Field Crops Research 154 (2013) 1–11

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Influence of water deficit and canopy senescence pattern on *Helianthus annuus* (L.) root functionality during the grain-filling phase

S. Lisanti*, A.J. Hall, C.A. Chimenti

IFEVA, CONICET/Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, C1417DSE, C.A. Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 16 August 2013
Accepted 18 August 2013

Keywords:
Sunflower
Root functionality
Drought
Canopy senescence

ABSTRACT

Root system size and functionality during the grain-filling phase are critical for water uptake and grain yield realization. In spite of its potential importance, root system functionality during this phase has received little attention. The study reported here aimed at documenting root system functionality dynamics between anthesis and physiological maturity, and its responses to water deficit, in two sunflower (*Helianthus annuus* L.) hybrids of contrasting intrinsic patterns of canopy senescence. In experiments repeated in two separate years, crops of the hybrids Aguará 6 (stay green [SG]) and CF 101 (fast dry down [FDD]) were exposed to two levels of soil water availability (irrigation and drought) during grain filling, and the temporal dynamics of root and crop system variables (live root length density [LRLD], root respiration, sap flow rate, green leaf area index [GLAI] and the percentage of live roots [LR%]) were followed. The highest values for all variables were observed close to anthesis, and these differed little or not at all between hybrids within water regimes; but decreased thereafter at rates that differed between hybrids, water supply treatment and response variable. Drought hastened the beginning of root and leaf senescence in of both hybrids. The start of the decline in GLAI in the SG hybrid was significantly ($p < 0.05$) delayed with respect to that of the FDD hybrid under both water regimes, so that the SG hybrid maintained a significantly ($p < 0.05$) greater canopy area for the duration of the experiments under both water regimes. The SG hybrid also exhibited significantly ($p < 0.05$) higher values of LRLD, and root respiration, as well as significantly ($p < 0.05$) lower rates of LR% decrease under both water treatments. On days of high evaporative demand the SG hybrid showed significantly ($p < 0.05$) greater rates of sap flow under both water regimes. These response patterns were consistent across the 2 years of experimentation. Importantly, when the dynamics of all measured variables were contrasted, it was found that root senescence preceded canopy senescence. We conclude that the SG syndrome in sunflower is associated with improved root functionality during grain filling under both irrigation and drought conditions.

1. Introduction

The need to understand the role of the root system in sustaining crop water uptake has been recognized for a long time. Barley (1970) focused attention on two main root system characteristics related to resource uptake, namely, root architecture and root longevity and functionality, and noted that both these attributes could vary substantially during the crop cycle.

The first of these two features has received most attention. The advantage of a prolific root system during early development phases for plant water status maintenance is widely recognized (e.g., Manschadi et al., 2008); as is the fact that the duration of the root system growth period and, hence, opportunity for exploring the soil profile, varies among species. Lilley and Fukai (1994) demonstrated that root growth stops during panicle emergence in rice (*Oryza sativa* L.); while in maize (*Zea mays*) Costa et al. (2002) observed that the greatest values of root density and depth were achieved at flowering; and similar behavior was found in sugar beet (Vamerali et al., 2009), where the root system stops growing between 80 and 130 days after sowing. In sunflower (*Helianthus annuus* L.) the root system also stops growing once flowering take place (Sadras et al., 1989), coinciding with the time that the greatest seasonal values for root density and depth are observed. Sadras et al. (1989) also observed that root system dynamics exhibits some similarity with those of leaf area per plant, with reductions in the values of both variables after anthesis.

* Corresponding author. Tel.: +54 011 4524 8000x8103.
E-mail address: lisanti@agro.uba.ar (S. Lisanti).

0378-4290/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.fcr.2013.08.009

The second characteristic described by Barley (1970) (root longevity and functionality), has received less attention. There have been only a few attempts to describe the dynamics of root system functionality, as reflected in crop water uptake capacity. In addition the contrast, evident in the literature, between the relatively meager information about root system structure and function dynamics during grain-filling phase, compared to the relatively better information about these issues during the vegetative phases of the crop, is particularly striking. In sunflower (Meinke et al., 1993; Dardanelli et al., 1997) and sorghum (*Sorghum bicolor* L.) (Robertson et al., 1993) the temporal patterns of root system growth and the variation in its water uptake capacity across the soil profile have been described, but this information was limited to the pre-flowering stage.

Variations in crop water uptake capacity during or shortly before the post-anthesis phase can have major effects on grain yield because grain number and grain weight are set during this phase. Water stress during grain filling reduces yield by its effects on both components of yield (grain number and size) in many species (Lilley and Fukai, 1994, rice; Chimenti et al., 2004, sunflower; Chimenti et al., 2006, maize; Kashiwagi et al., 2006, chickpea; Kirkegaard and Lilley, 2007, wheat; Hammer et al., 2009, maize), and there are strong evidences that increasing water uptake capacity during this phase may have significant positive effects on grain yield in water stressed crops (Chimenti et al., 2004, 2006; Kirkegaard and Lilley, 2007; Borrell et al., 2000; Hammer et al., 2009). It is inferred that improving crop water uptake, during this period, should provide an important adaptive advantage, making root system functionality a key attribute conferring tolerance to terminal water stress (Serraj et al., 2004). The importance of understanding the root system functionality dynamics during grain-filling is reinforced by some evidence which indicates that the root system senesces during this phase (Sadras et al., 1989), loses functionality (Lilley and Fukai, 1994) and undergoes a reduction in its rate of respiration (Hall et al., 1990).

Moreover, there is evidence linking higher water uptake during post-anthesis with, among other factors, greater canopy green area duration (Kirkegaard and Lilley, 2007; Mace et al., 2012). It has been shown that there is variability in canopy senescence patterns in sunflower (de la Vega et al., 2011) and other crops (Luquez and Guiamét, 2001, soybean; van Oosterom et al., 2010, sorghum; Lee and Tollenaar, 2007, maize), two extreme patterns being distinguished: delayed canopy senescence (stay-green, SG) and accelerated canopy senescence (fast dry down, FDD). In sunflower, these different leaf senescence patterns have a weak connection with grain yield in well watered crops, although post-anthesis biomass accumulation did respond to differences in canopy senescence pattern (de la Vega et al., 2011). By contrast, comparisons made between SG and FDD hybrids in sorghum under water stress (Borrell et al., 2000), have shown that SG hybrids had greater grain yield and water uptake capacity than FDD hybrids. Greater yields of SG phenotype have also been observed in well watered wheat (Christopher et al., 2008).

A decidedly non-trivial aspect of most root functionality and resource uptake studies is the duration of the intervals between successive measurements. In the experiments of Mengel and Barber (1974), Gregory et al. (1978), Lilley and Fukai (1994), Costa et al. (2002), Vamerali et al. (2009) and Dardanelli et al. (1997), observations were often carried out at intervals that varied from 12 to 30 days. While these first approximations are useful, it is evident that shorter intervals between observations would be advantageous, especially when focusing on the grain-filling phase, which may last 45 days in sunflower (Rondanini et al., 2007). This becomes even more evident if we consider that sunflower root respiration rates, measured by Hall et al. (1990), declined 50% during grain-filling and root length measured by Sadras et al. (1989) declined 60% during the same phase.

One of the major impediments to increasing the frequency of sampling of root architecture and functionality is that traditional techniques often require destructive sampling. Faster and nondestructive methods are therefore necessary (Johnson et al., 2001). Techniques such as root imaging using minirhizotrons (Majdi, 1996; Majdi et al., 2005) that allow the documentation of production and mortality of roots at short intervals; sap flow meters (Steinberg et al., 1989; Dugas et al., 1994; Kjelgaard et al., 1996), that permit soil water extraction dynamics to be followed; and the use of chambers to measure soil plus root respiration (Hall et al., 1990) can mitigate the drawbacks of traditional methods.

The present study reports the results obtained in experiments in which spatial and temporal variations in root system functionality were monitored during the grain-filling phase in sunflower hybrids of contrasting canopy senescence patterns (FDD and SG). The objective was to identify the timing of initiation and the rate of senescence of the root systems in both hybrids and also to determine the effects upon them of water stress in order to define a hierarchy of effects for these factors. Priority was given to the use of non-destructive techniques with short intervals between measurements, using destructive methods and/or extended intervals between observations to provide a general framework.

