CSIRO PUBLISHING

AUSTRALIAN JOURNAL OF PLANT PHYSIOLOGY

Volume 27, 2000

An international journal of plant function

www.publish.csiro.au/journals/ajpp

All enquiries and manuscripts should be directed to
Australian Journal of Plant Physiology
CSIRO PUBLISHING
PO Box 1139 (150 Oxford St)
Collingwood Telephone: 61 3 9662 7620
Vic. 3066 Facsimile: 61 3 9662 7611
Australia Email: laurie.martinelli@publish.csiro.au

Published by **CSIRO** PUBLISHING
for CSIRO and
the Australian Academy of Science

Aust. J. Plant Physiol., 2000, **27**, 463–471

Water constraints on the photoinduction of weed seed germination during tillage

Javier F. Botto[A], *Ana L. Scopel and Rodolfo A. Sánchez*

IFEVA, Consejo Nacional de Investigaciones Científicas y Tecnológicas and Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, (1417) Buenos Aires, Argentina.
[A]Corresponding author; email: botto@ifeva.edu.ar

Abstract. Germination of light-requiring seeds may be induced by very brief exposure to sunlight during soil disturbance through the very-low fluence (VLF) mode of phytochrome action. We studied the effect of soil water availability after cultivation on the photoinduction of seed germination in two important weed species, *Datura ferox* and *Chenopodium album*. In daily-irrigated plots, seedling density was 1- to 4-fold greater in plots cultivated during daytime than in those tilled at night. In contrast, when plots were not irrigated soon after tillage and rainfall was excluded, no significant differences were observed between seed germination in daytime *vs* night-time cultivated plots, although seedling emergence in night-time cultivated plots was higher than in non-cultivated controls. The average critical value of soil water potential required for the expression of VLF-induced germination was higher than –0.5 MPa (at 3-cm depth during the 6 d following cultivation). Dark germination was less sensitive to decreasing soil moisture than light-induced seed germination. The promotive effect of the light signal perceived by the seeds during daytime cultivation is maintained for several days (*ca* 6) in drying soil, even though laboratory data suggest that the far-red-light absorbing form of the phytochrome inducing the VLF photoresponse is unstable, disappearing in less than 24 h. These results reveal the complexity of interactions between the light signal and other environmental factors that control seed germination under natural conditions.

Keywords: *Chenopodium album*, cultivation, *Datura ferox*, phytochrome, seed germination, soil water content, very-low fluence response, weeds.

Introduction

Light induction of seed germination is mediated by phytochromes. These photoreceptors are a family of chromoproteins that exist in two photoconvertible forms, red-light absorbing form of phytochrome (P_r)* and far-red-light absorbing form of phytochrome (P_{fr}), with absorption peaks in red (R) and far-red (FR) regions of the light spectrum, respectively. Five phytochromes (phyA through phyE) have been found in *Arabidopsis thaliana*, the apoprotein of each being encoded by a different gene: PHYA to PHYE (Sharrock and Quail 1989; Clack *et al.* 1994). Also, five phytochrome genes are present in tomato (PHYA, PHYB1, PHYB2, PHYE, and PHYF) (Pratt *et al.* 1997). The germination of light-requiring seeds is induced by low fluence response (LFR) or very-low fluence response (VLFR) modes of action, depending on the sensitivity of seeds to P_{fr} concentrations (Casal *et al.* 1998). In *A. thaliana*, phyA is the photoreceptor mediating the VLFR (Botto *et al.* 1996; Shinomura *et al.* 1996), whereas phyB and at least another phytochrome other than phyA are involved in the induction of the LFR (Botto *et al.* 1995; Poppe and Schäfer 1997; Casal *et al.* 1998). Under field conditions the LFR, which is R/FR reversible, is involved in the germination of seed banks to canopy openings (Frankland 1981; Vázquez-Yanes and Orózco Segovia 1990; Deregibus *et al.* 1994; Insausti *et al.* 1995). The VLFR, which is not R/FR reversible (i.e. an FR pulse induces maximum response), operates when seed germination is triggered by very brief exposure to sunlight during soil disturbance (Scopel *et al.* 1991) or by the light filtered through very dense canopies (Botto *et al.* 1996).

Weed seeds can acquire an extremely high light sensitivity during burial, such that a few milliseconds of sunlight perceived by them during tillage may suffice to induce germination through a VLFR (Scopel *et al.* 1991; Botto *et al.* 1998*b*). Daytime cultivation with a mouldboard plough or disk harrow frequently enhances seedling emergence when compared with night-time cultivation (Hartmann and Nezadal 1989; Ascard 1994; Scopel *et al.* 1994; Buhler 1997; Botto *et al.* 1998*a*; Gallagher and Cardina 1998). In contrast, no promotion of seedling emergence following daytime (as compared to night-time) tillage has been observed in

*Abbreviations used: FR, far-red light; LFR, low fluence response; P_{fr}, far-red-light absorbing form of phytochrome; P_r, red-light absorbing form of phytochrome; R, red light; VLFR, very-low fluence response; VWC, volumetric water content; WSA, water-vapour-saturated atmosphere.

10.1071/PP99199 0310-7841/00/050463

soil cultivated with a chisel plough, which causes considerably less soil inversion than a conventional plough (Botto *et al.* 1998*a*).

The photoinduction of seed germination depends only on the light stimulus perceived *during* tillage in the field (Botto *et al.* 1998*a*). The magnitude of the photoresponse is affected by species composition (Scopel *et al.* 1994; Buhler 1997; Gallagher and Cardina 1998), the agricultural history of the seed bank (Botto *et al.* 1998*a*), and the season in which the cultivation is performed (Scopel *et al.* 1994; Botto *et al.* 1998*a*). Also, display of the photoresponse may be the result of other environmental conditions experienced by the seeds during and immediately after cultivation. Up until now, no attempts have been made to investigate the effects of environmental factors modulating the VLFR of weed seed banks.

