OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Rewatering Plants after a Long Water-deficit Treatment Reveals that Leaf Epidermal Cells Retain their Ability to Expand after the Leaf has Apparently Reached its Final Size

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• *Background and Aims* Leaves expand during a given period of time until they reach their final size and form, which is called determinate growth. Duration of leaf expansion is stable when expressed in thermal-time and in the absence of stress, and consequently it is often proposed that it is controlled by a robust programme at the plant scale. The usual hypothesis is that growth cessation occurs when cell expansion becomes limited by an irreversible tightening of cell wall, and that leaf size is fixed once cell expansion ceases. The objective of this paper was to test whether leaf expansion could be restored by rewatering plants after a long soil water-deficit period.
• *Methods* Four experiments were performed on two different species (*Arabidopsis thaliana* and *Helianthus annuus*) in which the area of leaves that had apparently reached their final size was measured upon reversal of water stresses of different intensities and durations.
• *Key Results* Re-growth of leaves that had apparently reached their final size occurred in both species, and its magnitude depended only on the time elapsed from growth cessation to rewatering. Leaf area increased up to 186% in *A. thaliana* and up to 88% in *H. annuus* after rewatering, with respect to the leaves of plants that remained under water deficit. Re-growth was accounted for by cell expansion. Increase in leaf area represented actual growth and not only a reversible change due to increased turgor.
• *Conclusions* After the leaf has ceased to grow, leaf cells retain their ability to expand for several days before leaf size becomes fixed. A response window was identified in both species, during which the extent of leaf area recovery decreased with time after the 'initial' leaf growth cessation. These results suggest that re-growth after rewatering of leaves having apparently attained their final size could be a generalized phenomenon, at least in dicotyledonous plants.

Key words: *Arabidopsis thaliana*, sunflower (*Helianthus annuus*), leaf expansion, leaf growth cessation, water deficit, rewatering.

INTRODUCTION

With only a few exceptions (e.g. the *Guarea* genus; Steingraeber and Fisher, 1986), leaves expand during a given period of time until they reach their final size and form. This is called determinate growth and it is considered a critical feature that distinguishes leaves from shoots (Tsukaya, 1995) and roots (Fitter, 2002). In pea, sunflower or sorghum, duration of leaf expansion differs between successive leaves of the plant, but each of these durations are constant over a large number of experiments, excluding stressing environmental conditions, when they are expressed in thermal time (Turc and Lecoeur, 1997; Granier and Tardieu, 1998*b*; Lafarge and Tardieu, 2002; Dosio *et al.*, 2003). In *Arabidopsis thaliana*, duration of expansion is similar for all leaf positions and stable when expressed in thermal time without any stress (Chenu *et al.*, 2005). As a consequence, it is often proposed that duration of leaf expansion is controlled by a robust programme at the scale of the plant, which mainly depends

on temperature and which is predictable when expressed in thermal time in non-stressing conditions.

Many studies on the plasticity of leaf expansion in response to abiotic stresses have shown the duration of leaf expansion to be consistently affected by stress. It was increased in response to a reduction in incident light, both in sunflower (Rawson and Dunstone, 1986; Granier and Tardieu, 1999*a*) and in *A. thaliana* (Chenu *et al.*, 2005; Cookson and Granier, 2006). In response to soil water deficit, duration of leaf expansion has been shown to either increase (Takami *et al.*, 1981, Rawson and Turner, 1982, Aguirrezábal *et al.*, 2006) or remain unaffected (Karamanos *et al.*, 1982, Granier and Tardieu, 1999*b*), depending on water deficit intensity and plant species.

At the cellular level, changes in duration of expansion by soil water deficit or shading are systematically accompanied by a decrease in epidermal cell number, whereas the epidermal cell area could be increased or decreased depending on the position and the intensity of the stressing treatment during leaf development (Aguirrezábal *et al.*, 2006; Cookson and Granier, 2006; Granier *et al.*, 2006*b*).

Knowledge of the mechanisms underlying leaf growth cessation is still scarce (in sunflower, Granier and

TABLE 1. *Description of water-deficit treatments imposed: soil water content and potential, dates of start of water deficit and rewatering, and number of fully expanded leaves at these moments*

Expt	Species	Treatment	Soil water content (g g^{-1})	Soil water potential (Mpa)	Days after emergence		Fully expanded leaves	
					Start of water deficit	Rewatering	Start of water deficit	Rewatering
1	*A. thaliana*	Early, long water deficit	0·22	−0·55	13	31	0	12
2	*H. annuus*	Early, long water deficit	0·20	−0·65	18	49	1	9
3	*H. annuus*	Early, long water deficit	0·20	−0·65	16	49	2	8
		Early, short water deficit	0·20	−0·65	16	30	2	4
		Late water deficit	0·20	−0·65	29	49	4	8
4	*H. annuus*	Early, long water deficit	0·20	−0·65	17	56	2	8
		Early, short water deficit	0·20	−0·65	17	28	2	4
		Late water deficit	0·20	−0·65	27	56	4	8
		Severe water deficit	0·18	−1·31	17	56	2	8

Control plants of *A. thaliana* were kept at a soil water content of 0·50 g g^{-1} (soil water potential = −0·3 MPa), and control plants of *H. annuus* at a soil water content = 0·30 g g^{-1} (soil water potential = −0·04 MPa).

