

Source limitations due to leaf rust (caused by *Puccinia triticina*) during grain filling in wheat

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Abstract. Late foliar diseases (especially leaf rust) reduce assimilate supply during post-anthesis, determining fewer assimilates per grain and thereby inducing grain weight reductions. Although the assimilate reduction hypothesis is the most accepted to explain decreases in grain weight due to late foliar diseases, it has not been clearly established whether those reductions could be completely ascribed to source limitations or whether diminished grain weight could be the consequence of reductions in grain weight potential. The objective of this work was to determine whether grain weight reductions due to leaf rust during grain filling could be associated with source–sink limitations. Two experiments (during 2007 and 2008 growing seasons) including healthy and diseased wheat crops were conducted under field conditions. Source–sink manipulation treatments and grain water content measurements were made to test the source- and sink- limitation hypotheses due to the appearance of late foliar diseases during grain filling. Leaf rust was induced to appear exclusively during grain filling, and in both years, it reduced grain yield and grain weight in both experiments. However, except for distal grains, there were no significant differences between healthy and diseased plots in maximum grain water content, indicating that late foliar diseases did not affect the potential size of the grains. The reserves stored in stems were remobilised to the growing grains in both healthy and diseased crops. However, the reserves remaining at physiological maturity were significantly reduced in diseased crops. Reduction in grain number by trimming the spikes increased the grain weight in diseased but not in healthy crops. Grain weight of trimmed spikes in diseased crops reached similar values to those of healthy crops. These results support the hypothesis that foliar diseases could cause source limitation for grain filling beyond differences in grain weight potential when the crops are severely affected by late foliar diseases such as leaf rust.

Additional keywords: grain weight, late foliar diseases, radiation interception/absorption, source–sink relationship.

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Introduction

Grain yield potential of cereal crops has been characterised by sink or source limitations (Borrás *et al.* 2004; Bingham *et al.* 2009). Sink limitations are defined by the grain-set capacity to use available resources, and source limitations are defined by the assimilate supply for yield determination. In some cases, source or sink limitations may operate during different developmental stages, as found in various crops. For wheat, research shows that the crop is mainly sink-limited (Jenner 1979; Savin and Slafer 1991; Slafer and Andrade 1991; Slafer and Savin 1994; Miralles and Slafer 1995; Kruk *et al.* 1997; Borrás *et al.* 2004), as little or no grain weight response has been observed when increases in resource availability per grain (i.e. detached treatments) or reductions in assimilate supply (i.e. defoliation treatments) were applied after grain weight potential had been defined. The capacity to use the assimilates in the stems as reserves, by translocation to growing grains, has been proposed as the main mechanism to compensate photosynthetic capacity reductions

during grain filling in wheat (Borrás *et al.* 2004; Serrago *et al.* 2011).

Leaf rust is one of the main foliar diseases reducing yield in the Argentinean Pampas region. Yield losses up to 55% have occurred, depending on the environmental conditions and cultivars used (Annone *et al.* 2001). The appearance of leaf rust during grain filling decreases assimilate supply during post-anthesis by reductions in radiation interception/absorption (Waggoner and Berger 1987; Bryson *et al.* 1997; Bancal *et al.* 2007; Serrago *et al.* 2009; Carretero *et al.* 2010). These reductions determine fewer assimilates supplied per grain, inducing grain-weight reductions (Cornish *et al.* 1990; Gooding *et al.* 1994, 2000; Dimmock and Gooding 2002; Ruske *et al.* 2003; Robert *et al.* 2004; Pepler *et al.* 2005; Serrago *et al.* 2011). Although the hypothesis of assimilate supply reduction is the most accepted to explain grain weight decrease due to the appearance of late foliar diseases during grain filling, it has not been clearly established whether those reductions could be completely ascribed to source

and/or sink limitations. Appearance of late foliar diseases (such as leaf rust) could reduce grain weight potential (determining sink limitations) and/or achievable grain weight (determining source limitations), even when grain weight potential remained unaffected. Both mechanisms reduce grain weight in wheat and could be confused in terms of the analysis of source and sink limitations.

