P1.1 ANALYSIS OF PHENOLOGICAL BEHAVIOUR OF SOME MEDITERRANEAN SHRUB SPECIES IN RESPONSES TO WARMING AND DROUGHT CONDITIONS.

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1. INTRODUCTION

Phenology is potentially a powerful tool for monitoring the response of plant and animal to climate change. In fact, phenological observations are a valuable source of information for investigating the relationship between climate and weather variation and plant and animal development (Kramer et al., 2000, Ahas et al., 2002, Menzel et al., 2003).

At mid-latitude the timing of phenological stages, such as leafing or flowering, and the onset of the growing season, after the dormancy is released, are highly dependent on air temperature (Fitter et al., 1995, Wiegolasky, 1999, Sparks et al., 2000).

Flowering is one of the most significant phenological stages to evaluate the sensitivity to climate variability (Spano et al., 1999). Spring flower phases are strongly influenced by the air temperature of the previous months (Maak and Storch, 1997). Higher air temperature values in spring induces an earlier start of plant development within the year (Chmielewski and Rotzer, 2001). However, the influence of temperature is not so pronounced for autumnal phonological phases (Estrella, 2000).

If temperature is considered the driving factor of phenological timing in most climate areas, the effect of temperature needs to be adjusted incorporating functions for water availability in Mediterranean regions (Kramer et al., 2000).

The use of standardized observation methods in phenology would help observer to accurately monitor plants and improve the quality of observations. In the Global Phenological Monitoring (GPM) network, phenological phases are recorded according to a BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) code (Hack et al., 1992), which classifies plant growth phases of most species according to a standardized system (Bruns et al., 2003).

The objectives of this paper are (1) to develop a detailed phenological scale, using the extended BBCH-scale system, for describing phenological behaviour of Mediterranean species growing in a Mediterranean-type climate, and (2) to evaluate the sensitivity of some Mediterranean species to climatic manipulations.

2. MATERIALS AND METHODS

Experimental design

Climatic manipulations were conducted on a Mediterranean macchia ecosystem that includes sclerophyll species, some scattered shrubs, and several herbaceous plants. Six experimental plots were manipulated by night-time warming and extending summer drought. The responses to the treatments were compared to three untreated control plots during the years 2001-2003. The warming treatment was performed covering the vegetation with an automated aluminum curtain at night. In the drought treatment, the curtain material is transparent to infrared radiation and the movement of the curtains is determined by rainfall events. The drought treatment was carried out for a 3-month period in autumn.

Study site

The study was conducted on a Mediterranean type ecosystem. The site is located in North-Western Sardinia, Italy, within a nature reserve (40° 37' N, 8° 10' E, 40 m a.s.l.) covering approximately 1200 ha. The soils are Luvi and Litosoils. The climate is semi-arid with a remarkable water deficit from May through September (mean annual rainfall 640 mm, mean annual temperature value 16.8 °C).

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Species description

The study was conducted on a Mediterranean ecosystem where some shrub species are prevailing (*Cistus monspeliensis* L., *Dorycnium pentaphyllum* L, *Helichrysum italicum* L. subsp. *microphyllum*).

Dorycnium pentaphyllum is a perennial legume native to the Mediterranean Basin. *Dorycnium* is considerably important for erosion control and re-vegetation due to its drought and frost tolerance, capacity to grow in acid and alkaline soils, prostrate growth habit, ability to sprout after a fire and settlement capacity (Sheppard and Douglas, 1986, Wills et al., 1989).

Helichrysum italicum subsp. *microphyllum* (Willd.) Nyman, a typical shrub of the Mediterranea islands (Sardinia, Corsica), grows frequently on dry cliffs and sandy soil along the coast. There is great interest for potential use of *Helichrysum* because of its peculiar ecological and pharmacological properties (Camarda and Valsecchi, 1990).

Cistus monspeliensis is a small shrub species typical of the whole Mediterranean Basin. This species is classified as drought semi-deciduous with leaf anatomical differences between summer and winter leaves (Correia et al., 1992, de Lillis and Fontanella, 1992).

Phenological observations

Phenological observations were made weekly on the main species of the Mediterranean-type ecosystem (*Cistus monspeliensis* L., *Dorycnium pentaphyllum* L., and *Helichrysum italicum* L. subsp. *microphyllum*) from June 2001 to June 2003. Observations were made on 20 terminal shoots for *Cistus* and on 3 plants for *Helichrysum* and *Dorycnium*, for each plot.