2. Materials and methods

2.1. Experimental design and crop growth conditions

The experiments were carried out at the Faculty of Agronomy, University of Buenos Aires (34°35′ S, 58°29′ W) during two growing seasons (2009/10 and 2010/11). Sowing dates were 19/09/09 and 16/10/10. Two sunflower hybrids (Advanta Semillas SC, Argentina), which do not differ in length of cycle or time to flowering but exhibit different canopy senescence patterns between flowering and physiological maturity, were used. Aguará 6 belongs to the "SG" type and has greater green leaf area duration while CF101 belongs to the "FDD" type and exhibits a greater canopy senescence rate during post-anthesis (pers. comm., Abelardo de la Vega). The crops were grown under field conditions at a density of 7.5 plants m^{-2} (achieved by over-planting and thinning at 4-true-leaf stage) in plots specially designed to impose drought conditions. The whole of the experimental area was protected from rain with a moveable rain-out shelter for the entire season. Every plot was isolated from its neighbors to a depth of 2 m to prevent sub-surface lateral water flow, and every plot fitted with an independent drip-irrigation system. This facility allowed soil water conditions in each plot to be controlled independently of the remaining ones and of rainfall. A split-plot experimental design with three replicates was used, with irrigation level as the main plot and hybrid as the sub-plot. Each sub-plot contained four 2-m long rows. Each hybrid was grown under two irrigation levels, defining four treatments: (1) SG-Irrigation, (2) SG-Drought, (3) FDD-Irrigation and (4) FDD-Drought. All main plots were irrigated every 3 days until anthesis, when the three of them were assigned to the drought treatment. The amount of water supplied differed between irrigations according to atmospheric demand, and aimed at maintaining soil water status close to field capacity. In the drought treatments, irrigation was suspended 8 days before the beginning of anthesis, a condition that persisted until the end of the experiment. In control treatments, soil water status was maintained close to field capacity until grain physiological maturity.

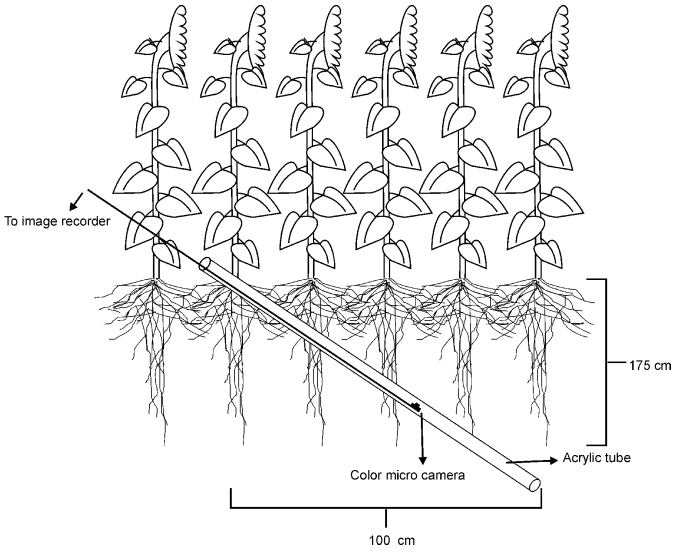

Fig. 1. Diagram of a minirhizotron tube installed under a row.

2.2. Response variables

2.2.1. Root senescence dynamics observed in minirhizotron images

Between 20 and 30 days before sowing, 4 transparent acrylic tubes were inserted into the soil of each sub-plot, under the row position and at a 60° angle with respect to the surface. The tubes were 2 m long, with outer and inner diameters of 50 mm and 44 mm, respectively. The tubes were inserted parallel to the row, their horizontal projection was 100 cm and the maximum depth attained was 175 cm (Fig. 1). The inlet port of the tube was at least 45 cm from the extremes of the row and at least one row away from the adjacent subplot. To monitor root senescence patterns, images were recorded, every 5 days on average, at 9 positions along the tube, separated by intervals of 0.20 m in depth. A SC302-2010 micro-camera (Cavadevices, Buenos Aires, Argentina, http://www.cavadevices.com/), supported on a probe which could be inserted in the tubes and which was connected to a laptop computer, was used to record images at each position.

Root color changes across time could be followed by contrasting successive images for a given position. Where present, a change in root color between two successive images from white to brown was abrupt, not gradual. In addition, all roots visible within each image changed color simultaneously. This behavior allowed the use of a binary classification for the state of the visible roots in each image, taking the brown as a qualitative indicator of senescence and white as a qualitative indicator of functional root. Vamerali et al. (2003) and Majdi et al. (2001) have followed similar criteria. It is important to note that we used color as an indicator of functionality, but do not claim that brown roots are completely unable to take up water. Each image showing the presence of some root was taken as a measurement unit, and only the minirhizotron tubes that exhibited roots in at least 5 of the 9 possible positions per tube were used. At the beginning of anthesis, roots visible in all images that exhibited roots were white. To estimate the % of live roots (LR%) value for each tube and observation date, images with white roots were expressed as a percentage of the total number of images exhibiting roots (either white or brown) for that tube. These values were averaged across all tubes within a sub-plot to obtain a mean value of the % of live roots (LR%) for each plot. The average number of tubes per sub-plot used to generate estimates of LR% was three.

2.2.2. Live root length density

Every 10 or 15 days from the beginning of anthesis (Stage R5.1, Schneiter and Miller, 1981) (anthesed florets cover 10% of the capitulum area) and through to physiological maturity (Stage R.9, Schneiter and Miller, 1981), three 5-cm diameter cores were extracted from the 0–20 and the 20–40 cm soil layers [a total depth containing more than 90% of roots in sunflower crops (Sadras et al., 1989; Angadi and Entz, 2002)] of each subplot. The sample was centered between two adjacent plants in the row, one row away from the adjacent subplot and at a minimum distance of 45 cm from the extremes of each row. Three transverse sub-samples (1.5 cm diameter and 5 cm long) were taken from each core (at distances of 5 cm between sub samples and from the upper and lower limits of the core) and roots were immediately separated from the soil using repeated washings and filtering through a 590-μm sieve. Roots recovered from the sub samples were incubated for 20 h at 20 °C in a 0.06 M phosphate buffer containing 0.6% (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC), and 0.05% (v/v) Tween 20, following the technique of Sturite et al. (2005), a procedure by which metabolically active roots are stained red. Once the incubation was completed, roots were scanned and the resulting color images were analyzed using the WinRHIZO Pro V 2008 (Regent Instruments, Inc., Canada) pixel classification method. Root length values for each category (active, inactive) were used to calculate the value of live root length density (LRLD, cm live root cm^{-3} soil). As was the case for root color in the previous section, we do not claim that roots that do not stain with TTC are completely non-functional. Rather, we take this condition as an indicator of progressive loss of root function.

2.2.3. Root respiration

Three measurements of (roots + soil) respiration ($g\,CO_2\,m^{-2}\,h^{-1}$) per sub-plot, were taken every 5–7 days after the start of the drought treatment using an SRC-1 soil respiration chamber (10-cm diameter and 15-cm high) and an EGM-4 infrared gas analyzer (PP Systems, UK). The chamber was centered between two adjacent plants in the row, at least one row away from the adjacent subplot and at a minimum distance of 45 cm from the extremes of the row. Paired samples were taken from areas of soil without plants, under the same irrigation regime as the plots, to estimate the value of root-free soil respiration. The CO_2 production rates of both samples were normalized using values of soil temperature and water content (to a depth of 15 cm) measured immediately after respiration measurements, following the methodology described by Hall et al. (1990). Root respiration (after standardizing the values to a soil temperature of 25 °C and the water content of the soil in the (soil + roots) measurement) was estimated as the difference between the values of respiration (soil + roots) and soil without roots.

2.2.4. Green leaf area index (GLAI)

Commencing 15 days before the beginning of anthesis and until all the leaves had reached full expansion, length and width of all leaves of four plants per subplot were measured, and individual leaf area estimated as the product of these two dimensions multiplied by 0.75 (Pereyra et al., 1982). Selected plants were representative, in perfect competition, non-contiguous and away from plot borders. Subsequently, every 6–8 days and until the end of the experiment, these same plants were observed and the areas of leaves with more than 50% of their surface yellow were discounted from plant leaf area.