Soil water potential frequently determines the timing of seedling emergence under field conditions (Benech-Arnold and Sánchez 1995, and references therein). In conventional tillage, the soil surface typically remains bare between seedbed preparation and crop seedling emergence. During this period, evaporation generates a dramatic decrease in water content in the upper centimetres of the soil profile, the region from which most weed seedlings emerge (Grundy *et al.*, 1996). In intensively replicated field experiments, Roberts and Potter (1980) showed that soil moisture was the over-riding factor determining seedling numbers in a British weed community. Water potential can also modify seed germination responses to light (Baskin and Baskin 1998, and references therein). Laboratory studies have convincingly shown that more negative water potential in the incubation medium before or after an R light pulse may block the P_{fr}-dependent processes leading to germination through a R/FR reversible response in several species (Berrie *et al.* 1974; Vertucci *et al.* 1987; de Miguel and Sánchez 1992). The escape time, i.e. the time during which the presence of P_{fr} is required to induce seed germination, has also been shown to be longer in seeds incubated at low water potential compared with those incubated in water (Hsiao and Vidaver 1971; Duke 1978). All these studies have investigated the effects of water availability on LF-induced seed germination; so far no work has been carried out studying water constraints on seed germination promoted by very low fluences (i.e. VLF).

In this paper we report the results of field and laboratory experiments with seeds of *D. ferox* and *C. album*, which were designed to address the following questions: (1) What is the relationship between soil water availability and seed germination induced by very brief exposures to sunlight during tillage? (2) In the case of water scarcity immediately following cultivation, for how long will the light stimulus last?

Materials and methods

Datura ferox L. is a troublesome weed in temperate areas around the world. Seeds of this species need light and alternated temperatures in order to germinate (Soriano *et al.* 1964). Seedling emergence of *D. ferox* take place after soil cultivation in summer field crops (Ballaré *et al.* 1988). This is possible because, after a period of burial of a few months, the seeds acquire a very high sensitivity to light, allowing the germination through the VLFR (Scopel *et al.* 1991; Botto *et al.* 1998*b*). *Chenopodium album* L. is a cosmopolitan weed whose seeds also require light to germinate (Cumming 1963), and field experiments indicate that seedling emergence fluxes of *C. album* are associated with the light perceived by seeds during tillage (Jensen 1995; Botto *et al.* 1998*a*).

Field experiments

Seed banks

Experiments were conducted at the experimental field of the Faculty of Agronomy, University of Buenos Aires, Argentina (34º35′ S, 58º29′ W). The experiments were carried out with seed banks of *D. ferox* and *C. album* buried in a lime loam soil classified as a vertic Argiudoll. Seeds of *D. ferox* were collected in commercial soybean fields in the autumns of 1995, 1996 and 1997 (Table 1). Seeds of *C. album* were collected in 1996. Depending on the seed bank, seeds were buried immediately after harvest or air-dried and stored for 1 year (Table 1). Seed banks A and B were obtained by burying 5000 seeds m^{-2} of *D. ferox* with a mouldboard plough (working depth 15–20 cm). Seed banks C and D were obtained by burying 2500 seeds m^{-2} of *D. ferox* and 75 000 seeds m^{-2} of *C. album* (only seed bank D) with a rototiller (working depth 10 cm). The seeds of both species had a high level of dormancy at the time of burial (germination was less than 10% after a saturating red pulse). In order to homogenise the distribution of buried seeds between experiments, the field that contained seed bank A was ploughed perpendicularly to the direction of the previous cultivation in the winter of 1996.

Tillage operations, soil water content and soil temperature

The experiments were conducted in December 1995, October and November 1996, and January 1998 using three different seed banks (Table 1). Daytime cultivations were performed around noon under full sunlight (photosynthetically active radiation > 1200 mol m^{-2} s^{-1}; LI 190SB quantum sensor, Li-Cor, Lincoln, USA). Night-time cultivations started more than 3 h after sunset. Primary tillage was carried out with a mouldboard plough, with a working width of 60 cm and a depth of 15–20 cm, followed by two passes of a rotocultivator during the night (on the same calendar day as the plough). Soil volumetric water content (VWC) at the beginning of each experiment was always *ca* 75% of field capacity. In December 1995 and October 1996 the plots were either irrigated daily after cultivation to maintain soil VWC near field capacity or left without further irrigation. In the experiments carried out in November 1996 and January 1998, an additional, intermediate level of irrigation was established.

The effect of soil water availability on the germination response to sunlight exposure was tested following two experimental approaches: (1) the plots were subjected to different levels of irrigation immediately after tillage, and (2) the irrigation treatment started at different times after cultivation. In the first approach, we established two or three levels of irrigation depending on the experiment: (i) full irrigation, plots irrigated daily to maintain soil water levels close to the initial values, (ii) medium irrigation, approximately 50% of the full irrigation treatment, and (iii) no further irrigation after tillage (non-irrigated treatment). In the second approach, we provided full irrigation immediately after cultivation (control treatment) or withheld water for different periods of time after tillage (i.e. 3, 5, 6, 8, 13 or 14 d) before irrigation began. Rain shelters (1.7 × 3.0 m), made of clear polyethylene (100 μm thick, Agropol, Buenos Aires, Argentina), were used to exclude natural rainfall. The height of the shelters was adjusted between 50 and 70 cm, so as to prevent an increase in temperature above the recorded air

Table 1. Seed burial, experimental cultivation dates, and seedling counts corresponding to each type of experiment
Irrigation level and timing of irrigation. [a] indicates data for *D. ferox* and *C. album*, [b] indicates data for *D. ferox*

	Seed bank	Harvest	Burial date	Cultivation date	Seedling count (Experiments of level)	Irrigation date	Seedling count (Experiments of timing)
Exp. 1	A (Df)	1995	August 1995	14 December 1995	27 December 1995	27 December 1995	9 January 1996
Exp. 2	A (Df)	1995	August 1995	14 October 1996	29 October 1996	—	—
Exp. 3	B (Df)	1996	July 1996	27 November 1996	5 December 1996	11 December 1996	16 December 1996
Exp. 4	A (Df)	1995	August 1995	23 December 1996	—	29 December 1996	3 January 1997
Exp. 5	C (Df)	1997	July 1997	5 December 1997	—	8 or 13 December 1997	23 December 1997
Exp. 6	D (Df, Ca)	1996	August 1997	8 January 1998	16 January 1998[a]	11 or 13 January 1998	23 January 1998[b]

temperature. Each experimental plot was irrigated using a mist sprayer (MA 360, API, Buenos Aires, Argentina), which covered an area of 80 × 80 cm. In all cases, irrigation treatments were applied until the date of seedling count; except in experiment 6, in which irrigation treatments were applied for only 5 d due to a heavy rainstorm and gusty winds that lifted part of the rain shelters.