Tardieu, 1998*a*; in *Festuca*, MacAdam and Nelson, 2002), but it is usually hypothesized that cell expansion becomes limited by an irreversible tightening of cell walls (e.g. Kutschera, 1996). Dimerization of ferulic acids could play a role in the control of some mechanical properties of the cell wall (Locher *et al.*, 1994). Such bonds result from the activity of cell wall peroxidases (Fry, 1986; Lin and Kao, 2001). Cell wall peroxidase activity increases at the end of leaf expansion in *Festuca* (MacAdam *et al.*, 1992) and is considered to be a likely candidate involved in growth cessation. Under this hypothesis, leaf size should be irreversibly fixed once cell expansion ceases.

The objective of this paper was to test whether leaf expansion could be restored by rewatering plants after a long soil water deficit period. Four experiments were performed in two different species (*Arabidopsis thaliana* and *Helianthus annuus*) in which leaves that had apparently reached their final size resumed growth upon modification of the environmental conditions (reversal of water stress). These experiments show that after the leaf has ceased to grow, leaf cells retain their ability to expand for several days before leaf size becomes fixed.

MATERIALS AND METHODS

Arabidopsis thaliana experiment (expt 1)

Two hundred plants of *Arabidopsis thaliana* (L.) Heynh ecotype Col-0 were grown in a growth chamber. Seeds were sown in cylindrical pots (9 cm high and 4·5 cm diameter) filled with a mixture (1:1, v/v) of a loamy soil and organic compost. Soil water content was determined before planting to estimate the amount of dry soil and water in each pot. Subsequent changes in pot weight were attributed to changes in soil water status after correction for plant weight (as described in Granier *et al.*, 2006*a*). This allowed the calculation and adjustment of soil water content to be precisely defined. This adjustment was made manually. The substrate was previously characterized by a water-release curve relating plant predawn water potential to soil water content (Granier *et al.*, 2006*a*).

The water-deficit treatment started at 13 d after emergence (Table 1 and Fig. 1A) when water was withheld until a soil water content of 0·22 g H_2O g^{-1} dry soil (soil water potential = −0·55 MPa) was obtained. Leaf 1 was not fully expanded at the start of the water-deficit treatment (Table 1). A batch of 70 control plants was kept under well-watered conditions (soil water content = 0·5 g H_2O g^{-1} dry soil, soil water potential = −0·3Mpa; Fig. 1A). At 31 d after emergence, when plants in the water-deficit treatment had 12 fully expanded leaves (Table 1), a batch of 115 of these was rewatered to a soil water potential similar to control plants (=0·5 g H_2O g^{-1} dry soil, −0·3 MPa, Fig. 1A). The experiment ended when leaf 15 reached its final leaf area.

Light in the growth-chamber was provided by a bank of cool-white fluorescent tubes and sodium lamps. Day length was maintained at 10 h (Table 2). Photosynthetic photon flux density (PPFD) was measured continuously at plant level, using a PPFD sensor (LI-190SB, LI COR, Lincoln, NV, USA). Air temperature and relative humidity were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland). All measurements of temperature, PPFD and relative humidity were averaged and stored every 600 s in a datalogger (Campbell Scientific, LTD-CR10 Wiring Panel, Shepshed, Leics, UK). Mean air vapour pressure difference (VPD) was calculated during the light period. Mean micrometeorological conditions during the experiment are presented in Table 1. Thermal time was calculated using a base temperature of 3 °C (Granier *et al.*, 2002).

Digital pictures of the plants were taken manually two or three times a week. The area of leaves 6, 8, 10 and 12 was measured on these pictures using an image-analysis software. A leaf was considered as fully expanded when three consecutive measurements with equal or decreasing leaf area were obtained, considering the first as the moment of leaf growth cessation.

