

# Grain Filling and Dry Matter Translocation Responses to Source–Sink Modifications in a Historical Series of Durum Wheat

F. Álvaro, C. Royo,\* L. F. García del Moral, and D. Villegas

## ABSTRACT

To understand yield limitations imposed by the source–sink relationship and the effects that breeding had on it, the source–sink ratio was modified on 24 durum wheat cultivars released in different periods. Field experiments were conducted during 3 yr, with treatments consisting of removing the flag-leaf blade, degrading half of the spike, and intact checks. Grains from near-basal spikelets tended to be the most sensitive to source–sink modifications. Degraining increased grain weight up to 15% in modern cultivars, which were more source limited and more responsive to source–sink modifications than the old ones. Our results suggest a source–sink colimitation for grain filling in Mediterranean environments. Translocation of pre-anthesis assimilates was responsible for 25, 27, and 33% of grain weight in old, intermediate, and modern cultivars, respectively. Defoliation did not alter dry matter translocation (DMT) from the main stem, as the reduction in translocation of leaf blades was compensated with translocation increases from the chaff. Sink demand determined DMT that was mostly regulated by the stem. Our results indicate that future yield improvements may be achieved by strengthening the source capacity.

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**Abbreviations:** CPA, contribution of preanthesis assimilates to grain yield; DMT, dry matter translocation; DMT<sub>c</sub>, dry matter translocation efficiency; GDD, growing degree-days.

**D**URUM WHEAT (*Triticum turgidum* L. var. *durum*) is an important crop in the Mediterranean basin, where terminal abiotic stresses are the main yield constraints. Under severe drought-stressed Mediterranean environments, the number of spikes per square meter becomes the most relevant component of durum wheat yield formation (García del Moral et al., 2005), while the contribution of grain weight to final yield increases as drought stress diminishes (Moragues et al., 2006; Royo et al., 2006).

Recent studies have associated genetic yield gains of durum wheat in Mediterranean countries with increases in the number of grains per unit area, while grain weight seems to have remained unchanged (De Vita et al., 2007; Royo et al., 2007). It has been reported that increases in the number of grains per unit area have resulted from increases in the number of plants per unit area, the number of spikes per plant, and the number of grains per spike, with relative contributions of 20, 29, and 51%, respectively (Royo et al., 2007).

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The number of grains per spike and final grain weight are established sequentially during plant development. The potential number of grains is determined during spike growth before anthesis, while grain weight is fixed during grain filling. The great increase in the number of grains per spike in modern cultivars seems to have been the result of the reallocation of assimilates from vegetative to reproductive organs (Loss and Siddique, 1994; Slafer et al., 1994), mostly as a pleiotropic effect of the introduction of dwarfing genes (Fischer and Stockman, 1986; Youssefian et al., 1992). There may be two reasons for the lack of increase in grain weight in modern cultivars: the insensitivity to gibberellic acid induced by the presence of dwarfing genes (Miralles et al., 1998), and postanthetic competition for assimilates (Flintham et al., 1997).

Numerous studies have been conducted in bread wheat to assess grain weight variations as a response to source–sink modification treatments, but contrasting results have been obtained according to the genotype and the environment (Fischer and HilleRisLambers, 1978; Slafer and Savin, 1994; Cruz-Aguado et al., 1999). However, in most cases the authors concluded that grain filling is colimited by both the availability of assimilates (source) and the capacity of the grains (sink) to use them (Borrás et al., 2004). It has also been reported that in bread wheat the limitation of the source has increased over time as a consequence of great improvements in the sink size (Fischer and HilleRisLambers, 1978; Koshkin and Tararina, 1989; Kruk et al., 1997). A decrease in the source–sink ratio would also be expected in durum wheat, given that modern cultivars have less biomass at anthesis than the old ones (Álvaro et al., 2008), but more grains per unit area. Nevertheless, Calderini et al. (2006) did not find significant differences in final grain weight of intact and degrading spikes of durum wheat grown in irrigated environments.

Grain filling in small-grain cereals occurs by acquisition of assimilates from current photosynthesis and translocation of stored nonstructural assimilates accumulated before anthesis. In Mediterranean environments, current photosynthesis during grain filling declines as a result of drought and heat stress, and the remobilization of stored nonstructural carbohydrates becomes particularly important for grain filling (Papakosta and Gagianas, 1991; Blum, 1998). Previous studies on durum wheat have shown that total dry matter translocated from the main stem to the filling grains is greater in modern cultivars than in those cultivated in the first half of the 20th century, and dry matter translocation efficiency has been greatly improved (Álvaro et al., 2008). Modern durum wheat cultivars reach values of up to 25% of the main stem biomass translocated (Álvaro et al., 2008), which are lower than those found in modern bread wheat cultivars (Ehdaie et al., 2006).