Soil volumetric water content was measured daily in the afternoon before irrigation. Measurements were obtained using probes (two 15-cm-long stainless steel guides) placed horizontally at 3-cm depth and connected to a TDR (Tektronic 1502C, Tektronic Inc., Beaverton, USA). The steel guides were buried immediately after soil cultivation and were left in that position throughout the experiment. At least four sets of probes were randomly assigned to each irrigation treatment. The VWC was calculated following the equation proposed by Topp *et al.* (1980). The amount of water applied each day varied according to soil water evaporation. The VWCs were converted to soil water potential using a previously determined relationship derived for the experimental soil using the method of Richards (1965). The obtained values for Ψ_w were –1.5 MPa at 12.7% VWC, –0.5 MPa at 15.1% VWC, –0.3 MPa at 18.6% VWC, –0.1 MPa at 24.3% VWC, –0.05 MPa at 29.6% VWC and –0.03 MPa at 31.9% VWC. Soil temperature was recorded at hourly intervals at 3-cm depth using thermistor probes (NTH 2074A, G.M. Electrónica, Buenos Aires, Argentina) attached to a micrologger (21X, Campbell Scientific, Logan, USA).

Experimental design and statistical analysis

The experimental design was a 'split-plot' with timing of cultivation (i.e. daytime or night-time) as 'main plots' and level of irrigation (i.e. full, medium and non-irrigated) or timing of irrigation (i.e. control, 3, 6, 8, 13 and 14 d) as 'subplots'. The size of the experimental areas varied from 7–20 m in width and 12–25 m in length (number of blocks was 4–7). Seedling emergence was evaluated in 50 × 50 cm quadrats placed at the centre of each plot (1.2 m × 1m). Seedlings counts were performed 8–15 d after cultivation in the irrigation level experiments (first count), or 11–26 d in the experiments in which the effect of the timing of irrigation was tested (second count) (Table 1). After emergence, the length of the etiolated hypocotyl of seedlings was measured to assess the depth of seed germination (5–37 seedlings per treatment/block). For experiments 2 and 6, frequency curves were constructed according to the germination depth of seeds.

Analyses of variance were performed using SAS statistical package (SAS 1988). Emergence data approximated normal distributions and generally met the assumptions of ANOVA. In experiment 6, data were square root transformed to meet the assumptions of normality and homoscedasticity. Means were back-transformed for presentation. The split plot analyses of variance were carried out to estimate the magnitude and significance of main effects (i.e. timing of cultivation and level or timing of irrigation) and interactions (i.e. timing of cultivation by level or timing of irrigation). Separation of means was tested by Waller-Duncan test at $P < 0.05$.

Laboratory experiments

Laboratory experiments were designed to test the stability of the light signal that triggers the VLF-induced germination in seeds of *D. ferox*. To evaluate the possibility that the photoreceptor involved in this type of response is unstable, we took advantage of the fact that in *D. ferox*, the action of P_{fr} requires alternating temperatures of 20/30ºC (Soriano *et al.* 1964). When the seeds of this species are incubated at constant temperature of 25ºC, only dark reversion and/or destruction of P_{fr} occurs (Casal *et al.* 1991). Therefore, seeds sensitive to very low fluences were kept at constant temperatures for 0, 1, 3 or 7 d following the light treatments, and then transferred to alternating temperatures, which allows the promotion of germination by the P_{fr}. Should an unstable phytochrome be the photoreceptor, even a short delay between the light pulse and the incubation at alternating temperatures would result in a substantial decrease in germination.

In order to obtain a sensitised seed population displaying VLFR, seeds were incubated in a water-vapour-saturated atmosphere (WSA) at constant 25°C (Scopel *et al.* 1989) for 12 d. After pre-treatment in WSA, samples of 25 seeds were placed on moist cotton and immediately given the irradiation treatment. Light treatments consisted of saturating (20–90 min, provided by R, R/FR mixtures or RG9 FR filters) and sub-saturating (10–100 s, provided by RG9 FR filter) light pulses that yielded different calculated P_{fr}/P (Scopel *et al.* 1991, Casal *et al.* 1991). Photon flux density varied from 11–35 μmol m^{-2} s^{-1} (R or R/FR mixtures) or 0.70–42 μmol m^{-2} s^{-1} (RG9 FR). After irradiation, the germination boxes were wrapped in black plastic sheets and incubated at constant 25°C for 0, 1, 3 or 7 d and then transferred to alternating temperatures of 20/30°C for 6 d. Radicle protrusion was used as the criterion for seed germination. The proportion of germinated seeds was transformed by arcsine for statistical analysis.

Results

Interaction between timing of cultivation and level of irrigation

In fully irrigated plots, daytime cultivation significantly promoted weed seedling emergence compared with night-time tillage. Depending on the seed bank, seedling density was 1- to 4-fold greater in plots tilled during daytime *vs* night-time tilled controls (Figs 1 and 2). In contrast, when no irrigation was applied after tillage, no significant differences in seedling emergence were found between daytime and night-time cultivation in four out of five experiments (Figs 1 and 2), although seedling emergence in night-time cultivated plots was higher than in non-cultivated controls (Figs 1*B* and 2*B*). We also failed to detect a significant photoresponse in two of the three experiments in which an intermediate

irrigation level was tested (Fig. 2). The detection of the photoresponse for *D. ferox* seedling emergence at the three levels of irrigation in experiment 6 may be due to the fact that irrigation treatments were effective for only 5 d (Fig. 3, *cf.* experiments 3 and 6 throughout the experimental period). In experiments 1 and 2, where only extreme irrigation conditions were analysed, the significant interaction between timing of cultivation and level of irrigation is a clear cancellation of the effect of timing of cultivation under non-irrigated conditions (Table 2). These results indicate that seedling emergence was significantly more affected by a decrease in soil water content in plots cultivated during the day than those tilled during the night.

