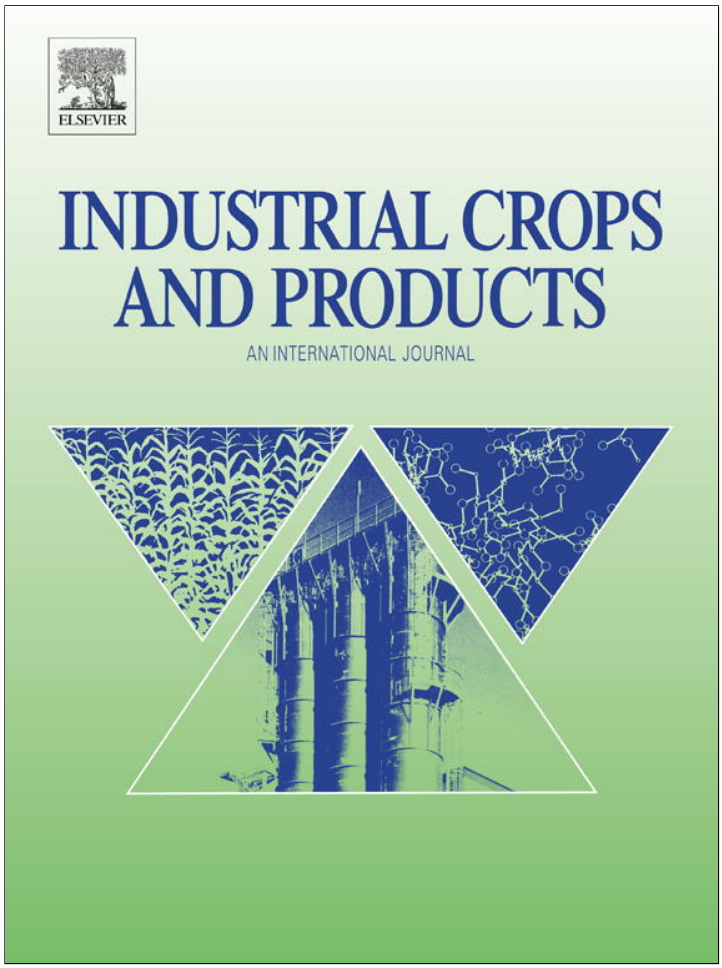

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The growth rate modulates time to first bud appearance in *Physaria mendocina*

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ABSTRACT

Physaria mendocina is under domestication because its seeds contain significant amounts of hydroxy fatty acids for several industrial uses, but displays a facultative biennial behavior which may represent a drawback in terms of production. Previous work revealed that the time to flower induction in this species is insensitive to temperature, photoperiod and vernalization, but suggested that this length of time could be determined by radiation, water and nutrients and/or the acquisition of a minimum growth rate. We aimed to determine whether the attainment of a threshold plant growth rate (GRt) triggers the initiation of the flowering phase in *P. mendocina*. Nutrient, water and radiation availability were manipulated to modify the timing of acquisition of that rate, expecting a concomitant modification of the time to flowering. We also explored the possibility that the stimulus is mediated by an accumulation of active gibberellins (GAs). Linear regressions were fitted between plant dry weight and time, and slopes of the relationships were considered as the growth rates. Radiation, water and nutrients constraints increased the duration of the phase between emergence and, concomitantly, first bud appearance (FBA). However, plants from all treatments reached FBA, after acquiring a growth rate of around 0.01 g d^{-1} pl^{-1} (GRt).When exogenous GAs was applied under limiting radiation, plants reached FBA despite the fact that they never acquired a GRt; conversely, when GAs biosynthesis was inhibited under high irradiances, the plants required more days to reach FBA than controls, despite the fact that they acquired a GRt. The information obtained allow us to conclude that the time to FBA, which is the first visible manifestation of floral induction in this system, is modulated by factors controlling growth mediated by an accumulation of (GAs) and suggest that the acquisition of GRt is the internal feature that triggers floral induction. This knowledge offers a frame within which cropping systems could be designed in order to avoid or not a biennial behavior.

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1. Introduction

Some species of the genus *Physaria* (formerly *Lesquerella* – Al-Shehbaz and O'Kane, 2002; O'Kane and Al-Shehbaz, 2004) are potential alternative crops for commercial oilseed production and are currently under domestication (Ploschuk et al., 2001; Dierig et al., 2006; Adam et al., 2007). For example, *Physaria fendleri* is a domesticated species characterized by an annual cycle; however, its low tolerance to water stress and low temperatures is well documented (González-Paleo and Ravetta, 2011a; Ploschuk et al., 2003; Dierig et al., 2006). This precludes its utilization in semi-arid environments as those that prevails in the central and southern parts of Argentina. In contrast, the perennial *P. mendocina*, native of the Monte Region of Argentina (Correa, 1984) also contains significant amounts of hydroxy fatty acids for several industrial uses (Thompson, 1990), but displays yield stability even under harsh environments, thus making it suitable for cropping areas that are too dry and/or cold for *P. fendleri* (González-Paleo and Ravetta, 2011a,b). It develops a perennial rosette with lateral spicate inflorescences every year with the central meristem remaining at vegetative stage.