Understanding the changes caused by breeding in the source–sink relationship, the limitations to grain weight

improvements, and the capacity to translocate dry matter to the filling grains is critical for orienting further yield and yield stability increases in durum wheat in the Mediterranean region, particularly in moderately stressed areas. In this study the source–sink relationship was modified in 24 durum wheat cultivars composing a historical series of cultivars grown in Italy and Spain during the 20th century. Our aim was to determine whether the constraints to grain filling—in terms of resources availability and sink size—have changed in the course of time and to establish their degree of dependence on the grain position within the spike. The effect of the source–sink modification treatments on the grain–filling curve coefficients, dry matter translocation (DMT), and the contribution of DMT to grain filling were also studied to determine the capacity of the source to respond to future increases in the sink size.

## MATERIALS AND METHODS

### Experimental Details

A collection of 12 Italian and 12 Spanish durum wheat cultivars representing the germplasm grown in these Mediterranean countries over the last century was used (Table 1). Based on their year of release, the cultivars were divided into three periods: old (before 1945), intermediate (from 1950 to 1985), and modern (released from 1988 to 2000). Old cultivars were landraces and, in the case of Italy, also selected genotypes from exotic populations such as Senatore Capelli. Within the intermediate cultivars some of the first semidwarf genotypes derived from the International Maize and Wheat Improvement Center materials, such as Mexa and Creso, were included. The modern cultivars were chosen among those recently released by local breeding programs. The presence of gibberellins-insensitive dwarfing genes, *Rht-B1* in durum wheat, had been previously determined (Royo et al., 2007) by analyzing the response of seedlings to gibberellic acid (Table 1).

Field experiments were conducted in Lleida, northeast Spain (41°40'N, 0°20'E) during three growing seasons, 2002–2003, 2003–2004, and 2004–2005, in a fine loamy, Mesic Calcixerolic Xerochrept soil. Each experiment consisted of a randomized complete block design with three replications and plots of 12 m<sup>2</sup> (8 rows 0.15 m apart), half of which were devoted to destructive samplings. Experiments were planted between November 26 and December 16 in all cases, with a sowing rate of 400 seeds m<sup>-2</sup>. Mean temperature during grain filling was around 21°C in the 3 yr, with averaged maximum temperatures of 29°C. Seasonal precipitations ranged from 130 to 250 mm, and flood irrigation provided 150 mm additionally every season. Plots were fertilized before planting with 500 kg ha<sup>-1</sup> of complex 15–15–15. At the end of tillering the plots were top-dressed with ammonium nitrosulfate at a rate of 35 kg N ha<sup>-1</sup>. Weeds were chemically controlled with diclofop-methyl

(methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate) at 3 L ha<sup>-1</sup>, bromoxynil (3,5-dibromo-4-hydroxybenzoxynitrile)+ ioxynil (4-hydroxy-3,5-diiodobenzoxynitrile) at 3 L ha<sup>-1</sup>, and thifensulfuron (3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoysulfamoyl)thiophene-2-carboxylic acid)+ tribenuron (2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoysulfamoyl]benzoic acid) at 45 g ha<sup>-1</sup>.

## Treatments and Curve Fitting

At anthesis, 150 main stems were tagged in each plot. Source–sink treatments consisting of (1) flag-leaf defoliation, and (2) degrading of spikes by trimming the spikelets of one side of the spike, were applied 8 d after anthesis on 50 spikes per plot each. The remaining 50 intact spikes were used as a control treatment. Three tagged spikes were removed at random twice per week from anthesis to physiological maturity. All the spikelets from one size of the spike were divided into near-apical, central, and near-basal, and the grains contained in each fraction were counted and oven-dried at 70°C for 48 h to determine dry weight. Grain dry weight data of each treatment and plot were fitted to the logistic curve proposed by Darroch and Baker (1990), and the coefficients of the grain-filling curves were determined for each treatment and plot. Final grain weight was estimated in milligrams. Grain-filling duration was considered to be the time in accumulated growing degree-days (GDD) required for grain weight to reach 0.95 final grain weight, using a base temperature of 9°C (Angus et al., 1981), and was derived from the curve. The maximum rate of grain filling was mathematically determined from the curve parameters, as described by Royo and Blanco (1999), and was expressed as milligrams of grain GDD<sup>-1</sup>.

## Biomass and DMT

The plants contained in a randomly chosen 0.5-m-long section of a central row of each plot were uprooted at anthesis and the number of plants counted. Leaf area of the total sample was measured from the projection of the leaves with a leaf-area meter (AT Delta-T-Devices Ltd., Cambridge, UK), excluding from the measurement yellow and dry leaves. Samples were oven dried at 70°C for 48 h to determine the dry weight per plant.

