plant showing early flowering is the Salix, which shows the well-developed aments from the 10th week. It is interesting to note that this phase coincides with the appearance of the first leaves. Compared to Corylus, this species shows a more uniform trend and concludes its entire cycle in less than 35 days, reaching complete withering in the 15th week.

The second plant showing early flowering is the Salix, which shows the well-developed aments from the 10th week. It is interesting to note that this phase coincides with the appearance of the first leaves. Compared to Corylus, this species shows a more uniform trend and concludes its entire cycle in less than 35 days, reaching complete withering in the 15th week.

For Crataegus, the bud appearance and opening was registered on the 17th week. Its reproductive cycle is concluded within 18 days. Therefore, the phases are extremely close, especially the two central phases, R4 and R5.

One day after the beginning of flower opening of Crataegus, the same process starts for Sambucus, which completes the entire cycle in 27 days. The cycle is characterized by well differentiated phases, at a distance of about one day from each other.

Cornus carries out its flowering phases in about 39 days: the opening of the flower head starts on the 19th week, the two successive phases occur with the frequency of about one week, while complete withering takes place on the 24th week.

The flowering of Robinia occurs two days later than in the Cornus. This species presents the shortest reproductive phase of only 11 days. The phases change at an interval of about 3 days, until reaching complete withering at the end of the 20th week.

Ligustrum is the last species to start flowering, 9 days later than Robinia. The phases are distant from one another of about one week, completing the entire cycle and reaching a withering phase in about 3 weeks.

In Figure 3 the mean values of vegetative and reproductive phases calculated considering contemporarily all plant species are shown. In Figure 3A the mean vegetative phases are simply described with the use of second degree polynomial trend lines which evidence different behaviours. The first phase (V3) shows a phenomenon of dates
delay with a minimum value in 2000. The second vegetative phase (V5) appears to be less variable with a last date in 2007 which shows a marked advance, probably linked to the particular meteorological values (high temperature) during the first months of the year. The last two vegetative phases (V7-V8) show a dual behaviour with a first advancing part from 1997 to 2003 and a second one delaying to 2007. In Figure 3B the reproductive phases evidence a higher homogeneity in comparison to the vegetative ones even if all the phases show their minimum values during 2002-2003 following the previous trends on a smaller scale.

To estimate the best GDD calculation method (single triangle, single sine) and the threshold temperatures (5-7-9-11-13°) which minimize the GDD amount variability over the years, the RMSE values (expressed in GDD) for the first vegetative (V3) and the first reproductive (R3) phases are shown in Table 1. The best results in terms of minor variance over the years were obtained with the use of single triangle in the GDD calculation considering all the plant species, and both the vegetative and reproductive phases. Only the Ligustrum species presented the best result (with a minimum value of RMSE) with the use of single sine for the R3 reproductive species (although the difference between the two GDD calculation methods was very low).

In quite all the cases analyzed the better threshold temperatures (for the amount of “useful warm temperature”) were represented by 7°C and 9°C, with the exceptions of Robinia vegetative phase and Sambucus reproductive phase, both with an optimal temperature of 11°C.

In addition a regression analysis was realized considering the best GDD method (triangle) and the best threshold temperature (9°C) related to vegetative phase (V3) considering all the studied species contemporarily. The regression was conducted to validate the best method for GDD calculation, carried out through RMSE evaluation, by using a first set of data (1997-2005). Moreover other two years of the historical series (2006-2007) were utilized to validate the regression results. In Table 2 the regression equation section is presented and both interpretative and predictability power of

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**Fig. 4 - Effective leaf assimilation (ELA) and flowering period (EFP) represented for all the plant species during the study period (the number indicate the vegetative and reproductive period in weeks).**

**Fig. 4 - Periodo vegetativo di crescita fogliare (ELA) e periodo di fioritura (EFP) per tutte le specie vegetali durante il periodo di studio (i numeri indicano la lunghezza dei periodi vegeto-riproduttivi in settimane).**

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<table>
<thead>
<tr>
<th>Meteorological variable summation</th>
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**Tab. 3 - Correlation results (r) between principal meteorological variable (Tmax, min and rain) amounts, calculated to vegetative (V3-V8) and reproductive (R3-R6) phases and ELA-EFP periods. Pearson correlation method (Prob-level * 0.05 - ** 0.01 - *** 0.001).**

**Tab. 3 - Risultati della correlazione (r) tra le principali variabili meteorologiche (Tmax, min e pioggia), calcolate fino alla comparsa delle fenofasi vegetative (V5-V8), riproduttive (R3-R6) e per i periodi ELA-EFP. Per le correlazioni è stato utilizzato il metodo Pearson (Prob-level * 0.05 - ** 0.01 - *** 0.001).**
GDD amounts are shown in relation to the vegetative phase of all the considered plant species. On the contrary, the regression analysis realized with reproductive phase (R3) did not reach significance standards probably because of the partial independence of reproductive processes respect to meteorological annual trends.

