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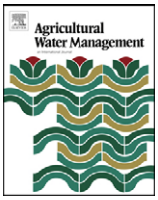

Water stress effects on growth, development and yield of opium poppy (*Papaver somniferum* L.)

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ABSTRACT

The effects of pre-anthesis water deficit and cycle length were examined in *Papaver somniferum* L., cultivated for alkaloid production, in two locations in southern Spain. The vegetative period was shortened by extending the photoperiod through supplemental lighting in the field, while water deficit in pre-anthesis was induced by avoiding irrigations and installing rain shelters. The treatments were: IN (irrigated-normal photoperiod), IL (irrigated-hastened flowering), DN (water deficit in pre-anthesis-normal photoperiod) and DL (water deficit in pre-anthesis and hastened flowering). The artificial photoperiod hastened the flowering by 15 and 21 days, for irrigated and deficit treatments respectively. Seasonal evapotranspiration (ET) ranged from 398 (DN) to 505 mm (IN). There was evidence of root water uptake deeper than 1.5 m. Stomatal conductance was reduced (16%) during water stress, and did not recover in post-anthesis after resuming irrigation. Head yields (capsule + seeds + 7 cm stem) ranged between 3.8 and 4.3 t ha^{-1}; water deficit and short vegetative period both reduced the biomass accumulated, although the effect on yields in these treatments was counterbalanced by a higher harvest index. Early flowering had a detrimental effect on alkaloid concentration in the capsule. Alkaloids yield ranged between 27 and 37 kg ha^{-1}. Water use efficiency (WUE) ranged between 0.78 and 0.96 kg m^{-3} ET for yield and between 63.4 and 73.7 g m^{-3} ET for alkaloids. Water stress increased slightly the Water Use Efficiency. A shorter vegetative phase had no effect on WUE for biomass or yield, but decreased the WUE for alkaloids production.

1. Introduction

Poppy (*Papaver somniferum* L.), is a plant of the dicot family Papaveraceae, cultivated for seed, oil and opium. It is an erect, annual herb, 30–170 cm tall; flower buds are ovate, dropping before anthesis (hook stage); the fruit is a capsule that usually contains a high number of very small seeds. Some cultivars contain up to 50% oil in seed, mainly in the form of oleic and linoleic acids and may be used as a source of linoleic acid. The oil is of high quality for human consumption because of its high amount of polyunsaturated fatty acids (Özcan and Atalay, 2006). In Europe, poppy seeds – that are free of alkaloids – have culinary uses in confectionery and bakery and are widely used in perfume, cosmetic and medicinal industries.

Poppy is also cultivated for opium production, the dried plant latex located mainly in the capsule walls. Opium is one of the oldest known painkillers, and the source of several alkaloids used for analgesic, antitussive and antispasmodic purposes in modern medicine (Peter, 2001). The global illicit cultivated area of opium poppy was estimated as 189,000 ha in 2008, mainly located in Afghanistan and followed by Myanmar and Lao PDR. Global opium production was about 8000 tons in 2008 (World Drug Report, 2009). Global licit cultivated area of opium poppy was about 175,000 ha. Opium poppy is one of the most important crops for the pharmaceutical industry for the production of natural opiate alkaloids, mainly morphine, codeine and thebaine, that are extracted mainly from the crushed dried capsules emptied of seeds. In Spain, the annual morphine demand of the pharmaceutical industry in 2000 was 5500 kg which was met by 5700 ha of cultivated opium poppy with more than 7000 kg of total morphine production (Muñoz-Ledesma, 2002).

The development of poppy can be divided into the following stages (Shuljgin, 1969): (1) growth of seedlings; (2) formation of

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rosette-type leaves and stalks; (3) budding (hook stage); (4) flowering; (5) technical maturity (readiness for cutting); and (6) biological maturity (ripening of dry seed). There are several climatic, agronomic and environmental factors affecting opium poppy growth and development. Photoregulation has an important role in the life cycle of poppy and the effects of light conditions on its germination (Bare et al., 1978), growth (Bernáth et al., 1988) and floral initiation are clear. While Wang et al. (1997a) concluded that temperature is not a major factor in flower development in opium poppy, they (Wang et al., 1997b) pointed to photoperiod as a main determinant of flower development in opium poppy and found a long-day response. Results of Acock et al. (1996) indicated that the effect of photoperiod on duration of emergence to flowering was observable after a period of 48 h of continuous light. Time to flowering was lowest in the 24-h photoperiod plants (30.2 days) and increased with photoperiods shorter than 14 h. Chung (1982) reported that longer sunshine duration seems to be more favorable for vegetative growth and morphine production in poppy. In the greenhouse, Mika (1955) showed that longer sunshine increased dry matter production in poppy. Gentner et al. (1975) showed that the long-day requirement is a main characteristic of poppy, although higher light intensities can compensate for inhibitory effects of short days (Bernáth, 1998). The critical daylength for flowering of opium poppy is between 14 and 16 h (Gentner et al., 1975).

There are few data on the effects of water supply on growth, development or yield of opium poppy. Chung (1987) showed that irrigation increased dry matter production and leaf area index and delayed leaf senescence. In this study, carried out in Australia (with 340 and 321 mm rainfall during the growing seasons), irrigation up to leaf senescence increased total morphine yield by 5–20 kg ha^{-1} as compared with nil irrigation which was attributed to the increase in the number and mass of lateral heads as well as the mass of terminal heads and morphine concentration. In comparison with no irrigation, Laughlin and Chung (1992) showed that full irrigation throughout the growing season increased head yield by 95%. Although excessive water may be detrimental due to possible morphine leaching (Hofman and Menary, 1984) and promotion of fungal diseases, previous studies have shown that water stress reduces total alkaloid production in opium poppy. However the relationship between yield and evapotranspiration (ET), which is critical for optimizing irrigation management, has not been determined for *Papaver somniferum*. The role of development on the response of *P. somniferum* to water stress has not been explored. For instance, in sunflower, Sadras and Connor (1991) showed that water limited yield is directly related to the post-anthesis transpiration. Thus manipulating the duration of the pre and post-anthesis periods may change the response to water stress.