2.2.5. Sap flow

A heat balance xylem flow sensor (Model Dynagauge SGB19, Dynamax, Inc., Houston, TX, USA) (Steinberg et al., 1989) was installed at the base of the stem of one plant per subplot,

following the manufacturer's recommendations and the indications of Steinberg et al. (1989). Selected plants were representative (verified by leaf area and stem diameter measurements), noncontiguous, under perfect competition and away from borders and the extremes of each row. Readings were taken every 60 s and the values, over periods of 15 min, were averaged using a CR10X datalogger (Campbell Scientific, Logan, UT, USA). Cumulative daily flux totals were used to estimate crop transpiration flow ($mm\,d^{-1}$), as the product of daily values of plant transpiration by crop population density. The duration of the measurement period extended from the beginning of anthesis to the time at which sap flow in each plant fell to zero.

2.2.6. Soil water content profile

Soil water content was measured using a neutron probe moisture gauge (model 4301/02, Troxler Electronics Laboratories, Inc., NC, USA) at intervals of 0.20 m to a depth of 1.80 m, with the exception of the water content of the first 0.20 m of soil, which was measured gravimetrically. The measurements took place at intervals of 6–9 days, began at the time when the irrigation was suspended and ended 50 days after the beginning of anthesis. Access tubes for the probe were equidistant from two neighboring plants in the row and sited at least one row away from adjacent subplots and 45-cm from row extremes. To illustrate the differences in soil water content profiles between treatments, values obtained at 3 different times are presented: when the drought treatment was imposed (i.e., 8 days before anthesis), when values for the main measured response variables began to change (20 [Year 1] and 25 [Year 2] days later) and at physiological maturity. A detailed analysis of the evolution of soil water content under these crops will be presented in a future publication.

2.2.7. Development

From the stage of visible flower-bud (Stage R1, Schneiter and Miller, 1981), three non-contiguous representative plants, in perfect competition and away from sub-plot borders and row extremes, were tagged in each sub-plot. Phenological stages of these plants were recorded twice-weekly using the Schneiter and Miller (1981) scale. The definition of "beginning of anthesis" used throughout this manuscript corresponds to the R5.1 stage of this scale.

2.3. Statistical analyses

Two different procedures were used to analyze the dynamics of the various response variables, to establish the significance of treatment effects and to make comparisons between the 2 years of variable responses to treatment. Sap flow and LRLD dynamics presented particular, albeit different, problems. Sap flow dynamics were strongly influenced by the inter-diurnal variations in atmospheric demand which differed between years, while LRLD dynamics could not be appropriately described using the simpler bilinear fits that could be used in most situations for the other responses variables. Mixed linear models were fitted to LRLD and sap flow dynamics using the *nlme* package implemented in Infostat/Professional V.2011 (Di Rienzo et al., 2010). Genotype, water supply regime, measurement dates and their interactions were the fixed terms, while the replicates were the random effects in the model. *Car-1* and *Ar-1*-1 error correlation structures were used for LRLD and sap flow, respectively. Multiple comparisons between means were performed using Fisher's LSD test. For the remaining response variables (GLAI, root respiration and live root (%) [minirhizotron images]), segmented linear regressions (also called bi-linear regressions) were fitted (least squares method) to the relationships with time of these variables, using GraphPad Prism software (version 5.00 for Windows, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com/).

In the cases of sap flow and LRLD dynamics, consistency across years was evaluated considering the maximum values achieved, the duration of the intervals during which high values were observed, the timing of achievement of low or zero values, and the consistency with which treatment effects could be statistically distinguished. To determine the consistency of treatment responses across the 2 years for variables that could be fitted with bilinear regressions, extra sum-of-squares (F-test) method was performed using GraphPad Prism (2007) (GraphPad Software, Inc., CA, USA) to compare the values of the parameters of the functions (i.e., slope of the initial segment, the value of the independent variable corresponding to the break point between the two segments, and the slope of the second linear segment) fitted to root respiration, GLAI, and % of live roots of both years. Bi-linear functions were preferred to polynomials, because they allow for simpler comparisons between treatments, and because visual inspection of the dynamics of these variables indicated that this approach was appropriate with description of the data. However, in a small number of cases (GLAI dynamics in FDD-drought for both years and the % of live roots FDD-drought for the second year) simple linear fits proved more appropriate than bilinear functions.

3. Results

3.1. Year–treatment interactions

The response patterns of the measured variables to treatment (canopy senescence pattern, irrigation level) were very similar in both years. Virtually no significant ($p < 0.05$) differences between years were found for the coefficients of the bi-linear functions fitted to the dynamics of % LR, GLAI and root respiration values for any of the water regime × canopy senescence pattern combinations (Table 1). In the case of sap flow values, the main characteristics of daily sap flow dynamics, such as the maximum values reached, the intervals during which these were observed, as well as the times at which flows tended to zero were consistent across both years (data not shown), despite differences in the detailed patterns between years due to dissimilar dynamics of inter-daily variations in atmospheric demand in the 2 years. The response patterns of LRLD showed no significant interaction ($p > 0.10$) with years. The timings of the highest and lowest values and the treatment response rankings for LRLD across time were consistent between years.

Physiological maturity (PM) (Stage R9, Schneiter and Miller, 1981) was reached, in Year 1, at 49 days from the beginning of anthesis (DFBA) for both hybrids under irrigated condition. Under drought, PM was reached at 46 and 39 DFBA for SG and FDD, respectively. Development patterns in Year 2 were similar to those of Year 1. There was no significant difference in the final grain number between hybrids within water regime in either year. However, unit grain weight was significantly ($p < 0.05$) greater in the SG hybrid, under both irrigation treatments in both years (data not shown).

Based on these general concordances between years in the responses to treatment of the measured variables, in crop development and in yield differences between SG and FDD hybrids, in what follows and in the interests of simplicity, data from a single year only will be presented to illustrate responses.

At the beginning of anthesis, there were no significant differences ($p > 0.10$) in GLAI, root respiration (Table 2), percentage of live roots and sap flow values between the four treatments, in either of the two years, indicating that any differences observed during the grain filling period were independent of the values of these variables at anthesis.

Table 1
Between-year contrasts for the functions fitted to the dynamics of live root length density, root respiration, GLAI and % of live roots. Values of the 3 coefficients of the bi-linear functions (1st stage rate, 2nd stage rate and timing of the change between rates) fitted to each variable/time relationship in both years are shown. Different letters after each value indicate significant differences ($p < 0.05$) between years for each canopy senescence × water regime pair. Empty cells and missing letters in the table correspond to situations where comparisons between years are inappropriate because the form of the fitted function varied between years or the fitted function was linear rather than bilinear. Measurements were taken from the beginning of anthesis until some days after physiological maturity. DFBA stands for "days from the beginning of anthesis".

Root respiration	1st stage decrease rate $g\,CO_2\,m^{-2}\,soil\,h^{-1}\,d^{-1}$	Timing of rate change DFBA	2nd stage decrease rate $g\,CO_2\,m^{-2}\,soil\,h^{-1}\,d^{-1}$	r^2
SG-Irrigation (Year 1)	−0.002 a	18 a	−0.011 a	0.99
SG-Irrigation (Year 2)	0.000 a	12 a	−0.015 a	0.97
FDD-Irrigation (Year 1)	−0.004 a	11 a	−0.014 a	0.98
FDD-Irrigation (Year 2)	−0.005 a	13 a	−0.016 a	0.98
SG-Drought (Year 1)	−0.017 a	18 a	−0.008 a	0.98
SG-Drought (Year 2)	−0.024 b	15 a	−0.008 a	0.97
FDD-Drought (Year 1)	−0.025 a	18 a	−0.007 a	0.97
FDD-Drought (Year 2)	−0.028 a	14 a	−0.007 a	0.99
GLAI	**1st stage decrease rate GLAI day^{-1}**	**Timing of rate change DFBA**	**2nd stage decrease rate GLAI day^{-1}**	**r^2**
SG-Irrigation (Year 1)	0.009 a	25 a	−0.140 a	0.98
SG-Irrigation (Year 2)	−0.006 a	23 a	−0.110 a	0.98
FDD-Irrigation (Year 1)	0.005 a	18 a	−0.155 a	0.98
FDD-Irrigation (Year 2)	−0.016 a	14 a	−0.131 a	0.96
SG-Drought (Year 1)	−0.003 a	14 a	−0.123 a	0.97
SG-Drought (Year 2)	−0.011 a	12 a	−0.114 a	0.99
FDD-Drought (Year 1)[a]	−0.105 a			0.98
FDD-Drought (Year 2)[a]	−0.096 a			0.99
Live roots	**1st stage decrease rate % d^{-1}**	**Timing of rate change DFBA**	**2nd stage decrease rate % d^{-1}**	**r^2**
SG-Irrigation (Year 1)	0.00 a	7 a	−2.21 a	0.98
SG-Irrigation (Year 2)	0.00 a	9 a	−2.36 a	0.99
FDD-Irrigation (Year 1)	−1.09 a	10 a	−2.60 a	0.99
FDD-Irrigation (Year 2)	0.00 b	8 a	−2.39 a	0.97
SG-Drought (Year 1)	−1.44 a	20 a	−2.66 a	0.99
SG-Drought (Year 2)	−1.31 a	20 a	−2.66 a	0.99
FDD-Drought (Year 1)	−2.60 a	16	2.91	0.99
FDD-Drought (Year 2)[a]	−1.67 b			0.99

[a] A simple linear model provided a better ($p < 0.05$) fit than a bi-linear function.