Ten main stems per treatment and plot were randomly sampled at anthesis and at physiological maturity in the second and third years of experiments. In the laboratory each stem was fractioned into its parts, the number of grains per spike was counted and dry weights of the 10 main stems were determined separately for the spikes, stems (including all leaf sheaths), flag-leaf blades, and remaining leaf blades. Dry matter translocation (mg) was computed for each fraction as the difference between the dry weight at anthesis and at physiological maturity, excluding grain weight in the case of the spike. Dry matter translocation efficiency (DMT<sub>e</sub>, %), and the contribution of preanthesis assimilates

**Table 1. Cultivars used in the study.**

Origin	Period	Cultivar	Year of release	<i>Rht-B1</i> presence	
Italian	Old	Balilla Falso	<1930	–	
		Carlojucci	1945	–	
		Razza 208	<1930	–	
		Senatore Capelli	1930	–	
		Adamello	1985	–	
	Intermediate	Capeiti 8	1955	–	
		Creso	1974	+	
		Trinakria	1970	–	
		Modern	Cirillo	1992	+
			Flavio	1992	+
			Simeto	1988	+
			Zenit	1992	+
Spanish	Old	Blanco Verdeal	<1930	–	
		Clarofino	<1930	–	
		Pinet	<1930	–	
		Rubio de Belalcázar	<1930	–	
		Bidi 17	1950	–	
	Intermediate	Camacho	1975	+	
		Esquilache	1976	+	
		Mexa	1980	+	
		Modern	Ariesol	1992	+
			Astigi	1999	+
			Boabdil	2000	+
			Senadur	1995	+

to the grain (CPA, %) were assessed following the methodology proposed by Papakosta and Gagianas (1991) as

$$DMT_e = (DMT/DM_{anthesis}) \times 100$$

$$CPA = (DMT/GWMS) \times 100$$

where GWMS was the grain weight of the main spike (g).

## Statistical Analysis

Analyses of variance were performed considering the growing season as a random factor. Least-square means of the variables between periods and spike positions were compared by a *t*-test at the 5% significance level. Linear regression lines were fitted to the relationships between the percentage of changes caused by each sink–source modification treatment on the grain-filling curve coefficients and the year of release of the cultivars. Pearson correlation coefficients between the percentages of change caused by the treatments on the grain-filling curve coefficients were also calculated by using the mean values of each cultivar across years. All analyses were performed with the SAS-STAT statistical package (SAS Institute, 2000).

## RESULTS

### Source–Sink Modification Treatments

Defoliation treatment, consisting in removing the flag-leaf blade of the main stems, reduced leaf area per plant

by around 20% in cultivars from the three periods (Table 2). The biomass of intermediate and modern cultivars was reduced by defoliation more than that of the old ones. The number of grains per spike was not affected by defoliation. Degraining one side of the spike caused a reduction of around 45% in the number of grains per spike in the cultivars of the three periods.

### Grain-Filling Curve Coefficients

The logistic equation proposed by Darroch and Baker (1990) provided an appropriate fit to the grain weight data from apical, central, and basal spikelets under the control and source-sink modification treatments (average  $R^2 = 0.98 \pm 0.02$ ). The environmental effect accounted for around 23, 25, and 49% of the variations in final grain weight, maximum rate of grain filling, and grain-filling duration, respectively. The analyses of variance of the coefficients of the grain-filling curves showed that final grain weight and maximum rate of grain filling were significantly affected by the source-sink modification treatments in grains from the three spike positions, (Table 3). Defoliation reduced final grain weight and maximum rate of grain filling of apical, central, and basal grains, while it had no effect on grain-filling duration. Degraining resulted in higher final grain weight, maximum rate of grain filling, and grain-filling duration values on grains from all the positions. The effect of degraining on the grain-filling curve coefficients was greater than that of defoliating in all the spike positions.

The coefficients of the grain-filling curves varied according to the grain position within the spike. In all treatments final grain weight and maximum rate of grain filling were greater in grains from central spikelets, followed by the basal and apical ones. Grain-filling duration was significantly longer in basal spikelets than in the apical or central ones (Table 3). The treatment  $\times$  position interaction was significant for final grain weight, the effect of both treatments being greater in the grains from basal spikelets. Defoliation reduced final grain weight from 4.6 to 5.8%, while degraining increased it from 8.7 to 12%.

The three grain-filling curve coefficients were similar for the cultivars released at different periods, but the treatment  $\times$  period interaction was significant, although noncrossover in nature, for final grain weight and maximum rate of grain filling. The lack of significance in the

treatment  $\times$  period  $\times$  position interaction indicates that the response to the treatments of cultivars released at different periods followed the same trend in apical, central, and basal grains. Mean values for the treatment  $\times$  period interaction on grain-filling curve coefficients, averaged over the three positions within the spike, are shown in Table 4. Defoliation reduced final grain weight and maximum rate of grain filling in cultivars of the three periods but did not affect their grain-filling duration. The reduction caused in final grain weight by defoliation increased from old to modern cultivars as shown by the significance of the relationship between the percentage of decrease in final grain weight due to defoliation and the year of release of Italian ( $R^2 = 0.60$ ;  $p < 0.01$ ) and Spanish cultivars ( $R^2 = 0.43$ ;  $p < 0.05$ ) (Fig. 1a). Genotype means across experiments of final grain weight reductions caused by defoliation ranged from -1.4 to -9.8%. Reductions in maximum rate of grain filling caused by defoliation were also greater in the intermediate and modern cultivars than in the old ones, but they were not associated with the year of release (Fig. 1b).