Like other herbaceous perennial species, *P. mendocina* can flower during the first year if a favorable growing environment prevails (Hirose and Kachi, 1982); otherwise the plant delays the entrance in the reproductive stage until the second year of life (Kelly, 1985; De Jong et al., 1986; Prins et al., 1990; Klinkhamer et al., 1991; Wesselingh and De Jong, 1995; Burd et al., 2006). This facultative biennial behaviour clearly may represent a drawback in

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terms of production (Windauer et al., 2004, 2006). Thus, knowing the way in which the environment determines time to flowering is of paramount importance if the species is intended to be domesticated. For this reason, phenological responses to environmental factors have been studied in *P. mendocina* under controlled and field conditions (Windauer et al., 2004, 2006). The results revealed that time to flower initiation in this species is relatively insensitive to temperature, photoperiod and vernalization but, at temperatures higher than 24 °C, *P. mendocina* displayed a qualitative response (i.e. no development progression was observed) (Windauer et al., 2004). Even though, field experiments showed that the rate of development was accelerated as sowing date was delayed although, under late spring sowing dates, a biennial behaviour was observed, possibly due to the prevalence of mean temperatures higher than 24 °C (Windauer et al., 2006). Taken together these results indicate that, while temperatures are lower than 24 °C, there is an unknown factor, other than the above-mentioned, whose inductive capacity increases throughout the growing season. According to these results (i.e. no response to photoperiod and temperature), *P. mendocina* is likely to fall in the category of "autonomous-flowering" plants. Plants falling in this category are usually sensitive to irradiance (Bernier et al., 1993). Hence, the hypothetical factor behind initiation of the flowering phase might have been the incident radiation which, as in the case of photoperiod, is strongly associated to sowing date. Developmental response to radiation has been reported for other crops (Salisbury and Green, 1991, in rapeseed; Rawson, 1993 in wheat; Bertero, 2001 in quinoa). In biennial or perennial species, the influence of incident radiation on time to flower induction can be attributed to its effect on growth and, therefore, plant size, threshold size or physiological minimum size and threshold growth rate (Wesselingh et al., 1997).

Although the attainment of a critical plant size as a trigger for floral induction has been suggested for several species (Wesselingh et al., 1997; Werner, 1975; Gross, 1981; Kachi and Hirose, 1983; Klinkhamer et al., 1987; Kagaya et al., 2009), recent studies in other facultative biennial crop revealed that the rate of development towards flowering under inductive photoperiods is strongly affected by rosette's growth rate and not by a critical size (Gimenez et al., 2013). Moreover, previous information in *P. mendocina* showed a great variation in plant size at the onset of flowering (Windauer, 2002), suggesting that the initiation of this stage might be related to the acquisition of a threshold growth rate rather than to the acquisition of a certain plant size. This "threshold growth rate, GRt" would trigger floral initiation. Since growth rate is strongly modulated by the availability of resources such as nutrients, radiation and water (Taiz and Zeiger, 2006), it seems reasonable to predict that any limitation in the availability of these resources (and not only incident radiation) would delay the onset of flowering in *P. mendocina*.

Genetic and physiological studies indicate that gibberellins (GAs) modulate the autonomous flowering pathway (Jacobsen and Olszewski, 1993). Moreover, there is genetic evidence for crosstalk between the autonomous and gibberellin-dependent flowering pathways (Mier et al., 2001). Hence, if in the end floral induction is indeed elicited once a threshold plant growth rate is attained, it might be expected that the stimuli is mediated by an accumulation of active GAs.

In this paper we tested the hypothesis according to which the attainment of a threshold plant growth rate triggers the initiation of the flowering phase in *P. mendocina*. To do this we experimentally manipulated variables as nutrient, water and radiation availability to modify the timing of acquisition of that rate, expecting a concomitant modification of the time to flowering. We also explored the possibility that the stimulus is mediated by an accumulation of active GAs.

2. Materials and methods

2.1. Plant material and management

Seeds of *P. mendocina* were collected from a native stand at Lihuel Calel, La Pampa, Argentina (37° 57′S, 65° 33′W). The seeds used for the experiments were reproduced and selected for morphological traits (i.e. plant size and seed size) under the same environmental conditions during five generations in our experimental field (Facultad de Agronomía, UBA; 34° 37′S, 58° 20′W). All experiments were carried out at the Facultad de Agronomía with seeds of the year (i.e. less than 1 year storage), and plants were grown under adequate water conditions and kept free from weeds, diseases and insects.

2.2. Experiment 1: radiation availability

A field experiment was carried out during 2007 on a salty clay loam soil (Vertic Argiudoll) with the aim to explore the possibility that radiation intensity (a factor strongly associated to sowing date) is behind the initiation of the flowering phase. Flower induction suggests an early event during which meristems commit to reproduction. It was beyond our possibilities to determine and to identify the first meristem changing to a reproductive stage. In addition, due to the architecture of this plant, the moment of floral initiation is not easily related to the number of leaves initiated. Therefore, first bud appearance (FBA, floral buds within the same inflorescence joined, still covered by the terminal leaves) was regarded as the first visible signal of floral induction, and time to FBA was recorded in calendar days; indeed, due to the absence of a relationship between developmental rate and temperature, thermal time for the phase emergence (EM)-FBA is meaningless for *P. mendocina* (Windauer et al., 2004). Plots were considered to be at a given phase when 50% of the plants reached that stage.

The experiment was hand sown on 5 June 2007 and urea (170 kg ha^{-1}) was applied before sowing. Treatments were arranged in three randomized complete blocks with three replicates (plots). Each plot consisted of eight rows, 0.2 m apart and 1 m long for a total density of 50 plants m^{-2}. Treatments consisted of a control (TC, 100% of incident radiation) and four treatments with low radiation (shaded treatment) imposed two weeks after EM for all plots over a period of increasing duration depending on the treatment: R1: 21 days, R2: 42 days, R3: 64 days and R4: maintained at low radiation until FBA. These treatments allowed for the accumulation of different amount of radiation. Low irradiance plots were shaded with black shade netting placed 0.20 m above the canopy. The shade netting intercepted 67% of incident radiation (i.e. 33% of incident radiation reaching the canopy) but did not modify light quality (i.e. R/FR ratio). Air temperature sensors, connected to a Data Logger (LI-COR model 1000, Lincoln, NE, USA) were placed into the canopy to test the impact of shading on canopy temperature. The nets used to reduce incident radiation (shaded treatment) reduced the canopy average daily temperature by only 0.7 °C.