3.2. Treatment effects on soil water profiles

At the time irrigation was suspended, soil moisture content did not differ significantly ($p < 0.05$) between SG and FDD hybrids throughout the measured soil profile in either of the two years (Fig. 2a) and water content values for the whole profile were close to field capacity (data not shown). The soil water content values corresponding to 20 and 25 days after suspension of irrigation (Year 1 and Year 2, respectively), clearly show a significant ($p < 0.05$) decrease in water content under drought in both hybrids compared to their controls (Fig. 2b). Large decreases in soil water content across the whole of the measured profile took place between 20 or 25 days after suspension of irrigation and achievement of physiological maturity (Fig. 2c), indicating that crops droughted grew under increasing levels of water stress during the whole of the grain-filling period. At PM and under drought conditions, the water content of the 130–170 cm soil layer was significantly ($p < 0.05$) lower for the SG than the FDD hybrid in both years. In Year 1 the SG hybrid also exhibited a lower soil water content in the 50–90 cm soil layer.

Table 2
Mean ($n = 3$) values of GLAI and root respiration at the beginning of anthesis for both hybrids and water supply levels. Values followed by different letters are significantly ($p < 0.05$) different from those of other means within each year-by-variable combination.

	Green leaf area index (GLAI)	Root respiration ($g\,CO_2\,m^2\,soil\,h^{-1}$)
Year 1		
SG-Irrigation	4.1 a	0.56 a
FDD-Irrigation	3.9 a	0.51 a
SG-Drought	4.1 a	0.59 a
FDD-Drought	4.2 a	0.60 a
Year 2		
SG-Irrigation	3.8 a	0.62 a
FDD-Irrigation	4.2 a	0.55 a
SG-Drought	4.3 a	0.56 a
FDD-Drought	4.1 a	0.56 a

3.3. Percentage of live roots (LR%) (minirhizotron images)

Root systems of all treatments had reached a minimum depth of 1.8 m at anthesis, as judged by the minirhizotron images (data not shown). The changes in soil water content between 20 or 25 days after suspension of irrigation and physiological maturity (Fig. 2) suggest that final rooting depth of these crops exceeded 1.8 m. New roots did not appear on the external face of the minirhizotrons in any treatment once anthesis occurred (data not shown) and, in both years, all images obtained using the minirhizotrons contained only white roots at anthesis. LR% values decreased during virtually the entire period of observation with some differences among

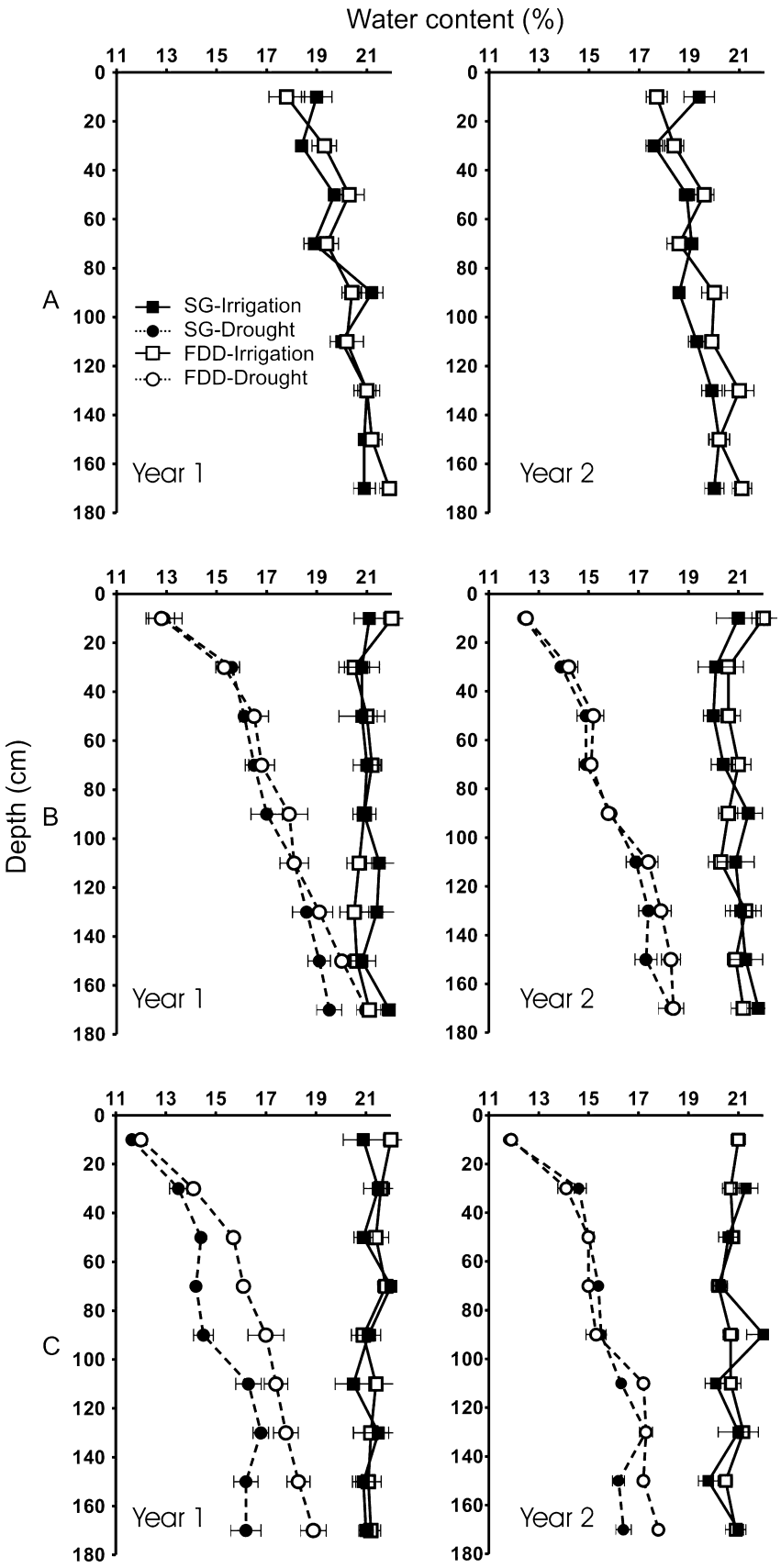

Fig. 2. Soil water content across the 0–180 cm profile for all treatments at the time of suspending irrigation (A), 20 or 25 days later (Year 1 and Year 2, respectively) (B), and at physiological maturity (C). Values for Year 1 are shown in the left-hand panels, those for Year 2 in the right-hand panels. Horizontal bars indicate ±1 S.E. ($n = 3$) and are not shown when smaller than the symbol.

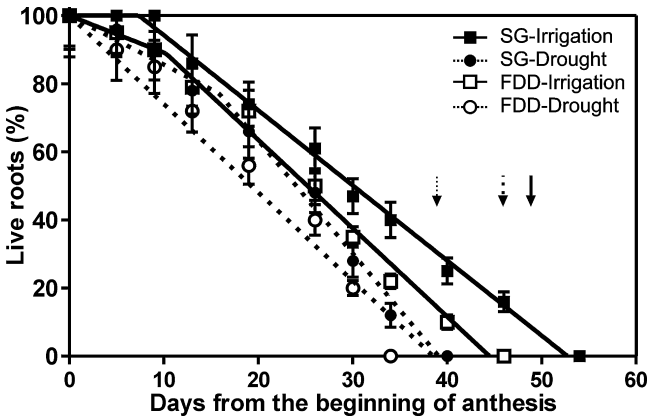

Fig. 3. Dynamics, during grain filling, of the percentage of live roots estimated from minirhizotron images for SG and FDD hybrids under two irrigation treatments. Irrigation in the drought treatment was suspended 8 days before anthesis. Physiological maturity (Stage R.9, Schneiter and Miller, 1981) is indicated with a full arrow (SG and FDD under irrigation), a wide dashed arrow (SG-Drought) and a narrow dotted arrow (FDD-Drought). Data for Year 1. Vertical bars indicate ±1 S.E. ($n = 3$). For parameter values of fitted functions, see Table 1.