Grain weight is determined from anthesis to physiological maturity or even earlier (i.e. from booting), as suggested by Calderini *et al.* (1999), as carpel size seems to be associated with grain potential size. The effective grain-filling period can be divided into two phases: lag phase, and active grain growth phase. During lag phase, endosperm cell division takes place defining the final number of endosperm cells per grain and, thereby, the potential grain weight (i.e. grain capacity to use available resources) (Brocklehurst 1977; Sofield *et al.* 1977; Nicolas *et al.* 1985; Schnyder and Baum 1992). During the active grain growth phase, rapid grain biomass accumulation takes place, finishing at physiological maturity (i.e. the time when grains reach maximum dry weight). Finally, grain weight remains stable and grain water content decreases until harvest time. In different crops, the maximum grain water content and final grain dry matter have shown to be closely related when grains are growing under potential conditions during grain filling (Schnyder and Baum 1992; Gambin *et al.* 2007; Alvarez Prado *et al.* 2013).

Understanding source limitation due to late foliar diseases appearing from around flowering to grain filling is important, as it could help in the design of more efficient foliar disease management programs. The underlying assumption is that if source limitation takes place during the active grain growth phase, achievable grain weight is affected by source limitation to growing grains, without effects on grain weight potential. However, if foliar diseases affect assimilates supply during the lag phase, the grain weight potential could be affected. This would determine lighter grain weight independent of the subsequent changes in source size (controlled by fungicide applications) or in remobilisation capacity, as the potential size of the grains was reduced early by diseases. In this case, grain weight reductions are linked with sink rather than with source limitations, because the sink size potential was affected early, and in these situations, fungicide applications to control late foliar diseases could have less effect. The objective of this paper was to determine whether grain weight reductions due to leaf rust appearance during grain filling could be associated with sink and/or source limitations.

Materials and methods

Treatments and experimental design

The experiments were carried out during 2 years (2007 and 2008) in the experimental field of the Department of Plant Production, University of Buenos Aires (34°35'S, 58°29'W). The treatments consisted of a combination of healthy and diseased plots and two levels of source–sink manipulation (i.e. control and trimmed spikes). Treatments were arranged in a split-plot design with three blocks, where the main plots represented the diseased treatments (i.e. control and diseased plots) and the subplot represented the source–sink manipulation

treatments (i.e. control and trimmed spikes). Each main plot consisted of ten 3-m-long rows, 0.175 m apart. The wheat cultivar used during both years was Klein Pegaso, sown on 24 July 2007 and 4 August 2008, at a density of 350 plants m⁻². In both years, fertilisation was applied during pre-sowing using diammonium phosphate (80 kg ha⁻¹) and urea during tillering (Z2.4, Zadoks *et al.* 1974) with the objective of reaching 300 kg N ha⁻¹. In both years, the insects were controlled through chemical spray application. During 2007, weeds were hand-removed, and during 2008, weeds were controlled by spray application of 4 g ha⁻¹ of metsulfuron + 400 cm³ ha⁻¹ of 2.4D applied at 31 days after sowing date.

Crops remained free of foliar diseases until emergence of flag leaf tips, as there was not enough inoculum in the field plots to promote necrotrophic and/or biotrophic diseases until that stage. At flag leaf emergence (Z3.9), the disease infection was promoted by artificial inoculation of spores of *Puccinia triticina* (causal agent of leaf rust). Inoculation was carried out by spray application of spores and water suspension with surfactant (polysorbate 20; Tween[®] 20, Sigma-Aldrich Corp., St. Louis, MO, USA) over the plots. After inoculation, plots (including healthy plots) were kept moist by covering them with plastic tents over three nights (removed during daytime) following inoculation. According to this procedure, leaf rust infection was uniformly distributed over the diseased plots. Healthy plots were maintained by spraying fungicide (750 cm³ ha⁻¹ of tebuconazole) every 15 days from inoculation to the end of grain filling in order to prevent leaf rust infections.