The objective of this study was to quantify the effects of water stress on the growth, development and yield of *Papaver somniferum* L. 'Nigrum'. To improve the understanding of these effects, the date of flowering and thus, the duration of the pre- and post-anthesis periods, were manipulated by extending the photoperiod in the field.

2. Materials and methods

2.1. Cordoba experiment

The experiment was conducted on a sandy loam, Typical Xerofluvent in the experimental farm of the Instituto de Agricultura Sostenible (IAS) in Cordoba, southern Spain (37.85°N, 4.8°W, 110 m). The soil has a drained upper limit (field capacity) of 0.26 m^3 m^{-3} and a lower limit (wilting point) of 0.10 m^3 m^{-3}. The experimental design was a randomized complete block with three replications. The treatments were 4 combinations of water supply and photoperiod extension as follows:

- Irrigation for the entire cycle and early flowering (extended photoperiod) (IL)
- Irrigation for the entire cycle and normal flowering (normal photoperiod) (IN)
- Water deficit (no irrigation) until flowering with early flowering (DL)
- Water deficit (no irrigation) until flowering with normal flowering (DN)

The crop was sown on 3 December 2003 at 0.2 m spacing between rows in 3 m × 4 m plots, with seeds of the Spanish cultivar "Madrigal". Fifty percent emergence occurred on 29 December 2003. Fertilization consisted of a pre-sowing application of 300 kg ha^{-1} of complex 15–15–15 ($N–P_2O_5–K_2O$) complemented by 150 kg N/ha at flowering as ammonium nitrate. The irrigation system was drip type with emitters spaced 0.4 m × 0.4 m. In irrigated treatments, irrigation was scheduled weekly by calculating crop evapotranspiration (ET) as:

$$\mathrm{ET} = K_c \mathrm{ET}_0 \tag{1}$$

where K_c is the crop coefficient and ET_0 is the reference ET. ET_0 was calculated using the Penman–Monteith FAO equation (Allen et al., 1998) with meteorological data (global radiation, air temperature, air humidity and wind speed) from the weather station of the IAS, located at 600 m distance from the plots. The crop coefficient was calculated assuming a linear relation with the ground cover with a maximum value of 1.2 for full ground cover.

In the treatments with water deficit until flowering, rainfall was excluded from the plots by metallic structures covered with transparent polyethylene sheet allowing manual covering depending on environmental conditions, trying to keep the polyethylene film extended only during rainfall events. These rain-shelters had no walls, to ensure good ventilation even when covered and the minimum possible alteration of the plants microclimate. Deficit treatments were started on 31 January 2004 to match the onset of light extension treatments.

In each "early flowering" plot, two sodium lamps of 200 W were installed at the north and south of the plot at a height of 0.6 m above the ground. The lamps were directed toward the interior of the plot and screened to avoid illumination of adjacent plots. With these lamps, photoperiod was extended to 24 h from 31 January 2004 until the appearance of flower buds (12 March). The lamps were automatically lit before sunset and turned off after sunrise.

The soil water content was measured with the gravimetric method with a Veihmeyer type sampling tube (Veihmeyer, 1929) in increments of 0.30 m to a depth of 1.50 m. The samples were weighed, dried in an oven at 105 °C for 48 h and then weighed again.

ET was calculated using the water balance method. Measurements of the soil water content were performed periodically (2 December, 2 February, 15 March, 5 April, 28 April and 15 June). The total evapotranspiration of the period between successive measurements was calculated as:

$$\mathrm{ET} = z(\theta_{(i-1)} - \theta_i) + I + R - D \tag{2}$$

where θ_i is the water content at date i, z the soil depth, I the irrigation amount and R the total rainfall and D is the drainage, estimated with a cascade layer modelling approach. Runoff was avoided by level plots surrounded by ditches, thus was not considered in Eq. (2).

Non-destructive observations of number of leaves (green and senescent), and phenological status in a group of 20 marked plants per plot were conducted weekly in 10 dates starting on 9 March and ending on 17 May.

Stomatal conductance was measured in the abaxial surface of the leaves (leaves of *P. somniferum* are hypostomatous) using a steady-state porometer (model PMR-4, PP Systems, U.K.). Data were taken in illuminated leaves (5 plants per plot) every week at noon on all plots (11/03, 16/03, 19/03) or only in the plots of the first block (05/04, 12/04, 21/04, 26/04, 08/05, 20/05).

Samples were taken for destructive biomass measurement in 6 April and 22 April (around flowering) from 3 plants per plot, and leaves, stalks, floral buds and heads were separated. The green leaf area as well as partially senescent LAI was measured using an electronic planimeter (model LI-COR 3100, LI-COR, Lincoln, NE, USA).

At harvest (8 June), an area of 2 m^2 was collected from the central part of each plot and dry mass of each organ was determined. A random sample of 40 heads (capsules, the contained seeds + 7 cm stem, to match with the standard harvested product) of each plot was collected and total dry mass of heads and seeds were measured. Thermal time was calculated using a base temperature of 3.2 °C according to opium poppy germination studies of Kamkar, Jami and Mahdavi (unpublished data).

2.2. Carmona experiment

In Carmona (37.4°N, 5.6°W, 79 m), seeds were sown in plastic pots on 10 January 2004 and then transplanted with 6 leaves into 3 m × 3 m plots on 12 February. The soil is a sandy clay loam with upper drained limit of 0.24 $m^3\ m^{-3}$ and lower limit of 0.13 $m^3\ m^{-3}$. In this experiment water deficit treatments were the same as in Cordoba (full irrigation vs. water deficit up to flowering) with three replications. Extended photoperiod was only implemented in two plots (one with water deficit before flowering and the other with irrigation for the entire cycle). Destructive samplings were made on 14 April (2 plants per plot) and 30 April (3 plants per plot) to determine the distribution of dry matter. The harvest was conducted on 18 June on the central square meter of each plot. A random sample of 5 heads (capsules with seeds + 7 cm stem) was collected from each plot and the total dry mass of seeds and other parts were measured. A single measurement of stomatal conductance was performed at noon on 14 April in the illuminated leaves of 5 plants per plot. The strain planted in Carmona was an high-thebaine variant of the cultivar used in the Cordoba experiment.