3. RESULTS

Environmental data

Rainfall amount recorded during the first year of the study was higher (599 mm) than during the second (411 mm). A very anomalous seasonal pattern of rainy events was observed from May to August 2002. In this period, the amount of rainfall was 160 mm against 10 mm registered for the same period in 2003 and 55 mm of mean climatic value. Drought treatment reduced annual rainfall by 15% and 36% on average, relative to control plots, in 2002 and in 2003 respectively.

The increase of daily minimum temperature of air and soil observed at 20 cm height and at 10 cm depth in the warming treatment was approximately equal to 0.5 °C relative to control.

The BBCH scale

In plant phenology integration of data can be difficult, since heterogeneity of plant growth, environmental conditions and phenotypes can led to obtain data set that are not consistent or easily comparable. The extended BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Hack et al., 1992). The decimal code, which is divided into principal and secondary growth stages, is based on the well-known cereal code developed by Zadoks et al. (1974) in order to avoid major changes from this widely used phenological key. The entire developmental cycle of the plants is subdivided into ten clearly recognizable and distinguishable longer-lasting developmental phases. The general scale forms the framework within which the individual scales are developed. It can also be used for those plant species for which no special scale is currently available (Meier et al., 2003).

Common growth stage definitions have been developed for many agronomically important crop, fruit trees and weed but, in our knowledge, no previous attempt to develop a key growth scale for Mediterranean shrubs was made. Adaptation of such a universal scale of phenological results would allow to easier data comparison between and within species, in different sites. Finally, the use of growth scale definitions will greatly facilitate information sharing and increase the value of individual research project.

In this paper the BBCH scale was used as a basis to define a series of developmental growth stages for the phenological descriptions of Mediterranean species. In Table 1, a list of growth stages adapted from the general BBCH scale for *Cistus monspeliensis* L., *Dorycnium pentaphyllum* L, and *Helichrysum italicum* L subsp. *microphyllum* are reported. In Table 2 the dates of each growth stage occurrence for the studied species during the experimentation are shown.

Four principal growth stages can describe the life cycle of *Cistus*. Vegetative growth in the BBCH scale (Hack et al., 1992) is defined as the time period between emergence and reproductive development. It includes four principal stages: Leaf development (stage 1), Formation of side shoot (stage 2), Stem elongation or shoot development (stage 3), and Development of harvestable vegetative part (stage 4). Vegetative growth for *Cistus* is described by stage 1 and 3. Leaf development occur either in autumn (Autumn leafing) or in spring (Spring leafing) when the first leaves are unfolded. Stage 3 is recorded when the elongation of main terminal shoots reached the maximum length. This stage is easily detectable because of the morphological changes on the leaves on the shoot apex. In addition, Senescence principal stage, with secondary growth stage 95, was used for identify the moment when plant shed their winter leaves, after flowering stage. Reproductive growth stages in BBCH scale are numbered from 5 to 9. For *Cistus*, Inflorescence emergence (stage 5), Flowering (stage 6), and Development of fruit (stage 7) stages were observed. Inflorescence emergence occurs when flower buttons are visible on the top of terminal shoot but they are still closed (stage 51). During the following stage the inflorescence elongates and individual flowers are visible but still closed (stage 55). At the beginning of flowering, 10% of flowers are open; other stages indicate how flowering is progressing until full flowering is reached when 50% of flowers are open and first petals may have fallen; flowering finishing is when majority of petals have fallen or dry; end of flowering is when fruit set is visible (secondary growth stages 61, 65, 67, and 69) (Table 1).

For describing *Dorycnium pentaphyllum* L. phenological sequence, four reproductive phases were used: Inflorescence emergence (stage 5), Flowering (stage 6), Development of fruit (stage 7), and Ripening or maturity of fruit (stage 8). For each of the principal growth stages, a different number of secondary stages were employed for describing the phases in detail. For *Dorycnium*, Inflorescence emergence occur when inflorescence appear at the top of the plants but they are still close and flowers are scarcely distinguishable. Afterwards, flowers start to develop and single flowers start separating (stage 55). Flowering stages are defined as for *Cistus*. When the development of fruits are completed and the fruits reach the final size (stage 7), Maturity of fruit (stage 8) starts with visible changes of colour. Beginning of fruit can be detected when 10% of fruits changes the colour from green to red or brown (stage 81). When 50% of fruits change colour is considered advanced fruit colouration (stage 85). In the final stage, Fully Ripe (stage 89), nearly all fruits show fully-ripe colour, and fruit abscission is beginning.

For *Helichrysum* only two principal growth stages were used: Inflorescence emergence (stage 5), and Flowering (stage 6). As described above for *Dorycnium*, Inflorescence emergence is when inflorescences appear at the top of the plants but they are still close and flowers are scarcely distinguishable. Flowering stage, from beginning to finishing, is described as for *Cistus* and *Dorycnium*.

