

Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant

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Summary

1. Summer drought stress is considered the primary constraint to plant performance in Mediterranean ecosystems. However, little is known about the implications of summer stress for plant reproduction under real field conditions and, particularly, for the regulatory mechanisms of maternal investment in reproduction.

2. The relationship between plant physiological status at different reproductive stages over the course of the summer drought period and final reproductive output was modelled in the Mediterranean semi-arid specialist *Helianthemum squamatum*.

3. Plant physiological status, assessed by the chlorophyll fluorescence-based parameter F_v/F_m , and soil moisture content beneath each plant, were determined in the field at five key phenological moments in a total of 88 plants. We used Generalized Linear Mixed Models to evaluate the effect of plant physiological status at those different dates on several components of reproduction (number of flowers and seeds per plant, fruit-set and intra-fruit seed abortion). We included soil moisture as an additional predictor to statistically control its potential effect on reproduction.

4. F_v/F_m measured at midday was a significant predictor of reproductive output, but its significance varied over time and with the specific reproductive response variable. F_v/F_m measured at the onset of flowering was positively related to the number of flowers and seeds per plant, whereas F_v/F_m at the fruiting peak positively affected fruit-set. Soil moisture content was only significant when measured before flowering, being positively related to total flowers and seeds. The effect of stress on reproductive output acted either at an early stage of the reproductive season, by varying the number of flowers produced and seed primordia initiated, or at a later stage, by adjusting the number or ripe fruits.

5. *Synthesis.* Our results show a direct relationship between physiological status and reproduction, and highlight the importance of the timing of stress for reproductive success. They also show that small departures from the physiological optimum at specific reproductive stages may cause significant decreases in the reproductive output. We suggest that the dynamic adjustment of reproduction in response to stress is adaptive in fluctuating and unpredictable Mediterranean semi-arid environments, where an adequate temporal distribution of maternal resources determines the species' ability to withstand severe environmental conditions.

Key-words: chlorophyll fluorescence, *Helianthemum squamatum*, Mediterranean, phenology, reproductive output, semi-arid, serial adjustment of reproduction, summer stress

Introduction

Summer drought stress in Mediterranean regions involves high temperatures and irradiances as well as water shortage,

and is considered the primary constraint to plant survival, growth and reproduction in these ecosystems (Fernández Alés *et al.* 1993; Larcher 2000). In fact, many studies have reported a reduced photochemical efficiency and very low photosynthetic rates during summer drought (Valladares & Pearcy 1997; Werner *et al.* 1998; Balaguer *et al.* 2002; Llorens

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et al. 2003b). Indeed, leaf temperatures can reach damaging values for the integrity of photosynthetic tissues (Epron 1997) and excessive levels of solar radiation can promote photo-inhibition (Long *et al.* 1994; Werner *et al.* 2002) during the Mediterranean summer.

Drought stress can also be detrimental for plant reproduction, shunting resources to reactions that increase stress tolerance or accelerating senescence of reproductive organs (Sun *et al.* 2004). It is known that water deficit and high temperature can affect plant reproductive output in multiple ways by, for example, interfering with processes such as microsporangogenesis, pollen grain germination or fertilization (Fox 1990, references therein), and reducing megagametophyte fertility (Young *et al.* 2004), which ultimately results in reduced fruit-set and/or seed-set. Furthermore, studies performed under controlled conditions have shown that failures in reproduction can be related to the moment in which the stress is experienced (Andersen *et al.* 2002; Erickson & Markhart 2002; Sun *et al.* 2004). For instance, reproductive development at the time of flowering, during early ovary growth, has been described as an extremely vulnerable stage especially sensitive to drought stress (Andersen *et al.* 2002). However, little is known about the implications of the environmental constraints imposed by summer drought for plant reproduction under real field conditions.

The influence of stress on plant reproduction is further complicated by the facts that the amount of maternal investment in reproduction is matched to the resources available under the particular circumstances of each plant, and that resource allocation to reproduction is thought to be adjusted at several hierarchical developmental stages (Lloyd 1980). Moreover, the regulating mechanisms of offspring production (e.g. seed abortion), have been found to be different under stress and non-stress conditions (Volis *et al.* 2004). The existence of these kind of mechanisms regulating maternal investment in reproduction is likely to be very important in Mediterranean habitats, where environmental factors are inherently variable and unpredictable (Blondel & Aronson 1999).