2.3. Experiment 2: water availability

A field experiment was hand sown on 18 June 2011 in rectangular boxes (1.2 m × 1.00 m × 0.12 m deep), containing a mixture of soil (80%) and sand (20%). Treatments were arranged in a completely randomized design with four replicates (microplots) per treatment and consisted of a control (W+, the microplots were maintained at field capacity) and one treatment of water stress; the microplots were restricted in the irrigation in order to generate water stress (water stress treatment, W-). This treatment was imposed two weeks after emergence and it was applied during 40 days approximately when the supply water was re-established

to finish the experiment. Each microplot consisted of eight rows, 0.2 m apart and 1 m long for a total density of 50 plants m^{-2}. All microplots were watered daily until the saturation of the soil and then the treatments were set up (two weeks after EM). Gravimetric moisture content of the soil (RH %), from sampling was monitored every 4–5 days. To quantify the water status of plants, plant water potential (Ψa) measurements were made weekly with a pressure chamber (Scholander pump) Biocontrol, model 6 (Biocontrol, Argentina). All measurements were made at noon in the main stem of entire plants.

2.4. Experiment 3: nutrient availability

A field experiment was hand sown on 18 June 2011 in rectangular boxes (1.2 m × 1.00 m × 0.12 m deep), containing a mixture of soil (20%) and sand (80%). In this case the proportion was altered in relation to that used in Experiment 2 to have more control on nutrient availability. Treatments were arranged in a completely randomized design with four replicates (microplots) per treatment and consisted of a control (F+), watered with nutrient solution (an equivalent to 150 kg ha^{-1} of N, 50 kg ha^{-1} of P and 25 kg ha^{-1} of K; Ploschuk et al., 2005) and water alternatively, from the moment in which the plants of the microplots reached two leaves until the end of the experiment, and one treatment with nutritional limitations (F−) that was irrigated with water only. The spatial arrangement was the same as that used for the Exp. 2.

Throughout Experiments 2 and 3, every time rains were predicted by the National Meteorological Service (SMN) plants were covered temporarily with plastic sliding roofs, to prevent the incidence of rainfall and avoid changes in the water content or in the concentration of nutrients in the microplots.

2.5. Experiment 4

The objective of this experiment was to elucidate whether the acquisition of a threshold growth rate truly triggers floral induction or if it is merely a consequence of floral induction. We also used this experiment to explore the extent to which the stimulus is mediated by the accumulation of active GAs. For this purpose, we attempted to uncouple both processes (i.e. acquisition of a minimum growth rate per plant and time to floral induction) by either i) inhibiting gibberellins (GAs) biosynthesis in plants growing under high irradiances or ii) supplementing with exogenous GAs plants growing under low (i.e. shaded) irradiance conditions.

A field experiment was carried out during 2006 at the Facultad de Agronomía, Universidad de Buenos Aires. Pre-germinated seeds of *P. mendocina* were sown on 17 July in pots 0.35 × 0.12 × 0.17 m containing a mixture of sand and mixed soil 1:1. Sixteen pots (four plants per pots) were assigned per treatment. Treatments were arranged in a completely randomized design with six replicates (plots) per treatment and consisted of a control (100% of incident radiation, C) and a low radiation treatment (shaded treatment, S) imposed two weeks after emergence until floral bud appearance. Low radiation treatment plots were shaded with a black shade netting (see Experiment 1) although with only 20% of incident radiation reaching the plants. Within each radiation regime, half of the plants remained as non-chemically treated (C and S) and the other half were treated in order to alter their endogenous gibberellin content. Shaded plants were treated with exogenous gibberellins (GA_4, GA_7, ProVide, Abbott Laboratories, Chicago, USA, SG gibberellin shaded treatment) and full radiation plants with paclobutrazol (Crestar, ICI, Buenos Aires, Argentina, an inhibitor of gibberellins biosynthesis, CP paclobutrazol control treatment). Chemicals were sprayed directly to shoot tips and young leafs as 100 μM of aqueous solution (GAs) and 4 ppm of aqueous solution (Paclobutrazol). Two weeks after emergence (23 August) until 10 October, plants were sprayed with the chemicals every two days until 10 October. Non-chemically treated treatments (C and S) plants were sprayed with distilled water each time the treated plants were sprayed with the respective chemical.

2.6. Measurements and statistical analysis

In all the experiments, phenological and development measurements were carried out at daily intervals, in order to establish an association between FBA and changes in the growth pattern of the plants For this purpose, two plants per plot were harvested weekly (except for 14 and 56 DAE that sampling was not carried out for operative reasons), dried at 65 °C for 72 h until constant weight and the whole plant dry mass was determined. Linear regressions were fitted to estimate the association between plant dry weight and time, and slopes were considered as the growth rate.

A piecewise linear regression model was used to estimate the association between plant dry weight and time.

$$\text{Dry weight} = a + b * \text{DAE if}(\text{DAE} = c) + d * (\text{DAE} - c)\text{if}(\text{DAE} = c) \quad (1)$$

where DAE are the days after emergence, a is the intercept, b and d are the different slopes of the linear regression (considered as growth rates) and c is a breakpoint of the function, that indicates the number of days until a change in the growth rate is detected. All the parameters were compared using one-way ANOVA. Tukey test comparisons were performed when significant differences between treatments were detected.

3. Results

3.1. The effect of resource limitation on plant phenology

3.1.1. Radiation (Experiment 1)

Time to FBA was affected by the available radiation level: the longer the duration of the shading treatment, the longer the period until FBA (Table 1, Fig. 1). However, when shading was given for 21 days, the duration of the period EM-FBA was not significantly different from that in the control (TC vs R1). Only when shading persisted for at least 42 days, phenology was significantly affected (R2, R3 and R4) (Table 1).