The average germination depth was not affected by timing of cultivation in those plots well irrigated throughout the experiment, although significant differences were found for daytime cultivated plots between different irrigation levels (Table 3). For *D. ferox*, the average depth of germination was 19.6–26.6 mm in well irrigated plots and 34.3–38.1 mm in non-irrigated plots. It is worthwhile to note that, in the plots maintained with higher soil VWC, over 35% of the seedlings emerged from shallow depths (0–19 mm), whereas only 10% of the seedlings emerged from that depth range in non-irrigated plots (Fig. 4). On the other hand, the absolute number of seedlings that emerged from depths higher than 40 mm was similar in all irrigation treatments (data not shown). *C. album* seedling emergence was restricted to the upper 5 mm of the soil profile. Nevertheless, significant differences were found in the relative frequency and average germination depth between well irrigated and non-irrigated plots (Fig. 4; Table 3).

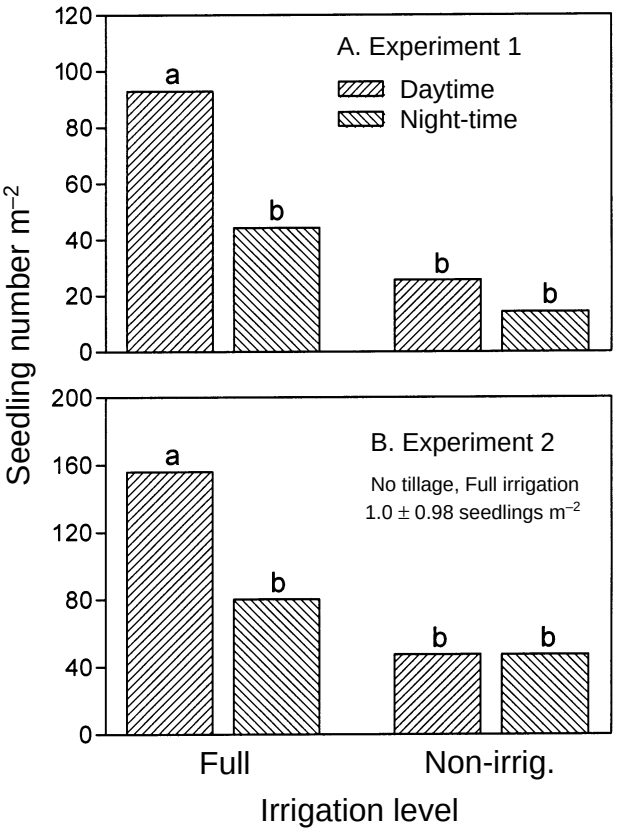

Fig. 1. Effect of irrigation on seedling emergence of *D. ferox* in plots cultivated during daytime or night-time. The plots were either irrigated daily to maintain soil water levels close to field capacity (Full), or left without further irrigation after soil cultivation (Non-irrig.). Different letters above bars indicate significance at $P < 0.05$.

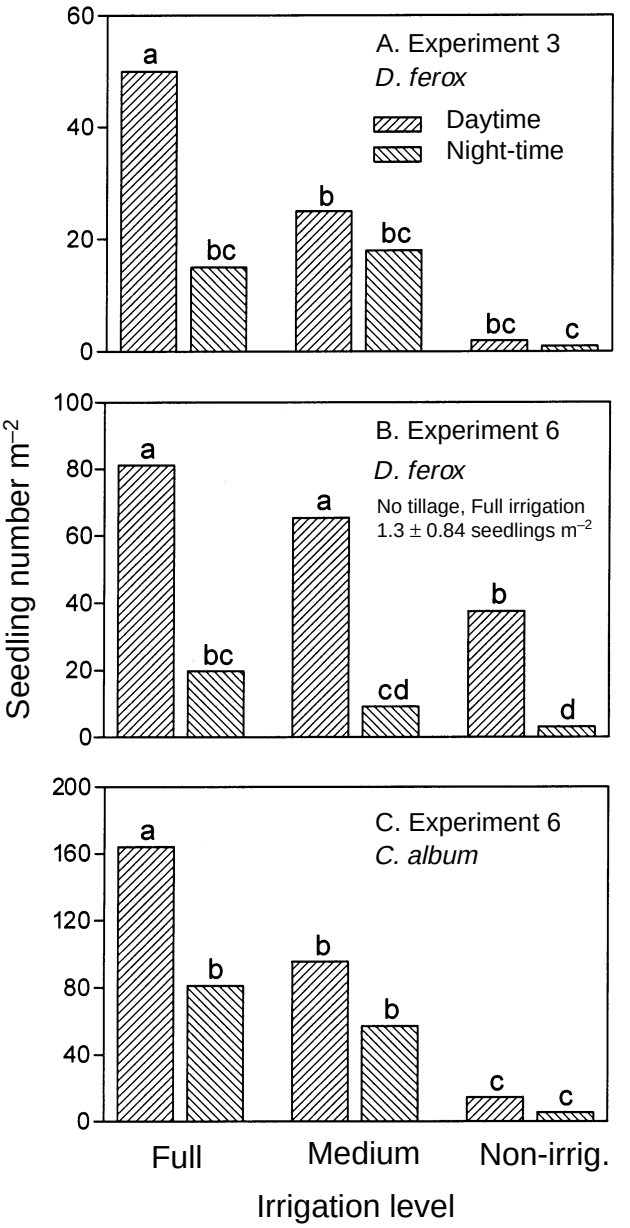

Fig. 2. Effect of irrigation on seedling emergence of *D. ferox* and *C. album* in plots cultivated during daytime or night-time. The plots were either irrigated daily to maintain soil water levels close to field capacity (Full), 50% of the full irrigation level (Medium), or left without further irrigation after soil cultivation (Non-irrig.). Different letters above bars indicate significance at $P < 0.05$.