In this work we wanted to model the relationship between plant physiological status at different reproductive stages and final reproductive output in the Mediterranean semi-arid specialist *Helianthemum squamatum*. The reproductive period of this species occurs during late spring and summer, when water becomes limiting (Aragón *et al.* 2007). This phenological pattern, uncommon among Mediterranean perennials which tend to avoid flowering under the unfavourable conditions of the Mediterranean summer (Herrera 1992), allows direct study of the influence of physiological status on reproduction over the course of summer drought stress, a topic that has received scant attention. We hypothesized that not only must there be a relationship between plant physiological status and final reproductive output but also that certain reproductive stages must be more sensitive to summer stress than others. Therefore, the consequences of stress for reproductive success should vary depending on the reproductive stage at which stress acts.

Methods

THE SPECIES AND THE STUDY SYSTEM

Helianthemum squamatum (L.) Dum. Cours (Cistaceae) is a small perennial subshrub (10–40 cm) that grows exclusively on gypsum soils in the semi-arid Mediterranean climate of the Iberian Peninsula. Its flowering period extends from the end of May to the end of July (Aragón *et al.* 2007), coinciding mostly with summer drought. Flowers are hermaphroditic, yellow, and are arranged in dense inflorescences at the tip of new branches. Fruits are small multi-seeded capsules (3 mm in diameter) (López-González 1993). Species' life span ranges between 4 and 6 years (Caballero 2006).

We performed the study in a gypsum landscape located close to Chinchón (40.08° N 3.26° W, 673 m a.s.l.), Central Spain. The area has a Mediterranean semi-arid climate, with an annual average rainfall of 373 mm, a severe summer drought and a mean annual temperature of 14.1 °C (31-year climatic data record from the thermopluviometric station of Arganda, 40.19° N, 3.26° W). Climatic conditions at the study site during 2004 were slightly more humid than average (Fig. 1a).

We selected two sites with contrasting slope aspect (south and north) as the extremes of the local stress gradient (see Aragón *et al.*

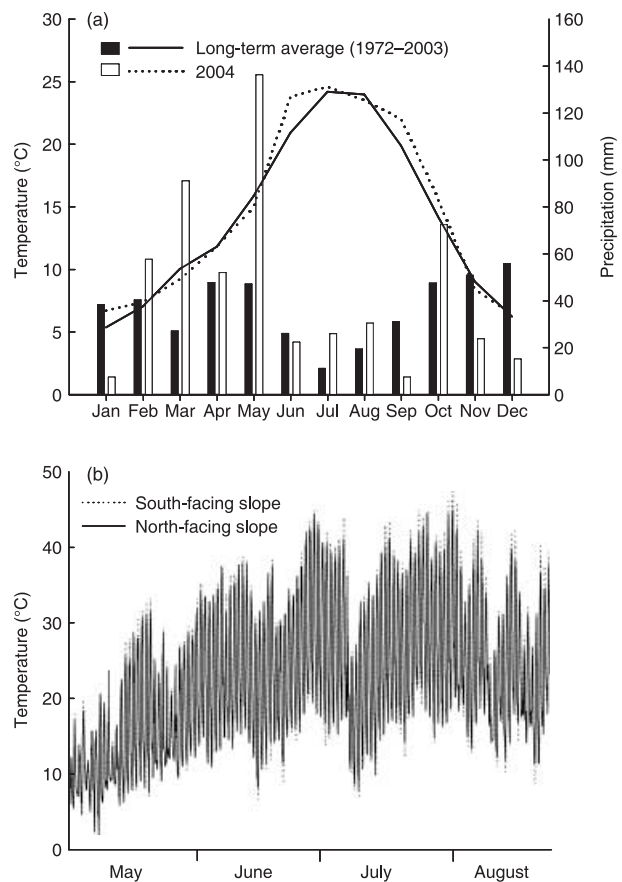


Fig. 1. Climatic conditions at the study site. (a) Bars and lines represent monthly mean precipitation and mean temperature, respectively, along the period 1972–2003 and in the studied year, 2004; (b) Lines show hourly air temperatures at plant height during the study period (May–August 2004) in the south- and the north-facing slopes.