Plants subjected to the longest radiation constraint (R4) displayed the longest delay to reach the reproductive phase (29 days), as compared to plants growing at full sunlight during the whole cycle (Table 1, i.e. from 68 to 97 DAE).

3.1.2. Water (Experiment 2)

Withholding water as described in Section 2 resulted in an effective water stress period between 14 DAE and 56 DAE (Fig. 2). Significant differences between treatments in Ψa started on 21 DAE ($P < 0.05$). The difference between treatments remained until irrigation was restored when Ψa reached a value of – 2.5 MPa in the stressed treatment. Time to FBA was affected by water availability to a similar extent than radiation did in Exp. 1: water stress caused a delay of 20 days to reach FBA, as compared to plants well supplied during the whole cycle (Table 1, Fig. 3)

3.1.3. Nutrients (Experiment 3)

A low nutrient availability also produced a significant delay to reach the FBA ($P < 0.05$, Fig. 4), The duration of the EM- FBA phase was increased with nutrients limitation, although not as much as with radiation and water constraint: less than 7 days of delay to reach FBA resulted from nutrient limitation as compared to the Control, (Table 1).

Table 1
Number of calendar days from emergence (EM) to floral bud appearance (FBA), and change in growth rate ("*c*" parameter) to FBA in plants under radiation, nutrients and water availability treatments (Exp.1, 2, 3 respectively). Different letters indicate significant differences for each parameter ($P < 0.05$). Means ± standard errors.

Experiment	Treatments	EM to FBA (Days)	"*c*" parameter to FBA (Days)
1. Radiation	TC	68 ± 1 b	26 ± 1
	R1	70 ± 2 b	27 ± 2
	R2	91 ± 3 a	23 ± 2
	R3	92 ± 3 a	24 ± 3
	R4	97 ± 3 a	29 ± 3
2. Water	W+	74 ± 2 b	24 ± 1
	W−	94 ± 4 a	31 ± 4
3. Nutrients	F+	74 ± 1 b	23 ± 1
	F−	81 ± 2 a	23 ± 2

3.2. Statistical analysis of growth parameters (Experiments 1, 2 and 3)

Resource limitation also altered the dynamics of plant biomass accumulation with phenology. The statistical analysis of the parameters of the piecewise regression model used to estimate the association between dry weight per plant and time (Figs. 1, 3 and 4) is shown in Table 2. The overall treatments of the three experiments displayed a common pattern: the initial growth rates (*b* parameters) were very low until "*c*" breakpoint (i.e. change of slope) and, from there on, there was an increase in growth rates (*d* parameter) which was well before any evident manifestation of FBA.

However, the time of acquisition of the second growth rate (parameter c, i.e. number of days until a significant change in the growth rate of individual plants) was clearly delayed with the application of low resource availability treatments, regardless of the limiting factor (radiation, water or nutrients). Under radiation constraint, the effect was observed when the shading period exceeded 21 days, around 36 DAE (Exp. 1, R2, R3 and R4 treatments, Fig. 1). Under restriction periods longer than 42 days, (i.e. R3 and R4), no additional changes were detected on the phenology nor in the time of acquisition of a higher growth rate, as compared to R2 treatment (Table 2 and Fig. 1).

Interestingly, the moment of FBA was strongly related to the breakdown of the function and the acquisition of a higher growth rate (*d* parameter in Table 2). Noticeably, the time interval between the moment of change of the growth rate (*c* parameter) and the occurrence of FBA was similar (25 ± 2 day) in all the treatments, and regardless of the nature of the limiting factor (Table 1). The constancy of this interval was reflected in the fact that the fit between "*c*" and FBA had a slope similar to 1, with an intercept similar to that deducted in Table 1 (Fig. 5). Once the change of slope had taken place, the growth rate (*d* parameter) was fairly similar in plants of all treatments in all experiments (Table 2). On average (considering all treatments), that growth rate had a value of 0.01 g d^{-1} pl^{-1}.

In spite of the remarkable difference observed for EM–FBA phase as a result of the different treatments carried out in the three experiments, all plants reached FBA only after acquiring a growth rate around 0.01 g d^{-1} pl^{-1} (GRt, average of all treatments). Concomitantly with the delay in FBA, the acquisition of this growth rate was delayed with radiation, water or nutrient constraints.

3.3. Experiment 4

As observed in the other experiments, plants from all treatments displayed a similar growth rate until *c* (i.e. number of days until significant change in the growth rate of individual plants) (Fig. 6; Table 3). The shading treatment (S and SG) also increased the value of "*c*", and their growth rates were always lower than that of treatments growing under full irradiance (C and CP). Moreover, S and SG plants never reached the reference (or minimum) growth rate value of 0.01 g d^{-1} pl^{-1} (*d* parameter), while C and CP plants clearly exceeded this value (Table 3). Concomitantly with these features, S plants never reached FBA (Table 4).

However, when these plants were supplemented with exogenous gibberellins (SG treatment) they reached FBA, despite the fact that they had neither acquired the reference growth rate found in Experiments 1, 2 and 3, nor did they display any significant change in growth rate. On the other hand, when GAs biosynthesis was inhibited through applications of paclobutrazol in plants growing

Table 2
Parameters of the piecewise linear regression model (Eq. 1) used to estimate the association between dry weight per plant and days after emergence (DAE) shown in Figs. 1, 3 and 4 for Experiments 1, 2 and 3 respectively.

