Dry matter and water dynamics of wheat grains in response to source reduction at different phases of grain filling

Claudia I. Harcha1*, and Daniel F. Calderini1

Agreement that wheat (Triticum aestivum L.) is scarcely limited by source of assimilates during grain fill has not been confirmed when source was drastically decrease in high yield potential environments. Dry matter (DM) and water dynamics of growing grains being possibly able to explain grain weight (GW) responses to source-sink ratios (S-S ratios) in these conditions. Objectives were to evaluate response of GW to S-S ratios at different phases during grain fill, and relationship between DM and grain water content in response to different S-S ratios. Wheat was sown at field conditions during 2004-2005 and 2005-2006 growing seasons. Four S-S ratios were assessed: Control without S-S modification (C), S-S ratios from anthesis (At) +12 d to physiological maturity (ShAll), S-S ratios from At +12 d to first half of grain fill (Sh1st) and from second half of grain fill to maturity (Sh2nd). Thousand grain weight (TGW), GW at individual positions (IGW), stabilized grain water content (SGWC) and grain filling rate (GFR) were measured. TGW sensitivity to S-S ratios varied according to length of treatment and its timing, i.e. ShAll, Sh1st, and Sh2nd treatments reduced TGW by 48%, 26%, and 22%, respectively. These reductions were little higher when IGW were evaluated in ShAll (i.e. 53%) and Sh1st (i.e. 33%) treatments and lower in Sh2nd (i.e. 12%). SGWC sensitivity was lower than that of IGW across S-S ratios (e.g. ShAll 27%, Sh1st 22%, and Sh2nd 5%). However, close association between IGW and SGWC (R² = 0.78, p ≤ 0.001) and between GFR and SGWC (R² = 0.98, p ≤ 0.001) was found regardless of S-S ratios and seasons.

Key words: Grain filling, grain water content, grain weight, shading, source-sink ratio, Triticum aestivum.

INTRODUCTION

The knowledge of plant constraints limiting yield potential during the grain filling period is necessary to develop breeding and management strategies aimed at increasing harvestable yields. Currently, the assessment of different source-sink ratios during grain filling has shown that wheat (Triticum aestivum L.) is mainly sink-limited (Slafer and Savin, 1994; Borrás et al., 2004; Zhang et al., 2010); however, Sandaña et al. (2009) working in a high-yield environment, found that wheat is more affected by the source shortage than generally assumed. The physiological bases accounting for the responses reported by Sandaña et al. (2009) are especially important in southern Chile, taking into account that crop production systems are based on temperate cereals, which are favored by high grain weight potential. Thus, a better understanding of the causes behind the higher-than-expected sensitivity of wheat-to-source reduction could improve the knowledge of physiological traits controlling grain weight of wheat in favorable environments.

Several studies have assessed the effect of different source-sink ratios on wheat yield and grain weight (Fischer and Laing, 1976; Fischer, 1985; Savin and Slafer, 1991; Slafer and Savin, 1994; Borrás et al., 2004; Sandaña et al., 2009; Serrago et al., 2013); but less information is available about the mechanisms involved in the source-sink response. Given that grain water content has been found to be a key trait in determining wheat grain weight (Egli, 1990; Saini and Westgate, 2000; Pepler et al., 2006, Hasan et al., 2011), and considering the close balance between water and DM in growing grains (Schnyder and Baum, 1992; Calderini et al., 2000; Saini and Westgate, 2000; Pepler et al., 2006), studying grain water content in response to the source-sink reduction during grain filling could provide useful information on grain growth and its responsiveness to source constraints of wheat as has been shown in maize (Borrás et al., 2003; Borrás and Westgate, 2006).

The sensitivity of wheat to source-sink ratios at different times during the grain filling has not been fully investigated in wheat. This aspect is clearly relevant considering that wheat could be affected by source shortage at different moments during grain filling, thereby forcing the crop to use reserves, which could be insufficient to fill the grains, particularly under high yield conditions (Calderini et al., 2006; Sandaña et al., 2009; Serrago et al., 2013). Therefore, the sensitivity of wheat to source reductions...
Entire bars show the crop season from emergence to physiological maturity in the control treatment. 1 January is assigned to 1 in the abscissa axis, representing consecutive days.