Degraining significantly increased final grain weight, maximum rate of grain filling, and grain-filling duration of cultivars from the three periods, with the exception of grain-filling duration of old cultivars (Table 4). Increases in final grain weight caused by degraining were associated with the year of release in both Italian ( $R^2 = 0.80$ ;  $p < 0.001$ ) and Spanish ( $R^2 = 0.52$ ;  $p < 0.01$ ) sets of germplasm (Fig. 1d), and were 10% greater in modern than in old cultivars. Genotype means across experiments of final grain weight increases caused by degraining ranged from 1.6 to 22%. Increases in maximum rate of grain filling and grain-filling duration caused by degraining were also greater in the intermediate and modern cultivars than in the old ones (Table 4). The relationship between grain-filling duration enlargement and the year of release was significant for the Italian cultivars ( $R^2 = 0.40$ ;  $p < 0.05$ , Fig. 1f).

The response to defoliation treatment of cultivars released at different periods measured in terms of final grain weight reductions was not associated with changes in maximum rate of grain filling or grain-filling duration, but changes in both coefficients were significantly and negatively correlated (Table 5). However, increases in final grain weight due to degraining proved to be significantly and positively correlated to changes in grain-filling duration.

**Table 2. Reduction caused in blade leaf area per plant (LAP) and dry weight per plant (DWP) at anthesis and in grains per spike determined at ripening, caused by defoliation and degraining treatments.**

	Control			Defoliated		Degrained
	LAP	DWP	Grains spike <sup>-1</sup>	Reduction in LAP	Reduction in DWP	Reduction in grains spike <sup>-1</sup>
	cm <sup>2</sup>	g		%		
Old	148a <sup>†</sup>	5.11a	40.0c	20.1a	2.82b	46.4a
Intermediate	122b	3.79b	43.4b	21.4a	3.57a	44.8a
Modern	124b	3.65b	45.4a	20.5a	3.31a	44.9a

<sup>†</sup> Means within a column followed by the same letter are not significantly different at 5% probability level.

## Dry Matter Translocation

Defoliation did not affect DMT,  $DMT_e$ , or the contribution of preanthesis assimilates to the main spike yield (CPA) when measured at main stem level (Table 6). Instead, defoliation reduced the DMT of leaf blades, increasing translocation from the chaff and causing increases in  $DMT_e$  of both leaf blades and chaff. The contribution of preanthesis assimilates to grain yield (CPA) from the stem and the chaff increased by removing the flag-leaf blade (Table 6). However, the CPA from all leaf blades did not decrease because their  $DMT_e$  increased significantly.

Degraining treatment reduced the DMT and  $DMT_e$  of the whole main stem, while maintaining CPA (Table 6). Reductions in the DMT and  $DMT_e$  were due to the decrease caused by degrading on the stem, as no changes were observed in the DMT and  $DMT_e$  of leaf blades, and both traits increased in the spike. The CPA to grain yield from the stem decreased by degrading by 32%, while that of the leaf blades and spikes increased by 53 and 103%, respectively.

The treatment  $\times$  period interaction was significant and crossover in nature for the CPA, suggesting that the effect of degrading on the CPA of main stem structures changed over periods (Fig. 2). Degraining did not affect the CPA of main stems of old and intermediate cultivars but caused a significant increase (4%,  $p < 0.05$ ), thus reducing the contribution of photosynthesis to grain filling in modern genotypes. Changes in the CPA of the stem mostly explained these differences, since degrading treatment increased the CPA of spikes and leaf blades in cultivars of all three periods (Fig. 2). Degraining reduced the CPA from the stem by 86 and 34% ( $p < 0.001$  and  $p < 0.05$ , respectively) in old and intermediate cultivars, while it did not affect modern ones (Fig. 2). Increases in the CPA of leaf blades caused by degrading were 46, 70, and 66% ( $p < 0.001$ ) in old, intermediate, and modern cultivars, respectively. The CPA from the flag-leaf blade to grain weight was not affected by degrading but, as result of the treatment the CPA from the spike, increased by 230, 81, and 90% ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively) in old, intermediate, and modern cultivars.