Experiment	Treatments	Parameters		
		b	*c*	*d*
1. Radiation	TC	0.0003 NS (0.00081)*	42 b (2.64)	0.009 b (0.00150)
	R1	NS (0.00099)	43 b (3.63)	0.008 b (0.00148)
	R2	NS (0.00038)	68 a (1.46)	0.015 a (0.00180)
	R3	NS (0.00033)	68 a (1.59)	0.011 ab (0.00191)
	R4	NS (0.00049)	68 a (2.91)	0.009 b (0.00150)
2. Water	W+	0.0008 NS (0.0004)	50 b (1.97)	0.010 NS (0.00107)
	W−	NS (0.0012)	63 a (5.66)	NS (0.00136)
3. Nutrients	F+	0.0007 NS (0.0009)	51 b (2.25)	0.017 (NS) (0.00219)
	F−	NS (0.0009)	59 a (2.75)	NS (0.0028)

NS = non significant.
*Numbers between brackets are standard errors of estimated parameters. Different letters indicate significant differences for each parameter ($P < 0.05$).
b: initial slope (growth rate); c: breakpoint of the function; d: final slope (growth rate).

Table 3
Parameters of piecewise linear regression model (Eq. 1) used to estimate the association between dry weight per plant and time (days after emergence) in Fig. 6 (Experiment 4). Parameter legends are the same than for Table 2.

Treatments	Parameters		
	b	*c*	*d*
C	0.0019 NS (0.00078)*	55 b (1.69)	0.029 a (0.00525)
CP	NS (0.00095)	54 b (2.77)	0.015 b (0.00234)
S	NS (0.00046)	84 a (3.57)	0.007 c (0.00206)
SG	NS (0.00049)	89 a (4.00)	0.002 c (0.00096)

Different letters indicate significant differences for each parameter ($P < 0.05$).
NS = non significant.
* Numbers between brackets are standard errors of estimated parameters.

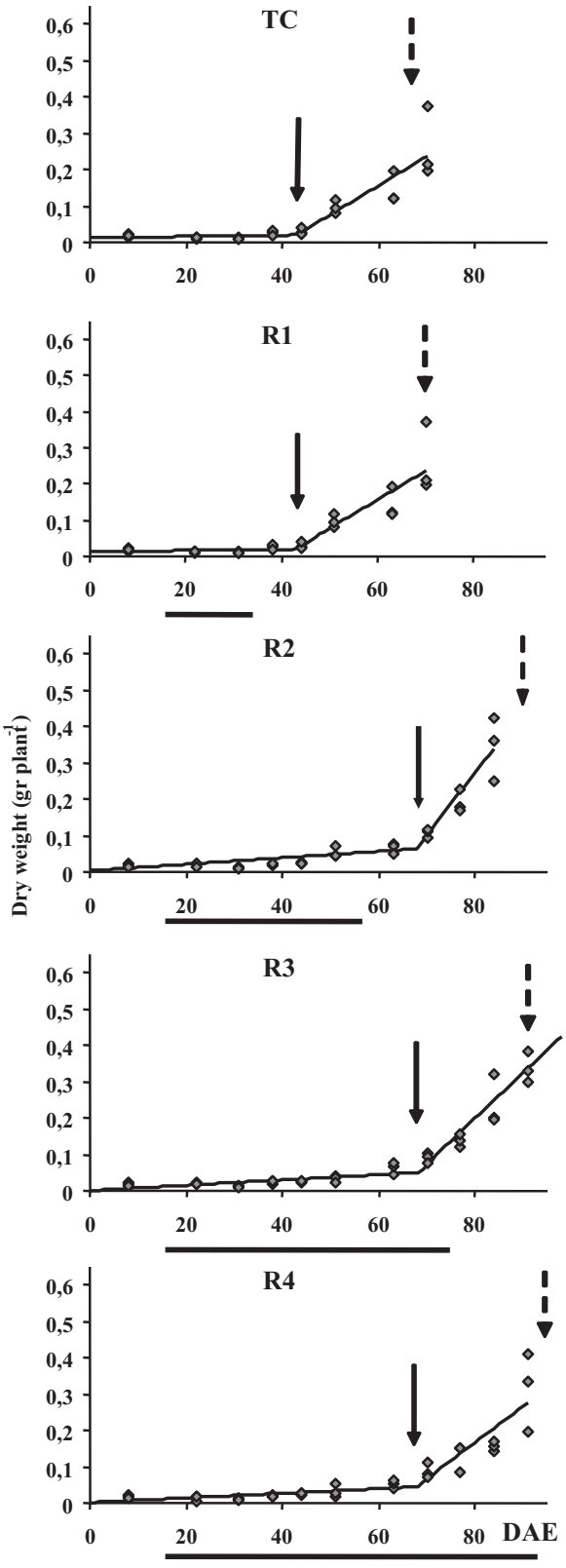

Fig. 1. Dry weight per plant in relation to days after emergence (DAE) for different shaded treatments (Experiment 1). Solid lines were fit using a conditional linear regression model (Eq. 1). The solid arrows indicate the "*c*" parameters of the model, and dotted arrows indicate the moment to first bud appearance (FBA). TC: 100% of incident radiation treatment, R1: 21 days, R2: 42 days, R3: 64 days and R4: maintained at low radiation until FBA (shaded treatments). Horizontals bars indicate the duration of the shading treatment.