Figure 1. Crop phenology of wheat ‘Otto Baer’ and ‘Huayún-INIA’ from emergence to physiological maturity. Bars show emergence to anthesis (grey bars), anthesis to beginning of source-sink reductions (white bars), and source-sink reductions in control (beginning of source-sink reductions to physiological maturity, until end bar), entire cycle (grated and black bar coupled), first half (grated bars), and second half (black bars) during 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons.

MATERIALS AND METHODS

Site, treatments, and experimental design
Three field experiments evaluating spring wheat were carried out at the experimental field of the Universidad Austral de Chile in Valdivia (39°47’ S, 73°14’ W, 19 m a.s.l.), Chile. Experiments were conducted in two successive seasons, during the 2004-2005 (at two sowing dates, called herein Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons in a Typic Hapludand soil. The treatments consisted of four source-sink ratios, i.e., control without source-sink ratio modification (C), lower source-sink ratio from anthesis (At) +12 d to physiological maturity (PM) (hereafter ShAt), source reduction from At +12 d to the first half of grain filling (Sh1st), and from the second half of grain filling to PM (Sh2nd). Figure 1 and Table 1 provide a complete picture of the timings of treatments and climatic variables. The experiments were arranged in a split-split-plot design with three replicates, where experiments were assigned to the main plots, cultivars to sub-plots, and source-sink treatments to sub-sub-plots.

Management of the experiments
Two wheat cultivars (Huayún-INIA and Otto Baer) were sown on 25 August 2004 (Experiment 1), 18 October 2004 (Experiment 2), and 26 September 2005 (Experiment 3). Plots consisted of six rows, 2 m long, and 0.15 m apart. Seed rates were 250, 320, and 350 seeds m⁻² in Experiments 1, 2, and 3, respectively. Between 1 and 3-mo prior to sowing, 7 Mg CaCO₃ ha⁻¹ were applied on plots to avoid Al toxicity, which is usual in the acidic soils of southern Chile (Typic Hapludand). According with soil
Table 1. Treatment duration (days and degree-days) and cumulative intercepted photosynthetically active radiation (CI-PAR) from anthesis to physiological maturity recorded under control and source-sink reduction treatments (S-S$_{sinks}$) during the entire (Sh$_{pm}$, first-half (Sh$_{1st}$) and second-half (Sh$_{2nd}$) phases of the grain filling period of wheat ‘Otto Baer’ and ‘Huayún-INIA’ during the 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons.

<table>
<thead>
<tr>
<th>Cultivar</th>
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<th>Treatment duration</th>
<th>CI-PAR</th>
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<tr>
<td></td>
<td></td>
<td>Experiment 1</td>
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</tr>
<tr>
<td>Otto Baer</td>
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<td>d °C d MJ m$^2$</td>
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<td>578</td>
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<tr>
<td>Sh$_{1fr}$</td>
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<td>646</td>
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<td>422</td>
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<tr>
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<td>22</td>
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<td>d °C d MJ m$^2$</td>
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analysis and based upon local recommendations (Instituto de Ingeniería Agraria y Suelos, Universidad Austral de Chile), plots were fertilized with 80 kg K$_2$O ha$^{-1}$ and 150 kg P$_2$O$_5$ ha$^{-1}$ at sowing and with 200 kg N ha$^{-1}$ split into three applications, 80 kg N ha$^{-1}$ at sowing; 60 kg N ha$^{-1}$ at tillering, and 60 kg N ha$^{-1}$ at stem elongation initiation in Experiments 1 and 2. In Experiment 3, 60 kg K$_2$O ha$^{-1}$ and 220 kg P$_2$O$_5$ ha$^{-1}$ were supplied at sowing and 250 kg N ha$^{-1}$ split into two applications, 140 and 110 kg N ha$^{-1}$ were added at sowing and tillering, respectively.