The source–sink ratio was modified by manipulating the number of spikelets per spike. Seven days after anthesis (during the end of the lag phase, when the grain weight potential is defined), main stem spikes per plot were selected and tagged, 50 in 2007 and 60 in 2008. Spike selection was made taking into account those spikes with the same number of spikelets per spike. Half of the selected spikes were randomly chosen and all the spikelets from one side of their spikes were removed by hand (trimmed spikes). This method did not appear to have any negative effects on grain characteristics, or on the susceptibility of these spikes to attack by saprophytic organisms.

Measurements and analyses

At maturity, the three central rows in each plot (1 m length) were manually harvested to avoid any major losses. In those samples, the yield and main numerical components (i.e. grain number and grain weight) were determined. Main stems and tillers were separated and both yield components were measured after oven-drying at 60°C for 72 h.

To estimate the presence of foliar diseases, samples of biomass (one row, 0.5 m length) were taken from the main plots during grain filling, 8 samples in 2007 and 7 in 2008. All samples were oven-dried at 60°C for 72 h and weighted. Before drying, subsamples of four plants per plot were taken, and leaf blades with a detectable green portion were separated from the stems, pasted on a white sheet of paper and scanned (300 ppi). Digital images were analysed using the Assess program[®] (Lamari 2002) to determine green and non-green leaf area. Green leaf area index (GLAI), non-green leaf area index (NGLAI) and green leaf area duration (GLAD) were obtained. Total leaf area index

(TLAI) was obtained by summing GLAI and NGLAI. The GLAD per unit grain was also calculated as an estimator of healthy source per grain.

Disease appearance was estimated as the percentage of non-green leaf area throughout grain filling (NGLA, %), and NGLA was estimated for each particular leaf layer as:

$$\text{NGLA}_i(\%) = (\text{NGLAI}_i / \text{TLAI}_i) \times 100 \quad (1)$$

where i indicates the vertical position of the corresponding leaf layer.

For the calculation of NGLA%, the upper three leaves of the main stems were considered (i.e. L1 was considered as flag leaf). When a particular leaf layer was absent in the plant, NGLA% of this layer was considered to be 100%, assuming that this particular layer had disappeared as a consequence of senescence (induced naturally or by leaf rust).

From anthesis onwards, two spikes were sampled from each treatment three times per week. The spikes were harvested early in the morning and immediately placed in plastic bags and taken to the laboratory for measurements. Dry weight and water content of particular grains from apical [grains 1 (G1) and 2 (G2)] and central [grains 1, 2 and 3 (G3), with grain 1 the most proximal to the rachis (basal grain)] spikelet positions into the spike were registered. To determine the dynamics of grain water content, 16 grains (from apical spikelets) and 24 grains (from central spikelets) per plot were removed from the spikes and placed into a humidified chamber, and grain fresh weight of all the grains was measured immediately after removing them from the spikes. Grain dry weight was measured after drying the grains in a forced-air oven at 60°C for at least 96 h. The grain water content for each assessment day was estimated as the difference between grain fresh weight and grain dry weight. Developing grains accumulate more water than reserves in their early development until they are stabilised during a period, named 'hydric plateau', when the maximum water content is attained. Once the hydric plateau is finished, grains start to lose water until harvest moisture is reached. The dynamics of the grain water content was characterised for each plot and spikelet position by fitting the following model (Eqn 2):

$$\begin{aligned} y &= a + bx(x \leq c) \\ y &= a + bc(x > c; x < e) \\ y &= a + bc + d(x - e)(x \geq e) \end{aligned} \quad (2)$$

The model considers grain water content (y) in relation to days from anthesis (x). The constant water content during the hydric plateau represents the maximum grain water content (MGWC).

