



Understanding grain yield responses to source–sink ratios during grain filling in wheat and barley under contrasting environments



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ABSTRACT

A better understanding of the effects of different source–sink ratio during the grain filling period on grain growth may be relevant in order to further increase cereal grain yield. The main objective of the present work was to determine the effect of different manipulations of the source–sink ratios in wheat and barley grown at four different environmental conditions on responsiveness of sinks (grain growth and yield) and sources (spike photosynthesis and water soluble carbohydrates in the stems). Four treatments were imposed 7 days after anthesis in two contrasting locations with low- and high-inputs conditions in wheat (cv. Soissons) and barley (cv. Sunrise): they were a control, a treatment removing all the spikelets from the upper half of the spikes (T_S), and shadings decreasing incident radiation by 75% on the whole canopy (S_W) or only on the leaves (having the top area of the meshes individual holes for each spike to be exposed to solar radiation, S_L). As expected grain yield was closely related to grain number per m^2 . Average grain weight was reduced by shading treatments far more markedly in S_W than in S_L . Interestingly, significant amounts of water soluble carbohydrates in the stems remained at maturity in S_L and S_W treatments and spike photosynthesis in S_L was consistently higher than in the unshaded controls in both species. These results may be an indication that wheat and barley are not source-limited during grain filling and that only when subjected to an extremely severe stress, grain size would be reduced due to lack of enough assimilates available to fill them.

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1. Introduction

Grain yield increases during the last century were almost fully (Slafer et al., 1990; Calderini et al., 1999; Fischer, 2007b), or at least mainly (Calderini et al., 1995; Sadras and Lawson, 2011), related to increases in grain number per m^2 . This is in line with the fact that grain growth is frequently sink-limited both in wheat (Jenner, 1979; Savin and Slafer, 1991; Slafer and Savin, 1994; Miralles and Slafer, 1995; Kruk et al., 1997; Borrás et al., 2004; Calderini et al., 2006) and barley (Dreccer et al., 1997; Savin et al., 2006; Bingham et al., 2007). This is seemingly true even in environments characterized by terminal stress (Cartelle et al., 2006; Pedro et al., 2011). In other words, post-flowering source-strength is commonly in excess to meet the demands of growing grains in cereals (Richards, 1996; Bingham et al., 2007). However, some reports disagree with this conclusion, and examples in the literature with grain weight

responding to source–sink manipulations during grain filling can be also found in wheat (Bremner and Rawson, 1978; Fischer and HilleRisLambers, 1978; Bindraban, 1996; Sandaña et al., 2009) and barley (Grashoff and d'Antuono, 1997; Voltas et al., 1997). Differences among experiments could be related to the timing at which the treatments were imposed. In the experiments made by Fischer and HilleRisLambers (1978) and Voltas et al. (1997) the source–sink manipulations treatments were imposed at anthesis. Then, the responses found in these experiments could be due to increases in grain weight potential, more than due to alleviation of source limitations during grain filling, as potential size of the grains is determined up to few days after anthesis (Brocklehurst, 1977). Having a clearer picture of the expected responsiveness of grain growth to source strength is relevant for identifying prospective alternatives for further increasing yields. Further increases in grain number could be critically relevant if grain growth is mainly sink-limited or totally ineffective if it were compensated by proportional grain weight reductions if grain growth were source-limited (Slafer et al., 2005; Reynolds et al., 2009; Foulkes et al., 2011). To determine whether there is a negative association between the number of grains per m^2 and their average weight could be of

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little help for clarifying the issue as such inverse relationship may be caused by competitive or non-competitive reasons (Miralles and Slafer, 1995; Acreche and Slafer, 2006). Therefore, direct determination of the degree of source- or sink-limitations to grain growth is critical. The most common approach to determine whether there is source- or sink-limitation has been the imposition of treatments in which either, source or sink strengths are manipulated ca. 7–10 days after flowering. Commonly, source manipulations are defoliations or shadings while sink manipulations are removals of grains (e.g. partially trimming the spikes), determining reductions or increases in the source–sink relationship, respectively.

During the grain filling period, the assimilate supply to fill the grains is the current canopy photosynthesis, of which spike photosynthesis may be a major contributor (Evans and Rawson, 1970; Araus et al., 1993; Tambussi et al., 2007), plus the translocation of non-structural reserves stored before the onset of grain filling (Ehdaie et al., 2008; Bingham et al., 2009). Commonly research reporting on whether grain growth is source- or sink-limited has been exclusively focused on grain weight responsiveness to treatments, disregarding to what degree changes in grain weight were explained by opposite changes in source-strength (either increased-reduced photosynthetic activity or accumulation-depletion of non-structural carbohydrate reserves). For instance, as the capacity of cereals to compensate for reductions in source–sink ratios is based on using stored assimilates in stems during grain filling (Setter et al., 1998; Richards et al., 2002; Borrás et al., 2004; Serrago et al., 2011), grain weight reductions in treated plants should be accompanied by a depletion of carbohydrate reserves to conclude that grain growth in the non-manipulated control was source-limited.

For interpreting the effects of different source–sink ratios on grain yield it is important to consider the background environmental conditions in which the responsiveness to the treatments is assessed (Borrás et al., 2004). Thus, differences in the environmental background in which source–sink ratios were manipulated could be responsible for the conflicting results mentioned above. Then, for conclusions of any study to be extrapolated, manipulations of source–sink ratios should be done under contrasting growing conditions. Therefore, the main objective of this study was to determine responsiveness of sinks (grain growth and yield) and sources (photosynthesis and non-structural reserves) to manipulations of source–sink ratio (trimming the spikes and fully or partially shading the canopy at the onset of grain growth) in wheat and barley grown at four different environmental conditions.