Plots were irrigated five times during grain filling with 12.5 L m$^{-2}$ to supplement rainfall (679, 310, and 562 mm during the crop season in Experiments 1, 2, and 3, respectively). Weeds were controlled by hand and mechanically in Experiments 1 and 2, whereas in Experiment 3, 35 g m$^{-2}$ of dazomet was applied to prevent weeds and fungi before sowing and 1.5 L ha$^{-1}$ of bentazon to prevent broadleaf weeds after emergence (Em). *Septoria tritici* was prevented with the subsequent addition of three active ingredients (fenpropimorph-kresoxim-methyl-epoxiconazole) in doses of 1.0 L ha$^{-1}$.

**Manipulation and calculation of the source-sink ratio**

Source-sink ratios were reduced by setting black nets that intercepted 90% of incident radiation at 20 cm above the top of the canopy covering plots in their entirety in all the experiments. Nets had little effect on air temperature as the average temperature under the nets was 1 °C lower than in controls (Sandaña et al., 2009). Aimed at reducing the source of assimilates only during the linear growth phase of the grain treatments, Sh$_{1fr}$ and Sh$_{2nd}$ began At +12 d (Fischer, 1985; Savin and Sláfer, 1991).

To calculate relative change of the source due to treatments, the methodology proposed by Sláfer and Savin (1994) and by Borràs et al. (2004), and used in Sandaña et al. (2009) was followed. Briefly, this method considers the fraction of cumulative intercepted radiation by nets as a proportion of the entire cumulative intercepted radiation during the grain filling period. The values of grain filling duration between anthesis and physiological maturity (PM) and the cumulative intercepted photosynthetically active radiation (CI-PAR, MJ m$^{-2}$) in the assessed treatments are shown in Table 1.

**Phenological, crop, and grain measurements and calculations**

Phenological stages were recorded in experiments using the scale proposed by Zadoks et al. (1974). Grain filling duration was calculated in thermal time units (°C d as in Monteith, 1984) at a base temperature of 0 °C as in previous studies (Hay and Kirby, 1991; Sláfer et al., 1994). Thermal time was estimated as the sum of daily average temperature ([maximum temperature + minimum temperature]/2) between At-PM.

In the three experiments the time course of grain DM and water dynamics was followed after anthesis by measuring fresh and dry weights by sampling plots twice a week from At-PM. Grains from positions 1 (G$_1$), 2 (G$_2$), and 3 (G$_3$) of two central spikelets of two spikes per plot were sampled; G$_1$, G$_2$ and G$_3$ were named considering the closest and furthest distance to the rachis, respectively. All these grains were measured to evaluate grain weight of individual positions of central spikelets (IGW) and stabilized grain water content (SGWC).

To standardize the timing of sampling, grains were harvested at noon (12:00 h) during the entire grain filling period. The follow-up of fresh weight of grain samples (GFW) was measured immediately after harvest and IGW was quantified after drying samples for 48 h at 60 °C. IGW was calculated using a bilinear model (0.80 ≥ r$^2$ ≥ 0.99) subjected to boundary conditions (i.e. GW is described by two equations with one break point) as in Calderini et al. (1999). Grain filling durations (GFD in °C d and days) were calculated from the break point, which is the time from At-PM, and grain filling rate (mg °C d$^{-1}$, GFR) was estimated from the regression slopes (Miralles et al., 1996).

To summarize the impact of source-sink treatments on grains, IGW within the spikelet was calculated as the average of all grain measured positions. In addition, the relative individual grain weight (RIGW) was calculated.
as the ratio between dry weights of grains measured at time 1 and IGW at PM.

The dynamic of grain water concentration (relative grain water content, RGWC) and the timing when water content stabilizes (SGWC) were calculated using a trilinear model similar to that described by Pepler et al. (2006) and used by Lizana et al. (2010). The hydric plateau duration (ºC d, HPD) between the time when SGWC is reached and grain PM was derived from this model. Furthermore, the trilinear model delivers the SGWC used to calculate relative (%) grain water content (RGWC on a fresh weight basis) as the ratio between absolute grain water content (AGWC) and SGWC expressed as percentage. The AGWC (mg) was calculated as the difference between grain fresh weight and individual grain dry weight (GFW - IGW). The bi- and trilinear models were fitted by using the iterative optimization routine of TBL Curve V 2.0 (Jandel Scientific, 1991).