2.3. Data analysis

The data were analyzed using analysis of variance (ANOVA) for block with two random factors (water stress and date of flowering in the experiment of Cordoba) and one factor (water stress in the experiment of Carmona). For analysis of percentages, an arcsine transformation was performed. In all cases the significance level of 95% (alpha = 0.05) was used.

For both experiments alkaloid were extracted by solvents and analysed by HPLC in the central laboratory of Alcaliber S.A. according to the standard procedures (Real Farmacopea Española, 2005).

The two sites, separated 130 km, are located in two different provinces (Cordoba and Seville), and are representative of the area of cultivation of poppy in southern Spain. The climate of the period December 2003–June 2004, when the experiment took place, was in general close to the average (Fig. 1), with a slightly colder-than-average May. The reference evapotranspiration (ET_0) was also inside the normal range (Fig. 1).

3. Results

3.1. Water balance

Profiles of soil water content are shown in Fig. 2 for different dates in Cordoba. Water deficit plots show a clear reduction of soil water content down to a depth of 60–90 cm at flowering (Fig. 1, 5 April). At harvest all profiles showed water extraction in the deepest horizon (120–150 cm, Fig. 1, 15 June).

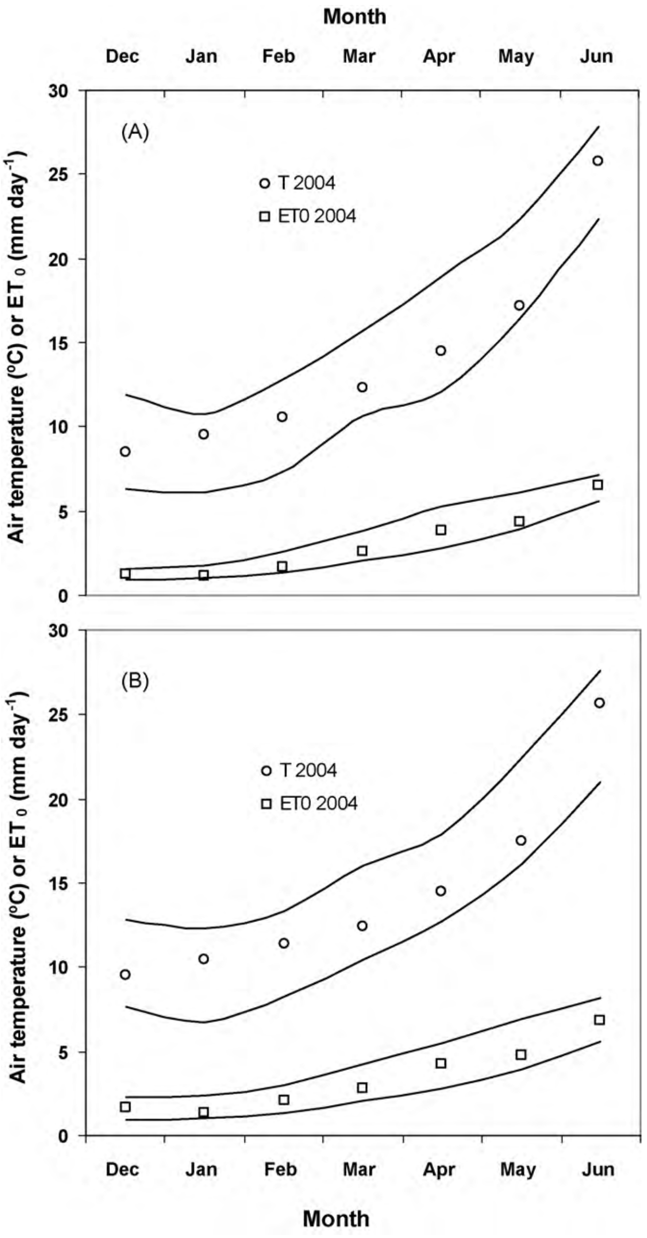

Fig. 1. Comparison of monthly temperatures and ET_0 of 2004 (the experimental year) with long term averages. The solid lines delimit the area of the 2000–2010 averages ± the standard deviation. (A) Cordoba site; (B) Carmona site.

The average values of ET and water applied are presented in Table 1 for various periods in Cordoba. The seasonal ET values ranged from 398 (DN) to 505 mm (IN), while the total amount of infiltrated water (irrigation + rain) ranged between 331 (DN) and 557 mm (IN and IL). The seasonal ET of the water deficit treatments was approximately 85% of that of the irrigated treatments. The estimated drainage from 03 February until the end of the cycle was 89 mm for the irrigated plots (16% of infiltrated water) and practically zero in water deficit ones.

3.2. Growth and development

The development phases considered in this work are: from sowing to emergence; from emergence to flower bud (hook) stage; from flower hook to flowering; and from flowering to maturity. The phase changes are taken at 50% flower hook and 50% open flowers. The timelines of development for the different treatments in Cor-