2. Materials and methods

2.1. General conditions, treatments and experimental design

Two field experiments were conducted on normal sowing dates and sowing densities for wheat and barley crops in Catalonia, NE Spain (Table 1). The experiments were conducted in two different locations within the province of Lleida during the 2009/10 growing season. One of the experiments was conducted at Agramunt (41°84'70" N, 01°80'60" E, 330 masl, within a region characterized by rainfed cereal production systems) and the other was conducted at Gimenezells (41°83'60" N, 00°81'30" E, 258 m, a region with most agriculture conducted under irrigation). The experiment at Agramunt was sown in a farmer's field, selected because of its low nitrogen fertility, with a soil classified as Typic xerorthent; while experiment at Gimenezells was sown in a more fertile soil, classified as Petrocalcic calcixerept.

Treatments in both experiments consisted in the factorial combination of (i) two species, bread wheat and two-rowed barley; (ii) two levels of inputs, "low" and "high", and (iii) four different

source–sink ratios imposed during grain filling. Treatments were arranged in a split-plot design with three replicates. In each replicate there were four main plots (6 rows 0.20 m apart and 6 m long) assigned to the combinations of barley and wheat under high and low inputs, and the sub-plots (6 rows 1.5 m long) were the source–sink manipulations.

The genotypes selected for wheat (cv. Soissons) and barley (cv. Sunrise) were chosen carefully to be well adapted cultivars representing those commonly sown in the region, and also used as standard controls by the GENVCE (group for the evaluation of the new cereals varieties in Spain) (Cossani et al., 2009). Thus results may be regarded to be safely extrapolable to "average" modern well-adapted cultivars of each of these species in the region. The water regimes were either rainfed or with supplementary irrigation in low and high inputs levels, respectively; while nitrogen (N) availability was that available in the soil in the low inputs treatment or with the additional supply from N fertiliser at sowing in high input treatments (Table 1).

Treatments modifying the source–sink ratios consisted of a control, a treatment increasing the ratio, and two treatments decreasing the ratio (Fig. 1). In all cases, the treatments were imposed 7 days after flowering. The flowering time was determined in both species when pollen liberation occurred inside the spike (Waddington et al., 1983). To increase source–sink ratios, all spikes in the central 4 rows of the corresponding sub-plots were hand trimmed, removing all the spikelets from the upper half of the spikes of every single shoot (T_S), to have an homogeneous treatment comprising the whole canopy (Acreche and Slafer, 2009) rather than manipulating isolated spikes within unmanipulated canopy (Fig. 1b). The two treatments to reduce the source–sink ratio consisted in heavily shading the sub-plots through installing black meshes (covering the experimental unit decreasing incident radiation by 75%) of the whole canopy (producing a "total shading" of leaves and spikes; S_W) (Fig. 1c) or making holes in the upper surface of the mesh enclosure so that spikes were exposed to solar radiation while the rest of the crop canopy (leaves, sheaths, stem) remained shaded (leaf shading treatment, SL) (Fig. 1a and c). The three source–sink manipulation treatments were performed in the four environmental conditions (location \times input level, which represented not only different conditions for grain filling but also different conditions of the crops at the time of imposition of treatments) and in both species; with the exception of the high-input treatment of barley growing at Agramunt, in which the shadings could not be installed (there were emergence failures in part of these main plots).

2.2. Measurements and analysis

The developmental stages of seedling emergence, jointing, booting, anthesis, and maturity were recorded when 50% of the plants in each plot reached them. Samples of 1 m of a central row of each sub-plot were taken at flowering and at maturity. In these samples above-ground biomass was determined after oven-drying the samples for 72 h at 60 °C. The samples were partitioned into the different organs (stems plus leaf sheaths, leaf lamina and spikes at flowering; and stems, leaves, chaff and grains at maturity) and counted the number of stems and fertile spikes at flowering and the number of spikes and grains at maturity. The yield and its components were also determined. The content of non-structural reserves was determined on stems (plus sheaths), by sequential extractions in ethanol and water followed by determination using the anthrone method (Galicia et al., 2009).

As the treatment T_S changed the normal distribution of grains (all spikelets of the upper half of the spikes were removed), in order to determine the effects of treatments on grain growth dynamics and on final grain weight, individual grains of the central spikelets

Table 1
Sowing date, crop plant population density at seedling emergence, soil nitrogen availability (nitrogen measured in the top 60 cm at sowing plus fertilizer addition), rainfall during the growing season and irrigation volume during the growing cycle of the experiments carried out in Gimenez and Agramunt (NE Spain).

Location	Sowing date	Condition	Density (plants m ⁻²)	Nitrogen (kg N ha ⁻¹)	Rainfall (mm)	Irrigation (mm)
Gimenez	November 23	Low inputs	212	114	274	0
		High inputs	212	195	274	400
Agramunt	November 17	Low inputs	225	47	365	0
		High inputs	225	190	365	32

from the bottom half of spikes were sampled, dried and weighed every 3–4 days after flowering from two randomly selected main shoot spikes in all treatments. The grains in wheat were divided into proximal (grains 1 and 2 counted from the base of the spikelet) and distal (grain 3) from the rachis, while in barley there is only one grain per spikelet. The dynamics were characterized by fitting the data to the following bi-linear model:

$$y = a + bx \quad (\text{if } x \leq c) \\ + bc \quad (\text{if } x > c) \quad (1)$$

where grain weight (y) is linearly increased through time (days after flowering, x) until it reaches a plateau. The parameters with biological interest are the grain filling rate (GFR; b) and the time after flowering when the maximum grain weight was attained or physiological maturity (PM; c). Final grain weight was calculated following the Eq. (1).