2007). Air temperature in each slope was recorded hourly during the study period with data loggers (Pro H8032, Onset Hobo, MA, USA) installed half a metre from the ground, to determine the microclimate at plant height (Fig. 1b). We randomly selected 100 plants per slope, distributed in two homogeneous plots. We also monitored a set of plants established in another study, from which past reproduction and growth were known. This set consisted of 97 plants, 39 and 58 plants in the south- and north-facing slope, respectively, also grouped in two plots (south: 18 and 21; north: 36 and 22). The information available for this old set of plants allowed us to control the potential effects of past reproductive or growth efforts on current reproduction.

DATA COLLECTION

We tagged the selected plants in April 2004, before the flowering period, and measured maximum height (h), maximum crown diameter (M) and maximum perpendicular length (m). Plant size was estimated as a function of these variables, as an approximation to the volume of an ellipsoid ($2/3 \times \pi \times h \times M \times m$).

We monitored flowering phenology in all the selected plants (139 and 158, in the south and the north-facing slopes, respectively) every 3–5 days throughout the reproductive season and counted at each census the number of inflorescences with at least one open flower. We calculated the following individual phenological variables: (i) flowering onset, as the number of days elapsed since the first of May to the appearance of the first flower in the plant; (ii) flowering peak, as the number of days elapsed since the first of May to the day in which the maximum number of inflorescences with open flowers was reached; (iii) flowering duration, as the number of days that the plant remained in bloom. To calculate phenological variables we only took into account plants that flowered and survived until the end of the reproductive period: 119 plants (85.6%) and 133 plants (84.2%) in the south- and the north-facing slope, respectively.

We evaluated plant physiological status and soil moisture content at five different phenological moments (see dates in Fig. 3): before flowering; at both the onset and the peak of flowering; at the peak of fruit ripening; and at the end of the reproductive period before seed dispersal. These measurements were made on a subsample of 88 plants, 44 plants per slope (15 plants per plot in the new plots, and 7 plants per plot in the old plots). We considered the onset of flowering at population level the moment in which 60% of the plants had at least one inflorescence with open flowers. The rest of the dates were chosen according to field observations and phenological data from previous years (Aragón *et al.* 2007). As an indicator of plant physiological status we determined the maximum efficiency of photosynthetic energy conversion of PSII, as the ratio of variable (F_v) to maximum fluorescence (F_m) of dark-adapted leaves ($F_v/F_m = [F_m - F_o]/F_m$, where F_o is the minimum or initial fluorescence; Maxwell & Johnson 2000). Measurements were taken from 4.00 to 6.00 GMT, under complete darkness (hereafter pre-dawn) and from 12.00 to 14.00 GMT, during maximum solar radiation (hereafter midday), in three mature healthy leaves per plant dark-adapted for 30 min prior to each measurement. All measurements were carried out with a portable pulse modulated chlorophyll fluorimeter (FMS2, Hansatech, Norfolk, UK) and dark adaptation achieved with appropriate leaf-clips. Since water is considered one of the most limiting factors for plant performance in semi-arid Mediterranean scenarios, we included soil moisture content as a potential predictor of reproductive output. Measurements of volumetric soil moisture content were taken at 6-cm depth in all the marked plants from 10.00 to 12.00 GMT, by using a soil moisture probe (Thetaprobe ML2X,

Delta T, Cambridge, UK). We took measurements in the northern and southern side of each plant within a 20-cm radius, and calculated the mean value. The sampling dates chosen to perform chlorophyll fluorescence and soil moisture content measurements were all clear, sunny days. Given our purpose of relating these parameters to the phenological stage, and considering that flowering phenology is not synchronized in both slopes (Aragón *et al.* 2007), all sampling dates were delayed one day in the north-facing slope.

At the end of the reproductive period (early August), we counted the total number of inflorescences per plant and collected 10 of them (when possible), in order to estimate the mean number of flowers and fruits produced per inflorescence and per plant. With these variables we calculated the proportion of flowers setting fruits (fruit-set). We dissected 10 fruits per plant to estimate the mean number of seeds (viable and aborted) produced per fruit and per plant. Intra-fruit abortion rate was calculated as the proportion of aborted seeds to total number of seeds per fruit.