## DISCUSSION

Defoliation treatment caused a decrease in final grain weight of about 5% across cultivars and spike positions, which was much lower than the decrease in leaf area per plant at anthesis (about 20%). This difference may be explained by the increase in translocation and photosynthesis of other main stem organs. Given that the DMT and the CPA of the whole main stem did not change either in absolute or in relative terms, defoliation may have caused an increase in the photosynthesis of the intact main stem structures, but it was not enough to compensate grain weight losses. These results suggest a source limitation in the germplasm used in this study, which was confirmed by

**Table 3. Adjusted means of the grain-filling curve coefficients: final grain weight ( $W$ ), maximum rate of grain filling ( $R$ ), and grain-filling duration ( $D$ ), determined from grains at three different positions along the main spike, under source-sink modification treatments. The percentage of change in relation to the control is in parentheses.**

	$W$ mg	$R$ mg GDD <sup>-1†</sup>	$D$ GDD
<b>Apical</b>			
Control	48.4b <sup>‡</sup>	0.228b	350b
Defoliated	46.0c (-5.0)	0.216c (-5.3)	350b (0)
Degrained	52.6a (8.7)	0.243a (6.6)	359a (2.6)
<b>Central</b>			
Control	52.3 b	0.246 b	354 b
Defoliated	49.9c (-4.6)	0.233c (-5.3)	352b (-0.6)
Degrained	57.8a (10)	0.261a (6.1)	367a (3.7)
<b>Basal</b>			
Control	51.5 b	0.239 b	366 b
Defoliated	48.5c (-5.8)	0.225c (-5.9)	363b (-0.8)
Degrained	57.6a (12)	0.257a (7.5)	377a (3.0)

<sup>†</sup>GDD, growing degree-days.

<sup>‡</sup>Means within a column and position followed by the same letter are not significantly different at 5% probability level.

**Table 4. Adjusted spike means of the grain-filling curve coefficients: final grain weight ( $W$ ), maximum rate of grain filling ( $R$ ), and grain-filling duration ( $D$ ) for durum wheat cultivars grown in three periods, under source-sink modification treatments. The percentage of change in relation to the control is in parentheses.**

	$W$ mg	$R$ mg GDD <sup>-1†</sup>	$D$ GDD
<b>Old</b>			
Control	52.1b <sup>‡</sup>	0.243b	353a
Defoliated	49.9c (-4.2)	0.236c (-3.2)	354a (0.3)
Degrained	54.8a (5.2)	0.254a (4.4)	358a (1.4)
<b>Intermediate</b>			
Control	50.6 b	0.236b	356b
Defoliated	48.0c (-5.1)	0.222c (-6.1)	356b (0)
Degrained	56.6a (12)	0.257a (8.7)	369a (3.6)
<b>Modern</b>			
Control	49.4b	0.235b	357b
Defoliated	46.2c (-6.5)	0.222c (-5.4)	352b (-1.4)
Degrained	57.0a (15)	0.251a (7.0)	380a (6.4)

<sup>†</sup>GDD, growing degree-days.

<sup>‡</sup>Means within a column and period followed by the same letter are not significantly different at 5% probability level.

the results of the degrading treatment. If the source had not been limited, grain weight would not have increased by degrading, but an increase of 11% was observed when half of the spikelets of the spike were removed. Nevertheless, the fact that grain weight increases caused by degrading were lower than double the weight of the control also suggests a sink limitation. Source-sink colimitation has been widely reported for bread wheat (Simmons et al.,