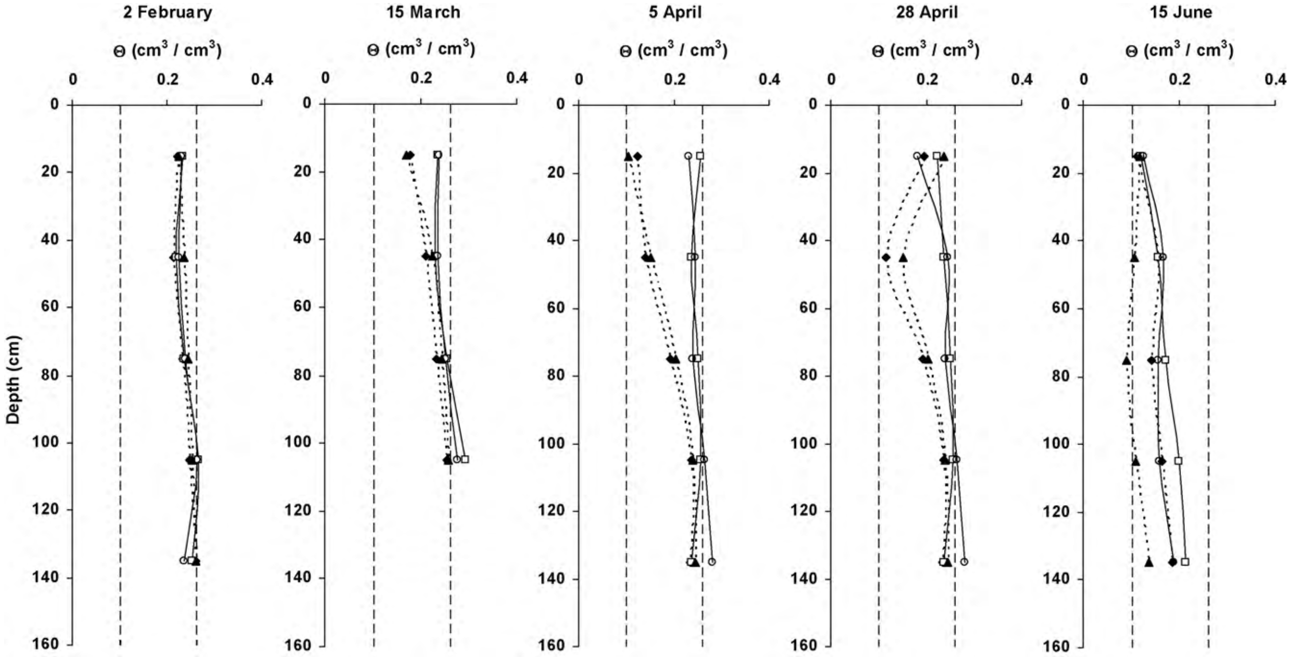

Fig. 2. Profile of soil water content, in the Cordoba experiment, at 5 dates for *Papaver somniferum* L. (♦) deficit irrigation-extended photoperiod (DL); (□) normal irrigation-extended photoperiod (IL); (▲) deficit irrigation-normal photoperiod (DN); (◊) normal irrigation-normal photoperiod (IN). The vertical long-dashed lines delimit the lower and upper level (wilting point and field capacity) of the soil.

doba are shown in Fig. 3. The minimum duration of both emergence to flower hook and flower hook to flowering phases corresponded to water deficit and early development (DL) and the largest to the irrigated with normal development (IN) (Fig. 3). All the treatments were harvested the same day (8 June). In Carmona, the shortest period to flowering was observed in extended light treatments (DL and IL), 2 weeks sooner than normal photoperiod (DN and IN). In both sites light extension hastened the appearance of flowering hooks and also shortened the hook-flowering phase.

The rate of leaf appearance was higher in treatments with extended light, but was not affected by water deficit. Thus, the phyllochron (the thermal time between the appearances of two consecutive leaves) was 40.5 °C$_d$ (degree days) for extended photoperiod and 50.5 °C$_d$ for normal photoperiod. In Carmona, the average number of leaves on the main stem was 21, without any significant effect of water deficit.

In Cordoba, green leaf area index (LAI) around flowering ranged between 4 (DL, 6 April) and 11.5 (IN, 22 April), while values in Carmona were between 2 and 3.7 in mid-April (Table 2). In the second sampling, the effect of extended photoperiod was significant on green LAI in Cordoba and normal flowering plants produced higher LAI than light extended plants. In the second sampling, the green LAI in Carmona was dramatically reduced (range 0.10–1.7). While plant height was decreased significantly by extension of photoperiod in Cordoba, plants that received more light in Carmona had higher heights. Water status did not affect plant height in either location.

3.3. Dry matter production

In the two samplings taken around flowering, water deficit reduced significantly the total biomass in Cordoba (Table 2). On 6 April, plants in IL and IN produced 4010 and 3660 kg ha^{-1} biomass, respectively, while the values for DL and DN were 1760 and 2870 kg ha^{-1}, respectively. The same trend was observed in the second sampling (22 April, Table 2) and irrigated plants with normal flowering (IN) produced 54% more biomass than plants that experienced water deficit with normal flowering (DN).

Total biomass values at harvest (Table 3) were much higher in Cordoba (range 943–1167 g m^{-2}) than in Carmona (355–734 g m^{-2}); normal irrigation treatments gave higher yields than deficit ones, although differences were not significant in Cordoba. Head dry matter production ranged between 381 and 433 g m^{-2} in Cordoba and between 135 and 221 g m^{-2} in Carmona. The harvest index (the ratio between the biomass of the heads and the total above-ground biomass) increased significantly with the hastening of flowering in Cordoba. In normal flowering, there was also an increase of harvest index by water deficit (0.39 vs. 0.33), though the difference was not significant. In Carmona, early flowering plants showed a similar trend and higher harvest indices (0.38

Table 1
Average values of ET, irrigation and rainfall for different periods for *Papaver somniferum* L. in Cordoba. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod.

Period	ET (mm)				Irrigation (mm)				Rainfall (mm)			
	DL	DN	IL	IN	DL	DN	IL	IN	DL	DN	IL	IN
3 December–2 February	52.0	52.0	52.0	52.0	10	10	27	27	106	106	108	108
3 February–14 March	22.6	27.6	81.9	85.6	0	0	0	0	8	8	110	110
15 March–4 April	61.1	63.5	110.1	93.2	0	0	20	20	0	0	69	69
5–27 April	113.7	70.9	123.7	172.4	82	57	67	67	17	10	17	17
28 April–15 June	173.6	184.3	122.5	102.1	40	40	40	40	99	99	99	99
Total	423.0	398.3	490.2	505.3	132	107	154	154	230	213	403	403

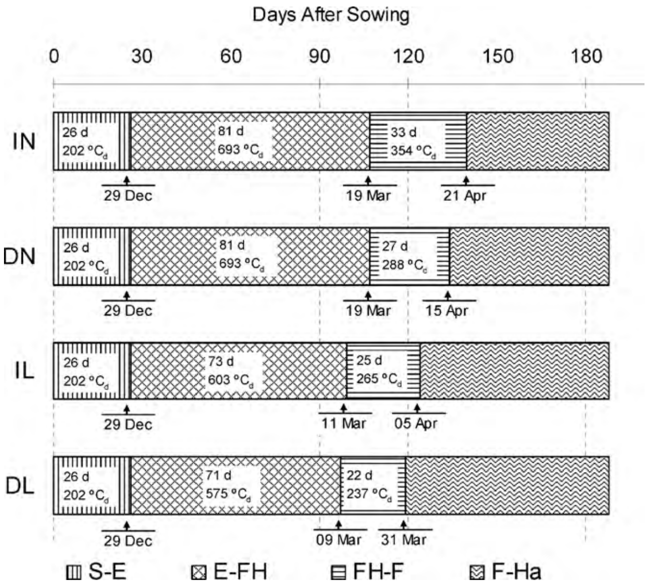

Fig. 3. Phenological timelines of the different treatments of *Papaver somniferum* L. in the Cordoba experiment. Plants were sown on 3 December 2003. S: sowing; E: emergence; FH: flower hook; F: flowering; Ha: harvest (8 June for all treatments). DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod. The phase changes were taken as 50% visible flower hooks (FH) and 50% visible open flowers (F). °C$_d$: degree days.

and 0.47) than the average values for normal flowering plots (0.32 and 0.29). The average number of heads per plant was very similar at both locations (2.75–3) despite differences in plant density (Table 3).