STATISTICAL ANALYSIS

We evaluated the effect of plant physiological status and soil moisture content at five different phenological moments on the reproductive output of *H. squamatum* by fitting Generalized Linear Mixed Models (GLMMs), via restricted maximum likelihood (REML; Patterson & Thompson 1971). We analysed the data following a multi-level approach, considering plants nested within plots and plots nested within slopes as random factors, and slope as a fixed factor. This approach allows us to overcome the problems arisen from the unbalanced and hierarchical structure of our data set. GLMMs provide a flexible way to model traits which do not satisfy the assumptions of a standard linear modelling, allowing at the same time the distinction between fixed and random factors in the model. Their use in this case is justified by the non-normal distribution of dependent variables under consideration and for the inclusion of random sources of variation, which represent an accurate estimation of the effect of environmental heterogeneity at several relevant scales on the modelled variables (for further details of GLMMs see Littell *et al.* 1996). Effects of random factors were tested using Wald *Z*-statistic tests, which are appropriate for large samples, and those of fixed factors were tested with *F*-tests, which are preferable when the structure of the variance–covariance matrix depends on an unknown scale parameter (Littell *et al.* 1996).

We modelled absolute and relative components of reproduction. The former set of components included number of flowers and number of seeds per plant; the relative components were fruit-set and intra-fruit seed abortion. We assumed a Poisson error distribution with log canonical link function for the two absolute response variables and a binomial distribution with a logit canonical link function for the two relative ones (McCullagh & Nelder 1989). The explanatory variables fitted in the model included the parameter F_v/F_m at midday (more variable than the pre-dawn value along time, see Results) and percentage of soil moisture content at the different sample dates, together with plant size. Due to the complexity of the model structure and the relatively large number of potential explanatory variables, we considered only main effects, excluding all possible interactions. We first checked for potential multicollinearity among predictors assessing variance inflation factor (VIF). Since maximum VIF was lower than seven, all the variables were included in the models. This modelling approach permits distinction of the effects due to every predictor considering and partialling out the rest of them and independently of the level where they were taken. This means that we evaluated how the chlorophyll–fluorescence based

Table 1. Effects of plant physiological status (F_v/F_m) and soil moisture content (SMC) at five different phenological moments (before flowering, at the onset and the peak of flowering, at the peak of fruiting and at the end of reproduction before dispersal) on the reproductive output of *Helianthemum squamatum*. The reproductive variables modelled were total number of flowers and seeds per plant, fruit-set and intra-fruit abortion rate. Data represent the Wald-type F -statistic (Wald Z -statistic for random factors) with the degrees of freedom as subindex, and the sign indicating the direction of the effects. The number of cases is given in the last row. Asterisks represent the level of significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

	Flowers per plant	Seeds per plant	Fruit-set	Abortion rate per fruit
Fixed factors				
Slope aspect	-1.54 _{1,2,3}	-0.59 _{1,1,9}	-1.48 _{1,2,3}	-1.22 _{1,2,1}
F_v/F_m Before flowering	+0.14 _{1,74}	+0.21 _{1,74}	+0.73 _{1,74}	-3.23 _{1,74}
F_v/F_m Onset flowering	+5.78 _{1,74} *	+4.66 _{1,74} *	-0.89 _{1,74}	+3.80 _{1,74}
F_v/F_m Peak flowering	+0.69 _{1,74}	+1.11 _{1,74}	-0.62 _{1,74}	-0.20 _{1,74}
F_v/F_m Peak fruiting	+0.25 _{1,74}	+0.89 _{1,74}	+15.28 _{1,74} **	-0.17 _{1,74}
F_v/F_m End	+0.05 _{1,74}	-0.00 _{1,74}	-1.04 _{1,74}	+0.08 _{1,74}
SMC Before flowering	+5.95 _{1,74} *	+8.02 _{1,74} **	-1.60 _{1,74}	+0.90 _{1,74}
SMC Onset flowering	-0.35 _{1,74}	-0.01 _{1,74}	+0.71 _{1,74}	-0.03 _{1,74}
SMC Peak flowering	-0.87 _{1,74}	-1.39 _{1,74}	-0.78 _{1,74}	-0.00 _{1,74}
SMC Peak fruiting	-0.93 _{1,74}	-1.85 _{1,74}	-1.02 _{1,74}	+3.44 _{1,74}
SMC End	+0.68 _{1,74}	+0.49 _{1,72}	+1.64 _{1,74}	-1.87 _{1,74}
Plant size	+2.98 _{1,74}	+2.34 _{1,72}	+1.03 _{1,74}	-0.82 _{1,72}
Random factors				
Plot	0.27	0.26	0.29	0.50
Residual (plant)	5.35***	5.61***	5.61***	5.45***
Number of cases	80	80	80	80