To evaluate the relationship between IGW and SGWC for each cultivar × source-sink ratio, a two-equation regression model was used:

\[ IGW = \alpha + \beta \text{ SGWC} \quad \text{if (SGWC } \leq \text{ GWC)} \quad [1] \]
\[ IGW = \alpha + \beta \text{ SGWC} + \gamma \text{ GWC} \quad \text{if (SGWC } > \text{ GWC)} \quad [2] \]

where \( \alpha \) is the intercept (mg), \( \beta \) is the grain filling rate per unit of GWC decrease (mg %\(^{-1}\)), \( \gamma \) is GWC stable grain water content when grain reaches IGW (%), and SGWC is stabilized grain water content (%).

At harvest, plants from the central row of each plot were sampled in 1 m long of the central row of plots only in Experiments 1 and 3. After that, spikes were threshed and their grains were weighed with an analytical balance (XP205DR, Mettler Toledo, Greifensee, Switzerland) to evaluate grain yield. Dry weights were measured after drying samples at 60 ºC for 48 h. Thousand grain weight (TGW) of each sample was estimated by three random subsets of grains (100 grains each) per plot and grain number (GN) was calculated as the ratio between grain yield and TGW. Unfortunately, the 1 m long plant samples were not taken at PM in Experiment 2 to avoid biased results due to bird damage.

Statistical analyses and transformations
ANOVA was performed on the experiments within each cultivar and source-sink ratio. Differences in TGW, IGW, SGWC, GFR, GFD, and HPD among source-sink ratios within each crop and experiment were compared using a Tukey test. In order to carry out a statistical analysis of the relative changes in yield, GN and TGW, these values were transformed by arcsin \( \sqrt{\%}/100 \) (Sokal and Rohlf, 1995).

RESULTS

Environmental conditions and crop phenology in Experiments 1, 2, and 3
Similar climatic conditions were registered in the experiments between emergence and At (12, 15, and 13 ºC, and 7, 10, and 9 MJ m\(^{-2}\) d\(^{-1}\) in Experiments 1, 2, and 3, respectively) and during At-PM (16, 18 and 17 ºC, and 11, 12, and 12 MJ m\(^{-2}\) d\(^{-1}\) in Experiments 1, 2, and 3, respectively). The control plots of cultivars showed similar phenology, anthesis dates were reached at 86, 58, and 73 d after emergence in Experiments 1, 2, and 3, respectively, averaged across the cultivars (Figure 1). The grain filling period lasted for 52 and 37 d in ‘Otto Baer’ and ‘Huayún-INIA’, respectively in Experiment 1, while 44 d were recorded in Experiments 2 and 3 (Figure 1 and Table 1).

Duration of shading treatments both in days and thermal time units are shown in Table 1. In the Sh\(_{All}\) treatment, black nets covered the plots between 61% and 78% of the grain filling period in the control treatment of ‘Otto Baer’ and between 65% and 77% in ‘Huayún-INIA’ (Table 1). When thermal time units were used instead of days, these treatments showed similar durations (Table 1). The Sh\(_{1st}\) treatment was imposed between 33% and 39% of days relative to the control in ‘Otto Baer’ and between 30% and 43% in ‘Huayún-INIA’. In thermal time units, the Sh\(_{1st}\) treatment accounted for 32% in Experiment 1 and 38% in Experiments 2 and 3 averaged across both cultivars regarding their similar phenology (Table 1). In Sh\(_{2nd}\), the relative period of source reduction was similar in days and thermal time units (i.e. between 30% and 41% of the grain filling period across cultivars) to Sh\(_{1st}\) (Table 1).

Cumulative intercepted radiation (CI-PAR) during grain filling was similar between cultivars in the control treatments except in ‘Huayún-INIA’ during the Experiment 1 as this cultivar reached PM earlier than ‘Otto Baer’ (Table 1). The Sh\(_{All}\) treatment decreased (CI-PAR) between 55% and 69% relative to the control in both cultivars and across the experiments, taking into account that the nets were set at At +12 d. The Sh\(_{1st}\) and the Sh\(_{2nd}\) treatments reduced this variable to a similar extent, i.e. between 30% and 33% relative to the controls, respectively, across cultivars and experiments (Table 1).