At the end of the experiment, transparent replication films of the adaxial epidermis of leaf 12 were obtained after evaporation of a varnish spread on the upper surface of the leaf. Films were placed under a microscope (Leica, Leitz DM RB, Wetzlar, Germany) coupled to an image
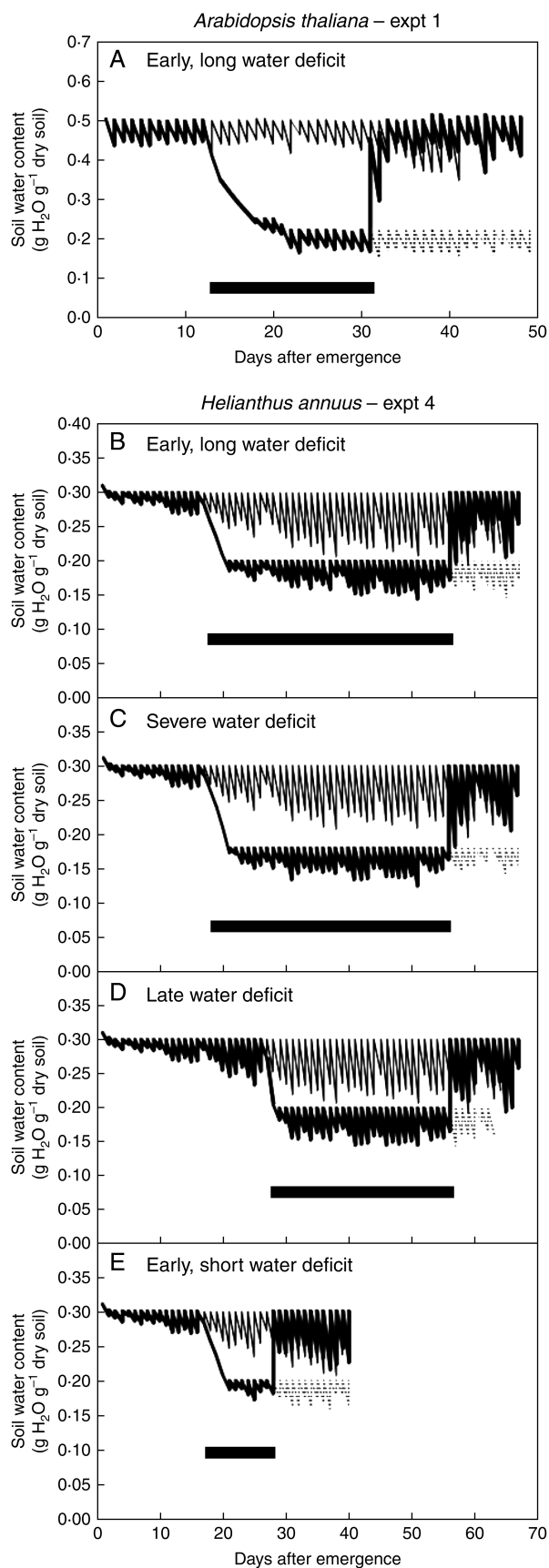
TABLE 2. *Growth conditions during the four experiments*

Expt	Species	Day length (h)	Mean air temperature (°C)	Daily incident light (mol m^{-2} d^{-1})	VPD (kPa)
1	*A. thaliana*	10·0	21·0	9·0	0·80
2	*H. annuus*	9·4	22·0	29·0	0·90
3	*H. annuus*	11·0	21·0	18·0	1·00
4	*H. annuus*	8·0	19·0	28·0	0·75

The effect of rewatering on leaf expansion was investigated in *Arabidopsis thaliana* grown in a growth chamber and *Helianthus annuus* grown in a greenhouse (expts 2, 3 and 4) For each experiment, mean micrometeorological conditions from sowing to end of rosette leaf development (expt 1) or from sowing to end of expansion of leaf 12 (expts 2, 3 and 4) are presented.

analyser. The area of 25 epidermal cells (excluding guard cells and trichomes) was measured at four different places on the leaf: near the base, near the tip and on each side of the mid-vein of the leaf.

Helianthus annuus experiments (expts 2, 3 and 4)

One hundred and fifty plants of *H. annuus* cv. HAR2 were grown in a heated greenhouse in three different experiments. Seeds were sown in cylindrical pots (35 cm high and 10 cm diameter) filled with soil (Typic Argiudoll, horizon A). Soil water content measurements and adjustments were similar to expt 1. The water release curve of the soil in the pots had been previously characterized (Pereyra-Irujo *et al.*, 2007).

Sunflower experiments included one to four water-deficit treatments (Table 1), consisting of two different imposition dates (when plants had one or two expanded leaves – 'early'; or when plants had four expanded leaves – 'late'), two different rewatering dates (when plants had four expanded leaves – 'short water deficit'; or when plants had eight or nine expanded leaves – 'long water deficit'), and two soil water potential levels (mild: soil water content = 0·20 g H$_2$O g^{-1}, soil water potential = −0·65 MPa, or severe: soil water content = 0·18 g H$_2$O g^{-1}, soil water potential = −1·31 MPa). In early water-deficit treatments, water deficit was imposed by using previously sown maize plants as described in Pereyra-Irujo *et al.* (2007). In late water-deficit treatments,

FIG. 1. Changes with time in soil water content: (A) data from expt 1 (*A. thaliana*) and (B–E) expt 4 (*H. annuus*). Plants were initially grown in well-watered conditions (soil water content adjusted daily to 0·5 g H$_2$O g^{-1} dry soil in *A. thaliana* and 0·3 g H$_2$O g^{-1} dry soil in *H. annuus*), followed by a water-deficit treatment (soil water content adjusted daily to 0·22, 0·20 or 0·18 g H$_2$O g^{-1} dry soil, depending on species and treatment), and then followed by rewatering to initial well-watered conditions (thick continuous line). Control plants were kept under well-watered conditions throughout the experiment (thin continuous line). In each water-deficit treatment, a batch of plants was kept under water deficit conditions after the moment of rewatering (dotted line). The thick horizontal bar indicates the length of each water-deficit treatment.

no maize plants were used since leaf area and rooting depth of sunflower plants were enough to reach the defined soil water content rapidly. In all treatments, only half of the plants were rewatered, keeping the rest under water deficit. In all cases, a batch of plants was kept as a control without water deficit throughout the experiment (soil water content = 0.3 g H_2O g^{-1}, soil water potential = -0.04 MPa; Fig. 1B–E). The change in time of soil water content in the four water-deficit treatments imposed in expt 4, as compared with the controls, is shown in Fig. 1(B–E).