parameter F_v/F_m (an estimate of physiological stress induced by multiple co-occurring stresses) explains our target reproductive variables taking into account a possible differential effect of water stress *per se*. All the computations were performed using the GLIMMIX Macro of SAS (SAS Statistical Package, 1990; SAS Institute, Cary, NC, USA). From the subsample of plants only 39 and 41 plants flowered and survived in the south- and the north-facing slope, respectively, and were included in the GLMMs.

The potential effects of past efforts in reproduction or growth on current reproductive output, which could mask contemporaneous effects, were evaluated by performing additional GLMMs for the response reproductive variables described above. We included as explanatory variables: total seed production per plant in 2003, plant growth from 2003 to 2004, current plant size and slope aspect as fixed factors, and the random factors plot and plant, following the same approach as used in the previous GLMMs.

To examine the variation in chlorophyll fluorescence and soil water content between slopes, we performed simpler models, considering exclusively the variables slope aspect, date, their interaction and plant size as fixed factors, and the random factors plant and plot as previously described. Plant size was included to avoid bias due to differences in plant performance among individuals, and was not fitted when modelling soil moisture content. We assumed a binomial error distribution with a logit link function in both cases. We used the DIFF option in the LSMEANS statement of the GLIMMIX procedure to identify the dates in which significant differences between slopes appeared.

Results

Flowering phenology was delayed by a minimum of 2 weeks compared to the 2 previous years, while flowering duration lasted some more days (Aragón *et al.* 2007). Average onset and peak of flowering happened in 20 June and 29 June, in the

south-facing slope, and in 22 June and 2 July in the north-facing slope (Fig. 3), and average flowering duration was 18.7 and 19.4 days in the south and north slope, respectively. The selected sampling dates (for chlorophyll fluorescence and soil moisture content measurements) matched the key phenological events over the course of reproduction. For instance, what we considered flowering peak coincided exactly with the calculated average flowering peak in the case of the north-facing slope and was only one day delayed in the case of the south-facing slope. The average phenological lag between the two slopes was 2.4 days.

Absolute and relative reproductive variables reached similar mean values when calculated from the total set of plants or from the subsample of plants selected for chlorophyll fluorescence measurement and did not vary significantly between slopes (Table 1). Plants produced on average (\pm SE) 175 ± 32 flowers and 257 ± 38 seeds. Mean fruit-set and intra-fruit seed abortion were about 75% and 30%, respectively.

Mean pre-dawn values of photochemical efficiency (F_v/F_m) were relatively constant along the reproductive period (always above the optimal value of 0.83; Maxwell & Johnson 2000). Average midday values, in contrast, were optimal only before flowering (mid-spring) but were below this optimal range during the rest of the study period (Fig. 2). Mean values of F_v/F_m were in general slightly higher in the south-facing slope, but significantly higher only at the onset of flowering ($F = 9.84$, $P = 0.0257$). Soil moisture content gradually diminished as the season advanced (Fig. 2), reaching minimum mean values close to 0% in both slopes at the end of reproduction (end of July). Average percentages of soil moisture content varied significantly between slopes only at the peak of flowering ($F = 12.04$, $P = 0.0178$).