In Cordoba, there were significant effects of the light treatment on the distribution of dry matter to green leaves and heads around flowering (6 April Table 4), with early flowering treatments showing a lower partitioning to leaves (accompanied by higher partitioning to heads). The same effect of early flowering on the dry matter partitioning to leaves was found on the 22 April sampling, as well as a significant effect of water deficit on the head partitioning.

In Carmona, the effect of irrigation was only significant for dry matter partitioning to leaves in the sampling dates of April 14 and 21 (Table 4). In both samples, the hastening of flowering caused a reduction in allocation to leaves and increased allocation to heads. Water deficit before flowering increased dry matter partitioning to heads.

In Cordoba, the average seed mass ranged from 0.53 mg (irrigation, no light) to 0.59 mg (irrigation, light), while the number of seeds per head varied inversely, i.e. between 3713 (Irrigation, light) and 6241 (Irrigation, no light) (Table 5). There was a significant effect of extended light on seed number, with early flowering associated with a smaller number of (bigger) seeds per head. Extended light affected significantly the mass of individual heads in Cordoba varying between 3.36 and 5.09 g and being proportional to the number of seeds. In Carmona, the mass of individual heads ranged from 3.48 to 4.09 g and was neither affected by light extension nor water deficit (Table 5). Capsule mass was lower with early flowering in Cordoba, but was not affected by water deficit in either of the two locations. Thus, for normal flowering, individual capsule mass after seed separation was 1.75–1.77 and 1.41–1.70 g in Cordoba and Carmona, respectively.

Distribution of head dry matter (Table 5) was not affected by extended photoperiod or water deficit. The seed/capsule ratio was 57/43 in Carmona and 63.5/36.5 in Cordoba. These values can be used to deduce an average yield of 2 t ha^{-1} of dry capsules in Cordoba while in Carmona the average capsule yield is 1 t ha^{-1} with water deficit and 1.4 t ha^{-1} with irrigation.

3.4. Alkaloid production

In Cordoba, there was a general trend towards reducing the concentration and yield of alkaloids with the hastening of flowering and water deficit before flowering (Table 6). These differences were significant (at the 0.05 level) for the concentration of codeine and thebaine, and nearly significant for the yield of codeine and thebaine. There were no significant effects on the concentration or on the yield of morphine. The concentration of total alkaloids was

Table 2

Average ($n = 3$) plant height, green and senescent LAI and biomass of different organs around flowering of *Papaver somniferum* L. in Cordoba and Carmona. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod.

Location & date	Treatment	Height (cm)	LAI		Biomass (g m^{-2})				
			Green	Senescent	Leaf	Stem	Floral bud	Head	Total
Cordoba (6 April)	DL	109	4.00	0.26	62	98	4	12	176
	DN	101	8.34	0.64	132	147	8	–	287
	IL	116	7.72	1.45	131	222	11	37	401
	IN	109	10.63	1.23	170	186	9	1	366
	Significance*	NS	NS	I	NS	I	NS	I, F, IF	I
Cordoba (22 April)	DL	116	4.54	1.03	112	282	3	110	508
	DN	134	10.55	1.96	170	261	19	13	463
	IL	127	6.16	3.78	163	318	10	140	631
	IN	136	11.53	1.98	227	394	15	77	713
	Significance	F	F	I, IF	F	I	F	I, F	I
Carmona (14 April)	DL**	94	2.30	0.14	138	219	6	46	408
	DN	37	2.48	1.01	211	114	4	–	329
	IL	96	2.02	0.36	119	193	7	29	348
	IN	45	3.69	1.03	255	160	6	–	422
	Significance	NS	NS	NS	NS	NS	NS	NS	NS
Carmona (30 April)	DL	99	0.12	0.30	118	297	2	185	602
	DN	69	0.47	0.55	171	166	10	25	373
	IL	95	0.10	0.91	60	177	2	127	366
	IN	88	1.73	1.36	282	255	17	13	567
	Significance	NS	I	I	I	I	NS	NS	I

* Significant effects ($\alpha = 0.05$) are denoted for irrigation (I), time of flowering (F) and irrigation-flowering interaction (IF); NS: not significant.

** In Carmona, extended light treatment covers one repetition and analysis of variance was calculated only for normal flowering.

Table 3
Biomass at harvest and yield components of *Papaver somniferum* L. in Cordoba and Carmona.

Location	Treatment	Plants (m^{-2})	Caps./$plant^{-1}$	Caps. (m^{-2})	Dry weight ($g\,m^{-2}$)				HI	HIc
					Stems	Leaves	Heads	Total		
Cordoba	DL	65	2.5	165.7	407.4	137.0	398.5	942.9	0.40	0.42
	DN	46	2.7	123.2	413.8	168.5	381.3	963.6	0.37	0.39
	IL	47	3.2	148.2	469.7	113.6	433.2	1016.5	0.40	0.42
	IN	38	2.6	97.3	592.3	178.5	396.0	1166.8	0.31	0.33
	Signifcance*	I,F	NS	I,F	I	F	NS	NS	F	F
Carmona	DL	30	1.9	56	179.3	41.5	134.7	355.5	-	0.38
	DN	24.7	3.0	73.0	158.03	136.37	135.63	430.03	-	0.32
	IL	23	3.9	89	182.3	67.4	221.2	470.9	-	0.47
	IN	27.0	3.0	82.0	324.73	193.50	215.87	734.10	-	0.29
	Significance	NS	NS	NS	I	I	I	I		NS

Significant effects ($\alpha = 0.05$) are denoted for irrigation (I), time of flowering (F), irrigation-time of flowering interaction (IF). NS: not significant. HI: harvest index, including 7 cm of stem; HIc: HI excluding 7 cm of stem. In Carmona, extended light treatment covers one repetition and analysis of variance was calculated only for normal flowering. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod; Caps.: no. of capsules.