PPFD, air temperature and relative humidity were measured continuously at plant level. Measurements were made by using, respectively, a PPFD sensor, a thermistor and a humidity sensor, and stored every 15 min in a data-logger (Cavadevices, Buenos Aires, Argentina). Sensors were placed in a ventilated shelter. All measurements were averaged daily. Mean air VPD was calculated from air humidity and temperature during the light period. Mean micrometeorological conditions during the experiment are presented in Table 2. Thermal time was calculated using a base temperature of $4.8°$C (Granier and Tardieu, 1998b).

Width and length of leaves 4, 5, 6, 7 and 8 were non-destructively measured every 2 or 3 d. Leaf area was calculated with a highly significant relationship ($R^2 > 0.99$, $n = 1755$, $P < 0.0001$) obtained from actual leaf area data from different leaves of control and water-stressed plants of 18 sunflower genotypes (G. Pereyra-Irujo and L. Aguirrezábal, unpubl. res.). This equation was checked against destructive measurements of leaf area at the end of the experiment. As in expt 1, a leaf was considered as fully expanded when three consecutive measurements with equal or decreasing leaf area were obtained, considering the first as the moment of leaf growth cessation.

At the end of the experiment, transparent replication films of the adaxial epidermis of leaf 8 were obtained as described for expt 1. Cell areas were measured as described for expt 1, except that 50 cells of three leaf zones (base, middle and tip) were measured in this case.

Statistical analysis

Leaves considered were those that were fully expanded at the moment of rewatering. The effect of treatments on the final leaf area of each leaf was assessed by analysis of variance (0.05 significance level). Treatment means were compared by least significant difference tests (0.05 significance level). The effect of water deficit on leaf area was quantified as: (final leaf area water deficit – final leaf area control)/final leaf area control. The increase in leaf area after rewatering was quantified as: (final leaf area rewatered – final leaf area water deficit)/final leaf area water-deficit.

RESULTS

In both species, water-deficit treatments significantly reduced leaf growth. In all water-deficit treatments, leaves continued to grow at reduced rates, and the duration of leaf expansion was maintained or even increased (Fig. 2).
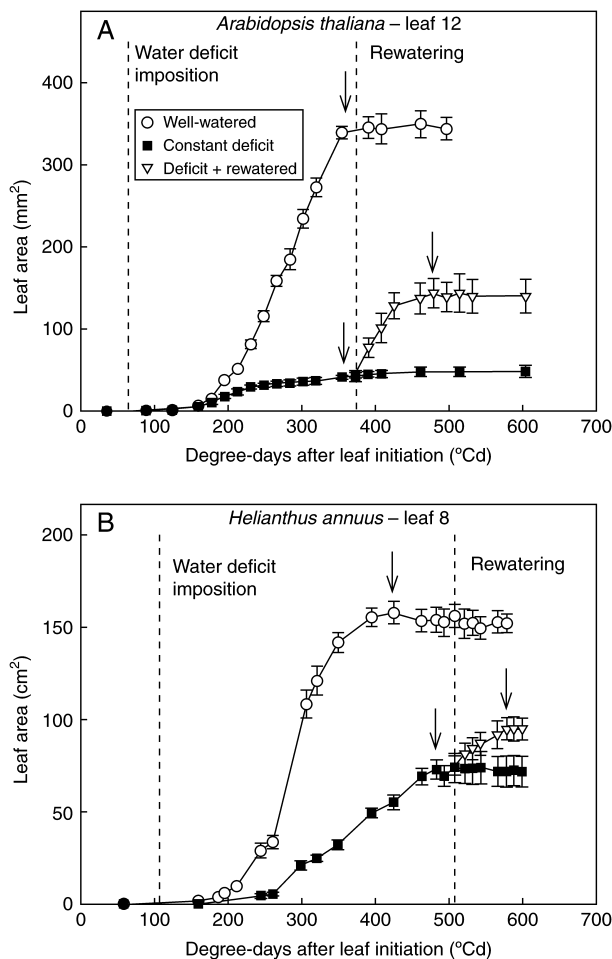

FIG. 2. Response of leaf area to water deficit and rewatering: growth of leaf 12 of *Arabidopsis thaliana* (A) or leaf 8 of *Helianthus annuus* (B), under well-watered conditions, constant water deficit, or a water deficit followed by a rewatering after the studied leaf had ceased to expand. Leaf area is plotted against growing degree days after the initiation of each leaf. Arrows indicate the date when the leaf was considered fully expanded in each treatment. Note the difference in area units between (A) and (B). Vertical bars represent ± s.e. ($n = 6$).

Upon rewatering, leaves that were still growing increased their expansion rates, and some of the leaves that had already ceased to grow resumed expansion (Fig. 2). In expt 1, leaf 12 of *A. thaliana* plants subjected to a water deficit expanded for 5 d ($88°$ Cd) after rewatering, increasing leaf area by 186 %. In expt 3, leaf 8 of *H. annuus* plants grown under mild water deficit resumed expansion for 6 d ($71°$ Cd) upon rewatering, increasing leaf area by 27 %.