Maximum net photosynthetic rate (A_{\max} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was determined on the spikes of the control and S_L subplots using an LCi Portable Photosynthesis System (ADC BioScientific, Hertz, UK). Measurements were made frequently (every 3–4 days) from 10 d after flowering to the onset of visual senescence of the spikes at solar noon of cloudless days, using a special closed chamber of 995 ml made for this purpose. As it was not realistic to bend the spikes within these chambers in plants of central rows of a field canopy to expose their area perpendicularly to the incident solar radiation, we cut the spikes and immediately introduced them into the chambers horizontally to maximize incident radiation. Beforehand we firstly tested that dynamics of photosynthesis was not altered for several minutes after detachment; we observed that, as in photosynthetic measurements of organs attached to the rest of the plant, there was a period of acclimation to the

chamber conditions in which photosynthesis increased rather linearly (ca. 2–3 min) until stabilizing at a particular rate that remained roughly constant for several minutes and considered the average of the rates measured during this stabilized period as the maximum net photosynthesis of the spikes. Then, we compared the observed values of A_{\max} of detached spikes with those reported in the literature for spikes attached to the rest of the plant (in individual potted plants in which bending the spikes is possible) and in all cases the values were in the range expected for spike net photosynthesis (Abbad et al., 2004). Therefore, for measurements two spikes were exposed during approximately 3 min (until maximum net photosynthesis was stabilized) and then the photosynthetic rate was determined every 15 s for approximately further 3 min. The overall spike photosynthesis was then corrected by the spike area including the awns to calculate the spike photosynthesis rate. The spike area was measured with a LI-COR LI-3100C Area Meter.

With the values of stem non-structural reserves at maturity we estimated a theoretical weight that grains would have achieved should all reserves be remobilized to, and accumulated by, the growing grains in each treatment (i.e. the weight that grains of the control would have achieved if all the reserves available in the stems at physiological maturity would have been remobilized to the growing grains). Then we compared this “theoretically achievable grain weight” with potential grain weight (assuming grains in the T_S treatment achieved their potential weight).

Statistical differences between treatments were tested through standard analyses of variance with first level interactions using INFOSTAT Version 1.1. When the analysis of variance revealed significant differences, the mean treatment values were compared using the Tukey test with a significance level of $p < 0.05$.



Fig. 1. Photographs of different treatments imposed to barley and wheat under low- and high-inputs at Gimenez and Agramunt: (A) spikes protruding through shade mesh in treatment (S_L); (B) trimmed spike (T_S); and (C) leaf (S_L) and whole canopy (S_W) shade treatments.

Table 2

Grain yield, relative change of grain yield in the treatments respect to the control and grain number (GN), for wheat and barley, grown under low- and high-inputs at Gimennells and Agramunt. The means are presented for the control (Ct), trimmed spikes at 7 days after anthesis (T_S), leaves shaded (S_L) and total shaded (S_W) treatments. Different letters indicate significant differences ($p < 0.05$) for the treatments within each experiment and crop.

Location	Crop	Treatment	Low-inputs			High-inputs		
			Grain yield		GN	Grain yield		GN
			(g m ⁻²)	(%)	(m ²)	(g m ⁻²)	(%)	(m ²)
Gimennells	Wheat	Ct	773 a		23,886 a	958 a		28,196 a
		T _S	501 b	-35	12,024 b	738 ab	-23	17,298 b
		S _L	469 b	-36	19,383 ab	869 a	-9	31,009 a
		S _W	408 b	-47	18,932 ab	612 b	-36	31,755 a
	Barley	Ct	771 a		16,919 a	855 a		19,900 a
		T _S	415 b	-46	9292 b	389 c	-55	8230 b
		S _L	586 ab	-24	15,527 a	648 b	-24	18,917 a
		S _W	422 b	-45	14,248 a	407 c	-52	18,187 a
Agramunt	Wheat	Ct	542 a		16,711 a	813 a		23,231 a
		T _S	345 b	-36	9057 b	485 b	-40	12,422 b
		S _L	493 ab	-9	15,742 a	720 ab	-11	22,886 a
		S _W	371 b	-32	14,279 a	635 ab	-22	21,082 a
	Barley	Ct	642 a		15,859 a	733 a		16,977 a
		T _S	338 b	-47	8508 b	531 b	-28	12,521 b
		S _L	528 a	-18	13,507 a			
		S _W	554 a	-14	16,422 a			

3. Results

3.1. Grain yield, grain number and average grain weight

Grain yield in control treatments varied from slightly over 500 to almost 1000 g m⁻², being on average similar for wheat and barley, although barley tended to outyield wheat in the lowest-yielding condition and wheat tended to outyield barley in the highest-yielding environments (Table 2). As yield differences were small, as expected wheat had more grains per m² than barley while barley had grains on average heavier than wheat (Table 2 and Fig. 2).

Reductions on source–sink ratio imposed during the effective grain filling period reduced grain yield both in wheat and barley; however, grain number was not modified significantly by shading treatments in any of the experimental situations (Table 2). Yield was reduced by ca. 35% when the whole canopy was shaded 75% throughout the whole grain filling period (S_W). But, when the spikes were released from the shading (S_L) yield was affected consistently less markedly by ca. 20%, though the magnitude and significance of the effect was different depending on the growing condition. It was evident the alleviation that represented for the yield of the crop having the spikes exposed to natural radiation when comparing the values achieved by these plots with those in which the

shading was imposed to the whole canopy (Table 2). As expected, trimming the spikes did reduce significantly yield in all cases (crop species and environmental conditions), though the magnitude of the reduction varied between conditions and in general was less than 50%, particularly in wheat (Table 2).