The dynamics of the grain growth was characterised by fitting the data to the following bilinear model (Eqn 3):

$$\begin{aligned} y &= a + bx(x \leq c) \\ y &= a + bc(x > c) \end{aligned} \quad (3)$$

The model considers grain weight (y) in relation to days from anthesis (x) as a bilinear relationship, with a plateau after parameter c is reached. Parameter b is the grain growth rate (GGR) and c indicates the time when physiological maturity stage was reached (i.e. the time when grain weight stops growing).

Thus, the period between anthesis and physiological maturity corresponds to grain-filling duration (GFD). As during the lag phase, there is no important increase in grain weight, and the effective duration of grain filling (EGFD) was estimated as (Eqn 4):

$$\text{EGFD} = \text{GFD} - (a/\text{GGR}) \quad (4)$$

Water-soluble carbohydrate (WSC) content was determined on stems plus sheaths in the samples taken during grain filling, after oven-drying at 60°C for 72 h, by sequential extractions in ethanol and water followed by determination using the anthrone method of Yemm and Willis (1954). The dynamics of WSC content was followed during grain filling to establish the maximum value of WSC content and the WSC remaining at physiological maturity. Both points were calculated by fitting the data to the following bilinear model (Eqn 5):

$$\begin{aligned} y &= a + bx(x \leq c) \\ y &= a + bc + d(x - c)(x > c) \end{aligned} \quad (5)$$

The model considers WSC content (y) in relation to the days after anthesis (x) as a bilinear response.

During 2008, the number of endosperm cells in grains from central spikelets was determined. Two spikes were harvested at 6, 10 and 13 days after anthesis, and grains from central spikelets positions (i.e. G1 and G2) were stored on formaldehyde. Endosperm was dissected from grains, fixed in acetic acid/ethanol (1:3) and stored for one night. Endosperm was then treated with cellulose and amylase before staining and resuspending, and counting nuclei with a haemocytometer (Singh and Jenner 1982). The area under the curve of the cell number dynamics was estimated by direct interpolation during the first part of grain filling (i.e. lag phase, when the number of endosperm cells is being defined).

Statistical differences among treatments were tested using standard analyses of variance with first-level interactions. Statistically significant differences were then determined with the overall error rate being $\alpha = 0.05$.

Results

Diseases pressure, grain yield and numerical components

As expected, the predominant foliar disease was leaf rust and the diseased plots showed higher values of NGLA% than the healthy plots during grain filling (Fig. 1). Greater differences between diseased and healthy crops regarding the percentage of NGLA due to leaf rust were observed on the flag leaf (L1) and the leaf immediately below (L2) (Fig. 1). Generally, leaf rust pressure during the pre-anthesis period in these leaf layers was very low (data not shown) and it increased from 10 days after anthesis (Fig. 1). In fact, at anthesis in both years, the two upper leaf layers were completely healthy, as the NGLA% at that stage was zero. The differences in the NGLA between diseased and healthy plots in L3 (the lowest leaf layer measured) were lower than observed on the top leaf layers, especially during 2008 (Fig. 1).

Although the total leaf area duration (TLAD) through grain filling was lower in diseased than healthy crops, there were no significant differences between treatments (Fig. 2). In 2007, TLAD was 117 and 79 cm² leaf cm⁻² soil day for healthy and