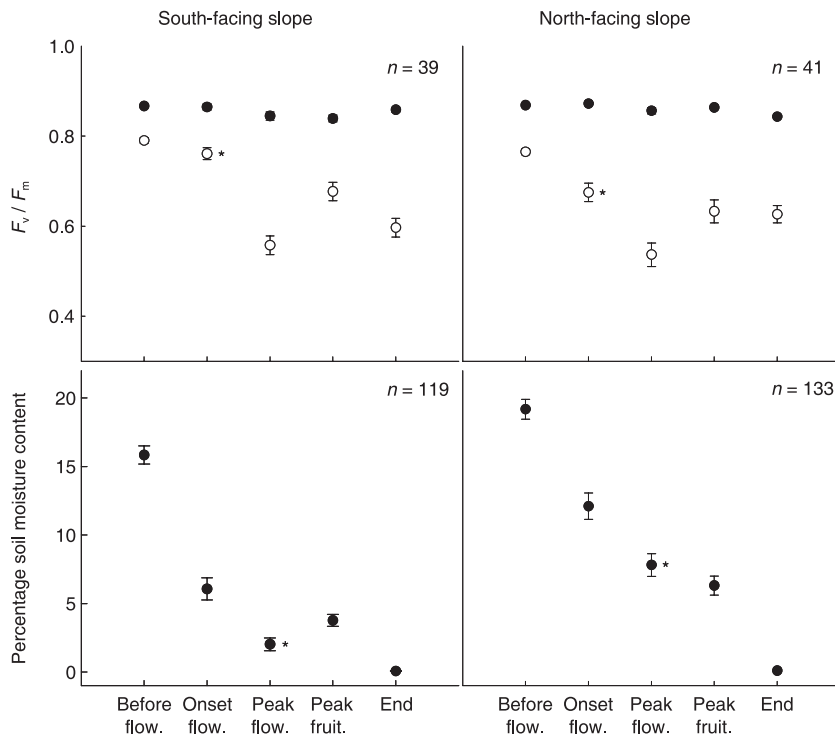


Fig. 2. Maximum photochemical efficiency of PSII (F_v/F_m) at pre-dawn (solid circles) and midday (open circles) and percentage of soil moisture content at different phenological stages (before flowering, at the onset and the peak of flowering, at the peak of fruiting, and at the end of reproduction before dispersal) during the reproductive period of *Helianthemum squamatum* in the south- and the north-facing slopes. Data points represent mean \pm SE. Sample size (n) is given in the upper right corner of each graph. Asterisks indicate significant differences ($*P < 0.05$) between slope aspects on a given date.

Table 2. Effects of past reproduction and growth on current reproductive variables (number of flowers and seeds per plant, fruit-set and intra-fruit abortion rate). The explanatory variables included were total seed production per plant in 2003, plant growth from 2003 to 2004, current plant size and slope aspect as fixed factors, and the random factors plot and plant. Data represent the Wald-type F -statistic (Wald Z -statistic for random factors) with the degrees of freedom as subindex, and the sign indicating the direction of the effects. The number of cases is given in the last row. Asterisks represent the level of significance ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$)

	Flowers per plant	Seeds per plant	Fruit-set	Abortion rate per fruit
Fixed factors				
Slope aspect	-0.03 _{1,1.7}	+0.19 _{1,1.6}	-1.48 _{1,1.8}	-0.06 _{1,1.8}
Seeds per plant 2003	+1.82 _{1,94}	+2.10 _{1,88}	+2.13 _{1,87}	+1.13 _{1,87}
Plant growth 2003–2004	+2.90 _{1,94}	+1.81 _{1,88}	+0.65 _{1,87}	-2.45 _{1,88}
Plant size 2004	+9.68 _{1,94} **	+3.72 _{1,94}	+2.64 _{1,89}	-3.54 _{1,88}
Random factors				
Plot	0.65	0.05	0.56	0.24
Residual (plant)	2.89**	3.08**	2.89**	2.97**
Number of cases	97	95	95	95

The parameter F_v/F_m measured at midday and to a lesser extent also the percentage of soil water content were significant predictors of final reproductive output, their significance being affected by the phenological stage and the response variable considered in each case. Absolute components of reproduction (flowers and seeds per plant) were positively related to F_v/F_m measured at the onset of flowering, whereas fruit-set was positively affected by the F_v/F_m measured at the fruiting peak (Table 1). In other words, plants producing more flowers and seeds were less stressed (higher F_v/F_m values) at the onset of flowering and, similarly, plants with higher fruit-sets were less stressed at the peak of fruit ripening. Soil moisture was a significant predictor of reproductive output only before flowering, when high values improved the production of total flowers and seeds per plant.

The effect of plant size was included in all the models, and no significant effect was found in any case. While the random factor plot had no significant effect on any reproductive variable, the plant factor (residual) had always a highly significant effect. We found no significant effects of previous year's reproduction or growth on any of the reproductive variables modelled (Table 2).