Trimming the spikes by half did naturally reduce the number of grains per m² (Table 2 and Fig. 2). Overall, yield was significantly related to the number of grains per m² in both cereals, but wheat explored a larger range of grain number than barley (Fig. 2). The data-points of the plots in which shading was imposed on the whole canopy did consistently fall well below the adjusted function ($p < 0.05$ and $p < 0.001$ for barley and wheat, respectively). On the other hand, data-points of the treatment severely shading the rest of the canopy but releasing the spikes from shading did only slightly affect the relationship. Trimming the spikes modified the relationship between grain yield and grain number differently in barley and wheat with respect to the untrimmed controls, as the slopes were significantly different in wheat (grey circles; $p < 0.01$), but not in barley ($p > 0.05$), which remained around the line fitted by controls (see distribution of grey squares; Fig. 2). Probably, these effects were due to direct effects on average grain weight. It was reduced by the shading treatments more consistently and markedly in S_W than in S_L, in both crops and in all environmental conditions (Fig. 3). Average grain weight was significantly increased in wheat and virtually unaffected in barley by reducing strongly the number of grains being fed by the non-manipulated source in the T_S treatment (Fig. 3). The increases on the average weight of the grains in wheat tended to be higher in the low- than in the high-inputs conditions (Fig. 3). However, in all cases the magnitude of the responses of average grain weight was smaller than the magnitude of the treatments. The range of grain weight reduction in response to reducing the photon flux density during the whole period of effective grain filling by 75% was between 15 and 45% in both wheat and barley. Reducing the number of grains through trimming the spikes roughly by 45% resulted in an increase of average grain weight between 10 and just over 20% in wheat, and did not affect grain weight in barley.

3.2. Grain weight, grain growth rate and duration of individual grain positions

Final grain weight and its components (rate and duration of grain filling) were obtained for the grains sampled in the bottom

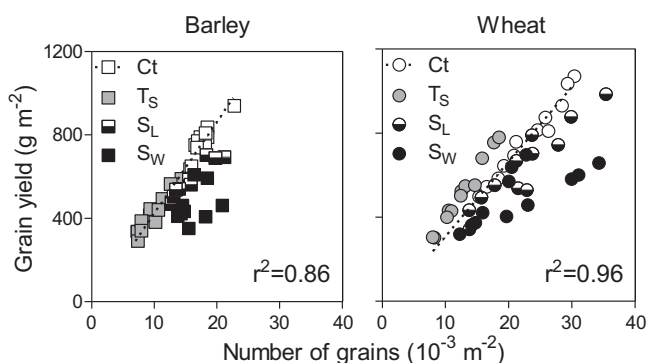


Fig. 2. Relationship between grain yield and grain number in barley and wheat crops (left and right panels, respectively) for control (Ct), trimmed spikes at 7 days after anthesis (T_S), leaf (S_L) and whole canopy (S_W) shade treatments. The regression line (dotted) is fitted to Ct data. Each data-point represents a replicate within each of the growing conditions.

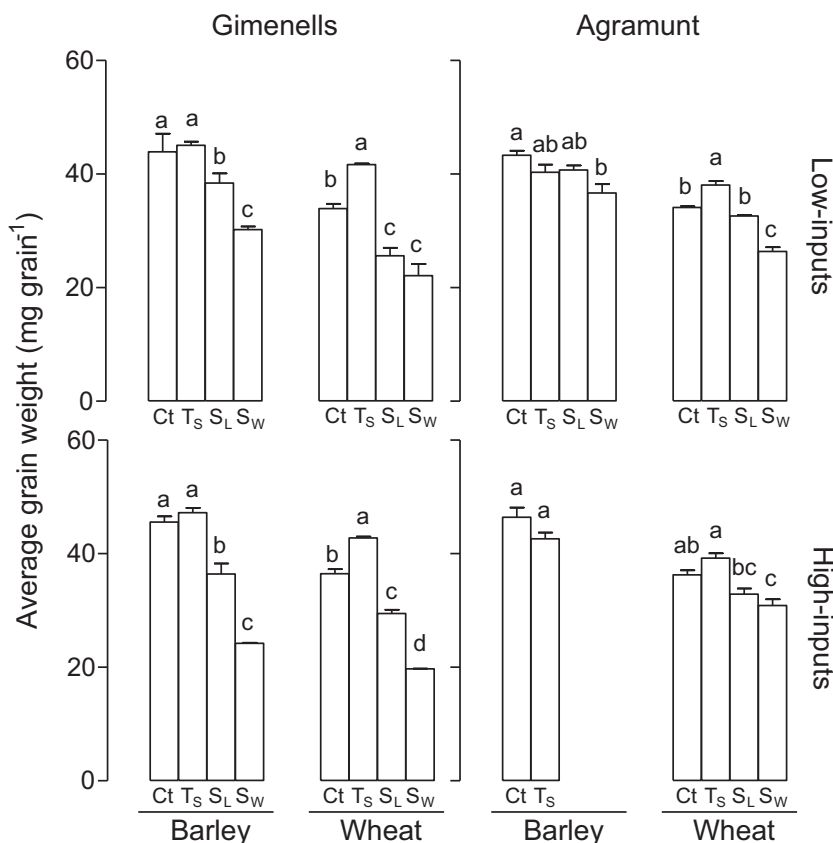


Fig. 3. Average grain weight of barley and wheat crops at Gimennells and Agramunt growing under low- and high-inputs for control (Ct), trimmed spikes at 7 days after anthesis (T_s), leaf (S_L) and whole canopy (S_W) shade treatments. Different letters indicate significant differences ($p < 0.05$) among treatments within each experiment and crop. Bars indicate the standard error of the means.

half of main-shoot spikes from the bi-linear regression of grain weight over time after flowering. In each of the cases analyzed (two species \times four environments \times four source–sink ratios) and for each of the grain positions considered (proximal or distal grains in wheat) the data were very well fitted by the model, with coefficients of determination ranging from 0.94 to 0.99 (in all cases $p < 0.001$). The abscissa intercept of the grain weight data fitted by the model is an estimate of the onset of the effective grain filling period and roughly represents the “lag phase” of post-anthesis development. The values observed were 4.60 ± 0.82 and 6.41 ± 0.51 d after flowering in barley and wheat, respectively; confirming that the source–sink treatments were actually imposed soon after the onset of effective grain filling.