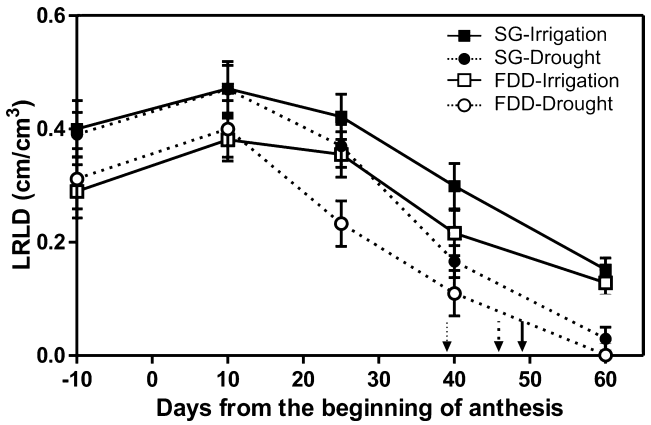

Fig. 4. Dynamics, during grain filling, of live root length density (LRLD) in the 0–40 cm soil layer for SG and FDD hybrids under two irrigation treatments. Irrigation in the drought treatment was suspended 8 days before anthesis. Physiological maturity (Stage R.9, Schneiter and Miller, 1981) is indicated with a full arrow (SG and FDD under irrigation), a wide dashed arrow (SG-Drought) and a narrow dotted arrow (FDD-Drought). Data for Year 1. Vertical bars indicate ±1 S.E. ($n = 3$).

treatments (Fig. 3). In the control treatments, the SG hybrid maintained maximum values of LR% until 7 DFBA, after which time LR% fell continuously. The FDD hybrid showed a root senescence rate of $-1.09\%\,d^{-1}$ from anthesis until 10 DFBA, after which time the LR% values for this hybrid fell at a greater rate. Once rapid rates of senescence became established in both hybrids, these did not differ significantly ($p > 0.10$) between hybrids ($-2.60\%\,d^{-1}$, FDD vs. $2.21\%\,d^{-1}$, SG). A similar pattern was observed in Year 2 (Table 1). The outcome of these different root senescence patterns, however, was that under irrigation the (LR%) of the SG hybrid was significantly ($p < 0.05$) greater than that of the FDD hybrid during the whole observation period (Fig. 3). Under drought conditions, the FDD hybrid showed a senescence rate of $-2.60\%\,d^{-1}$ during the entire measurement period, a value 80.6% greater than that observed in the SG hybrid ($-1.44\%\,d^{-1}$) in the 0–20 DFBA interval. After this time, root senescence rates for both hybrids did not differ significantly ($p > 0.10$). However, the SG hybrid maintained greater LR% values than the FDD hybrid during the entire measurement period due to these significant ($p < 0.05$) differences in the senescence patterns (Fig. 3).

3.4. Live root length density (LRLD)

Samples from the 0–20 cm and the 20–40 cm soil layers showed similar time trends and values for LRLD, so data for both layers were pooled and pooled values for the 0–40 cm layer are shown (Fig. 4). The greatest values were achieved, in both years, at 10 DFBA in both control and drought treatments, and in both years the SG hybrid showed a significantly ($p < 0.05$) greater LRLD than the FDD hybrid [$0.47 \pm 0.048\,cm\,cm^{-3}$ for SG vs. $0.38 \pm 0.036\,cm\,cm^{-3}$, FDD, a 23.7% greater value, data for Year 1]. From this time and until 40 DFBA (a time close to physiological maturity), LRLD decreased more rapidly in the drought treatments than in the controls in both years. The greater values in LRLD for the SG hybrid were also significant ($p < 0.05$) at 25 and 40 DFDA both under irrigation and drought conditions.

3.5. Root respiration

The highest rates of root respiration were observed at anthesis (Fig. 5), with no significant differences ($p > 0.10$) between hybrids or water availability treatments in either of the two years (Table 2). From that time, the respiration rate dynamics of control and water stressed treatments showed very different patterns (Fig. 5 and Table 1). Under control conditions, respiration values remained statistically indistinguishable from initial values until 11 DFBA (FDD) or 18 DFBA (SG), a difference in timing between hybrids that was significant ($p < 0.05$). From these times onwards, respiration began to decrease at a rate that was not significantly different ($p > 0.10$) between hybrids (SG-irrigation = $-0.011\,g\,CO_2\,m^2\,soil\,h^{-1}\,d^{-1}$ and FDD-irrigation = $-0.014\,g\,CO_2\,m^2\,soil\,h^{-1}\,d^{-1}$). Under drought conditions, root respiration rates showed a steep decrease from the beginning of anthesis (a temporal pattern very different from that of the controls), with a significantly ($p < 0.05$) higher rate of decrease in the FDD ($-0.025\,g\,CO_2\,m^2\,soil\,h^{-1}\,d^{-1}$) compared to SG ($-0.017\,g\,CO_2\,m^2\,soil\,h^{-1}\,d^{-1}$) until 18 DFBA. From this time onwards, the rate of decrease in root respiration rate was similar in both hybrids ($-0.007\,g\,CO_2\,m^2\,soil\,h^{-1}\,d^{-1}$). The hastening of the start in the decrease of FDD root respiration under irrigation and the greater initial slope of the decrease in this hybrid under drought, determined that during much of the period from 11 DFBA onwards, SG root respiration was significantly ($p < 0.05$) greater than that of the FDD hybrid, under both levels of water supply.

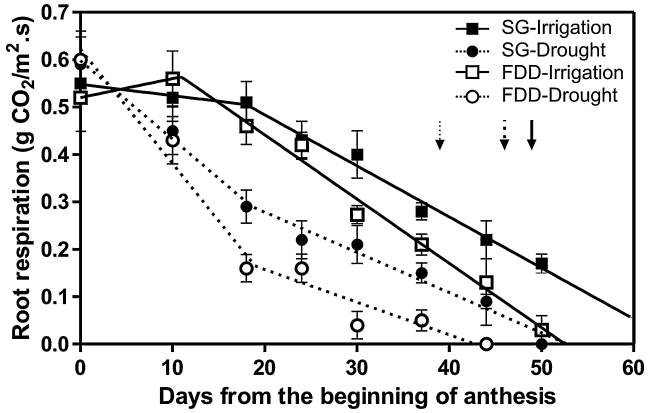

Fig. 5. Dynamics, during grain filling, of root respiration (values standardized to 25 °C) during grain-filling in SG and FDD hybrids under two irrigation treatments. Irrigation in the drought treatment was suspended 8 days before anthesis. Physiological maturity (Stage R.9, Schneiter and Miller, 1981) is indicated with a full arrow (SG and FDD under irrigated situations), a wide dashed arrow (SG-Drought) and a narrow dotted arrow (FDD-Drought). Data from Year 1 ($n = 3$). Vertical bars show ±1 S.E. For parameter values of fitted functions, see Table 1.

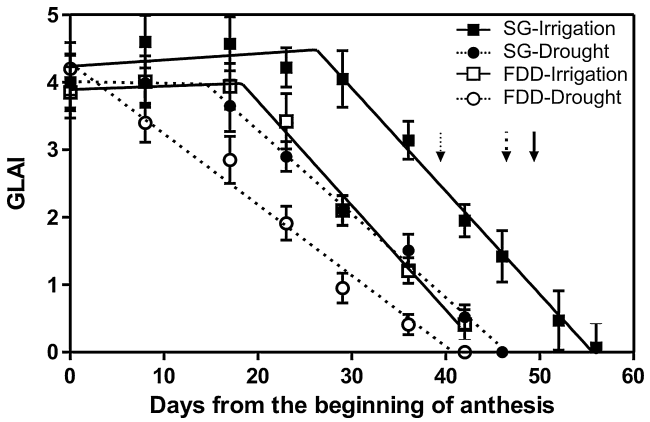

Fig. 6. Dynamics, during grain filling, of GLAI in SG and FDD hybrids grown under two irrigation regimes. Irrigation in the drought treatment was suspended 8 days before anthesis. Physiological maturity (Stage R.9, Schneiter and Miller, 1981) is indicated with full arrow (SG and FDD under irrigation), a wide dashed arrow (SG-Drought) and a narrow dotted arrow (FDD-Drought). Data from Year 1 ($n = 3$). Vertical bars show ±1 S.E. For parameter values of fitted functions, see Table 1.