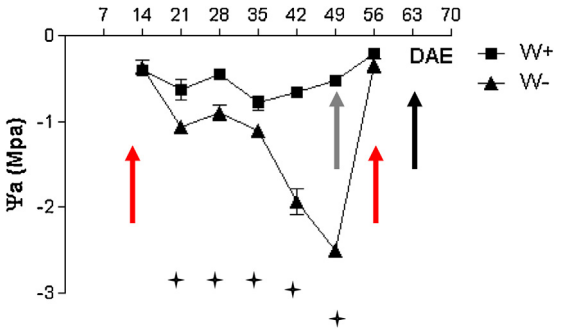

Fig. 2. Plant water potential (Ψa) of *P. mendocina* in the control treatments (W+) and water stress treatments (W−) measured at midday. The red arrows indicate the beginning and the end of water stress treatment. The grey and black arrows indicate the "*c*" parameters in the linear regression model for W+ and W− respectively. Vertical segments indicate the standard errors and only appear when larger than symbols. The asterisks indicate significant differences ($P < 0.05$) between treatments for each moment of sampling.

under full irradiance conditions (CP treatment), plants required significantly more days to reach FBA than C plants, despite the fact that both treatments acquired a growth rate higher than the reference one (0.01 g d^{-1} pl^{-1}) at the same time (Tables 3 and 4, Fig. 6). With this experiment, we were able to alter the tight association between the time until the plants' growth rate changed (and reached the reference value of at least 0.01 g d^{-1} pl^{-1}) and the time required to reach FBA that we found in Exp. 1, 2 and 3 (Fig. 6). Hence, a significant change in growth rate does not appear to be a consequence of

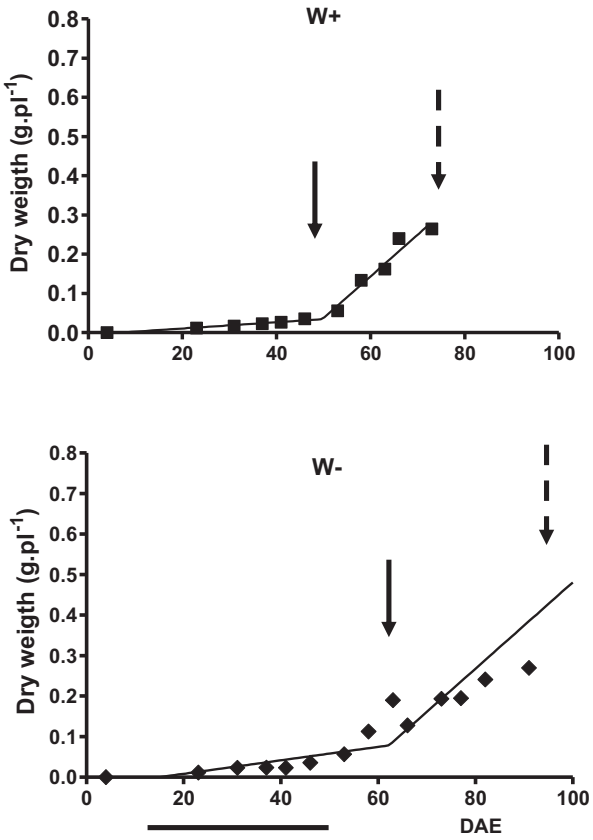

Fig. 3. Dry weight per plant in relation to days after emergence (DAE) for different water availability treatments (Experiment 2). Solid lines were fit using a conditional linear regression model (Eq. 1). W+: control treatment and W−: water stress treatment. The solid arrows indicate the "*c*" parameters of the model, and dotted arrows indicate the moment to first bud appearance (FBA). Horizontals bars indicate the duration of the water stress treatment.

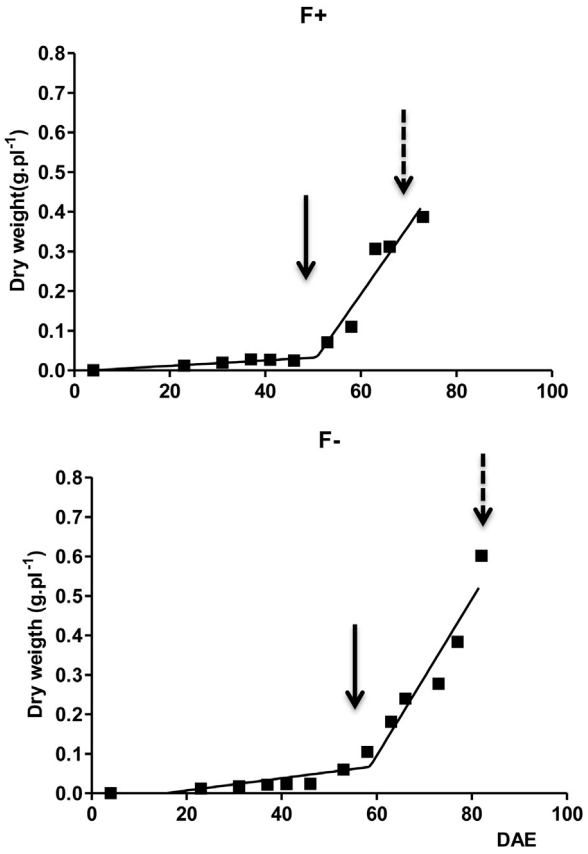

Fig. 4. Dry weight per plant in relation to days after emergence (DAE) for different nutrient availability treatments (Experiment 3). Solid lines were fit using a conditional linear regression model (Eq. 1). F+: control treatment with nutrient solution and F−: without nutrient solution treatment. Solid arrows indicate the "c" parameters of the model, and dotted arrows indicate the moment to first bud appearance (FBA).

floral induction. In addition, these results suggest a role for GAs in mediating a primary stimulus for floral induction in this species.