After rewatering, the increase in area of leaves having apparently attained their final size (Fig. 2) was paralleled by an increase in the area of epidermal cells, in both species (Fig. 3). Rewatering did not affect epidermal cell number with respect to plants that remained under water deficit, neither in *A. thaliana* nor in *H. annuus* (insets in Fig. 3A, B). In *H. annuus*, all the rewatered plants showed a re-growth of some of the leaves which had ceased to expand, regardless of the duration (Fig. 4A vs. C), the intensity (Fig. 4A vs. B) or the moment of
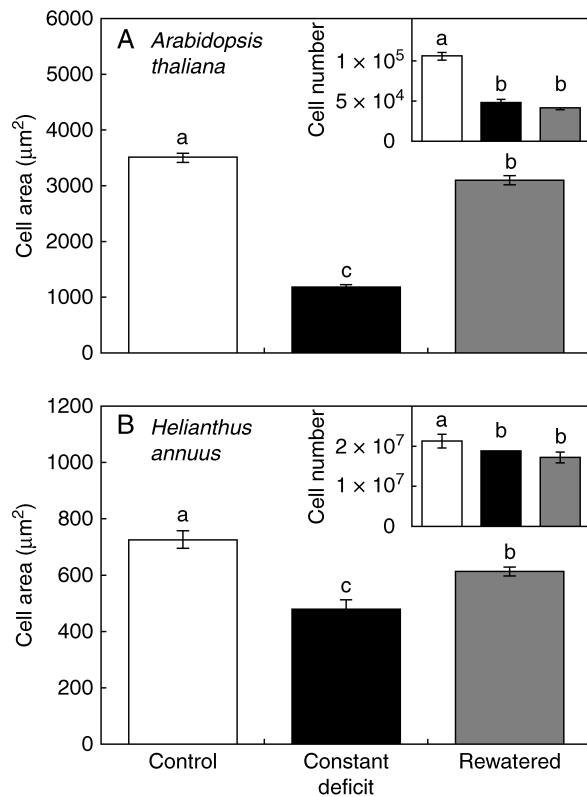
FIG. 3. Response of epidermal cell area and cell number to water deficit and rewatering: final average cell area of leaf 12 of *Arabidopsis thaliana* (A) or leaf 8 of *Helianthus annuus* (B); under well-watered conditions (white); constant water deficit (black); or a water deficit followed by a rewatering after the studied leaf had ceased to expand (grey). Insets: final cell number per leaf calculated from final leaf area (Fig. 2) and cell area data. Data represent the mean value of measurements in three leaf zones of at least four plants. Vertical bars represent ± s.e. and different letters indicate significant differences between means (*P* < 0·05).

imposition (Fig. 4C vs. D) of the water-deficit treatment. In *A. thaliana*, all the measured leaves which had ceased to expand at the moment of rewatering, drastically increased their area (Fig. 5, top). In the case of leaf 6, re-growth occurred despite plants were rewatered 11 d after cessation of expansion. In *H. annuus*, only the leaves that had recently ceased to expand (<5 d: leaves 7 and 8 in expt 3) responded significantly to rewatering (Fig. 5, bottom).

The decrease in final leaf area under water deficit was greater in younger leaves. Among leaves which had reached their final size, re-growth was also greater in the younger ones. No clear relationship was found, however, between both effects (*P* > 0·08). The effect of rewatering in leaves having apparently attained their final size was inversely related to the time elapsed between the end of leaf expansion and the moment of rewatering, which was lower in younger leaves (Fig. 6). In sunflower, this relationship accounted for the effect of rewatering (R^2 = 0·79; *P* < 0·001), regardless of the moment of imposition of water deficit, its duration, its intensity or the moment of rewatering. This relationship determines a time window during which a leaf which has ceased to expand under stress is able to resume growth upon rewatering. In sunflower,
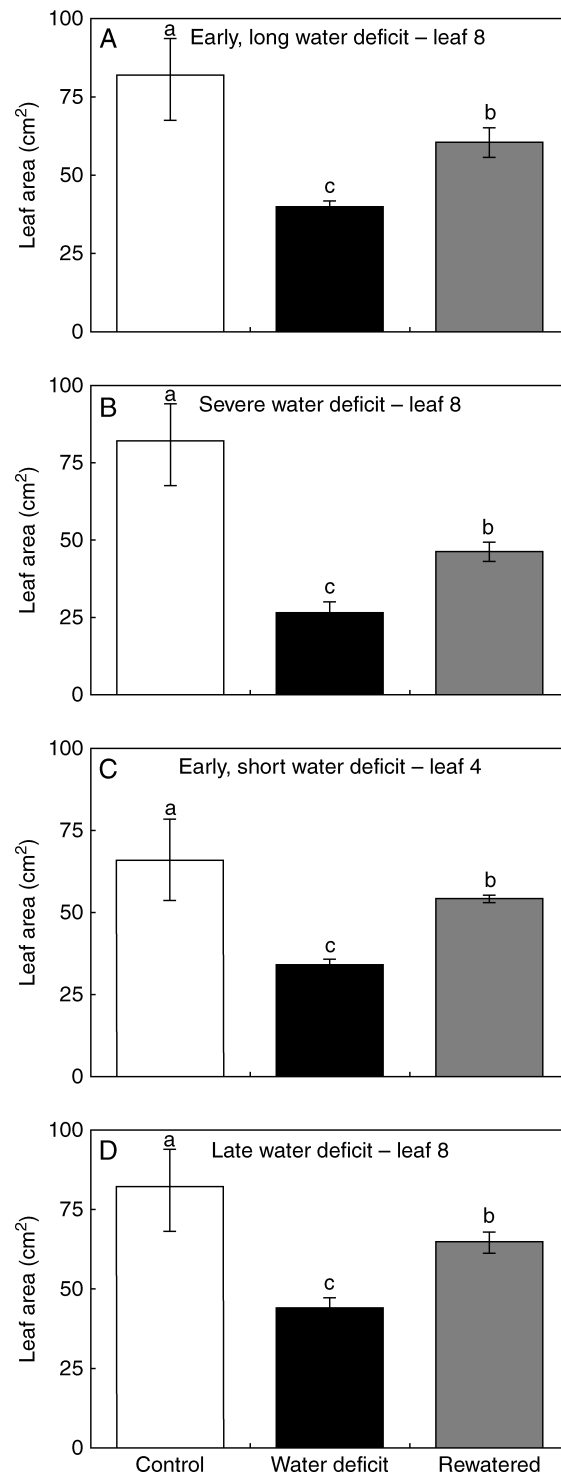