Discussion

Our results confirm our hypothesis and connect physiological status and reproduction under real field conditions. We have shown the existence of critical stages of reproduction during which the physiological stress negatively affected final reproductive output. In an environment such as the one studied

here where stress is considered chronic, a detrimental effect of drought stress on plant performance is always to be expected. But our results go further, recognizing especially sensitive reproductive stages and detecting that even small departures from the physiologically optimum condition (e.g. F_v/F_m midday mean values at the onset of flowering, Fig. 2) have short-term consequences for plant fitness. As we hypothesized, the consequences for the reproductive output were different depending on the stage at which the stress was experienced. It is worth noting that plant stress was assessed by F_v/F_m , an indicator of plant physiological status particularly sensitive to high temperatures and excess light (Long *et al.* 1994) but rather insensitive to dehydration (Gamon & Pearcy 1990; Havaux 1992; Epron 1997; Llorens *et al.* 2003a). Low values of F_v/F_m at the onset of flowering were related to a lower production of flowers per plant, and consequently, to total seeds per plant, while low values of F_v/F_m at the peak of fruit ripening were related to lower fruit-sets. The latter was probably due to the fact that many plants invest in ripening the best fruits and abort the rest (Stephenson 1981; Albert *et al.* 2001). On the other hand, the percentages of soil moisture content recorded during reproduction were not significant predictors of final reproductive output. Only soil moisture measured before the flowering season (mid-spring) and well in advance of the summer drought period was found to be significantly related to certain absolute components of reproduction (flowers and seeds per plant). These results suggest that the regulating mechanism of maternal investment is environmentally sensitive (Volis *et al.* 2004) and they agree with the hypothesis of serial adjustment of maternal investment proposed by Lloyd (1980). The latter postulates that the amount of maternal resources invested in reproduction is regulated through a series of sequential developmental stages, including flower and ovary production and fruit maturation, and that this is also controlled by extrinsic factors. Soil moisture content in mid-spring appeared to be an important factor for final reproductive output, when this late-flowering species is probably starting to mobilize resources and prepare for the upcoming reproductive season. Surprisingly, no other measure of soil moisture content during reproduction was a significant predictor of reproductive output. This response to the extreme water shortage cannot be explained by great root depth, as has been suggested for other woody Cistaceae in similar scenarios (Castro-Díez *et al.* 2005), given the relatively shallow root system of *H. squamatum* (Romão 2003). Furthermore, plant physiological status was near optimum even with almost no water in the soil (Fig. 2). Pre-dawn values of maximal photochemical efficiency of PSII (F_v/F_m) remained above the optimum level (0.83 according to Maxwell & Johnson 2000) during all of the reproductive season and, in spite of the generalized diurnal decline in F_v/F_m , midday values also remained considerably high, indicating a relatively mild photo-inhibition even under conditions where it is usually very pronounced (Valladares & Pearcy 1997). Moreover, this diurnal decline was always reversible, even in the hottest and driest times of the year, suggesting that there was no chronic damage to photosystem II (according to Werner *et al.* 2002). These

results parallel the high resistance to summer stress exhibited by other Mediterranean species (Zunzunegui *et al.* 1999; Llorens *et al.* 2003a; González-Rodríguez *et al.* 2005). The existence of scales on the leaves of *H. squamatum* (López-González 1993) could help to moderate leaf temperatures and provide photoprotection (Sandquist & Ehleringer 1998). Protection against excessive irradiance and heat can also be achieved in this species by the modification of the leaf angle during the day, reaching the steepest angle (about 70°) at midday (C.F. Aragón, personal observation). Steep leaf angles have been described as a structural mechanism to avoid overheating and photoinhibition (Ryel & Beyschlag 1995; Valladares & Pearcy 1997). Despite the relatively high F_v/F_m midday mean values in our study plant we have found that even small reductions in this parameter, likely to be caused by the co-occurrence of multiple stresses, can lead to reductions in certain components of reproduction. The moment (phenological stage) at which this stress is experienced by the plant was even more important than its intensity. For example, the lowest midday mean values of F_v/F_m were found at the peak of flowering and had no direct effect on the reproductive variables studied. The effect of stress, regardless of the factors causing this stress and estimated here by the integrated F_v/F_m parameter, was significant for the final reproductive output when it took place either at an early stage of the reproductive season, by varying the number of flowers produced and seed primordia initiated, or at a later stage, by adjusting the number of ripe fruits (Fig. 3).