4. Discussion

For the introduction of a wild species into cultivation it is important to understand crop phenology and its control by the environment mostly for avoiding the coincidence of critical

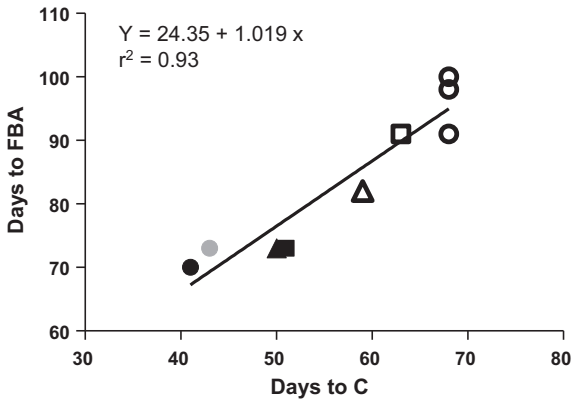

Fig. 5. Number of days from emergence to FBA in relation to change of plant growth rate ("c" parameter of the piecewise linear regression model, Eq. 1) for radiation (●, Experiment 1), water (▲, Experiment 2) and nutrient (■, Experiment 3) availabilities. Black and empty symbols represent control and stressed treatments respectively. Grey symbol indicates days for R1 treatment (Exp. 1).

Table 4
Number of calendar days from emergence (EM) to significant change in individual plant growth rate ("c" parameter of the piecewise linear regression model, Eq. 1), from EM to floral bud appearance (FBA), and the proportion of plants reaching FBA in Experiment 4.

Treatments	Days from EM to change in growth rate (c)	Days from EM to FBA	Proportion of plants reaching FBA (%)
C	55 b	74 b	100
CP*	54 b	92 a	72
S	84 a	**	0
SG*	89 a	93 a	73

C: full radiation control, CP: full radiation with paclobutrazol, S: shaded treatment, SG: shaded treatment with gibberellins.
* The hormonal treatment begins August 23 until October 10.
** Never reached FBA.

periods (i.e. flowering) for yield determination with conditions that can limit potential and actual yield in each particular area (Richards, 1991; Windauer et al., 2006). *P. mendocina* is under domestication but displays a facultative biennial behaviour which clearly may represent a drawback in terms of production. For all these reasons, phenological responses to environmental factors have been studied (Windauer et al., 2004, 2006). According to these studies *P. mendocina* can be considered as an "autonomous-flowering" plant, since it does not respond to photoperiod or temperature to flower (Boss et al., 2004). Even though, previous experiments had demonstrated that the rate of development was accelerated as sowing date was delayed (i.e. from early winter to early spring) (Windauer et al., 2006), suggesting the influence of an un-known factor on development, whose inductive capacity increases throughout the growing season.

The results of the present study allow us to conclude that the time to FBA, which is the first visible manifestation of floral induction in this system, is modulated by factors controlling growth such as radiation, water and nutrients. The negative impact of the limitation of radiation, water and nutrients on the growth rate is well known (Azcón-Bieto and Talón., 2003). Through this experimental approach, we were able to delay first bud appearance (FBA) in 29 ± 2 days; 20 ± 2 days and 7 ± 2 days (Table 1, Figs. 1, 3 and 4). Moreover, in Experiment 4, where radiation interception by neutral shadings was more intense than in Experiment 1, shaded plants never reached FBA. Although it has been demonstrated that time until FBA is not influenced by temperature (Windauer et al., 2004, 2006), an effect of an alteration in the thermal regime produced by the shadings, on time to FBA, cannot be ruled out. However, a difference of $-0.7\,^{\circ}C$ with respect to the unshaded controls is too small to explain a delay of up to 29 days in reaching FBA or, moreover, no completion of the phase as it was observed in Experiment 4. Taken together these results strongly suggest that time to FBA is modulated by environmental factors that are know to control growth. In particular, radiation intensity increases with the growing season thus positioning this factor as a strong candidate to explain our previous results showing an acceleration of phenology with a delay in sowing date (Windauer et al., 2006).

The results demonstrated that a limitation in the availability of three environmental factors (i.e. radiation, nutrients, water) influenced the dynamics of bilinear adjustment of the dry weight-time relationships. Thus, the time at which the growth rate increased to values around $0.01\ gd^{-1}\ pl^{-1}$ (i.e. the threshold growth rate value (GRt), defined here as the growth rate that an individual plant must reach or exceed to progress towards flowering), was clearly delayed when the plants were subjected to deprivation of radiation, water or nutrients. This delay, in turn, determined a concomitant delay in the onset of flowering, with an interval between the time of break of slope and FBA that was remarkably constant across experiments (average was 25 days in all of the experiments) (Table 3, Fig. 6).

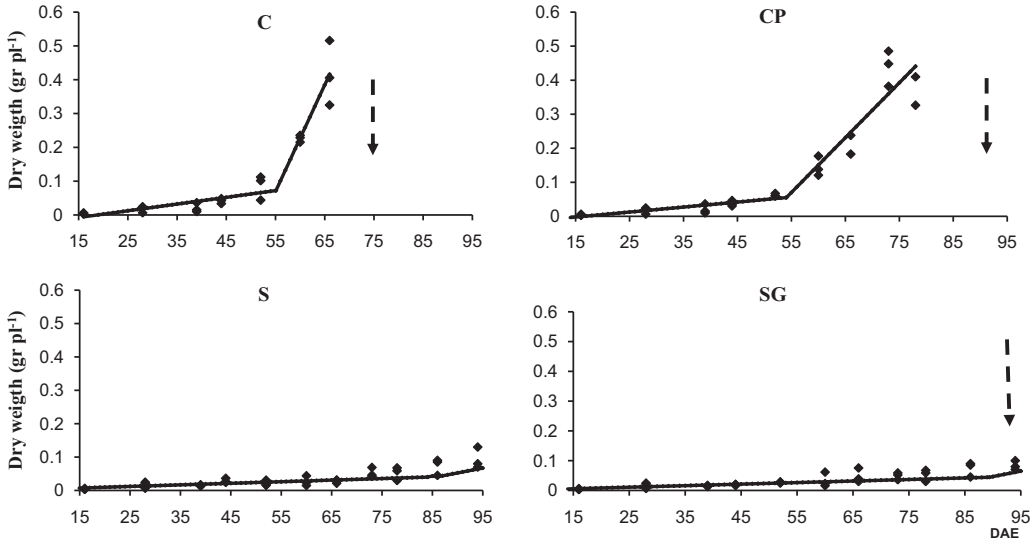

Fig. 6. Dry weight per plant in relation to days after emergence (DAE) for different treatments (radiation regimes were combined with hormonal treatments, Experiment 4). Solid lines were fit using a conditional linear regression model (Eq.1). The dotted arrows indicate the moment to first bud appearance (FBA). C: 100% radiation control; S: shaded treatment; SG: shaded treatment with exogenous gibberellins; CP: 100% radiation with paclobutrazol.