It is important to note that the conclusions of these experiments seem to hold for a wide range of soil environmental conditions (Table 4). During the 6 days following cultivation, average soil water potentials at 3-cm depth ranged from –0.16 MPa to –0.5 MPa in fully irrigated plots, and maximum soil temperatures were between 25.8 and 36.6°C. In non-irrigated plots, soil water potential was always lower than –0.72 MPa (minimum values < –1.5 MPa) and maximum soil temperatures ranged from 28.6 to 41.4°C.

Duration of the effect of the light signal

In field experiments, the enhancing effect of daytime cultivation on seedling emergence was abolished when irrigation was differed for more than 6 d after tillage (Fig. 5). When the plots were watered within the first 6 d after tillage, in two out of three experiments the effects of the exposure to sunlight persisted (Fig. 5, *cf.* experiments 4 and 6 *vs* experiment 5). The effects of timing of cultivation and timing of irrigation were significant in almost all experiments (Table 5). The average depth of germination was similar for plots cultivated during the day or the night, and for irrigation treatments starting on day 1 or 3; significant effects on germination depth were only found when irrigation was deferred for longer periods (data not shown).

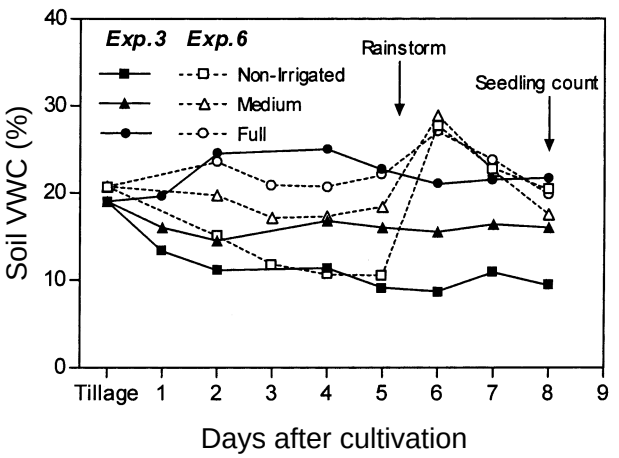

Fig. 3. Soil VWC at 3-cm depth following cultivation for experiments 3 and 6. In experiment 6 the rise in VWC was associated with a heavy rainstorm (thick arrow). Seedling counts were performed on d 8 after cultivation.

The stability of phytochrome signal for the VLF-induced germination was tested in laboratory experiments with seeds that had been sensitised by a WSA pre-treatment. After 12 d

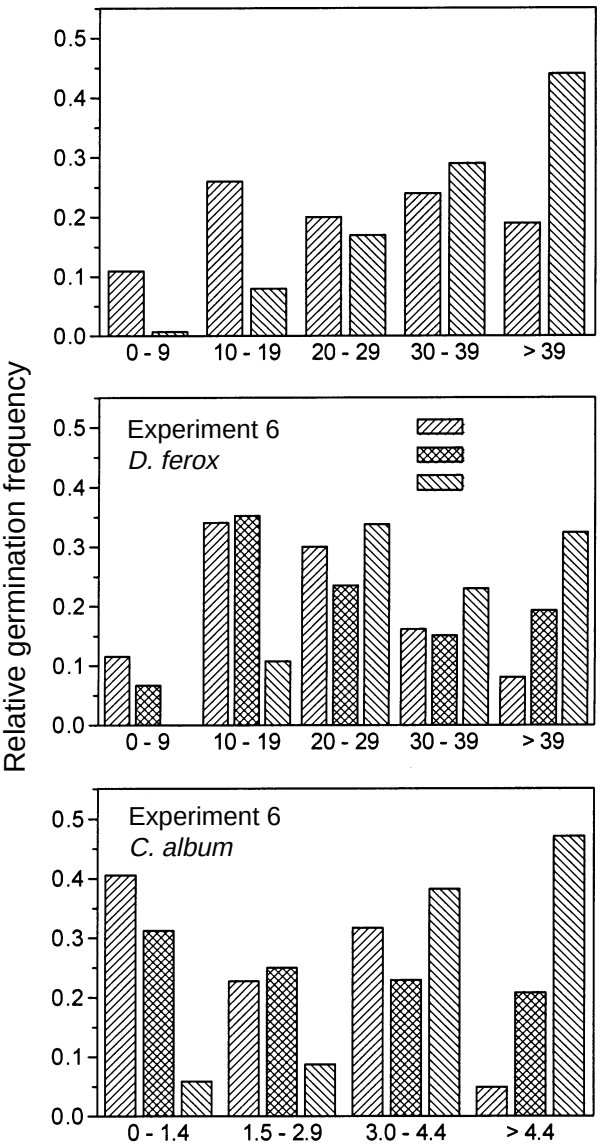

Fig. 4. Effect of irrigation on the relative frequency of germination depth *of D. ferox* and *C. album* seeds.

Table 2. Split plot analysis of variance for the experiments testing the effect of soil water availability on the photoinduction of seed germination

	Exp. 1			Exp. 2			Exp. 3			Exp. 6 (*D. ferox*)			Exp. 6 (*C. album*)		
Main effects/interaction	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value
Block	5	3.64	0.0390	4	1.50	0.2882	3	0.95	0.4485	6	2.80	0.0339	6	9.31	0.0001
Timing of cultivation	1	3.19	0.1340	1	1.75	0.2568	1	6.06	0.0907	1	51.62	0.0004	1	43.56	0.0006
Level of irrigation	1	23.66	0.0007	1	26.25	0.0009	2	7.67	0.0072	2	10.17	0.0007	2	45.03	0.0001
Timing by level	1	3.48	0.0917	1	7.51	0.0254	2	2.56	0.1190	2	0.19	0.8321	2	1.00	0.3828

of incubation in a WSA at 25°C, 50% of the seed population displayed a VLFR (Fig. 6*A*). Sensitised seeds were exposed to different irradiation treatments and subsequently incubated for 0, 1, 3 or 7 d at 25°C (non-inductive temperature) before being transferred to alternating temperatures of 20/30°C for 6 d (inductive temperatures). A saturating R pulse induced the germination of 80% of the seeds, and this was independent of the period of time spent by the seeds at non-inductive temperature. Conversely, an FR pulse only promoted germination (compared with the dark control) when the seeds were immediately transferred to inductive, alternating temperatures (Fig. 6*B*). An additional control in which sensitised seeds were irradiated with an FR pulse after 24 h at constant 25°C showed that this temperature treatment did not produce a decline in the sensitivity to light and in the capacity to display a VLFR (data not shown).