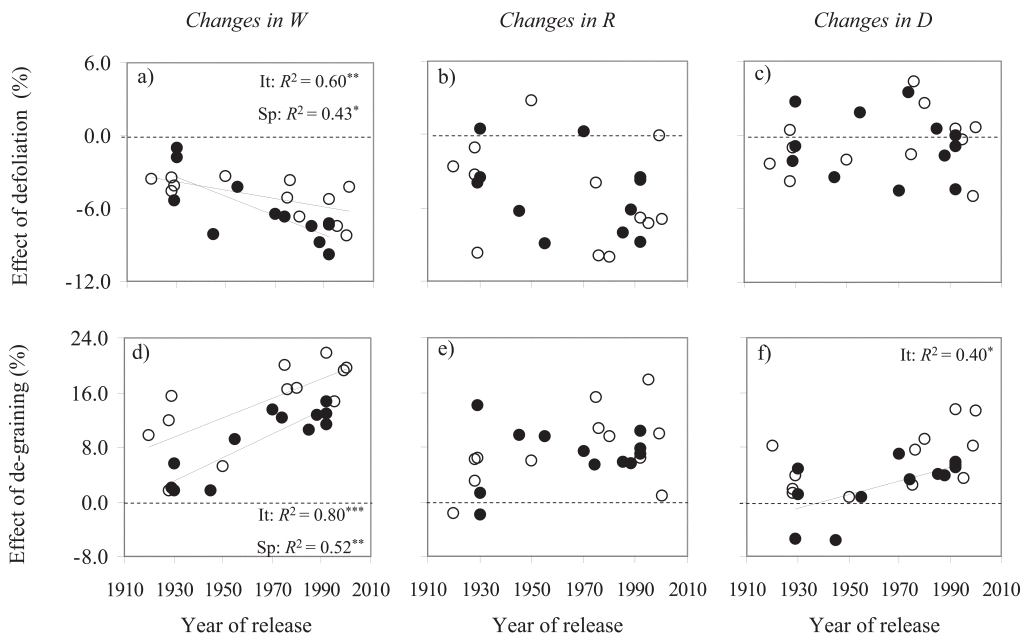


Figure 1. Relationships between the percentage of change caused by each sink–source modification treatment on the grain-filling curve coefficients: final grain weight ( $W$ ), maximum rate of grain filling ( $R$ ), and grain-filling duration ( $D$ ), and the year of release of Italian (filled circles) and Spanish (open circles) cultivars. Regression lines are fitted only to significant relationships. \*Significant at the 0.05 probability level; \*\*significant at the 0.01 probability level; \*\*\*significant at the 0.001 probability level.

**Table 5. Pearson correlation coefficients between the percentages of change, with regard to the control, caused by source–sink modification treatments on grain-filling curve coefficients.**

Treatment†	Italian	Spanish
	———— (n = 12) ————	
Defoliating		
W change – R change	0.33	0.14
W change – D change	0.46	0.13
R change – D change	-0.59*	-0.77**
De-graining		
W change – R change	0.05	0.37
W change – D change	0.80**	0.66*
R change – D change	-0.45	-0.27

\*Significant at the 0.05 probability level.

\*\*Significant at the 0.01 probability level.

†W, final grain weight; R, maximum rate of grain filling; D, grain-filling duration.

1982; Slafer and Savin, 1994; Kruk et al., 1997) and other cereals (Voltas et al., 1997; Royo et al., 2000; Borrás et al., 2004). However, our results do not completely agree with the conclusions of Calderini et al. (2006), who failed to find source limitation in the grain filling of durum wheat. Differences between the two studies may be due to the contrasting environmental conditions in which the experiments were conducted, as well as in the plant material used. The conclusions of Calderini et al. (2006) were obtained in two durum wheat cultivars grown in optimal environments, while our study was conducted in water-

limited Mediterranean conditions and involved a wider range of genetic variability. Reductions in plant biomass of close to 40% have been reported in durum wheat as a consequence of drought (Villegas et al., 2001), and this may be the cause of a source limitation in our experiments.

Reductions in final grain weight caused by defoliation were exclusively due to changes in the maximum rate of grain filling, since grain-filling duration remained constant after the treatment. However, increases in final grain weight due to de-graining resulted from the combination of increases in both the maximum rate of grain filling and grain-filling duration, the former being the most responsive to the treatment.

The increase in grain-filling duration could be explained by the increase in the availability of assimilates to filling grains in the de-graining treatment, which did not occur when the flag-leaf blade was removed. Our results are in agreement with previously reported effects of source–sink modification treatments on grain-filling curve coefficients in bread wheat (Simmons et al., 1982; Miralles and Slafer, 1995) and triticale ( $\times$  *Triticosecale* Wittmack) (Royo et al., 2000), which found slighter or even no responses of grain-filling duration to the changes in the source–sink ratio.

Source–sink modification treatments affected final grain weight in all the positions along the spike, but grains from near-basal spikelets tended to be the most sensitive to changes in the availability of assimilates. The fact that final weight of apical grains, the smallest of the spike, was not affected as much as that of central and basal grains by both source–sink modification treatments indicates that their smallest size within the spike was not due to competition for assimilates during grain growth, as reported for bread wheat (Slafer and Savin, 1994). Their position in the spike, distal from the peduncle, the main route for incorporation of assimilates in the spike, likely reduced their chance of taking advantage of an increased source size in the de-graining treatment. However, the greater response of grains from basal spikelets may be explained by their long grain-filling period, and also by the great effect that the treatments exerted on their grain-filling rate, which could be due to their proximity to the main source of assimilates.

The treatments affected final grain weight of cultivars from all periods, but the modern ones responded more to source–sink modifications than the intermediate ones, which in turn were more affected than the old ones. This trend was followed by both Italian and Spanish cultivars. Our results suggest that durum wheat yield gains achieved by breeding during the 20th century led to an increasing limitation of the source of assimilates, probably caused by both the striking increase in the number of grains per unit area (Royo et al., 2007) and the reduction in biomass caused by the introduction of dwarfing genes (Álvarez et al., 2008).