3.6. Green leaf area index (GLAI)

Under irrigation, both hybrids showed similar GLAI dynamics during grain filling, although these were significant shifted in time (Fig. 6). In the FDD hybrid, GLAI began to decrease at 18 DFBA, while the start of GLAI decrease was delayed until 25 DFBA in the SG hybrid, with similar senescence rate between hybrids. Under drought conditions, GLAI in the FDD hybrid began to decrease as from the beginning of anthesis, at a rate of -0.105 GLAI d^{-1}, while in the SG hybrid this decrease was delayed until 14 DFBA, and thereafter took place at a rate of -0.123 GLAI d^{-1}. These senescence rates did not differ significantly ($p > 0.10$) between hybrids, however, the timing of the beginning of GLAI decrease was significantly ($p < 0.05$) different between hybrids. In the FDD hybrid, water deficit significantly ($p < 0.05$) hastened by 18 days the beginning of GLAI decrease compared with that of the irrigation treatment [0 DFBA (drought) vs. 18 DFBA (irrigation)]. In the SG hybrid, drought significantly ($p < 0.05$) hastened by 11 days the beginning of GLAI decrease [14 DFBA (drought) vs. 25 DFBA (irrigation)]. The final result of these differences in patterns of canopy senescence, was that the SG hybrid showed significantly ($p < 0.05$) greater values of GLAI than FDD, during the entire measurement period under stress conditions and, as from 25 DFBA, under irrigation.

3.7. Sap flow

Under irrigation and in both years (as exemplified for the first year in Fig. 7), the greatest values of sap flow (between 8 mm d^{-1} and 12 mm d^{-1}) occurred during the first 18 DFBA. During this interval, there were significant ($p < 0.05$) differences between hybrids on only 3 days, with the SG hybrid exhibiting the greater sap flow rate. From 18 DFBA and until the end of the measurements, the differences between hybrids tended toward becoming consistently and significantly ($p < 0.05$) favorable to the SG hybrid, with the exception of days with low atmospheric demand. Under drought conditions (Fig. 7b), the highest values for the SG hybrid were registered during the first 17 DFBA, with values between 9 mm d^{-1} and 11.5 mm d^{-1}, while for the FDD hybrid the highest values ranged between 7.0 mm d^{-1} and 10.6 mm d^{-1}. Water deficit generated significant ($p < 0.05$) differences between hybrids on high atmospheric demand days during the 0–17 DFBA interval, increasing (with respect to control values) the number of days in that interval for which SG sap flow rate was significantly ($p < 0.05$) higher than that of the FDD hybrid (contrast Fig. 7A and B). After 17 DFBA and under drought, significantly higher flow rates in the SG hybrid were registered in the majority of the remaining days of the observation period (Fig. 7B).

When the differences between irrigation and drought treatments are compared separately for both hybrids (Fig. 7C and D), it is clear that water stress had a greater impact on the FDD hybrid, because from 4 DFBA, the drought treatment showed a significantly ($p < 0.05$) lower sap flow rate than the corresponding control (Fig. 7D). By contrast, in the SG hybrid (Fig. 7C), significantly ($p < 0.05$) lower sap flow rates under drought in days of high atmospheric demand only became evident after 15 DFBA.

4. Discussion

Values for soil water content in the drought treatment, measured at 20 days (Year 1) or 25 days (Year 2) after the interruption of irrigation, showed a sharp decline in most of the profile (Fig. 2). These dates are close to the times when most of the other measured response variables began to show significant differences between irrigation and drought treatments, and/or between hybrids, and/or marked the beginning of sharp changes in value with respect to initial values (Figs. 3–7 and Table 1). In addition, the patterns of canopy senescence under both drought and irrigation conditions showed clear differences between SG and FDD hybrids (Fig. 6). Finally, under irrigation, there were no developmental differences between genotypes in time to flowering or duration of grain-filling which might have confounded the dynamics of the various response variables that were followed. Thus, the differences in the response patterns of the various variables between treatments can be ascribed, with confidence, to the effects and interactions between water supply regime and hybrid canopy senescence pattern.

The response patterns for LR% (Fig. 3), LRLD (in the 0–40 cm layer) (Fig. 4) and root respiration (Fig. 5) were consistent across variables and treatments, and indicate that both water regime and hybrid canopy senescence patterns had substantial effects on the dynamics of these variables. If the dates of physiological maturity (PM) and the timings at which each of these response variables fell to zero are compared (Figs. 3–5) in all treatment combination, these two dates are, in general, quite close in time, although in the SG-irrigation treatment GLAI and root respiration fell to zero some days after PM.

Our data, for irrigation conditions, are consistent with those of Sadras et al. (1989), but these authors made their root system measurements on only two occasions during grain filling. With respect to root respiration (Fig. 5), the post-anthesis patterns registered in the present experiments are consistent with those observed by Hall et al. (1990) for their irrigation and drought treatments. Thus, our results expand these previous findings by demonstrating differing responses in hybrids with contrasting canopy senescence patterns. The responses to drought of root respiration became evident before those of LR% and LRLD (Figs. 3, 4 and 6); this may reflect the rapid drying of the first 0.20 m soil layer. However, the overall temporal pattern of root respiration dynamics was similar to those exhibited by the other root variables.

The GLAI dynamics during the grain-filling phase under irrigation for both hybrids was similar to those found by de la Vega et al. (2011) under non-limiting water supply conditions for hybrids exhibiting SG and FDD canopy senescence patterns. Our results extend those of de la Vega et al. (2011) by demonstrating that drought accelerates the onset of canopy senescence and by showing that the difference between hybrids is maintained under this condition (Fig. 6). Similar results were observed in sorghum (Borrell et al., 2000) where higher leaf areas were recorded in a SG hybrid under water stress conditions during the grain-filling phase.