Effect of the source-sink ratio on grain yield, grain number, and thousand grain weights
When plant samples were harvested at PM, i.e. in Experiments 1 and 3, grain yield of controls ranged from 1025 to 1243 g m\(^{-2}\) (‘Otto Baer’ from 1025 to 1177 g m\(^{-2}\) and ‘Huayún-INIA’ from 1243 to 1035 g m\(^{-2}\) in Experiments 1 and 3, respectively). The sensitivity of grain yield depended on the source-sink treatment (\( p \leq 0.001 \)) as Sh\(_{All}\) was the treatment with the greatest impact (52% of grain yield reduction), followed by Sh\(_{1st}\) (29%) and the least impact was found under Sh\(_{2nd}\) (23%), averaged across cultivars and experiments (Figure 2). These responses were consistent across cultivars taking into account that no interactions among cultivars, experiments or source-sink treatments were found (\( p \geq 0.05 \)); however, ‘Otto
found between these traits ($R^2 = 0.60, p \leq 0.001$) including the commented exception of ‘Otto Baer’ under Sh$_{All}$ in Experiment 1. Control treatments reached higher TGW ($p \leq 0.001$) in Experiment 1 (47 g) than in Experiment 3 (37 g). Between cultivars, differences in TGW ($p \leq 0.05$) were found in both experiments, where ‘Otto Baer’ reached TGW of 45 and 39 g, while ‘Huayún-INIA’ reached 50 and 35 g in Experiments 1 and 3, respectively. Similar to grain yield, the effect of source reduction on TGW by Sh$_{All}$ showed the greatest impact (48% averaged across cultivars and experiments), whereas Sh$_{1st}$ and Sh$_{2nd}$ had lower effect (26% and 22%, respectively, averaged across cultivars and experiments). In the Sh$_{All}$ treatment, ‘Otto Baer’ was more sensitive (58%) than ‘Huayún-INIA’ (39%) when the source reduction treatments were averaged across experiments (Figure 2). The Sh$_{1st}$ treatment decreased TGW by 29% and 23% in ‘Otto Baer’ and ‘Huayún-INIA’, respectively, while Sh$_{2nd}$ had a similar or lower impact depending on the cultivar, i.e. 30% and 14% in ‘Otto Baer’ and ‘Huayún-INIA’, respectively (Figure 2).

Individual grain weight and water content responses to source reduction

Grain weight and water dynamics were measured at each grain position of the central spikelets showing similar behavior (data not shown). To facilitate the view of data of individual grains, DM and water dynamics as well as their relationships are shown averaged across grain weight of individual positions of central spikelets ($G_1$, $G_2$, and $G_3$, hereafter named IGW). Source-sink treatments decreased ($p \leq 0.001$) IGW in both cultivars (Table 2) but, similarly to TGW, no interaction ($p \geq 0.05$) between cultivar and the source-sink ratios was found. Thus, Sh$_{All}$ (the most influential source-sink treatment) decreased IGW by 57% in ‘Otto Baer’ and 48% in ‘Huayún-INIA’ averaged across experiments. These results confirm, in turn, the high sensitivity of wheat under strong source shortage (Table 2, Figure 3). The negative effect of Sh$_{All}$ on IGW was due to both the lower ($p \leq 0.001$) grain filling rate and the shorter ($p \leq 0.001$) duration of grain filling shown by both cultivars (Table 2, Figure 3). When the crop was shaded during the first half of the grain filling period (Sh$_{1st}$) the impact on IGW was higher (33%) than in the second half (Sh$_{2nd}$) as IGW decreased by 12%. Under Sh$_{1st}$, ‘Otto Baer’ was also more sensitive than ‘Huayún-INIA’ since IGW decreased by 37% and 28%, respectively, averaged across experiments (Table 2, Figure 3). However, and more important, grains of both cultivars under Sh$_{1st}$ were able to continue their growth after the nets were removed from the plots (Figure 3). It is important to highlight that there were different magnitudes of IGW change between experiments after the Sh$_{1st}$ treatment ended. The removal of nets in Sh$_{1st}$ was earlier in Experiment 1 than in Experiments 2 and 3 (Figure 3), i.e. in Experiment 1 ‘Otto Baer’ and ‘Huayún-INIA’ was more sensitive (58%) than ‘Huayún-INIA’ (39%) when the source reduction treatments were averaged across experiments (Figure 2). The Sh$_{1st}$ treatment decreased IGW by 29% and 23% in ‘Otto Baer’ and ‘Huayún-INIA’, respectively, while Sh$_{2nd}$ had a similar or lower impact depending on the cultivar, i.e. 30% and 14% in ‘Otto Baer’ and ‘Huayún-INIA’, respectively (Figure 2).
Table 2. Grain weight of individual positions of central spikelets (IGW), grain filling duration (GFD), and grain filling rate (GFR) of wheat ‘Otto Baer’ and ‘Huayún-INIA’ under control and source-sink reduction treatments (S-Sratiost) during the entire (Sh1st), first-half (Sh2nd) and second-half (Sh2nd) periods in the 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons.