Discussion

Weed seedling emergence is strongly dependent on soil disturbance (Wesson and Wareing 1969). In agreement with previous studies (Scopel *et al.* 1994; Buhler 1997; Botto *et al.* 1998*a*; Gallagher and Cardina 1998), this report shows that seedling emergence was significantly larger after daytime than after night-time cultivation (Figs 1 and 2). The novel result of our field experiments is that the light-promoted seedling emergence is significantly more affected by the quantity and timing of irrigation than seedling emergence induced by night-time cultivation (Figs 1, 2 and 5). This result suggests that, although for a significant proportion of the dark-germinating seeds the soil water content at the cultivation day was enough for germination and seedling growth up to the soil surface, a sizeable proportion of the light-stimulated seeds required the maintenance of higher soil water content for a longer period.

Seedling emergence is the final event of a complex process that involves a series of concatenated steps. For photosensitive seeds, these steps include the transformation of P_r to P_{fr} and the completion of the transduction chain of the phytochrome signal that induces radicle protrusion. Finally, etiolated seedlings should grow to reach the soil surface. All of these steps could be affected by water availability. Our experiments were not designed to distinguish the extent to which each of these processes was affected and which was more likely to explain the differences observed (differences between daytime and night-time cultivated plots in the effects of irrigation on seedling emergence). However, some points can be discussed on the basis of existing data and our own experimental results. Several observations argue against the possibility that differential seedling survival could explain the greater response to soil water potential in plots cultivated during the day. Firstly, at the same level of irrigation, the seedlings emerged from similar soil depths whether the plots were tilled during daytime or night-time (Table 3). Secondly, the temporal patterns of seedling emergence were similar between treatments of timing of cultivation (data not shown). On the other hand, it has been documented that sensitivity to water potential increases with the level of seed dormancy (Bradford 1995; Christensen *et al.* 1996). In *D. ferox*, de Miguel and Sánchez (1999) observed that seeds with different degrees of dormancy germinate similarly at high water potentials, but the germination of seed batches with a higher degree of dormancy were more sensitive to a decrease in the water potential of the substrate. Based on these results it may be suggested that germination of VLF-induced seeds (i.e. more dormant) would be more

Table 3. Depth of germination (mm) of *D. ferox* and *C. album* seeds as affected by timing of cultivation and irrigation level

Each value represents the average ± s.e. For each experiment, values followed by the same letter are not significantly different at $P = 0.05$; nd, no data. [a] indicates data for *D. ferox*, [b] indicates data for *C. album*

	Full		Medium	Non-irrigated
	Daytime	Night-time	Daytime	Daytime
Exp. 2	26.6 ± 0.8a	22.4 ± 3.0a	nd	38.1 ± 3.1b
Exp. 3	22.0 ± 2.1a	22.4 ± 3.3a	23.6 ± 1.2a	nd
Exp. 6[a]	24.2 ± 2.5a	19.6 ± 2.6a	27.2 ± 2.1ab	34.3 ± 4.3b
Exp. 6[b]	2.4 ± 0.3a	2.0 ± 0.2a	2.6 ± 0.3a	4.8 ± 0.5b

Table 4. Average maximum and minimum temperatures, percentages of soil volumetric water content (VWC) and soil water potentials (ψ_{soil}) in full, medium and non-irrigated plots

Each temperature and VWC value was calculated from the average of daily records at 3 cm of depth during the days immediately following the experimental cultivation (i.e. 6 d or 5 d for experiment 6). Each value represents the average of temperature or VWC ± SE. Soil water potentials (MPa) were estimated from a drying curve obtained for the experimental field site (see 'Materials and methods'); nd, no data available. In Exp. 3, without covers, the average maximum temperature was 38.6 ± 2.4 and the average minimum temperature was 18.8 ± 1.3

	Full				Medium				Non-irrigated			
	T_{max}	T_{min}	VWC	ψ_{soil}	T_{max}	T_{min}	VWC	ψ_{soil}	T_{max}	T_{min}	VWC	ψ_{soil}
Exp. 1	36.6 ± 0.8	17.4 ± 0.9	20.7 ± 1.7	–0.5	nd	nd	nd	nd	41.4 ± 0.5	19.7 ± 1.0	6.7 ± 1.8	< –1.5
Exp. 2	25.8 ± 0.8	14.4 ± 0.9	26.1 ± 2.8	–0.16	nd	nd	nd	nd	28.6 ± 1.9	14.1 ± 1.3	17.2 ± 6.6	–0.72
Exp. 3	33.5 ± 1.6	21.1 ± 0.9	21.4 ± 1.8	–0.46	34.0 ± 1.6	20.7 ± 0.9	15.2 ± 0.6	–1.12	36.2 ± 1.6	22.5 ± 0.9	12.1 ± 1.1	< –1.5
Exp. 6	30.5 ± 1.4	20.1 ± 0.5	23.9 ± 0.6	–0.3	31.4 ± 1.2	20.6 ± 0.6	20.8 ± 1.3	–0.49	34.1 ± 1.4	22.2 ± 0.5	15.7 ± 2.6	–0.93