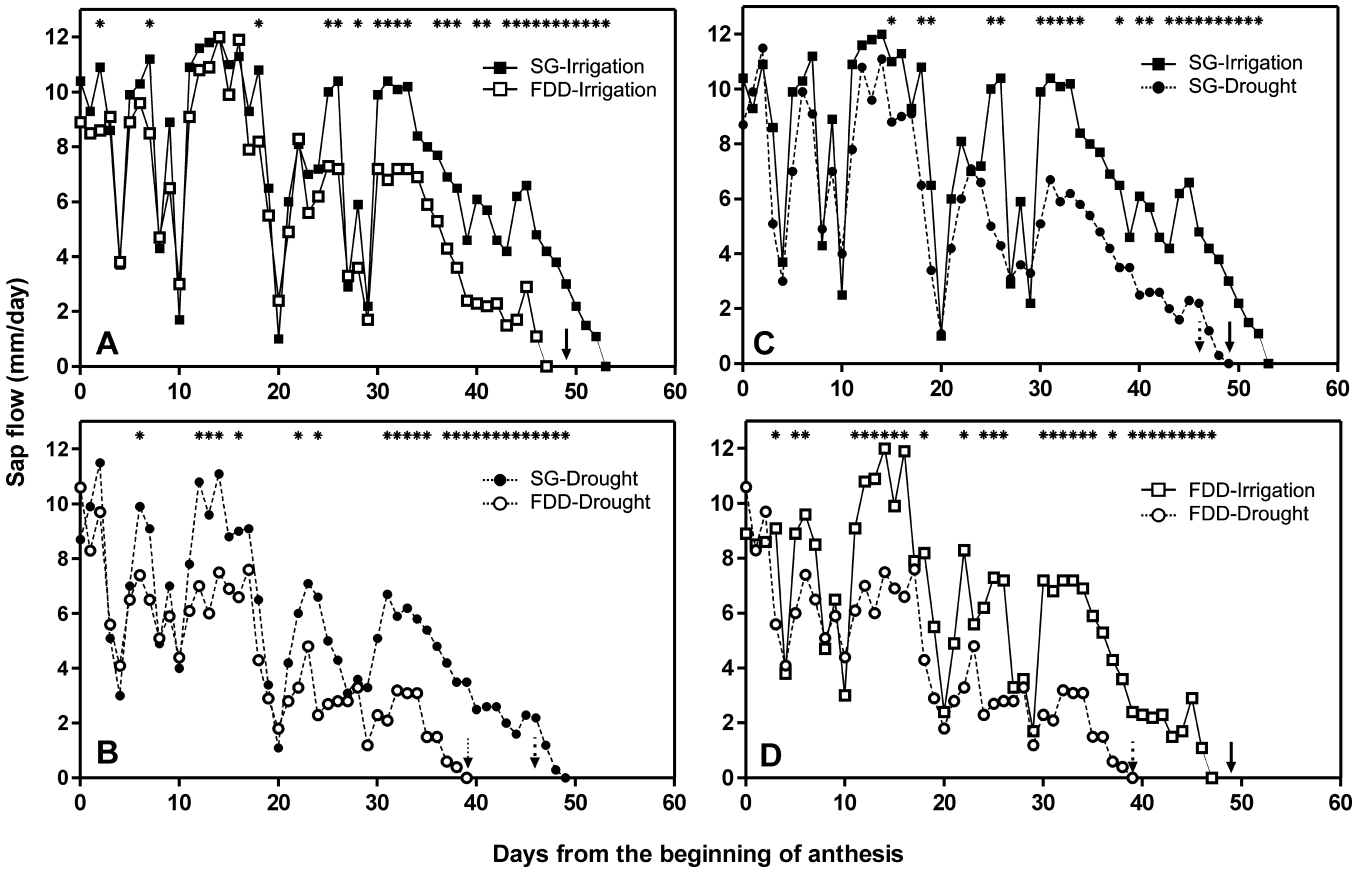

Fig. 7. Dynamics, during grain filling, of sap flow in SG and FDD hybrids grown under two irrigation regimes. Irrigation in the drought treatment was suspended 8 days before anthesis. Left-hand panels contrast different hybrids for the same water supply regime (A, irrigation; B, drought) and right-hand panels contrast different water supply regimes for the same hybrid (C, SG; D, FDD). Physiological maturity (Stage R.9, Schneiter and Miller, 1981) is indicated with a full arrow (SG and FDD under irrigated situations), a wide dashed arrow (SG-Drought) and a narrow dotted arrow (FDD-Drought). Data for Year 1. * indicates significant ($p < 0.05$) differences between treatments for values obtained on the indicated days.

Contrasting the timings of the beginning of canopy senescence (Fig. 6) and root senescence (Figs. 3–5) shows that the second precedes the first by an interval, which ranged from 3 to 14 days in these experiments, depending on water supply regime and hybrid. This precedence in root senescence initiation leads to the question of whether such senescence might be involved in the triggering of canopy senescence mediated, for example, by reductions in the synthesis of cytokinins in root tips (Smigocki, 1991). Another question raised by these results is whether the delay in the onset of root senescence in the SG hybrid could be influenced by a greater contribution of photo-assimilates from the canopy to the roots. This might help sustain N uptake during grain filling, which could imply less N mobilized from leaves, resulting in lower leaf senescence rate (Rajcan and Tollenaar, 1999; van Oosterom et al., 2010). The answers to these questions await further research focused on resolving them.

Our measurements of daily sap flow throughout the grain-filling phase are the first obtained for sunflower. This methodology has already been used in annual crops such as cotton (Ham et al., 1990; Dugas et al., 1994) and maize (Jara et al., 1998). The data of Fig. 7 clearly show the higher water uptake capacity of the SG hybrid throughout the grain-filling period, both under irrigation and drought. The highest values reached in our experiments are consistent with estimates, made by Karam et al. (2007) using lysimeters sited within 1-ha plots, of transpiration values for sunflower hybrids under irrigation of up to 13 mm d^{-1} around anthesis.

The relationship between estimations of the relative (to the maximum) proportions of apparently living roots in the first 40-cm layer obtained using minirhizotron images and those obtained using the 2,3,5-triphenyl tetrazolium chloride method could be fitted with a linear and significant ($p < 0.05$) regression ($r^2 = 0.89$), with a slope and an intercept which did not differ significantly ($p < 0.05$) from 1 and 0, respectively (Fig. 8). This result is important because the images taken with the minirhizotron are nondestructive, allow sampling at shorter time intervals and are much less laborious than the destructive sampling method followed by staining the roots. While this degree of agreement between the two techniques is encouraging, it is important to note that it is not a full proof that brown roots or roots that do not stain with TTC are completely non-functional.

To summarize, our results have served to confirm and strengthen previous observations by Sadras et al. (1989) and Hall et al. (1990) relating to root functionality loss after anthesis in sunflower. Sap flow rate was the variable which most rapidly and dramatically responded to the four treatments (especially when comparing irrigation vs. drought), preceding the changes observed in other root functionality indicators (LRLD, the change of root color in images taken with the minirhizotrons and root respiration). It is important to note that the sap flow rate in the FDD hybrid under drought decreased much more (in proportion) than GLAI (compare Figs. 6 and 7b for the periods 8–15 and 20–30 DFBA). These differences are an incentive to study the intrinsic effects of post-anthesis canopy dynamics on grain yield of sunflower under drought conditions. Although these response variables undoubtedly interact with each other, xylem flow is clearly the most sensitive and the best integrator of the whole effect. To our knowledge, this is the first

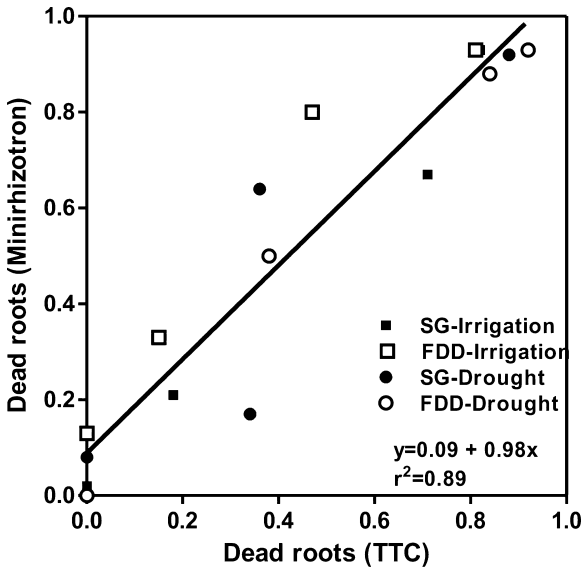

Fig. 8. Relationship between the proportions (relative to the maximum value reached) of dead roots estimated by the TTC and the minirhizotron image techniques for the 0–40 cm soil layer. Each point is the mean of three replicates. Data for Year 1.

study to demonstrate an advantage, throughout the grain-filling phase and on a daily basis, of the SG habit over the FDD habit in terms of water capture by the root system under both irrigation and water deficit conditions. Heretofore, the little information in literature about the SG effects on drought tolerance used, as indicators, water extraction over long periods, grain yield and/or green leaf area (e.g., Borrell et al., 2000). Finally, this study shows that the process of root senescence precedes canopy senescence, a fact consistent with the possibility that the roots may play a key role in controlling leaf senescence and in determining differences between SG and FDD hybrids. Further studies, focused on the relationships and signaling between roots and shoots are needed in this context.

Acknowledgements

We thank Advanta Semillas SAIC for access to seed of the hybrids used in the experiments. This research was supported by grants from UBACyT (UBA G064) and FONCyT (PICT 2006-02098). SL was in receipt, successively, of FONCyT and CONICET scholarships and AJH is a member of CONICET, the National Research Council of Argentina.