<table>
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<th>Cultivar</th>
<th>S-Sratiost</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
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<th>Exp. 2</th>
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<td>Control</td>
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<td>747</td>
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<td>Sh1st</td>
<td>17.4 (-66)</td>
<td>22.7 (-46)</td>
<td>19.3 (-59)</td>
<td>592</td>
<td>606</td>
<td>573</td>
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<td>26.7 (-36)</td>
<td>24.4 (-49)</td>
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<td>40.4 (-29)</td>
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<td>722</td>
<td>706</td>
<td>0.095</td>
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</table>

Values in parenthesis indicate percentage values (%) show grain weight decrease under the source reduction treatments relative to the controls.

*: **: ***: significant at 0.05, 0.01, and 0.001 probability levels, respectively.

sem: Standard error of the mean; ns: non significant; E: experiment; Cv: cultivar; S-S: source-sink ratio.

INIA’ accumulated 67% and 60%, respectively, of their final IGW after removing the nets in Sh1st; whereas in Experiments 2 and 3 the accumulation of IGW was lower (i.e. 31% in ‘Otto Baer’ and 41% in ‘Huayún-INIA’, averaged across Experiments 2 and 3). IGW reduction under Sh2nd was higher in ‘Otto Baer’ than in ‘Huayún-INIA’ across experiments; i.e. 14 and 10%, respectively (Table 2, Figure 3).

The main driver of IGW reduction under the source-sink treatments was the grain filling rate as a linear relationship

Vertical bars show the standard error of the mean. The DM dynamics of C and Sh1st were built with data measured during the entire grain filling period in these treatments; whereas in Sh1st and Sh2nd data corresponding to C and Sh1st were used until the shades were taken out or set in Sh1st and Sh2nd, respectively. Left and right vertical lines show the start of the Sh1st and Sh2nd treatments, respectively.

Figure 3. Time-course in thermal time units of individual grain weight (IGW) from central spikelets of wheat ‘Otto Baer’ and ‘Huayún-INIA’ under control (C, open triangles) and source-sink reduction treatments during the entire (Sh1st, closed triangles), first half (Sh1st, gray circles), and second half (Sh2nd, open circles) phases of the grain filling period, during 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons.
(R² = 0.81, p ≤ 0.001; Figure 4a) was found between these variables. Conversely, IGW showed no association with grain filling duration (R² = 0.014, p ≥ 0.05). Interestingly, final IGW was associated with the cumulative intercepted radiation across cultivars and source-sink treatments, and partially with the length of the source-shortage treatment (Figures 4b and 4c).

Similar to dry matter, grain water dynamic was also affected by the source reduction but less than GW. Thus, Sh_All decrease (p ≤ 0.001) the SGWC 25% in ‘Otto Baer’ and 29% in ‘Huayún-INIA’ averaged across experiments (Table 3). The Sh_1st decreased SGWC 21% in Otto Baer and 23% in ‘Huayún-INIA’. Under this treatment, grain water dynamic was maintained even after the nets were removed (Figure 5), but unlike grain DM, SGWC was not recovered after the removal of nets (Figures 3 and 5). The lowest impact of the source-sink treatments on SGWC was recorded in Sh_2nd, where SGWC decreased only 5% in both cultivars across experiments (Table 3) and grain water dynamics were almost not affected when compared with the control treatments (Figure 5). The source-sink treatments, cultivars and experiments affected (p ≤ 0.001) the hydric plateau duration (HPD); however, no clear trends were found for HPD among cultivars and source-sink treatments (Table 3).