The lack of a significant effect of slope aspect on the final reproductive output concurs with the reproductive flexibility previously reported for this species (Aragón *et al.* 2007). Moreover, we only found significant differences in plant physiological status between slope aspects in one sampling date (at the onset of flowering) with slightly improved values of F_v/F_m in the south-facing slope. This finding is contrary to the widely accepted idea of north-facing slopes as more favourable scenarios for plant performance in Mediterranean environments in the northern hemisphere (Kutiel 1992; Escós *et al.* 2000; Bellot *et al.* 2004). The competitive ability of this species in the south-facing slope might be related to the existence of a well-developed biological soil crust in this exposure (Martínez *et al.* 2006), where only a few number of genuine gypsophyte species are able to germinate and establish. In contrast, the north-facing slope, lacking this typical gypsum crust, is covered by a richer and denser shrub community, where together with some strict gypsophytes, calcicole plants become dominants. Consequently, a higher competition for resources may explain to some extent a slightly worse plant physiological status in the north-facing slope, similar to that which has been reported in shaded vs. sunny Mediterranean habitats (Valladares & Pearcy 2002).

In conclusion, our results suggest that even minor changes in plant physiological status can have direct and significant consequences for plant reproduction and highlight the importance of the reproductive stage at which these physiological changes occur. Adjusting reproduction and resource allocation during drought stress could be a mechanism to optimize plant resources, and thereby, to maximize plant

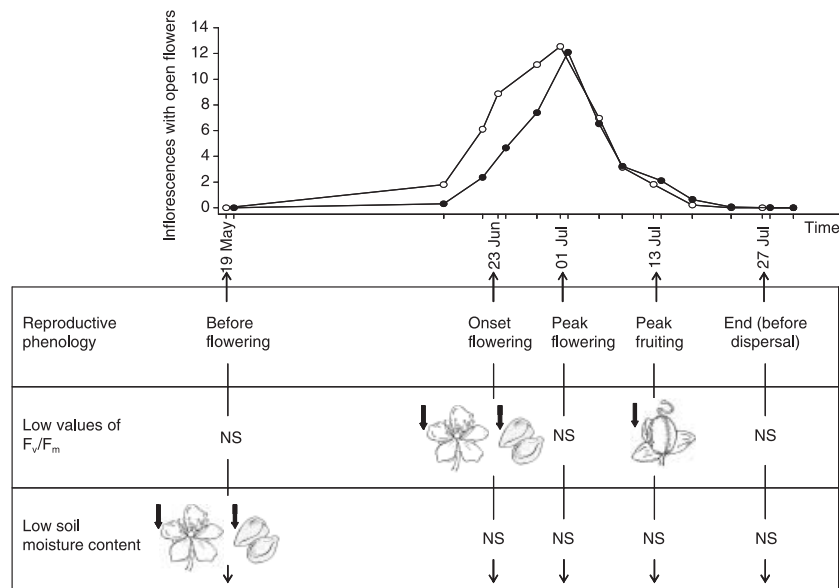


Fig. 3. Diagram summarizing the effects of plant physiological status (F_v/F_m) and soil moisture content on the reproductive response variables (flowers, seeds and fruit-set) at different phenological stages: before flowering, onset of flowering, peak of flowering, peak of fruiting and end of reproduction before dispersal. Drawings of flowers, seeds and fruits are placed at phenological stages at which stress (F_v/F_m) or soil moisture had a significant negative effect (represented by the arrow facing down) on these fitness components. Low values of soil moisture content in mid-spring, before the flowering period, and high levels of stress (low values of F_v/F_m) at the onset of flowering, likely due to high temperatures and irradiances, negatively affected the total production of flowers and seeds per plant. On the other hand, high levels of stress at the peak of fruit ripening led to reductions in plant fruit-set. (NS, no significant effect). The upper graph shows flowering phenology data of *Helianthemum squamatum* in the south- and the north-facing slopes (lines with open and solid circles, respectively). Data points represent the mean number of inflorescences with open flowers per plant on a given date. The specific dates at which the measurements of F_v/F_m and soil moisture were taken are shown only for the south-facing slope (in the north-facing slope measurements were always taken one day later).

fitness. We suggest that this dynamic strategy is adaptive in fluctuating and unpredictable Mediterranean semi-arid environments, where an adequate temporal distribution of maternal resources determines the species' ability to withstand severe environmental conditions.

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