Responses to treatment of grain weight for positions G1, G2 and G3 on the main shoot spike provided a fair representation of the mean response of all grains in the crop (Fig. 4). Naturally, there was a clear trend for the grains of the bottom half of the main-shoot spikes to be heavier than the average of all grains (from all spikelets and from main-shoot plus tiller spikes). The relative performance and responsiveness to treatments of both populations of grains was rather similar (Fig. 4, inset).

The weight of individual grains was significantly affected by source–sink manipulations. Shading the whole canopy did significantly reduce grain weight at all positions analyzed both in wheat and barley and either under high- or low-inputs conditions in both locations (Table 3). On the other hand, shading only the leaves significantly reduced the weight of individual grains in ca. 50% of the grains at Gimennells and in none of the cases at Agramunt (Table 3). Furthermore, when there was a significant effect at Gimennells, its magnitude was much smaller than that of shading the whole canopy (Table 3). In wheat, the reductions due to shading

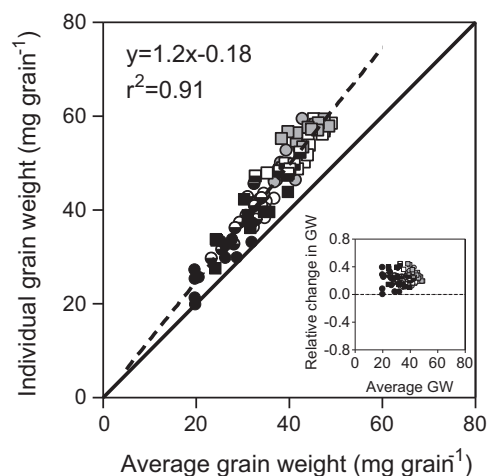


Fig. 4. Relationship between the weight of individual grains in the main-shoot spike (G1–G3 in wheat) and the average weight of all grains for all crops \times treatments combinations. Inset: Relative change in grain weight (calculated as the difference between individual and average grain weight divided average grain weight) related to average grain weight. Square and circles represent barley and wheat crops, respectively. Open symbols represent the control, grey symbols represent spikes trimmed 7 days after anthesis, pied black and white symbols represent the leaf shading (S_L) and black symbols represent the whole canopy shading (S_W) treatments. The solid line represents the 1:1 relationship and the dotted line the regression fitted to the observed data. Each data-point represents a replicate within each of the growing conditions.

Table 3

Grain weight and relative change in grain weight in the treatments respect to the control (%), for wheat and barley grown under low- and high-inputs at Gimenez and Agramunt. Values are for grains from central spikelets. In wheat, values for grain 1 (G₁) and grain 2 (G₂) were pooled (as proximal grains). The means of the different variables are presented for the control, trimmed spikes at 7 days after anthesis (T₅), leaves (S_L) and whole canopy (S_w) shade treatments. Different letters indicate significant differences ($p < 0.05$) between treatments within each experiment and crop.

Location	Crop	Position	Treatment	Low-inputs		High-inputs	
				Grain weight		Grain weight	
				(mg)	(%)	(mg)	(%)
Gimenez	Wheat	G ₁ –G ₂	Ct	42.4 b		46.9 b	
			T ₅	51.5 a	+21	57.7 a	+23
			S _L	34.3 c	–19	40.9 b	–13
		G ₃	S _w	29.6 c	–30	25.3 c	–46
			Ct	35.4 b		39.6 ab	
			T ₅	46.7 a	+32	49.8 a	+26
	Barley	G ₁ –G ₂	S _L	25.8 c	–27	30.8 b	–22
			S _w	21.8 c	–38	18.1 c	–54
			Ct	54.4 ab		57.4 a	
		G ₃	T ₅	57.8 a	+6	58.6 a	+2
			S _L	49.1 b	–10	48.2 b	–16
			S _w	39.1 c	–28	31.7 c	–45
Agramunt	Wheat	G ₁ –G ₂	Ct	44.1 a		45.7 a	
			T ₅	50.8 a	+15	51.9 a	+14
			S _L	43.6 ab	–1	44.9 ab	–2
		G ₃	S _w	35.5 b	–20	35.9 b	–21
			Ct	36.2 bc		40.1 a	
			T ₅	47.5 a	+31	42.8 a	+7
	Barley	G ₁ –G ₂	S _L	37.6 b	+4	38.2 a	–5
			S _w	28.8 c	–20	28.9 b	–28
			Ct	51.5 a		55.0 a	
		G ₃	T ₅	55.2 a	+7	56.2 a	+2
			S _L	49.7 a	–3	–	–
			S _w	41.1 b	–20	–	–

were similar in the distal (G₃) and in the proximal (G₁–G₂) grain positions (Table 3). Trimming the spikes did significantly increase the weight of the wheat grains in Gimenez but not in Agramunt (Table 3). In barley, no differences were found in the weight of the individual grains in any of the two locations (Table 3). The effects reported on individual grain weight were more related to the rate of grain growth than to the duration of grain filling (Fig. 5).