FIG. 4. Response of leaf area to rewatering after water deficits of different duration, intensity, or applied at different moments: final area of leaves of *Helianthus annuus*, under well-watered conditions (white), constant water deficits of different duration, intensity, or applied at different moments (black), and the same water deficits followed by a rewatering after the studied leaf had ceased to expand (grey). Water deficits in (A) and (B) are long water deficits (34 d) of different intensity (−0·65 and −1·31 MPa, respectively). Water deficits in (C) and (D) are shorter (11 and 26 d), mild (−0·65 MPa) water deficits, applied at 17 and 28 d after emergence. Vertical bars represent ± s.e. (*n* = 6) and different letters indicate significant differences between means (*P* < 0·05)
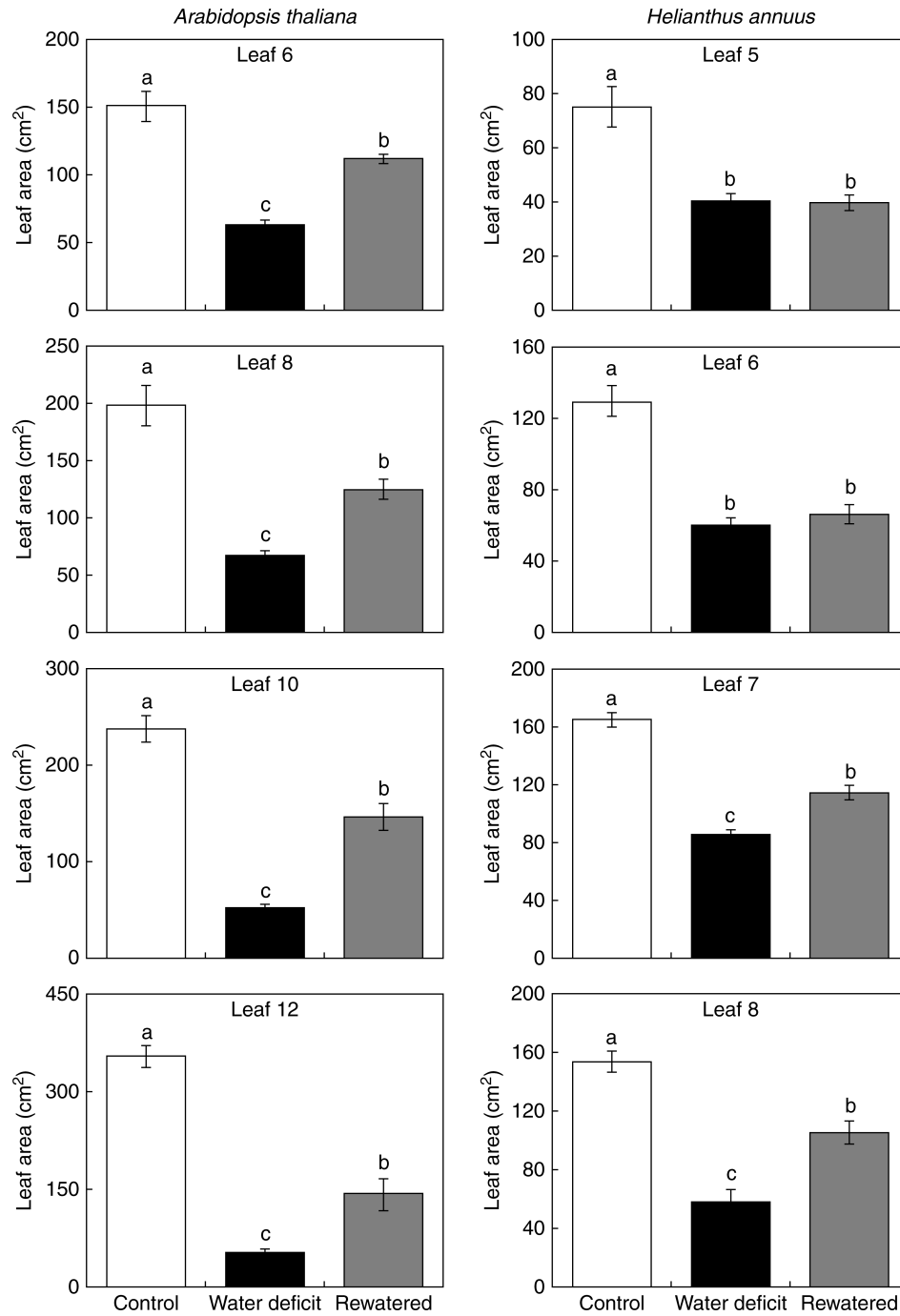
FIG. 5. Response of leaf area to water deficit and rewatering in different leaves of a plant: final area of leaves 6, 8, 10 and 12 of *Arabidopsis thaliana* or leaves 5, 6, 7 and 8 of *Helianthus annuus*, under well-watered conditions (white), constant water deficit (black), or a water deficit followed by a rewatering after the studied leaves had ceased to expand (grey). Vertical bars represent $\pm$ s.e. ($n = 6$) and different letters indicate significant differences between means ($P < 0.05$)