Phenological observations

Cistus monspeliensis L.

For this species, continuous vegetative growth was observed throughout the year, with a slower growing rhythm during the drought season. At the end of the summer, when a higher water availability was recorded, an Autumnal Leafing was observed. No break for this stage was observed until the end of the winter. Afterward (February-March) principal shoots started growing and, lately, when temperature raised, a certain number of lateral shoots from the principal ones elongated. In spring, leaf unfolding and shoot elongation stages were observed simultaneously. Maximum principal shoots length was reached when flower buds appeared at the apex of shoots. The early stage of flower buds development could be easily recognized from morphological signs: the external leaves at the shoot apex, which became swollen, assumed a red color along the ribs. Flowering started in average during the first two weeks of May and it was concluded by the beginning of June when the stage fruit set visible was observed. Afterward, began the development of fruit until nearly all fruits had reached the final size. The maturation was completed by the end of the summer. During this period, seeds were released progressively from the dehiscent fruits. Most of the leaves were shed after the flowering phase. During summertime, plants maintained only spring leaves, which are generally smaller and thicker than autumnal leaves.

Dorycnium pentaphyllum L.

Dorycnium vegetative growth did not show significant breaks during the year, with a peak of activity during spring months when plants seemed to have their maximum leaves growth and shoots elongation. For this reason, leafing was observed several times during the period between September to the next June but the major flush of shoot growth was in spring. Only during summertime vegetative growth seemed to have a period of stasis. Reproductive stages (flowering, development of fruit, ripening) were observed between April and June. Fully Ripe was reached when fruit showed the typical dark-reddish brown colour and some of the fruit fell.

Helichrysum italicum L. subsp. *microphyllum*

Helichrysum showed a period of vegetative break during the dry season. The vegetative activity period started in autumn after the first precipitations events. A more intense vegetative activity was observed at the end of the winter. *Helichrysum* developed in spring three distinct vegetative stages: (i) a moderate shoot elongation which occurred starting from terminal shoots, (ii) a pronounced shoot growth with a visible elongation of internodes portions, and (iii) a visible unfolding of leaves. The last two steps were widely overlapping. When inflorescences appeared plants were at their maximum peak of vegetative activity. Flowering started at the beginning of June and it was completed in two weeks.

Climatic manipulations and weather effects on vegetation

In Figures 1-3, the pattern of mean phenological stages observed during the period 2002-2003 in warming and drought plots is compared with observations from control by species. As it was expected, slight differences in the phenological stages sequences were observed. For *Cistus* (Figure 1), the growing season started few days in advance (5 days) in warming treatment than in non-treated plots whereas Autumn leafing was observed about 1 week later in the drought treatment (Table 2). The end of the growing season was recorded at the same date (doy 177). The flowering phase duration seemed to be more sensitive to climatic manipulation: it lasted 35 days long in warming treatment and 30 days in the control. In the drought conditions, flowering period was shorter of about one week (24 days). Vegetative growth showed to be less sensitive to different environmental conditions. In fact, the beginning of spring growing season and the end of shoot elongation period were observed almost at the same date.

In Figure 2 phenological stages pattern for *Helichrysum* is shown. Also in this case, flowering stage showed the same pattern than in *Cistus*. In warming treatment beginning of flowering was recorded five days in advance in comparison with drought treatment (Table 2).

On the contrary, *Dorycnium pentaphyllum* (Figure 3) did not show any effect of treatment as phenological pattern sequence was similar in all plots (Table 2). Major effects were found by comparing phenological behavior of *Dorycnium* in 2002 and 2003 (Figure 4). The anomalous rainy period in May - August 2002 seemed to have affected the length of reproductive season for *Dorycnium*. Actually, beginning of flowering was recorded two weeks in advance (April 16). In total, the reproductive season was a decade longer in 2002 than in 2003. This species showed to have a higher sensitivity to water availability than a difference in temperature condition.

Also *Cistus* showed some differences in the phenological behavior of 2002 compared to 2003. In fact, the start of growing season was slightly advanced (5 days) for 2002 (Table 1).

No relevant differences in the phenological stage sequence were observed for *Helichrysum* (Table 1).

CONCLUSIONS

In this work site phenological scales based on BBCH standardize system and phenological behaviours for some Mediterranean species were described. In addition, the phenological response of the same species was described in relation to climatic manipulations and variability in weather conditions.