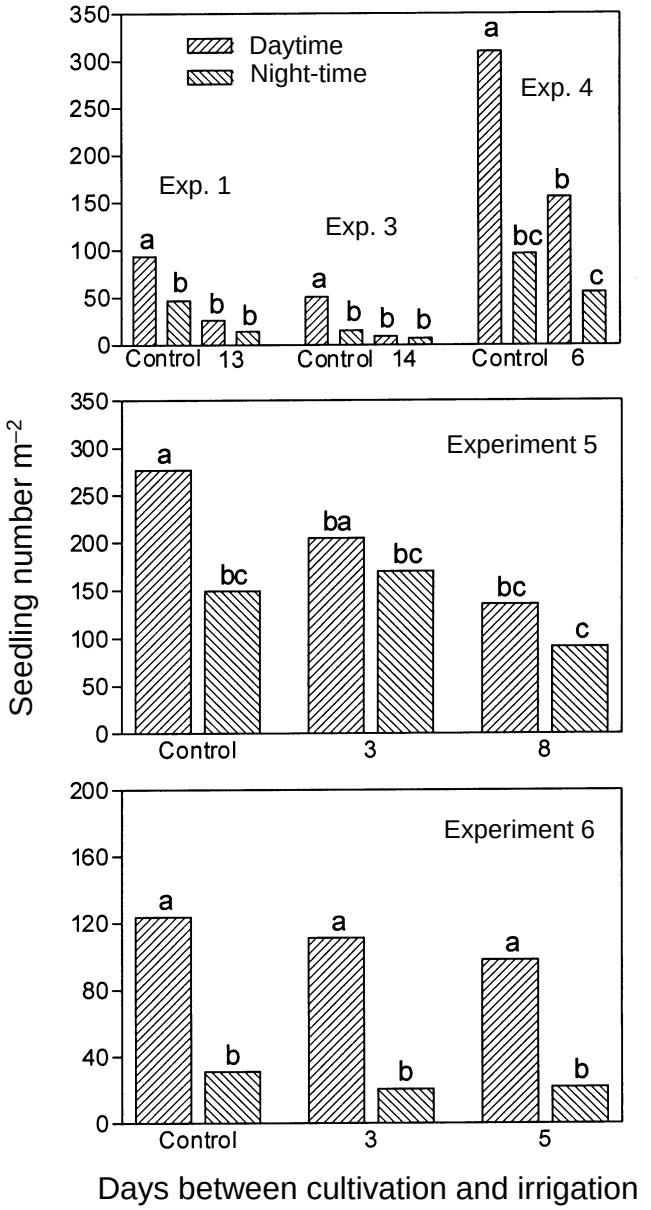

Fig. 5. Effect of delaying irrigation on the emergence of *D. ferox* seedlings in plots cultivated during daytime or night-time. Daily-irrigation started on the same day of cultivation (control) or at different times after the tillage operation (i.e. 3, 5, 6, 8, 13 and 14 d). Seedling counts were performed between 11 and 26 d after the cultivation. Different letters above bars indicate significance at $P < 0.05$.

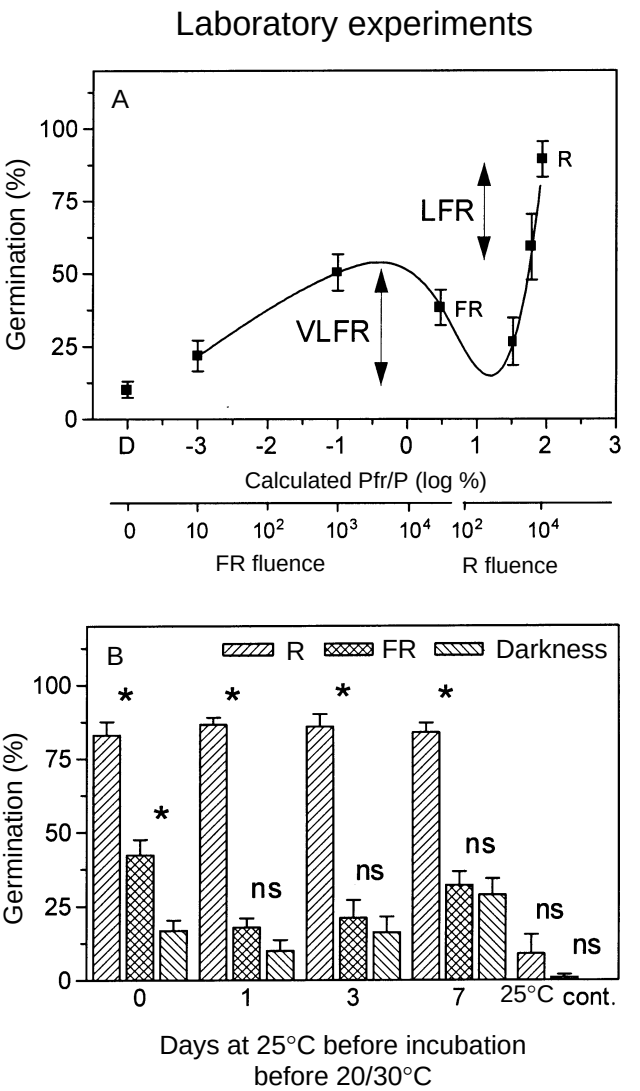

Fig. 6. (*A*) Relationship between germination of *D. ferox* seeds and the P_{fr} percentages and fluences (μmol m^{-2}) established by various irradiation treatments. See 'Materials and methods' for details of treatments. D = darkness, FR = saturating pulse of FR, and R = saturating pulse of R. Each value represents the average of 9 independent samples of 25 seeds ± s.e. (*B*) Effect of lengthening the incubation at non-inductive temperature (continuous 25°C) on germination responses after R or FR pulse. Each bar represents the average of 4 to 10 independenst samples of 25 seeds ± s.e. The asterisks above bars indicate significant differences between adjacent treatments ($P < 0.05$); ns: not significant.