this response window was found to be of approx. $60\,^{\circ}\mathrm{Cd}$ (approx. 4 d), during which the response decreases from 80 % to nearly zero (Fig. 6B). In *A. thaliana*, this window appears to be longer than $200\,^{\circ}\mathrm{Cd}$ (approx. 11 d), and the magnitude of the response is about twice that of sunflower (Fig. 6A).

DISCUSSION

The effect of rewatering on plants under water deficit has received a lot of attention as plants are often exposed to variations in soil water availability. Most of previous works mainly focused on the changes in different plant processes after rewatering, such as photosynthesis (Galmés
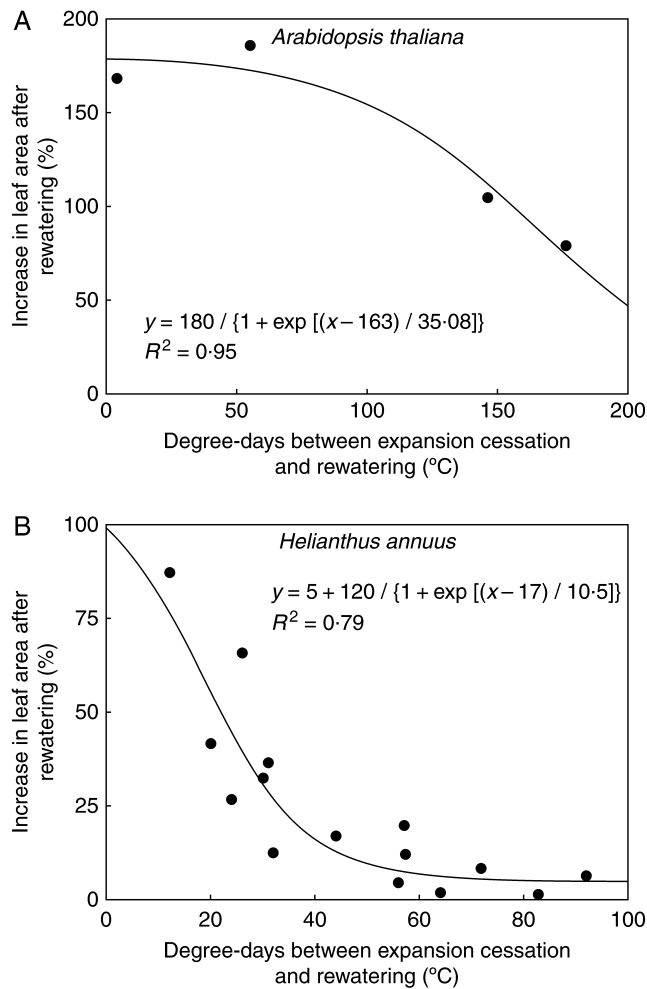
FIG. 6. Response window of leaf area to rewatering: percentage increase in final leaf area of the rewatered treatments, relative to the final leaf area in the corresponding water-deficit treatment, in *Arabidopsis thaliana* (A) or *Helianthus annuus* (B). Data are plotted against the moment of rewatering, relative to the moment of cessation of leaf expansion in the water-deficit treatment. Data include all the experiments and treatments described.

et al., 2007), photoassimilate partitioning (Dai *et al.*, 2007), and plant water relations (Secchi *et al.*, 2007). The effect of rewatering on leaf expansion has been investigated on growing leaves of plants subjected to short water deficit periods (Singh *et al.*, 2000; Alves and Setter, 2004). This paper focuses on re-growth after rewatering of leaves which have apparently attained their final size, a phenomenon that has not received attention in the literature. The main result is that this process could be significant in the recovery of plant leaf area after a water-deficit treatment. As an example, in expt 1, *A. thaliana* leaves of rewatered plants recovered to an average of 60 % of the area of well-watered plants whereas plants that remained under water deficit reached only 28 % of the area of the controls (calculated from Fig. 5).