3.3. Stem reserves and spike photosynthesis

Important amounts of reserves had been stored in the stems at anthesis in most combinations of crops species by environmental conditions (Fig. 6), though wheat always stored more reserves in the stems (from slightly over 100 to more than 200 g m^{–2}) than barley (ca. 20–130 g m^{–2}). Using the difference in stem reserves between anthesis and physiological maturity as a proxy of the remobilization of the reserves stored in stems before flowering to fill the grains, it seemed that the most general condition for the

controls is that yield was rather independent of the contributions of these reserves. The three cases departing clearly from this pattern, and in which the reserves stored in stems at flowering seemed to have large contributions to yield, were the two barley crops grown in Agramunt (roughly 100 g m^{–2} of yield might have been contributed by remobilization of these reserves) and the wheat crop under low inputs in Gimenez (roughly 150 g m^{–2}; Fig. 6). Furthermore, the latter was the unique case in which there were virtually no reserves left available in the stems at maturity.

Shading treatments increased the utilization of pre-flowering reserves both in wheat and barley, but even in the extreme cases of severe shadings there were noteworthy amounts of reserves left non-remobilized in the stems in most cases (Fig. 6). In wheat there were non-structural reserves still available for remobilization in the stems at maturity in three out of the four environments (all but that of Gimenez with low-inputs) (Fig. 6). In barley this depletion of non-structural reserves in shaded plots was also clear in two of the conditions in which this was measured (Gimenez with high-inputs and Agramunt with low-inputs) (Fig. 6). Trimming the spikes increased the reserves stored in the stems at physiological maturity compared to the control in all the analyzed cases (and in most of them, the reserves at maturity in T₅ were also higher than at flowering) (Fig. 6).

The rate of photosynthesis of the spikes during grain filling was measured in the control and in the plots in which the leaves but not the spikes were severely shaded (i.e. S_L). It was clear that the photosynthetic rate of the spikes was consistently increased by shading the rest of the canopy (Fig. 7). The trend was general for all growing conditions and through grain filling (earliest measurements taken ca. 10 d after flowering are on the top right of the figure and latest measurements, taken when the spikes started to lose green colour on the bottom left of the figure). The trend was also similar for wheat and barley, the increase in spike photosynthesis due to leaf shading was between ca. 60% and 75% (Fig. 7). This means that spike photosynthesis rate in the controls was

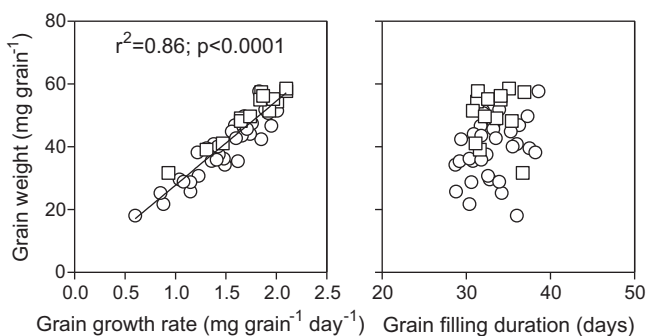


Fig. 5. Relationship between grain weight and (a) grain growth rate and (b) grain filling duration for barley (squares) and wheat (circles) grown at Gimenez and Agramunt (NE Spain) under low- and high-inputs.

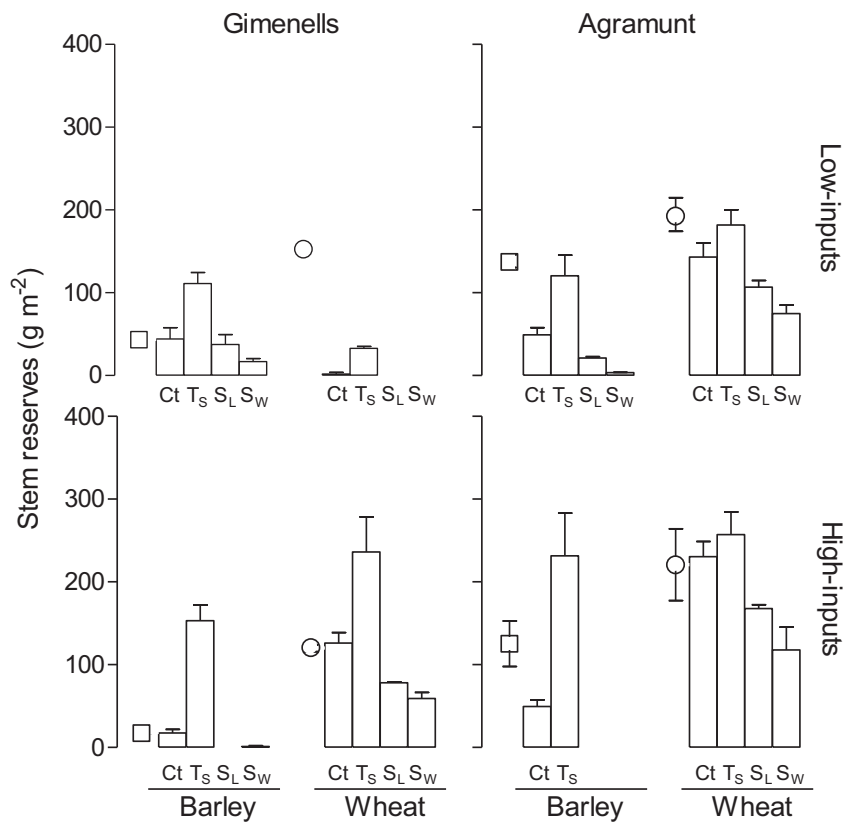


Fig. 6. Stem reserves (water soluble carbohydrates) at anthesis (squares, barley; circles, wheat) and at maturity (bars) for barley and wheat grown under low- and high-inputs at Gimenells and Agramunt for control (Ct), trimmed spikes at 7 days after anthesis (T_S), leaf shading (S_L) and whole canopy shading (S_W) treatments. Crossed vertical lines indicate standard error.

down-regulated with respect to their potential value both in wheat and barley.