Table 5. Split plot analysis of variance for the experiments testing the stability of light signal

	Exp. 1			Exp. 3			Exp. 4			Exp. 5			Exp. 6		
Main effects/interaction	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value
Block	5	3.28	0.0520	3	0.53	0.6808	5	1.51	0.2789	5	1.61	0.2139	6	5.46	0.0026
Timing of cultivation	1	2.80	0.1551	1	7.56	0.0706	1	29.04	0.0030	1	13.62	0.0141	1	19.80	0.0043
Timing of irrigation	1	23.95	0.0006	1	9.52	0.0215	1	8.76	0.0160	2	4.04	0.0380	2	3.10	0.0713
Cultivation by Irrigation	1	2.95	0.1164	1	4.38	0.0812	1	6.15	0.0350	2	1.22	0.3203	2	0.31	0.7348

affected by lower water potentials than the dark-germinating seeds (i.e. less-dormant).

The effectiveness of P_{fr} in promoting seed germination decreases when seeds are incubated in an osmotic medium (Baskin and Baskin 1998 and references therein). Several authors have shown that low water potential arrests P_{fr}-dependent processes in light-induced seed germination (Duke 1978; Vertucci *et al.* 1987; Pons 1991; de Miguel and Sánchez 1992). In *D. ferox,* a species in which dormancy is imposed by the tissues surrounding the embryos, de Miguel and Sánchez (1992) found that endosperm cell wall softening (and seed germination) decreased dramatically when water potentials dropped from 0 to –0.8 MPa after the seeds were irradiated with an R pulse. Results from our field experiments show that soil water availability on the days immediately following soil tillage should be high (–0.5 MPa) to allow the expression of the VLF-induced germination in *D. ferox* seeds.

In agreement with previous reports, the promotion of *D. ferox* seed germination by a saturating R pulse (i.e. LFR) persisted for several days at constant, non-inductive temperature indicating the participation of a stable phytochrome pool (Casal *et al.* 1991). In contrast, the effect of an FR pulse disappeared even if the exposure to alternating temperatures was delayed for only 1 d (Fig. 6*B*). This result suggests the action of a labile form of phytochrome in the promotion of VLF-germination of *D. ferox* seeds, which would be consistent with phytochrome A being the photoreceptor involved in this type of response in *Arabidopsis* (Botto *et al.* 1996). Interestingly, however, in the field experiments, the effect of daylight tillage on seedling emergence was detected even when irrigation was withheld for periods as long as 6 d (Fig. 5). These results indicate that, during short periods and depending on soil water potential, the induction of VLF-seed germination may persist for longer periods in natural conditions compared with controlled conditions in the laboratory. This induction would not depend on the storage of phytochrome as P_{fr}, but rather in the form of some component/s of the signal transduction chain that is/are downstream the initial P_{fr}-dependent stage.

It is important to note that, in our field experiments, daytime cultivation always promoted seedling emergence compared to night-time cultivation when soil VWC was maintained high throughout the experimental period (Figs 1 and 2, full irrigation treatment). This result seems to be contradictory with the large variation in photoresponse observed in experiments carried out in agricultural fields with natural seed banks (Scopel *et al.* 1994; Buhler 1997; Botto *et al.* 1998*a*; Gallagher and Cardina 1998). This discrepancy should not be surprising if we consider that in our experiments seed banks were homogeneous regarding species composition (one or two species), and soil water availability was a controlled factor. In contrast, seed banks in commercial fields are typically diverse both in species composition and physiological status, and water availability in the soil has never been manipulated.

Experiments carried out in commercial field plots have shown that the magnitude of photoresponse depends on the agricultural history of the seed bank and time of the year when tillage is performed (Scopel *et al.* 1994; Botto *et al.* 1998*a*; Gallagher and Cardina 1998). Our results suggest that soil water availability after tillage controls the expression of the VLF-induced seed germination and, hence, it may modulate weed species composition after soil tillage. Preliminary data (J. F. Botto *et al*., unpublished results) do indeed suggest that, depending on the timing of cultivation, not only seedling density but also species composition can be dramatically affected by differences in soil water availability after soil disturbance.

Acknowledgments

The authors thank Dr William Batista and Dr Carlos Ballaré for the critical review of this manuscript and Mr Andrés Berney for his help in the field experiments. This research was financially supported by grants from the University of Buenos Aires (AG024) and the Antorchas Foundation (A–13532/1–85).

References

Ascard J (1994) Soil cultivation in darkness reduced weed emergence. *Acta Horticulturae* **372**, 167–177.

Ballaré CL, Scopel AL, Ghersa CM, Sánchez RA (1988) The rate of *Datura ferox* seeds in the soil as affected by cultivation, depth of burial and degree of maturity. *Annals of Applied Biology* **112**, 337–345.

Baskin CC, Baskin JM (1998) (Eds) 'Seeds: ecology, biogeography, and evolution of dormancy and germination.' (Academic Press: San Diego)

Benech-Arnold RL, Sánchez RA (1995) Modelling weed seed germination. In 'Seed development and germination'. (Eds M Negbi, G Galili and J Kigel) pp. 545–566. (Marcel Dekker: New York)

Berrie A, Paterson J, West H (1974) Water content and the responsivity of lettuce seeds to light. *Physiologia Plantarum* **31**, 90–96.

Bradford KJ (1995) Water relations in seed germination. In 'Seed development and germination'. (Eds M Negbi, G Galili and J Kigel) pp. 351–396. (Marcel Dekker: New York)

Botto JF, Sánchez RA, Casal JJ (1995) Role of phytochrome B in the induction of seed germination by light in *Arabidopsis thaliana. Journal of Plant Physiology* **146**, 307–312.

Botto JF, Sánchez RA, Whitelam GC, Casal JJ (1996) Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in *Arabidopsis*. *Plant Physiology* **110**, 439–444.

Botto JF, Scopel AL, Ballaré CL, Sánchez RA (1998*a*) The effect of light during and after cultivation with different tillage implements on weed seedling emergence. *Weed Science* **46**, 351–357.

Botto JF, Sánchez RA, Casal JJ (1998*b*) Burial conditions affect the light responses of *Datura ferox* seeds. *Seed Science Research* **8**, 423–429.

Buhler DD (1997) Effects of tillage and light environment on emergence of 13 annual weeds. *Weed Technology* **11**, 496–501.