In Figure 4 the Effective Leaf Assimilation (ELA) and the Effective Flowering Period (EFP) are shown for all plant species and their different trends during the whole study period. The ELA had a decreasing trend from 1999 to 2005, while during the two last years (2006-2007) the leaf assimilation period increased to the maximum values of about 160 days considering contemporary all the plant species. The minimum complex duration of ELA was recorded in 2005 with a value of about 100 days. This characteristic trend of ELA is very similar to those evidenced by the minimum T° and rain summations recorded during the 20th and 30th week of the year. A specific correlation analysis among ELA and the temperature and rain summations showed the highest values with rain sums, which cumulated at the 10th, 20th, 30th and 40th week (the highest value was r=0.71 with the rain sum at 30th week).

On the other hand, the EFP trend constantly increased from 1999, when the complex period of flowering (considering all the species) was of about 15 days. In the last study years (2006-2007) this complex duration arrived at 30 days and even more. This phenomenon is due to the lengthening of the Corylus flowering, which was not registered in the first two years. The EFP trend seems to be influenced by internal (physiological) processes of the plants, which in their first years of development are still not quite mature and stabilized.

The meteorological variable amounts, calculated from 1st January to the dates when vegetative and reproductive phases (V5-V8 and R3-R6) were recorded yearly, were related to the two phases of “ELA” and “EFP”.

In Table 3 the influences of principal meteorological variable amounts, calculated to the dates of vegetative (V5-V8) and reproductive (R3-R6) phases, on ELA and EFP are shown. The correlation results show a high influence of Tmax amount calculated to the V5 phase (r=0.9) on the V5 realization dates, but a low influence on ELA. All the temperature variables and rain amounts calculated to the V8 phase are highly related (r values are always higher than 0.5) to V8 realization dates and with the same ELA.

Considering the reproductive phases, it can be noted that only the temperature variable amounts (above all, Tmax) calculated until the last phase (R6) are related to the EFP, but the correlation values are lower than those calculated for the vegetative phases.

**CONCLUSIONS**

The observations realized during the study period let us say that climate is subject to more or less evident fluctuations, depending on the period of the year. Regarding the seasons, a lengthening of typical summer parameters with detriment of the spring ones was observed, while the winter and autumn seasons were practically unchanged. The observed variations can influence the plants’ bio-rhythms determining their adaptation.

The results obtained during the study period demonstrated that all the examined species had a similar behaviour: they showed, in different degrees, a similar shift of phases (anticipation / delay), depending on climatic conditions. In general, we can note a slight delay in the manifestation of the open bud phase and a parallel anticipation of the young open leaves phase, with a shortening of the period of leaf opening. On the contrary, the V7 (adult leaves) and the V8 (autumn leaf colouring) phases tend to remain constant, with the exception of some species such as Crataegus which shows a shortening of these phases, confirming that, in contrast to spring events, the signal for leaf colouring in fall is quite ambiguous and less evident (Menzel et al. 2006).

In dependence of meteorological features, the adult leaves show the tendency to remain longer on the trees in consequence of the fact that warm season and mild temperatures favour the delaying of the successive vegetative phases, postponing the plant’s seasonal rest period.

These behaviours were confirmed by the high correlation values calculated between ELA and rain amounts recorded at the 40th week which show as the rain events retard the V8 phase extending in this manner the ELA period. Moreover, the influences of temperature and rain on the V8 dates confirmed that the ELA duration is above all linked to a delaying phenomenon of the V8 phase.

The reproductive phases are quite stable; however, they show a gradual lengthening of duration. The flowering period follows the development of the first vegetative phases, to which it seems strictly connected, while the weather influences more markedly the extension of single phases.

Observing the single species, we can note that the plant which suffered fewer modifications was the Corylus. Sambucus had a weak tendency to delay
the phases, excepting the last two years. Cornus, Crataegus, Robinia and Salix manifested slow oscillations, depending on the variations of atmospheric conditions. Ligustrum also showed these oscillations, but with greater modifications.

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