Remarkably, although SGWC was less sensitive to the source reductions than grain DM, a close association between final IGW and SGWC was found across cultivars, source-sink treatments and experiments (Figures 6a and 6b). SGWC was also found to be an accurate predictor of the grain filling rate as a positive association (R² = 0.89, p ≤ 0.001) was obtained when both traits were plotted together (Figure 6b). On the other hand, no associations were found when IGW was tried with either the grain

![Figure 4. Relationships between individual grain weight from central spikelets and (a) grain filling rate, (b) cumulative intercepted radiation (CI-PAR), and (c) source-sink treatment duration of wheat ‘Otto Baer’ (black symbols) and ‘Huayún-INIA’ (white symbols) under control (C, diamonds) and source-sink reduction treatments during the entire (Sh_All, circles), first half (Sh_1st, triangles), and second half (Sh_2nd, squares) phases in 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons.](image-url)

![Table 3. Stabilized grain water content of individual positions of central spikelets (SGWC) and hydric plateau duration (HPD) of wheat ‘Otto Baer’ and ‘Huayún-INIA’ under control and source-sink reduction treatments (S-S) ratios during the entire (Sh_All), first-half (Sh_1st), and second-half (Sh_2nd) phases during 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons.](table-url)
The evaluation of wheat response to different source-sink ratios has been widely attempted and it is generally accepted that this crop is scarcely limited by the source of assimilates during grain filling (Slafer and Savin, 1994; Borrás et al., 2004). However, in a previous experiment assessing only ‘Otto Baer’, greater sensitivity than expected was found when this cultivar was exposed to a severe source reduction (Sandaña et al., 2009). Therefore, the present study aimed to evaluate simultaneously the sensitivity of two wheat cultivars to strong source reductions during grain filling and to improve the understanding of the causes associated with the greater-than-expected sensitivity of grain weight found previously (Sandaña et al., 2009). Moreover, two additional questions of the present study previously unexplored to wheat were: (i) whether the wheat sensitivity to source reduction depends on the time at which the source-sink ratio occurs and (ii) whether differences in grain weight in response to the source shortage are associated with grain water dynamics as was reported for maize (Borrás et al., 2003).

In the present study the assimilate availability of wheat was reduced by 65%, 33%, and 33% across experiments for Sh_{All}, Sh_{1st}, and Sh_{2nd}, respectively, following the quantification proposed by Slafer and Savin (1994) and Borrás et al. (2004). These source reductions were similar to those carried out in previous assessments of wheat where the calculated assimilate availability per grain was decreased by up to 70% (Borrás et al., 2004).

Under the Sh_{All} treatment, TGW and IGW reductions across cultivars and experiments were 48% and 53%, respectively (Figures 2 and 3, and Table 2). The sensitivity of TGW and IGW found in the present study confirms previous findings that under very high source reduction wheat grain weight decreases to a greater extent than generally reported (up to 32%, Borrás et al., 2004). By contrast, Beed et al. (2007) found decreases of TGW by only 27% when the source was reduced by setting nets intercepting 80% of incident radiation from two weeks (i.e. 14 d) after At until PM (i.e. 27 d).

Differences between the present study and that of Beed et al. (2007) could be ascribed to different water soluble carbohydrates (WSC) stored in the stems by the crops as genetic variability has been found in wheat (Ehdaie et al., 2008; Dreccer et al., 2009) and the demand by grains, which would explain differences between ‘Otto Baer’ and ‘Huayún-INIA’. This is consistent with that shown by Serrago et al. (2013) where the mobilization of reserves from stems was an important source of assimilates for growing grains in wheat and barley. Indeed, increases in

**DISCUSSION**

The time-course in thermal time units of individual grain water content (GWC) from central spikelets of wheat ‘Otto Baer’ and ‘Huayún-INIA’ under control (C, open triangles) and source-sink reduction treatments during the entire (Sh_{All}, closed triangles), first half (Sh_{1st}, gray circles), and second half (Sh_{2nd}, open circles) phases of the grain filling period, during 2004-2005 (Experiments 1 and 2) and 2005-2006 (Experiment 3) growing seasons. Vertical bars show the standard error of the mean. The water dynamics of C and Sh_{All} were built with data measured during the entire grain filling period in these treatments; whereas in Sh_{1st} and Sh_{2nd} data corresponding to C and Sh_{All} were used until the shades were taken out or set in Sh_{1st} and Sh_{2nd}, respectively.