Modern degraigned spikes yielded heavier grains than the old ones, suggesting that the potential grain weight of modern cultivars may be even greater than that of old cultivars. Potential grain weight is determined early during grain development, when the number of endosperm cells is fixed (Brocklehurst, 1977). It may be hypothesized that the shorter duration of the period from sowing to anthesis of modern cultivars compared with the old ones (Álvarez et al., 2008) could encourage the cell division to occur under cooler and wetter conditions, thus enhancing the potential grain weight. Moreover, the increased partitioning of assimilates to the spike of modern cultivars may have also improved the potential grain size. This assumption is supported by the findings of Youssefian et al. (1992), in the sense that carpel size has been improved as a pleiotropic effect of dwarfing genes.

Modifications of the maximum rate of grain filling caused by both treatments were also greater in modern than in old cultivars, in agreement with the changes observed in final grain weight. The percentage of reduction caused by defoliation in final grain weight was not associated with the percentage of changes in its components, the maximum rate of grain filling, and grain-filling duration. The explanation may be found in the significant and negative relationship between the percentages of change in the maximum rate of grain filling and grain-filling duration resulting from the defoliation treatment. This indicates that, in those genotypes suffering the greatest reduction in grain-filling rate, the duration of grain filling was less affected, and vice versa. Our results suggest that there was a differential genetic response to leaf area reductions.

The sensitivity of grain-filling duration to the degraing treatment roughly increased over the periods. The positive and significant association between the percentage

**Table 6. Adjusted means across cultivars of dry matter translocation (DMT), dry matter translocation efficiency (DMT<sub>e</sub>), and the contribution of preanthesis assimilates to grain yield (CPA) for the whole main stem and its fractions under source–sink modification treatments. The percentage of change in relation to the control is in parentheses.**

	DMT	DMT <sub>e</sub>	CPA
	mg	%	
Whole main stem			
Control	576a <sup>†</sup>	17.7a	27.7a
Defoliated	590a (2.4)	18.8a (6.2)	30.8a (11)
Degrained	378b (–34)	11.7b (–34)	28.5a (2.9)
Stem			
Control	362a	17.1a	17.6b
Defoliated	397a (9.7)	18.4a (7.6)	20.7a (18)
Degrained	163b (–55)	8.03b (–53)	11.9c (–32)
Leaf blades (including flag leaf)			
Control	174a	33.0b	8.58b
Defoliated	145b (–17)	36.8a (11)	7.66b (–11)
Degrained	160ab (–8.0)	30.3b (–8.2)	13.1a (53)
Flag-leaf blade			
Control	25.5a	18.2a	1.27b
Degrained	21.5a (–16)	15.2b (–16)	1.66a (31)
Chaff			
Control	35.7b	6.03b	1.72c
Defoliated	47.0a (32)	7.86a (30)	2.36b (37)
Degrained	48.3a (35)	8.96a (49)	3.49a (103)

<sup>†</sup>Means within a column and main stem component followed by the same letter are not significantly different at the 0.05 probability level.

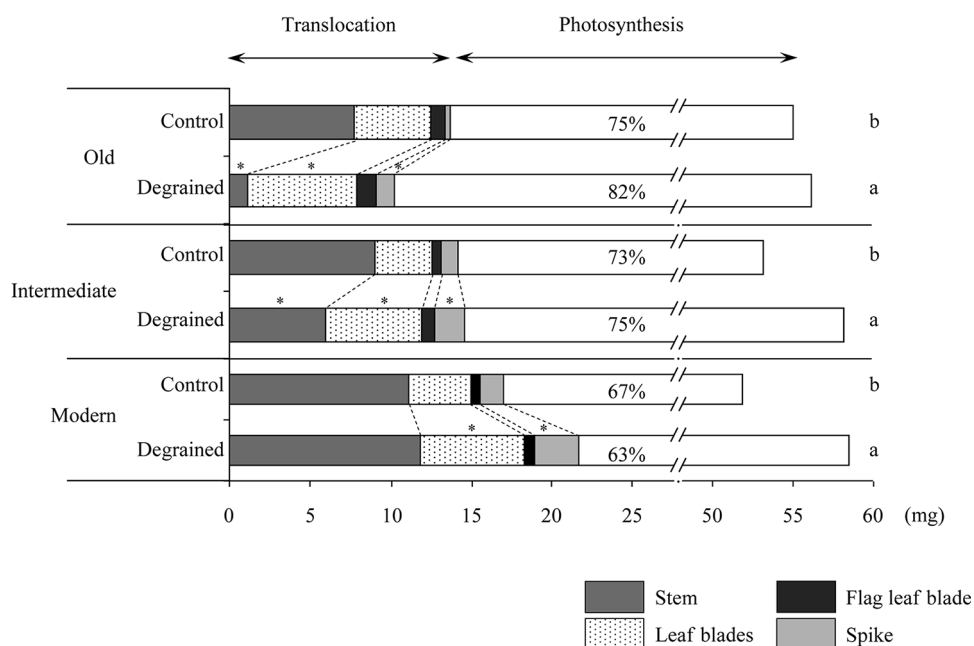


Figure 2. Contribution of preanthesis assimilates from main stem fractions to the grain weight of control and degraigned spikes of old, intermediate, and modern durum wheat cultivars. Letters indicate differences between mean grain weight of control and degraigned spikes within each period at  $p < 0.05$ .