This tight association between the time taken to reach GRt and time to FBA as observed when growth was limited by different factors, suggest that flowering in this species might be triggered by the acquisition of this GRt and not by the acquisition of a threshold plant size. Indeed, several studies had suggested that the influence of resource limitation on time to flowering can be attributed to its effect on growth and, therefore, signals associated with the plant size, threshold size or physiological minimum size (King and Evans, 1977; Lacey, 1986; Reekie, 1997; Reekie et al., 1997; De Jong et al., 1986; Levy and Dean, 1998; Pfeifer et al., 2006; Castro Marín et al., 2011; Gimenez et al., 2013). However, recent studies in other facultative biennial crops revealed that the rate of development towards flowering under inductive photoperiods is strongly affected by rosette's growth rate and not by a critical size (Gimenez et al., 2013). Moreover, previous information in *P. mendocina* showed a great variation in plant size at the onset of flowering (Windauer, 2002).

We also carried out experiments to demonstrate that the increase in plant growth rate preceding FBA was not merely a consequence of floral induction by uncoupling both processes (i.e. acquisition of a minimum growth rate per plant and floral induction). This was done with Experiment 4 by either i) inhibiting gibberellins (GAs) biosynthesis in plants growing under "inductive" irradiance conditions or ii) supplementing with exogenous GAs plants growing under non-inductive (i.e. shaded) irradiance conditions. As mentioned before, the shading treatment performed in Experiment 4 was more intense (i.e. lower irradiance reaching the canopy) than that established for Experiment 1, thus determining a qualitative effect on floral induction (i.e. they never reached FBA during the experimental period) that resulted in a biennial behaviour. This could be explained through the fact that shaded plants never displayed a significant change in individual growth rate and, consequently, never reached the reference growth rate for triggering floral induction. Later in the season, temperatures became higher than 22–24 °C and, consequently, prevented advancement towards flowering (Windauer et al., 2004), even after irradiance levels became inductive as a result of shading. However, when exogenous application of gibberellins was performed under limiting radiation, the plants reached the floral stage, without experiencing any change in their growth rate with respect to that observed for untreated plants under the same radiation regime. On the other hand, floral induction was significantly delayed, in plants that having reached the minimum growth rate because they have been growing under full irradiance had been treated with paclobutrazol. It has been shown for other rosette species that de novo synthesis of GAs occurs in plants perceiving an inductive photoperiod and/or exposure to low temperatures (vernalization) (Hedden and Phillips, 2000). Also, GAs have been implicated specifically in the autonomous pathway of flowering (Jacobsen and Olszewski, 1993; Blázquez et al., 1998; Mier et al., 2001). The role for these GAs has been suggested in triggering the series of events that lead to floral induction (Tan and Swain, 2006; Simpson and Dean, 2002).

According to these and previous results, a biannual behaviour in this species might be expected when, as a consequence of resource deprivation, the acquisition of the GRt is delayed in the growing season until temperatures become too hot (i.e. higher than 24 °C) as to permit development to advance (Windauer et al., 2004). Under these circumstances, plants would stay as a rosette until the next season. A similar explanation was given for *Oenothera biennis*, another alternative oil species with facultative biennial behavior (Gimenez et al., 2013).

The information obtained with this work sheds light on the environmental factors that modulate time to flowering in this species. Consequently, this knowledge offers a framework within which cropping systems could be designed. According to our results, development should proceed towards flowering after sowing during the first growing season if sowing takes place at mid-winter, long before temperatures become higher than 24 °C, and provided the crop is being grown under good irradiation conditions and well supplied with nutrients and water. The later the crop is sown, the higher the irradiance to which the crop will be subjected and, consequently, the faster the crop will proceed towards flowering. But, at the same time, late sowings would increase the risk of exposing the crop to temperatures above 24 °C thus preventing it to advance towards flowering and determining biannuality. This situation would be worsened if, in addition to have been sown late in the season, the crop is exposed to water and/or nutrient deprivation. The latter would delay the acquisition of the threshold growth rate thus increasing even further the risk of exposing the crop to temperatures above 24 °C.

5. Conclusions

The results of the present study allow us to conclude that the time to FBA, which is the first visible manifestation of floral induction in this system, is modulated by factors controlling growth such as radiation, water and nutrients. The results demonstrated that a limitation in the availability of three environmental factors influenced the dynamics of bilinear adjustment of the dry weight-time relationships. The time at which the growth rate increased to values around 0.01 g d^{-1} pl^{-1} (GRt), was clearly delayed when the plants were subjected to deprivation of radiation, water or nutrients. This delay determined a concomitant delay in the onset of flowering.

These studies revealed that a threshold plant growth rate is associated to initiation of the flowering phase and that GAs are specifically involved in the route of the activation of the series of events that lead to the floral induction in *P. mendocina*. The information obtained with this work offers a frame within which cropping systems could be designed in order to avoid or not a biennial behavior. Indeed, a biennial behavior might be detrimental under some production systems but beneficial under others. If flowering during the first year is aimed, these cropping systems should include early sowing dates and good availability of water and nutrients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2013.04.049.

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