3.4. Source–sink balance during grain filling period

The source–sink balance was estimated through the relationship between a theoretically achievable grain weight (the weight that grains of the control would have achieved if all the reserves

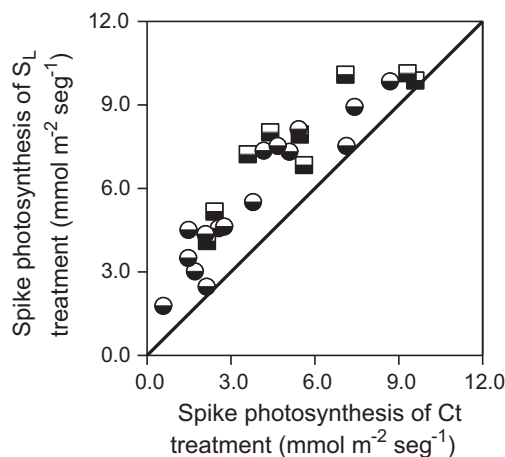


Fig. 7. Relationship between spike photosynthesis rates measured at solar noon on cloudless days in spikes of the exposed spike/shaded canopy treatment (S_L) and those measured in control (Ct) spikes. Barley (squares) and wheat (circles). The line represents the 1:1 relationship. The measurements were made over a period stretching between ca. 10 d after flowering to the onset of visual senescence of the spikes.

available in the stems at physiological maturity would have been remobilized to the growing grains¹) and the potential grain weight (assumed to be represented by grain weight in T_S). In this analysis, values of theoretically achievable grain weight higher than those of potential grain weight (above the 1:1 line in Fig. 8) would imply that the availability of assimilates (those actually allocated to the grains plus those that could have been remobilized to the grains) was more than sufficient to fill the grains to their potential size. Conversely, if data points fell below the 1:1 line, there would be a limitation to reach the potential grain size due to a lack of assimilates being available. In barley, the theoretically achievable grain weight exceeded the potential grain weight in virtually all growing conditions, with a single exception (Gimenells under high inputs), where the source availability and the sink demands appeared to be closely balanced (Fig. 8). At least under the range of growing conditions explored in the present study, no source limitations were observed in barley crops (Fig. 8). In wheat, the scenario depended upon the environmental conditions; there seemed to have been a degree of source-limitation at Gimenells under low inputs, as the potential weight of the grains could not be reached by the grains of the control even if all reserves available at maturity in the stems would have been allocated to the grains. In the same location, but under high inputs, there seemed to have been no source-limitation provided all assimilates available would have been allocated to and used by the growing grains. In Agramunt the data-points fell well above the 1:1 line indicating sink-limited grain growth under low- and high-input in this site for wheat as well (Fig. 8).

¹ This assessment would actually slightly overestimate the theoretically achievable grain weight disregarding remobilization costs.

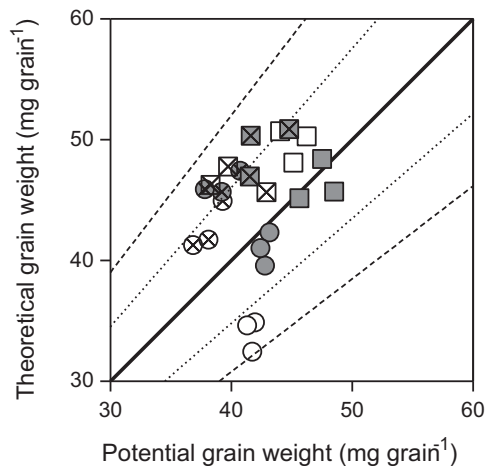


Fig. 8. Relationship between theoretical grain weight (grain weight of control grains plus estimated increase attributable to remobilization to grain of stem WSC available at maturity) and potential grain weight (weight of grains in the trimmed spike treatment). Squares and circles represent barley and wheat; white and grey symbols represent low and high inputs and crossed and solid symbols represent Agramunt and Gimenez locations, respectively. The solid line represents the 1:1 relationship and the dotted line represents the iso-responses of 15 and 30%.

4. Discussion

The choice of prospective parents for a crossing, the identification of sound selection criteria and, ultimately, the design of ideotypes require having a picture as clear as possible of what sort of attributes must be modified in particular developmental stages. During the ‘critical period’ (that when major reductions in yield are evident in response to reductions in crop growth), crops are strongly source-limited (Slafer and Savin, 2006; Serrago et al., 2008). In cereals, this critical period for yield determination is when grain number is being determined (Fischer, 1985; Slafer, 2003; Reynolds et al., 2012), through sensitivity of floret development, pollination and grain setting to environmental conditions during the spike growth phase of few weeks immediately before anthesis (Fischer, 2011; Gonzalez et al., 2011). That is why yield is far more related to grain number than to grain weight (Fischer, 2007a; Miralles and Slafer, 2007), as grain weight is less plastic and more heritable than grain number in cereals (Sadras and Slafer, 2012). It is less clear whether during the effective period of grain filling the capacity of the source is in excess of the grain-growing demands, as it might be evolutionarily expected for grain crops (and for other organisms not taking care of the offspring; Sadras, 2007). To clarify this, many studies have focused on establishing the degree in which grain growth would be limited by the source strength. What our study contributes over most others already published includes that (i) we did not limit responsiveness to grain growth but also included source responses in terms of reserves and spike photosynthesis, (ii) we analyzed responsiveness of sources and sinks to a range of treatments removing sink- and source-strengths (one of which, shading the canopy below the spikes on a crop basis was rather original), (iii) we quantified the responses under a wide range of conditions which affected the status of the crop at the timing of the imposition of the treatments as well as during grain filling, and (iv) we compared simultaneously and under the same range of conditions and treatments responses of wheat and barley. In contrast, the most common approach to determine whether yield is source- or sink-limited during grain filling has been to determine grain weight changes in response to post-anthesis source-sink manipulations in a particular species and under restricted range of conditions. Significant changes in grain weight are usually taken as the unique indicator of source-