The effects of the environmental conditions on plants are referred to a short time experiment of climatic manipulations and further analyses are desirable. The main effort was put in the phenological data collection starting from a different key scale, and in the determination of growth stage scale based on international standard. The use of such standardize scales would improve the value of research project in term of monitoring and data-collection which are crucial to global change studies.

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Table 1. Principal and secondary growth stage descriptions and codes based on the BBCH scale system for *Cistus monspeliensis* L., *Dorycnium pentaphyllum* L., and *Helichrysum italicum* L. species.

BBCH code	Principal growth stages	BBCH code	Secondary growth stages	Figure code
	***Cistus monspeliensis* L.**			
1	Leaf development	11 P	Leafing (Autumn): first leaves unfolded in Autumn	AL
1	Leaf development	11 P	Leafing (Spring): first leaves unfolded in Spring	SL
3	Shoot development	39	Shoot Elongation: maximum shoot length reached	SE
5	Inflorescence emergence	51	Inflorescence visible	
5	Inflorescence emergence	55	First individual flowers visible	
6	Flowering (main shoot)	61	Beginning of Flowering: 10% of flowers are open	
6	Flowering (main shoot)	62	20% of flowers open	
6	Flowering (main shoot)	63	30% of flowers open	
6	Flowering (main shoot)	64	40% of flowers open	
6	Flowering (main shoot)	65	full Flowering: 50% of flowers open, first petals may have fallen	F
6	Flowering (main shoot)	67	Flowering Finishing: majority of petals fallen or dry	
6	Flowering (main shoot)	69	end of flowering: Fruit Set visible	FS
7	Development of fruit	75	fruits have reached 50 % of final size	
7	Development of fruit	79	development of fruit: nearly fruits have reached final size	FR
9	Senescence	95	50% of leaves fallen	
	***Dorycnium pentaphyllum* L.**			
5	Inflorescence emergence	51	Inflorescence visible	
5	Inflorescence emergence	55	First individual flowers visible	
6	Flowering (main shoot)	61	Beginning of Flowering: 10% of flowers are open	BF
6	Flowering (main shoot)	62	20% of flowers open	
6	Flowering (main shoot)	63	30% of flowers open	
6	Flowering (main shoot)	64	40% of flowers open	
6	Flowering (main shoot)	65	full Flowering: 50% of flowers are open, first petals may have fallen	F
6	Flowering (main shoot)	67	Flowering Finishing: majority of petals fallen or dry	FF
7	Development of fruit	79	development of fruit: nearly fruits have reached final size	FR
8	Ripening or maturity of fruit	81	Beginning of fruit colouration	
8	Ripening or maturity of fruit	85	Advanced fruit coloration	
8	Ripening or maturity of fruit	89	Fully Ripe: fruits show fully-ripe colour, beginning of fruit abscission	R
	***Helichrysum italicum* L.**			
5	Inflorescence emergence	51	Inflorescence visible	
5	Inflorescence emergence	55	First individual flowers visible	
5	Inflorescence emergence	59	First flower petals visible	
6	Flowering (main shoot)	61	Beginning of Flowering: 10% of flowers open	BF
6	Flowering (main shoot)	62	20% of flowers open	
6	Flowering (main shoot)	63	30% of flowers open	
6	Flowering (main shoot)	64	40% of flowers open	
6	Flowering (main shoot)	65	full Flowering: 50% of flowers open, first petals may have fallen	F
6	Flowering (main shoot)	67	Flowering Finishing: majority of petals fallen or dry	FF

Table 2. Mean phenological dates of studied species for drought, warming, and control thesis.

	Drought		Warming		Control	
***Cistus monspeliensis* L.**	**2001 -2002**	**2003-2003**	**2001 -2002**	**2003-2003**	**2001 -2002**	**2003-2003**
Autumn Leafing	23/10	22/10	8/10	9/10	13/10	15/10
Spring Leafing	13/2	19/2	14/2	19/2	13/2	19/2
end Shoot Elongation	27/3	23/3	26/3	26/3	27/3	24/3
Flowering	9/5	10/5	4/5	9/5	9/5	9/5
Fruit Set visible	31/5	26/5	2/6	1/6	31/5	2/6
end Fruit development	18/6	26/6	18/6	26/6	18/6	26/6
***Dorycnium pentaphyllum* L.**	**2002**	**2003**	**2002**	**2003**	**2002**	**2003**
Beginning of Flowering	16/4	30/4	17/2	30/4	15/4	29/4
Flowering	23/4	6/5	25/4	7/5	22/4	6/5
Flowering Finishing	3/5	14/5	8/5	13/5	6/5	14/5
end Fruit development	31/5	4/6	5/6	30/5	5/6	1/6
Fully Ripe	9/6	13/6	11/6	13/6	11/6	11/5
***Helichrysum italicum* L.**	**2002**	**2003**	**2002**	**2003**	**2002**	**2003**
Beginning of Flowering	3/6	4/6	3/6	29/5	3/6	1/6
Flowering	10/6	9/6	10/6	9/6	10/6	10/6
Flowering Finishing	18/6	17/6	18/6	16/6	18/6	16/6