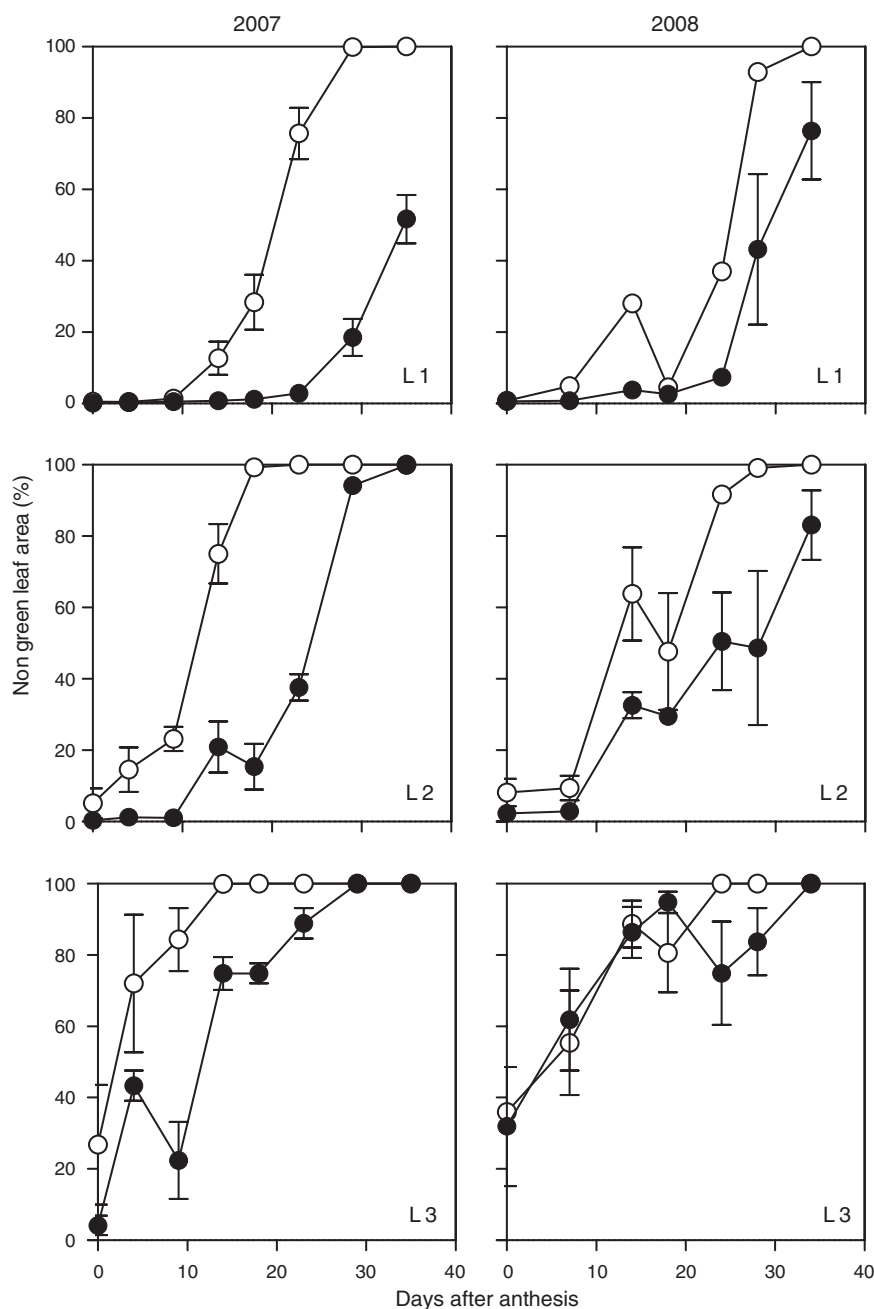


Fig. 1. Non-green leaf area measured during grain filling for 2007 and 2008 experiments. Diseased crops (○); healthy crops (●); L1, flag leaf; L2, layer immediately below; L3, lowest leaf layer measured. Capped lines are standard deviation of the mean.

diseased crops, and in 2008, TLAD was 88 and 70 cm^2 leaf cm^{-2} soil day for healthy and diseased crops, respectively. However, the GLAD was significantly reduced by leaf rust (Fig. 2). In 2007, GLAD was 93 and 49 cm^2 leaf cm^{-2} soil day for healthy and diseased crops, and in 2008, GLAD was 70 and 48 cm^2 leaf cm^{-2} soil day for healthy and diseased crops, respectively (Fig. 2).