sink-limitation to grain growth. In the present study, we found that this seems inappropriate to reach sound conclusions as even when there was a significant reduction in grain weight due to shading, crops did not exhaust its stem reserves at maturity in 9 out of 14 cases analyzed. Thus, it seems that the results were occasionally paradoxical: grain weight might have been reduced by shading or increased by trimming whilst there were noteworthy amounts of soluble carbohydrates left unused in the stems at maturity and control spike photosynthesis seemed to have been down-regulated. In those specific cases, it could not be indisputably interpreted that grain growth would be source- or sink-limited. However, even acknowledging the occasional paradoxical results, all in all, it seemed that grain growth would hardly be source-limited in the controls, with wheat in Gimenez under low-inputs being a likely exception. When applying very severe shadings throughout grain filling a clear reduction in final grain weight may be easily reached, as we occasionally found in the present study with the S_W treatments. Sandaña et al. (2009) found that shading a wheat crop with a mesh allowing only 10% of the incoming radiation to reach the canopy (90% shading) produced a remarkable reduction of grain weight, in line with reductions observed earlier when 80% shading was imposed (Beed et al., 2007). But reductions in grain weight due to very severe shading may be wrongly interpreted as an evidence that grain growth in the control (the realistic situation in which we are truly interested) was source-limited.

The most common approach carried out to determine the actual contribution of the spikes to grain growth is through direct reduction on spike photosynthesis (i.e. shading). However, our treatment consisted on shaded the canopy below the spikes (S_L) and then determining the spike capacity to increase its photosynthetic activity. The results shown in our study highlighted the importance of the spike photosynthesis to grain growth (Tambussi et al., 2007), especially in poor grain filling conditions. More importantly in the context of the present study, it was clear that when shading was imposed to the leaves but not to the spikes (i) the reductions in grain weight were not significant or only minor and, in addition, (ii) spike photosynthesis was consistently increased compared to that of the control with un-shaded leaves. Then, grain weight under S_L was largely unresponsive due to compensations produced by remobilization of reserves and increased spike photosynthesis. Our results are in agreement with few studies in the literature showing the relevance of spike photosynthesis on grain growth both in bread wheat (Maydup et al., 2010) and durum wheat (Araus et al., 1993), reinforcing the conclusion that there could hardly be any source-limitation to grain growth if spike photosynthesis in the control plots was down-regulated (as it increased by shading the leaves).

Different papers linked grain yield losses due to different stresses occurring during grain filling period with the source-sink ratio established by the crops (Bingham et al., 2009; Serrago et al., 2011). However, according to our results, abiotic or biotic stresses occurring during grain filling would only affect final grain weight if they are rather severely affecting the strength of the source (e.g. Acreche and Slafer, 2011; Serrago et al., 2011) or if they affect grain weight potential (e.g. Slafer and Miralles, 1992; Calderini and Reynolds, 2000). For example, leaf diseases (Serrago et al., 2011) or lodging (Acreche and Slafer, 2011) would only produce a source-limited grain growth when they are very severe, implying that mild stresses would not affect grain size. This is only compatible with a situation in which grain growth in the controls is dominantly limited by the capacity of the sinks to grow. During the grain filling period, assimilate supply can be provided by three different sources: (i) post-anthesis leaf photosynthesis, (ii) remobilization of stems reserves (Schnyder, 1993; Serrago et al., 2011), and (iii) current post-anthesis spike photosynthesis (Maydup et al., 2010). As mentioned above, light-limited conditions, foliar diseases or lodging reduced the incident/intercepted radiation during

grain filling (Beed et al., 2007; Serrago et al., 2011). The sharp reduction on the incident radiation during the grain filling period due to S_W treatment reduced the assimilates provided by post-anthesis overall photosynthesis decreasing grain weight in both, wheat and barley crops, as it was previously shown by Beed et al. (2007) and Sandaña et al. (2009). In this growing condition, the remobilization of reserves from stems was an important source of assimilates for growing grains. These results are in agreement with previous reports where wheat crops exposed to different stresses during grain filling period increased the remobilization of reserves (Zilberstein et al., 1985; Gaunt and Wright, 1992; Serrago et al., 2011).

There are few evidences comparing source–sink limitation in cultivars of wheat and barley growing together. However, it has been proposed that barley would be less source-limited than wheat, at least in light-limited environments (Bingham et al., 2007, 2009). Recognizing that only one cultivar per species was tested, our results are in agreement with previous reports (Dreccer et al., 1997; Savin et al., 2006); barley crops did not seem to be source-limited, as the source availability (expressed as the theoretical grain weight) exceeded the sink capacity (expressed as grain weight potential). However, in wheat crops (cv. Soissons) the degree of source-limitation was somewhat different than that found in barley (cv. Sunrise). In the low-input conditions at Gimennells, sink requirements exceeded the source availability during the grain filling period, causing an increase in grain weight in the T_5 treatments respect to the control (ca. 20%). However, this response seemed exceptional, as at the other environmental conditions no clear evidences of source-limitation for grain growth in wheat were found. Furthermore, we did not find any clear indication of source-limitation to grain growth in barley in any of the environmental conditions.

These results highlight the need to consider the source-limitation for cereals grain growth as a crop condition only occasionally taking place, highly dependent on the growing conditions during the grain filling period. Thus, wheat and barley would hardly be source-limited during grain filling, and only when subjected to an extremely severe stress occurring during grain filling (in the case of our study, reducing the radiation levels during the whole grain filling by 75%) grain size would be reduced due to lack of enough assimilates available to fill them.

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