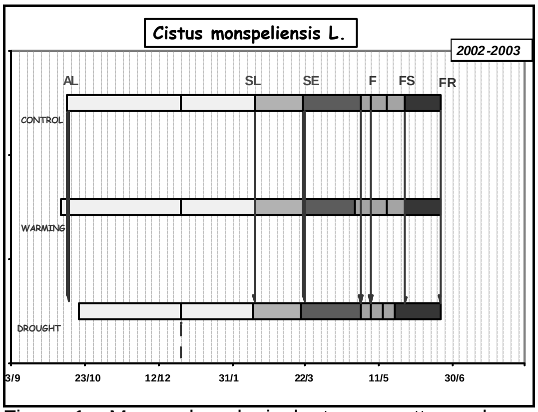

Figure 1. Mean phenological stages pattern observed during the period 2002-2003 in warming and drought treatment compared to control for *Cistus monspeliesis* L. (AL=Autumn Leafing, SL=Spring Leafing, SE=Shoot elongation, F=full flowering, FS=fruit set visible, FR= fruit development, see also Table 1).

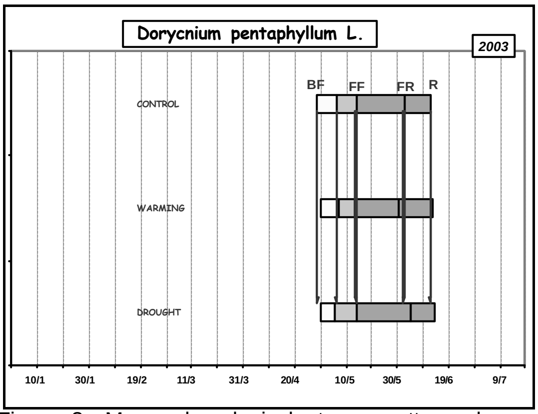

Figure 3. Mean phenological stages pattern observed during the period 2002-2003 in warming and drought treatment compared to control for *Dorycnium pentaphyllum* L., (BF=beginning of flowering FF=l flowering finishing, FR= fruit development, R= fully ripe, see also Table 1)

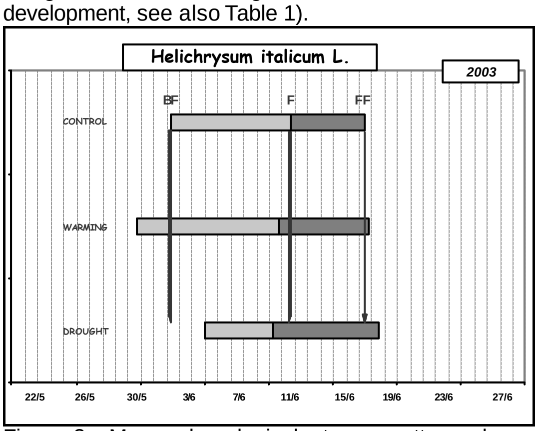

Figure 2. Mean phenological stages pattern observed during the period 2002-2003 in warming and drought treatment compared to control for *Helichrysum italicum* L., (BF=beginning of flowering F= full flowering, FF=l flowering finishing , see also Table 1)

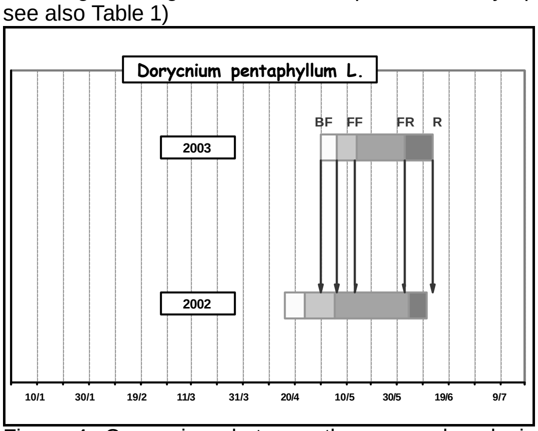

Figure 4. Comparison between the mean phenological pattern observed during 2002 and 2003 for *Dorycnium pentaphyllum* L. (BF=beginning of flowering FF=l flowering finishing, FR= fruit development, R= fully ripe, see also Table 1)