Re-growth after rewatering of leaves having apparently attained their final size occurred both in the model species *A. thaliana* and the oil crop *H. annuus*. Both species are relatively genetically unrelated (Dominguez

et al., 2003). Despite the wide range of conditions previous to rewatering explored in *H. annuus*, no clear effect of timing, duration or intensity of water deficit was observed. The magnitude of re-growth depended only on the time elapsed from the cessation of growth to rewatering. These results suggest that re-growth after rewatering of leaves having apparently attained their final size could be a generalized phenomenon, at least in dicotyledonous species, and a quite unspecific response of plants to variations in water availability.

The present results support the idea that the increase in area of leaves which had previously ceased to expand represents a new growth and not a reversible change due to increased turgor after rewatering. (*a*) Re-growth of leaves having attained their apparent final area occurred during several days depending on the species and the time elapsed from cessation of leaf expansion. The duration of re-growth ranged between 87 and 144 °Cd for leaves 6–12 of *A. thaliana*, and between 12 and 71 °Cd for leaves 6–8 of *H. annuus*. (*b*) Leaf area increased up to 186% in *A. thaliana* and up to 88 % in *H. annuus* after rewatering, with respect to the leaves of plants that remained under water deficit. In *H. annuus* plants, leaves which had ceased to expand >50 °Cd before rewatering showed only a slight increase in area upon rewatering (5 % on average, Fig. 6B); this age-independent increase is presumably due to increased turgor. (*c*) the phenomenon was observed under water deficit levels previously characterized as moderate (Aguirrezabal *et al.*, 2006; Granier *et al.*, 2006*a*) for the ecotype Columbia of *A. thaliana*. Collectively, these results question the commonly accepted idea that leaf size should be irreversibly fixed once cell expansion ceases.

In both species, cell division in the leaf epidermis ceases well before the end of leaf expansion, when the leaf has attained approx. 50 % of its final area (Pyke *et al.*, 1991; Granier and Tardieu, 1998*a*). During the phase with cell division in the sunflower leaf, epidermal cell area is lower than 200 μm², whereas at the end of expansion all cells fall outside this range (Granier and Tardieu, 1998*a*), suggesting that no cells remain in the epidermis with a potential to re-enter the division cycle. As expected, in the present experiments cell proliferation was not detected upon rewatering in leaves that had previously ceased to expand. In both species, cell expansion accounted for growth of leaves having previously ceased to expand. A developmental gradient within the leaf determines that cells closer to the tip cease to expand before those closer to the base, both in *A. thaliana* (Pyke *et al.*, 1991) and in *H. annuus* (Granier and Tardieu, 1998*a*), making it likely that re-growth be larger in basal than apical cells.

Biochemical and molecular mechanisms underlying re-growth of cells having ceased to expand are unknown. Cell expansion depends on cell turgor and rheological properties of cell wall (Lockhart, 1965), and both could theoretically be points of regulation of the re-growth process. It is currently considered that cell turgor is the driving force of cell expansion but it does not play a key role in the control of growth (e.g. Spollen and Sharp, 1991), the constitution of the cell wall and the activity of cell wall enzymes being the most likely points of regulation of this

process. For instance, expansins are proteins involved in non-reversible cell wall extension (Catalá *et al.*, 2000; Li *et al.*, 2002; Belfield *et al.*, 2005). Cell wall peroxidases could play a key role in cell wall tightening as their activity drastically increases after the end of leaf expansion in several species (MacAdam *et al.*, 1992; Thompson *et al.*, 1998). Consistently with the duration of the response window in *H. annuus*, a delay of 2–4 d between leaf growth cessation and the completion of cell wall deposition was found in two *Festuca* genotypes (MacAdam and Grabber, 2002). In the present experiments, the response of leaf area to rewatering depended on the time elapsed from the end of leaf expansion to the moment of rewatering and not on the magnitude of the change in soil water potential, making it unlikely that cell turgor alone regulates this response. Insufficient cell turgor for cell wall displacement could, however, be responsible for the 'initial' growth cessation. Processes linked to cell wall tightening seem to be delayed under water deficit, and the end of the response window for growth resumption could therefore represent the release from this condition. While more research is needed to clarify the biophysical and biochemical mechanisms underlying the re-growth after cessation of leaf expansion, studying this phenomenon could probably be useful to elucidate the control of leaf growth cessation.

A response window of leaf area to rewatering was identified in both species. Determining a response window could be useful to incorporate the effect of a recovery in plant water conditions into developmental models of whole-plant leaf area (e.g. Dosio *et al.*, 2003). The results presented here show that cessation of leaf expansion in plants subjected to water deficit is a process which can be reversed by an increase in water availability. This attribute of leaf cells allows for the duration of the expansion of the leaf to increase drastically if the plant goes through a drying/rewatering cycle. Thus, the duration of leaf expansion under well-watered conditions or under constant water deficit was lower than the maximum ('potential') duration of growth. This result clearly shows the limitations of the conventional curvilinear models to describe the growth of leaves under non-constant water conditions. Moreover, while the study of rate and duration of leaf expansion under constant homogeneous water deficit was useful to identify clearly the response of different genotypes to water conditions (e.g. Aguirrezábal *et al*, 2006), results presented here show that extrapolation of these results would be of little value in predicting leaf growth of plants growing under fluctuating water availability.

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