Table 4
Dry matter distribution (%) around flowering of *Papaver somniferum* L. in Cordoba and Carmona.

Location & date	Treatment	Leaves			Stems	Floral bud	Heads
		Green	Senescent	Dead			
Cordoba (6 April)	DL	25.1	3.4	3.9	55.9	2.5	9.1
	DN	35.7	4.7	5.7	51.1	2.6	0.1
	IL	28.3	1.4	4.5	56.4	2.0	7.4
	IN	36.0	2.3	5.6	53.2	2.8	0.0
	Significance*	F	NS	NS	NS	NS	F
Carmona (14 April)	DL	26.4	4.0	3.2	53.6	1.4	11.3
	DN	37.0	11.5	16.8	33.7	1.1	0.0
	IL	27.0	4.3	2.7	55.5	2.1	8.5
	IN	44.9	9.1	11.6	33.3	1.0	0.0
	Significance	I	NS	NS	NS	NS	NS
Cordoba (22 April)	DL	15.4	6.4	3.7	51.2	1.7	21.6
	DN	24.0	3.2	4.8	55.1	2.0	11.0
	IL	16.7	2.9	2.9	55.3	0.7	21.4
	IN	29.5	3.5	3.7	56.6	3.7	3.0
	Significance	F	NS	NS	NS	F	IF
Carmona (30 April)	DL	0.9	2.3	16.4	49.4	0.4	30.7
	DN	6.9	7.0	34.1	43.6	2.9	5.5
	IL	1.4	7.0	8.1	48.4	0.5	34.7
	IN	15.2	9.3	26.0	44.5	3.1	2.0
	Significance	I	NS	NS	NS	NS	NS

[*] In Carmona, analysis of variance was calculated only for the treatment of normal flowering. Significant effects ($\alpha = 0.05$) are denoted for irrigation (I), time of flowering (F) and water-light interaction (IF); NS: not significant. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod.

Table 5
Average mass ($n = 3$) of seeds and heads of *Papaver somniferum* L. in Cordoba and Carmona.

Location	Treatment	Seed mass (g)	Number of seeds/capsule	Mass of one head (g)		
				Head	Seed	Capsule
Cordoba	DL	0.590	3713	3.36	2.19 (63.5)	1.17 (36.5)
	DN	0.534	6241	5.09	3.34 (64.3)	1.75 (35.7)
	IL	0.559	5344	4.02	2.63 (64.6)	1.39 (35.4)
	IN	0.549	5539	4.83	3.06 (61.4)	1.77 (38.6)
	Significance*	F	F	F	F (NS)	F (NS)
Carmona	DL	0.465	3133	2.42	1.46 (59.1)	0.97 (40.9)
	DN	0.665	3308	3.96	2.20 (57.4)	1.75 (42.6)
	IL	0.512	4057	3.48	2.07 (54.2)	1.41 (45.8)
	IN	0.480	4938	4.09	2.39 (56.6)	1.70 (43.4)
	Significance*	NS	NS	NS	NS (NS)	NS (NS)

Significant effects ($\alpha = 0.05$) are denoted for irrigation (I), time of flowering (F), irrigation-time of flowering interaction (IF). NS: not significant. In Carmona, extended light treatment covers one repetition and analysis of variance was calculated only for normal flowering. In parentheses is the % of head dry weight. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod.

Table 6
Alkaloid contents of different treatments of *Papaver somniferum* L. in Cordoba and Carmona.

Location	Treatment	Concentration (‰)					kg/ha^{-1}				
		Morphine	Codeine	Thebaine	Oripavine	Total	Morphine	Codeine	Thebaine	Oripavine	Total
Cordoba	DL	17.31	1.55	0.43	ND	19.29	24.06	2.16	0.59	ND	26.81
	DN	19.54	2.04	0.89	ND	22.46	25.26	2.71	1.18	ND	29.15
	IL	18.22	1.99	0.91	ND	21.11	27.21	2.94	1.45	ND	31.60
	IN	20.31	3.35	1.42	ND	25.08	30.49	4.80	1.94	ND	37.23
	Significance*	NS	I,F	I,F		F	NS	NS	NS		NS
Carmona	DL	0.93	ND	16.24	ND	17.17	0.50	ND	8.72	ND	9.22
	DN	ND	0.18	17.83	3.72	21.73	ND	0.07	9.63	1.85	11.55
	IL	ND	ND	16.51	9.18	25.69	ND	ND	16.21	9.01	25.22
	IN	0.80	ND	15.86	ND	16.66	0.75	ND	13.27	ND	14.02

Significant effects ($\alpha = 0.05$) are denoted for irrigation (I), time of flowering (F), irrigation-time of flowering interaction (IF). NS: no significant. Analysis of variance was not calculated in Carmona. ND: not detected. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod.

only significantly affected by flowering date, while the differences in total yield of alkaloids were not significant. In Carmona, where the plants were high-thebaine variants, water deficit significantly affected the concentration of thebaine and the total yield of alkaloids (Table 6). Notably, oripavine was detected only in Carmona; although only represented by one plot, a significant concentration and yield of oripavine was detected in the early flowering plants under irrigation.

3.5. Stomatal conductance

Conductance at mid-day was reduced in Cordoba during the cycle from more than 1000 mmol m^{-2} s^{-1} in March down to 300 mmol m^{-2} s^{-1} during May (Fig. 4). The water deficit plants had generally lower conductance at pre- and post-anthesis. In general, there was greater conductance in extended photoperiod plots after flowering. The seasonal average values of conductance (noon) ranged from 641 (DL) to 758 mmol m^{-2} s^{-1} (IL).