of changes in final grain weight and grain-filling duration indicates that the cultivars in which grain-filling duration was most enlarged by degrading treatment were those in which grain weight increased the most. This was the case of modern cultivars, in which the greatest grain-filling duration and grain weight increases were observed as a consequence of degrading. These results indicate once more the greater source limitation of modern cultivars and suggest that they have a superior capacity to increase grain-filling duration than the old ones, probably because of their earlier anthesis. The augmentation of the availability of assimilates resulting from degrading in modern cultivars led to an increase in grain-filling duration and probably a delay in plant senescence. This is in agreement with the observed reduction in DMT, since a negative relationship has been reported between leaf area duration and DMT (Blum, 1998).

As mentioned above, DMT did not increase on defoliated plants. This was due to the compensation between the reduction in translocation of leaf blades and the increase in translocation from the chaff, which was not sufficient to compensate for the decrease in grain weight caused by the removal of the flag leaf. Although not significant, DMT from the stem tended to rise. Considering that the stem provided more than 60% of the assimilates translocated to the filling grains, the release of improved cultivars with an increased translocation capacity may lead to future yield gains.

Sink reduction decreased translocation of preanthesis assimilates from the main stem to the filling grains by 34%. This reduction was smaller than the 45% decrease in the size of the sink imposed by the degrading treatment. The difference between the two (11%) coincides with the increase observed in the grain weight of degraded spikes, suggesting that the reduction in sink demand only affected translocation, without having any effect on current photosynthesis. These results support the conclusions on bread wheat of Kühbauch and Thome (1989) and Wang et al. (1997), who consider that sink demand determines stem reserve mobilization without affecting the net production of photo-assimilates during grain filling.

Given that the number of grains per spike of modern cultivars is much greater than that of intermediate and old ones (Royo et al., 2007), we analyzed the relative contribution of translocation and photosynthesis to grain filling considering the mean weight of one grain. When the grain weight of intact spikes of different periods is compared, our results demonstrate that translocation of assimilates accumulated before anthesis was responsible for 25, 27, and 33% of grain weight in old, intermediate, and modern cultivars, thus indicating that the role of translocation has been enhanced in modern cultivars. Moreover, durum wheat breeding has also modified the effects of degrading on the photosynthesis/translocation ratio in cultivars of different periods. In old and intermediate cultivars the

relative contribution of photosynthesis and translocation was not affected by the increase in the source of assimilates by degrading, while the contribution of translocation to the grain weight increased significantly by 4% in modern cultivars. This result indicates that modern cultivars expressed a greater plasticity in terms of increasing translocation as a response to improvements in the source size. Old cultivars responded to the increases in the availability of assimilates by drastically raising translocation from the spike and the leaf blades, while reducing translocation from the stem. Modern cultivars also responded to the degrading treatment by increasing translocation from the spike and the leaf blades, but maintaining translocation from the stem, which contributed the most to grain filling. Intermediate cultivars had a response between old and modern. These results may be interpreted in the framework of the increased demand from the sink of modern cultivars, which seems to bring about an enhancement of translocation from the different main stem structures. The most important difference between the response of old and modern cultivars relies on the role played by translocation from the stem, which seems to be the structure that regulates the plant's reaction to the availability of assimilates. Our results seem to indicate that the potential grain weight of modern cultivars has also been improved over time, the stem being the plant structure that shows the greatest plasticity to cover the increased requirements.

## CONCLUSIONS

Grain yield of durum wheat grown in Mediterranean environments is limited by both the availability of assimilates during grain filling and the size of the sink. Colimitation occurred in grains located in all the positions along the spike, the basal ones being the most responsive to source-sink modification treatments. Durum wheat breeding activities conducted in Italy and Spain during the last century resulted in sink size increases but, at the same time, in larger constraints imposed on yield by the source. Modern cultivars seem to be more source limited than the old ones, thus being more responsive to source-sink modification treatments. Reductions in the sink limitation of modern cultivars may have been due not only to the improved number of grains per unit area (De Vita et al., 2007; Royo et al., 2007), but also to a larger grain weight potential. Modern cultivars also showed a higher plasticity than the old ones in modifying grain-filling duration and the photosynthesis/translocation ratio in response to the treatments. Remobilization of carbohydrates accumulated in preanthesis seems to be regulated mostly by modifications in the contribution from assimilates accumulated in the stem. Our results indicate that future yield improvements in durum wheat could be achieved by increasing the source capacity or strengthening both source and sink sizes.



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