References

Angadi, S.V., Entz, M.H., 2002. Root system and water use patterns of different height sunflower cultivars. Agron. J. 94, 136–145.
Barley, K.P., 1970. The configuration of the root system in relation to nutrient uptake. Adv. Agron. 22, 59–201.
Borrell, A.K., Hammer, G.L., Henzell, R.G., 2000. Does maintaining green leaf area in sorghum improve yield under drought? Dry matter production and yield. Crop Sci. 40, 1037–1048.
Chimenti, C.A., Giuliano, J., Hall, A., 2004. Osmotic adjustment, its effects on yield maintenance under drought in sunflower. In: 16th International Sunflower Conference, pp. 261–266.
Chimenti, C.A., Marcantonio, M., Hall, A.J., 2006. Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. Field Crops Res. 95, 305–315.
Christopher, J.T., Manschadi, A.M., Hammer, L., Borrel, K., 2008. Developmental and physiological traits associated with high yield and stay-green phenotype in wheat. Aust. J. Agric. Res. 59, 354–364.
Costa, C., Dwyer, L.M., Zhou, X., Dutilleul, P., Hamel, C., Reid, L.M., Smith, D.L., 2002. Root morphology of contrasting maize genotypes. Agron. J. 94, 96–101.
Dardanelli, J.L., Bachmeier, O.A., Sereno, R., Gil, R., 1997. Rooting depth and soil water extraction patterns of different crops in a silty loam Haplustoll. Field Crops Res. 54, 29–38.
de la Vega, A., Cantore, M., Sposaro, M., Trápani, N., López Pereira, M., Hall, A., 2011. Canopy stay-green and yield in non-stressed sunflower. Field Crops Res. 121, 175–185.
Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2010. InfoStat version 2010. InfoStat Group, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba, Argentina.
Dugas, W.A., Heuer, M.L., Hunsaker, D., Kimball, B.A., Lewin, K.F., Nagy, J., Johnson, M., 1994. Sap flow measurements of transpiration from cotton grown under ambient and enriched CO_2 concentrations. Agric. Forest Meteorol. 70, 231–245.
GraphPad Software, Inc., 2007. GraphPad Prism v 5.00 for Windows. GraphPad Software, Inc., San Diego, CA, USA.
Gregory, P.J., McGowan, M., Biscoe, P.V., Hunter, B., 1978. Water relations of winter wheat. 1. Growth of the root system. J. Agric. Sci. (Camb.) 91, 91–102.
Hall, A.J., Connor, D.J., Whitfield, D.W., 1990. Root respiration during grain filling in sunflower: the effects of water stress. Plant Soil 121, 57–66.
Ham, J.M., Heilman, J.L., Lascano, R.J., 1990. Determination of soil water evaporation and transpiration from energy balance and stem flow measurements. Agric. Forest Meteorol. 52, 287–301.
Hammer, G., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Pszkiewicz, S., Cooper, M., 2009. Can changes in canopy and/or root system architecture explain historical maize yield trends in U.S. corn belt? Crop Sci. 49, 299–312.
Jara, J., Stockle, C.O., Kjelgaard, J., 1998. Measurement of evapotranspiration and its components in a corn (*Zea mays* L.) field. Agric. Forest Meteorol. 92, 31–145.
Johnson, M.G., Tingey, D.T., Phillips, D.L., Store, M.J., 2001. Advancing fine root research with minirhizotrons. Environ. Exp. Bot. 45, 263–289.
Karam, F., Lahoud, R., Masaad, R., Cabalan, R., Breidi, J., Chalita, C., Rouphael, Y., 2007. Evapotranspiration, seed yield and water use efficiency of drip irrigated sunflower under full and deficit irrigation conditions. Agric. Water Manage. 90, 213–223.
Kashiwagi, J., Krishnamurthy, L., Crouch, J.H., Serraj, R., 2006. Variability of root length density and its contribution to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Field Crops Res. 95, 171–181.
Kirkegaard, J.A., Lilley, J.M., 2007. Root penetration rate: a benchmark to identify soil and plant limitation to rooting depth in wheat. Aust. J. Agric. Res. 47, 590–602.
Kjelgaard, J., Stockle, C.O., Black, R.A., Campbell, G.S., 1996. Measuring sap flow with the heat balance approach using constant and variable heat inputs. Agric. Forest Meteorol. 85, 239–250.
Lee, E.A., Tollenaar, M., 2007. Physiological basis of successful breeding strategies for maize grain yield. Crop Sci. 47, 202–215.
Lilley, J.M., Fukai, S., 1994. Effect of timing and severity of water deficit on four diverse rice cultivars. I. Rooting pattern and soil water extraction. Field Crops Res. 37, 205–213.
Luquez, V.M., Guiamét, J.J., 2001. Effects of the 'stay green' genotype GGd1d1d2d2 on leaf gas exchange, dry matter accumulation and seed yield in soybean (*Glycine max* L. Merr.). Ann. Bot. 87, 313–318.
Mace, E.S., Singh, V., Van Oosterom, E.J., Hammer, G.L., Hunt, C.H., Jordan, D.R., 2012. QTL for nodal root angle in sorghum (*Sorghum bicolor* L. Moench) co-locate with QTL for traits associated with drought adaptation. Theor. Appl. Genet. 124, 97–109.
Manschadi, A.M., Graeme, L., Hammer, L., 2008. Genotypic variation in seedling root architectural traits and implication for drought adaptation in wheat (*Triticum aestivum* L.). Plant Soil 303, 115–129.
Majdi, H., 1996. Root sampling methods – applications and limitations of the minirhizotron technique. Plant Soil 185, 255–258.
Majdi, H., Damm, E., Nylund, J.-E., 2001. Longevity of mycorrhizal roots depends on branching. New Phytol. 150, 195–202.
Majdi, H., Pregitzer, K., Morén, A., Nylund, J.E., Agreen, G., 2005. Measuring fine root turnover in forest ecosystems. Plant Soil 276, 1–8.
Meinke, H., Hammer, G.L., Want, P., 1993. Potential soil water extraction by sunflower on a range of soils. Field Crops Res. 32, 59–81.
Mengel, D.B., Barber, S.A., 1974. Development and distribution of the corn root system under field conditions. Agron. J. 66, 341–344.
Pereyra, V.R., Farizo, C., Cardinali, F., 1982. Estimation of leaf area on sunflower plants. In: Proc. 10th Int. Sunflower Conf. Surfers Paradise, Australia, March 14–18, 1982, International Sunflower Association, Paris, pp. 21–23.
Rajcan, I., Tollenaar, M., 1999. Source:sink ratio and leaf senescence in maize. II. Nitrogen metabolism during grain filling. Field Crops Res. 60, 255–265.
Robertson, M.J., Fukai, S., Ludlow, M.M., Hammer, G.L., 1993. Water extraction by grain sorghum in a sub-humid environment. II. Extraction in relation to root growth. Field Crops Res. 33, 99–112.
Rondanini, D.P., Savin, R., Hall, A.J., 2007. Estimation of physiological maturity in sunflower as a function of fruit water concentration. Eur. J. Agron. 26, 295–309.
Sadras, V.O., Hall, A.J., Trapani, N.T., Vilella, F., 1989. Dynamics of rooting and root length:leaf area relationships as affected by plant population in sunflower crops. Field Crops Res. 22, 45–57.
Schneiter, A.A., Miller, J.F., 1981. Description of sunflower growth stages. Crop Sci. 21, 901–903.
Serraj, R., Krishnamurthy, L., Kashiwagi, J., Kumar, J., Chandra, S., Crouch, J.H., 2004. Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. Field Crops Res. 88, 115–127.
Smigocki, A.C., 1991. Cytokinin content and tissue distribution in plants transformed by a reconstructed isopentenyl transferase gene. Plant Mol. Biol. 16, 105–115.
Steinberg, S., van Bavel, C.H.M., McFarland, M.J., 1989. A gauge to measure mass flow rate of sap in stems and trunks of woody plants. J. Am. Soc. Hortic. Sci. 114, 466–472.

Sturite, L., Henriksen, T.M., Breland, T.A., 2005. Distinguishing between metabolically active and inactive roots by combined staining with 2,3,5-triphenyltetrazolium chloride and image color analysis. Plant Soil 271, 75–82.
Vamerali, T., Bona, S., Mosca, G., 2003. Fibrous root turnover and growth in sugar beet (*Beta vulgaris* var. *saccharifera*) as affected by nitrogen shortage. Plant Soil 255, 169–177.
Vamerali, T., Guarise, M., Ganis, A., Mosca, G., 2009. Effect of water and nitrogen management on fibrous root distribution and turnover in sugar beet. Eur. J. Agron. 31, 69–76.
van Oosterom, E.J., Chapman, S.C., Borrell, A.K., Broad, I.J., Hammer, G.L., 2010. Functional dynamics of the nitrogen balance of sorghum. II. Grain filling period. Field Crops Res. 115, 29–38.