The only measured mid-day conductance in Carmona (April 14) showed much lower values (range 89–325 mmol m^{-2} s^{-1}) than those of Cordoba (412–683 mmol m^{-2} s^{-1}) in the nearest date (April 12, Fig. 4). Furthermore, the differences between water deficit and irrigation were much more pronounced in Carmona. Stomatal conductance in normal flowering plants was 89 mmol m^{-2} s^{-1} in water deficit as compared to 310 mmol m^{-2} s^{-1} in irrigated plots.

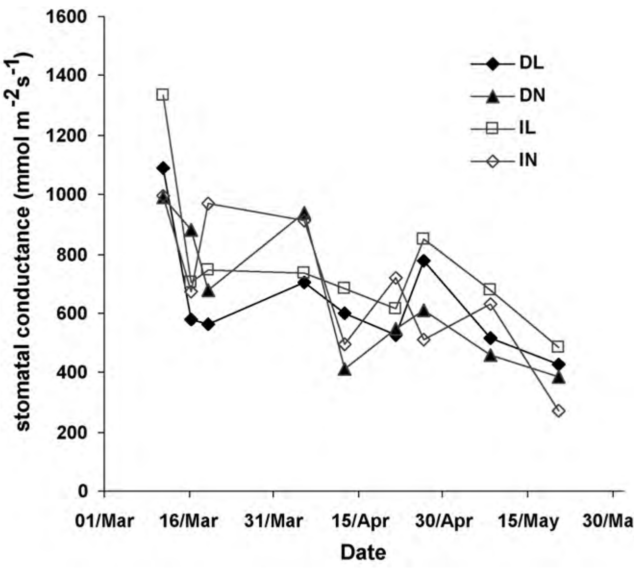

Fig. 4. Mean values of stomatal conductance at noon in *Papaver somniferum* L. in Cordoba. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod.

3.6. Water use efficiency

To evaluate the productivity of water in the determination of crop yield, a useful tool of analysis is the ratio between the water used and the dry biomass obtained at the end of the cycle, which is commonly called seasonal Water Use Efficiency (WUE). This ratio usually takes some different forms, depending on which part of the crop biomass and which fraction of the water used by (or applied to) the crop are considered. The poppy WUE obtained experimentally in Cordoba is presented in Table 7, calculated for different numerator/denominator options. The ratio of final biomass and ET ranged between 2.07 and 2.42 kg m^{-3}, while water use efficiency as head yield/ET was between 0.78 and 0.96 kg m^{-3}, with an advantage for water deficit plots (Table 7). The same applies to the water use efficiency calculated as capsule/ET (0.72–0.85 kg m^{-3}), morphine/(irrigation + rainfall) (48.9–76.3 g m^{-3}), yield/irrigation (2.58–3.57 kg m^{-3}) and head yield/total infiltrated water (0.71–1.15 kg m^{-3}).

4. Discussion

4.1. Water balance

At harvest, all profiles showed water extraction in the deepest horizon (120–150 cm, Fig. 2) which implies that effective root depth may exceed 1.5 m which was the sampling depth for soil water. Thus, our estimates of ET using the water balance may underestimate the true values. This crop is generally considered shallow-rooted, although no information on the subject can be found in the scientific literature. The evidence (Fig. 2) that this crop is able to extract water from 1.5 m depth is relevant to calculate irrigation requirements and scheduling. Deep rooting of this crop may be the source of problems observed in transplanted poppies in Carmona which showed dramatic symptoms of water stress. ET values in Cordoba showed that there was a 15% reduction in ET for water deficit plots which is very close to the average reduction in stomatal conductance at noon (Fig. 4). In soybean (*Glycine max*), Liu et al. (2005) argued that similarity in the sensitivity of stomatal conductance and transpiration to soil drying indicates that any decrease in transpiration during soil drying was mainly due to a decrease of stomatal conductance which is also reported for vegetable amaranth (*Amaranthus* sp.) (Liu and Stützel, 2002). These results indicate an overall moderate water stress in Cordoba and thus cannot be generalized to situations of more severe water deficits. In Carmona where no data are available for ET, stomatal

Table 7
Average values of water use efficiency of *Papaver somniferum* L. in Córdoba, calculated as different numerators and denominators.

Treat.	B/ET (kg m^{-3})	Y/ET (kg m^{-3})	Caps./ET (kg m^{-3})	Morph./ET (g m^{-3})	Alkal./ET (g m^{-3})	Y/I (kg m^{-3})	Y/I + R (kg m^{-3})	Caps./I + R (kg m^{-3})	Morph./I + R (g m^{-3})
DL	2.23	0.94	0.78	56.9	63.4	3.03	1.10	0.90	66.3
DN	2.42	0.96	0.83	63.5	73.2	3.57	1.15	1.00	76.3
IL	2.07	0.88	0.72	55.5	64.5	2.82	0.78	0.63	48.9
IN	2.31	0.78	0.85	60.4	73.7	2.58	0.71	0.77	54.7

B: total biomass; ET: evapotranspiration; Y: head yield; Cap: capsule yield; Morph: morphine yield, I: irrigation; R: rainfall. DL: deficit irrigation-extended photoperiod; IL: normal irrigation-extended photoperiod; DN: deficit irrigation-normal photoperiod; IN: normal irrigation-normal photoperiod; Caps.: no. of capsules.

conductance measured in irrigated plots was less than half of those of Cordoba (325 mmol m^{-2} s^{-1} vs. 683 mmol m^{-2} s^{-1}), which suggests a more severe water stress and may have contributed to the lower values of biomass and yield of Carmona.

Water deficit before anthesis led to a reduction in stomatal conductance which continued after anthesis, despite irrigation restart.

4.2. Growth and development

Extending the photoperiod to 24 h was effective in advancing the onset of flowering, without any reduction in leaf number (mean 18). Mika (1955) reported that the number of leaves per plant varied between 23 and 25 and was not affected by photoperiods greater than 15 h. Gentner et al. (1975) reported up to 40 leaves in opium poppy that is much larger than our observations. Such differences may be explained by variability among cultivars, like those reported for biomass (Bernáth and Tétényi, 1982) and alkaloid yield (Kaicker et al., 1978) in *Papaver somniferum*. Our data also indicated that there is proportionality between the duration of the phases “emergence-flower hook” and “flower hook-flowering”, so the difference between normal and extended photoperiod in the time needed to reach the appearance of flower hooks (maximum 11 days) is further increased to reach flowering (maximum 21 days). At flowering in Cordoba (6 April, Table 2), plants under normal photoperiod had generally higher green LAI (10.6 and 8.3) than plants in extended light plots (7.7 and 4.0) and the same trend was recorded 2 weeks after flowering (Table 2). Irrigation only increased LAI in Carmona approximately at the time of flowering (30 April). The maximum LAI in Carmona was 3.7 which indicate a strong water stress even in irrigated plots, probably due to a poor establishment of transplanted plants. Chung (1982, 1987) reported maximum LAI of 7.4 and 7.9 in two growing seasons.