**Figure 5**
WSC supply to grain yield and TGW have been reported for wheat cultivars released in the UK from 1972 to 1995 (Foulkes et al., 2002; Shearman et al., 2005). In addition, Beed et al. (2007) evaluated winter wheat cultivars, while in our study spring cultivars with a shorter crop cycle were investigated, i.e. 72 d from emergence to anthesis averaged across cultivars and growing seasons (Figure 1). Likely, spring wheats could accumulate fewer soluble carbohydrates up to anthesis. Therefore, the sensitivity of grain yield to high source shortage during the grain filling of spring cultivars in high-yield environments seems to respond similar to maize as Sala et al. (2007) found grain weight reductions by 55% and 62% when 85% and 100% leaves were removed at 20 d after silking, respectively. However, in wheat the spike photosynthesis could be an important source of assimilates to the grains as was found by Serrago et al. (2013) when these organs were not shadowed.

Shading imposed during the Sh1st treatment strongly affected IGW. In Sh1st, both cultivars showed reductions of 26% in TGW, while IGW decreased from 13% to 49%.

The Sh2nd treatment had lower impact on both TGW (i.e. 2% decreases) and IGW (12%), although the latter trait showed a wide range of reduction, i.e. between 5% and 33%. Our results support that source reduction earlier in grain filling has higher impact on final grain weight and yield than later ones, thus confirming the hypothesis of Borrás et al. (2004).

The IGW was positively associated with grain filling rate across all source-sink treatments, which is consistent with previous studies where grain weight has been found to be closely related with the grain filling rate (Chowdhury and Wardlaw, 1978; Calderini et al., 1999; Sandaña et al., 2009; Serrago et al., 2013). Nevertheless, no association was found between IGW and grain filling duration; however, the source-sink treatments reduced (p ≤ 0.001) grain filling and in turn decreased cumulative intercepted radiation available for plants. For this reason, the effect of grain filling duration on IGW cannot be completely discounted. In addition, cumulative intercepted radiation was more descriptive of the IGW sensitivity than the treatment duration (Figures 4b and 4c). The latter is in line with the source limitation to the growing grains found in the present experiment.

The sensitivity of SGWC under source-sink treatments was lower than that of IGW. This contrasting sensitivity is in agreement with that found by Sala et al. (2007) in maize (TGW decreased 59%, while GWC 15%) and contrary to the response reported by Borrás et al. (2003) for the same crop, where the maximum water content was more sensitive (33% reduction) than grain weight (22% reduction). Despite different sensitivities found between dry matter and water in our study, both dry matter and maximum grain water content correlated strongly, as in maize (Borrás et al., 2003; Sala et al., 2007).

The dry matter and water dynamics of grains are not independent each other in crops like wheat, maize and sunflower (Rondanini et al., 2009). The present study demonstrate that the relationship between dry matter and water content of grains is maintained even when the source-sink ratio was decreased either early (Sh1st and Sh2nd) and later (Sh2nd) in the grain filling period.

CONCLUSION

In wheat grain yield and grain weight showed a high response to reduced source-sink ratios under the assessed conditions of this study, i.e. very high decreased...
assimilate availability evaluated in a high yield potential environment. Grain yield penalties were mainly due to the sensitivity of IGW. IGW sensitivity to source reduction varied according to the length of the treatment and the timing of setting, i.e. high, moderate and mild impact were found in Sh24t, Sh1st and Sh2nd treatments, respectively. This is particularly important because wheat sensitivity to source reduction during grain filling could be higher than previously reported. Cultivars assessed in this study were able to increase IGW when assimilate availability was enhanced during the second half of grain filling; however, IGW was highly decreased compared with the controls. Importantly, the stabilized grain water content was found to be a good estimator of the final IGW across the source-sink treatments and seasons assessed.

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LITERATURE CITED