The plants were taller in Cordoba (136 cm) than in Carmona (99 cm) (Table 2). The extension of the photoperiod resulted in a reduction of the height in Cordoba that can be attributed to shorter growing time before flowering. Acock et al. (1996) reported that height of poppies increased with short photoperiods, especially for photoperiods less than 14 h. In Carmona, however, the taller plants were the deficit irrigated in extended photoperiod. Water status did not affect plant height in either location.

4.3. Biomass, dry matter and alkaloid production

The measured values of biomass in Cordoba (9400–11,600 kg ha^{-1}) are consistent with those of previous reports in Tasmania (9800–12,200 kg ha^{-1}, Chung, 1982 and 10,000–15,500 kg ha^{-1}, Chung, 1990). Chung (1982) attributed higher head yields to the higher total dry matter which was also observed in Cordoba.

In both locations, the hastening of flowering caused an increase in biomass allocation to the heads at the expense of leaves (Table 3). This effect on biomass distribution is reflected in the harvest index which increased significantly with earlier flowering in Cordoba (0.42 vs. 0.39 and 0.33 for the 2 irrigation treatments). This trend was also observed in Carmona. The values of HI are similar to other reported values for irrigated (0.32–0.34; Chung, 1982) and non-irrigated (0.35; Chung, 1987) poppies.

Acock et al. (1996) suggested that the number of photosynthetically active days between plant emergence and flowering is the primary determinant of poppy biomass. Results of this study showed that IN plants produced the highest biomass and had the longest days from emergence to flower initiation and flowering (Fig. 3). The advance of about 2 weeks of the time of flowering increased the HI from 0.33 to 0.42. Although the artificial extension of the photoperiod is applicable only for experimental purposes, the use of early flowering varieties could be beneficial in some cases; for example to escape water stress (or cut down irrigation needs) in climates and years with dry spring, or to reduce the probability of crop lodging, a common problem for poppy growers. Our data suggest that a moderate reduction in crop total biomass due to a shorter vegetative period may be counterbalanced by an increased harvest index, thus the total yield could remain unaffected, although alkaloids concentration would be reduced to some extent. The pros and cons of using – or breeding for – early flowering varieties should be examined with further research. Earlier flowering did not affect seed production, although reduced the seed number per head.

In Cordoba, alkaloid production per surface unit tended to increase by irrigation as a result of higher concentrations of alkaloids as well as higher head yields (Table 6); which is in accordance with the experiments of Chung (1987) in two seasons in Tasmania. Table 6 indicates that water deficit has two different effects on alkaloid production in opium poppy. Moderate water deficit before flowering reduced capsule mass and caused a reduction in the concentrations of codeine and thebaine indirectly. Under severe stress (in Carmona), however, water deficit caused the appearance of oripavine while reducing total alkaloid production.

To compare the WUE of poppy with another winter crop in the same environment, Gallardo and Fereres (1993), in an experiment with winter wheat in Cordoba, found WUEs around 3.3 and 1.4, as biomass/ET and grain yield/ET, respectively. In a more recent review, Katerji et al. (2008) report for winter wheat WUE values ranging between 0.11 and 2.5 in various Mediterranean locations.

In general, WUE is higher if the crop is kept below its maximum transpiration rates, i.e. under deficit irrigation; this behaviour is also observed for poppy in the Cordoba experiment (Table 7). Nevertheless, the mild levels of water stress and the low evaporation demand (as a winter crop, the most part of poppy cycle takes place during spring, when less transpiration water is required to assimilate carbon) resulted in a little effect of water stress on WUE. No higher WUE was observed in the water deficit treatments for morphine/ET (55.5–63.5 g m^{-3}) as well as total alkaloids/ET (63.4–73.7 g m^{-3}). On the contrary, these water use efficiencies for alkaloids are negatively influenced by the shortened vegetative period. The reduction in alkaloids concentration that was observed in the hastened flowering treatments (Table 6) was the cause. Shortening the vegetative period seems to have no other effect on the water use efficiencies calculated as biomass/ET, yield/ET or capsule/ET.

4.4. Stomatal conductance

Water deficit reduced mid-day poppy stomatal conductance in Cordoba by 10% in average, while ET decreased by 15%. Stomatal conductance values for Carmona were much lower than those observed in Cordoba, and the reduction in Carmona conductance in response to water deficit was relevant (64%, data not presented).

The slightly lower conductance of stressed plants was maintained during the post-flowering period, although irrigations were resumed. This is a common response of stomata as an acclimation to moderate water stress, but did not cause an acceleration of leaf senescence (Table 4).

5. Conclusions

Artificial extension of photoperiod since crop emergence caused an advance of 8–10 days in flower initiation and 15–21 days in flowering. The final leaf number, however, was the same for all treatments, which is explained by a higher rate of appearance of leaves with extended photoperiod. Water stress caused a 13% reduction in the biomass production without apparent reduction in capsule yield, which implies an increased harvest index for plots under water deficit. In Carmona, deficits were more severe, leading to lower yields and reduced harvest indices.

Water deficit reduced noticeably the ET and stomatal conductance of the water deficit treatments; however, the improvement of harvest index can compensate for the lower observed ET and biomass production with considerable water savings.

The benefits of deficit irrigation before flowering are saving water, reducing leaf growth and possible reduction of N leaching losses, which may improve the N supply in post-flowering. The practical possibility of applying a moderate deficit before flowering depends on the holding capacity of soil and technical level of the farmer. Less retentive soils would not be advisable to deficit irrigation. If necessary, it would be implemented by reducing the recommended doses of irrigation by 15% before flowering and maintaining the normal schedule after flowering.

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