There were clear differences in grain yield between years, the healthy crops yielding $\sim 800 \text{ g m}^{-2}$ during 2007 and $\sim 400 \text{ g m}^{-2}$ during 2008. A wide range of grain yields was observed in the

different treatments during 2007 and 2008, from $\sim 300 \text{ g m}^{-2}$ (diseased crops in 2008) to $\sim 800 \text{ g m}^{-2}$ (healthy crops in 2007). Differences between years were mainly explained by differences in grain number per unit area, as that yield component varied between $\sim 23\,000$ grains m^{-2} (2007) and $\sim 13\,500$ grains m^{-2} (2008). Apart from the large differences between years, grain yield was significantly reduced by leaf rust in both years. During the 2008 experiment, the difference between healthy and diseased crops was $\sim 30\%$ ($P < 0.01$) (i.e.

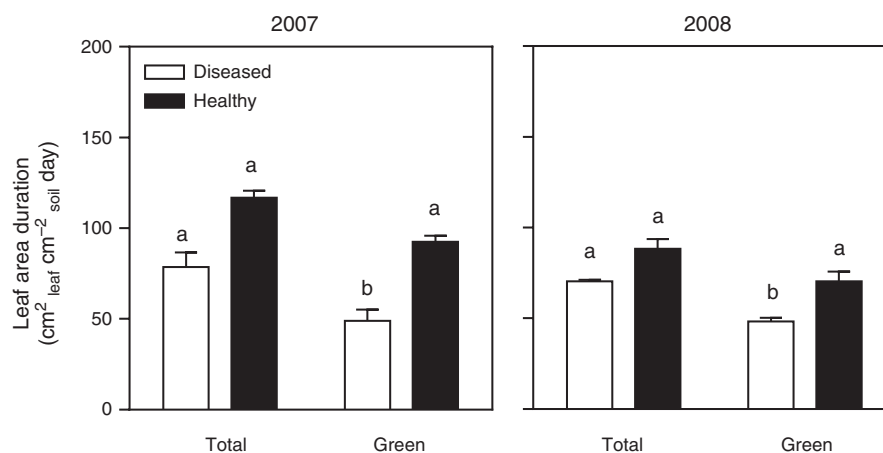


Fig. 2. Total and green leaf area duration for 2007 and 2008 experiment for diseased crops and healthy crops. For each diseased–healthy pair, bars (means) with the same letter are not significantly different at $P=0.05$. Capped lines are standard deviation of the mean.

420 g m⁻² in the healthy crop and 299 g m⁻² in the diseased crop). Similarly, in 2007, grain yield was significantly affected ($P<0.06$) by leaf rust (~25%). In that year, grain yield was 789 and 586 g m⁻² for healthy and diseased crops, respectively; those differences were only partially explained by grain number per unit area. In fact, foliar diseases significantly reduced grain number in 2008 only (13 540 and 10 818 grains m⁻² for healthy and diseased crops, respectively), whereas in 2007, grain number per unit area was not significantly affected by diseases (22 978 and 20 943 grains m⁻² for healthy and diseased crops, respectively). However, leaf rust significantly reduced the mean grain weight in both years. Grain weight reductions, due to leaf rust, were ~20% and ~12% in 2007 and 2008, respectively.

Grain weight, grain water content and endosperm cell number for particular grain positions

Leaf rust appearance during grain filling differentially reduced the particular grain weight positions in the spikes in both years (Table 1). Thus, grain weight of apical spikelet positions was reduced ~22% and 14% for 2007 and 2008 experiments, respectively. In central spikelets, grain weight of G1 and G2 positions was reduced due to leaf rust by ~18% and 22% in 2007 and 2008, respectively. However, the greatest reductions in grain weight due to leaf rust were observed in those grains on the distal position of central spikelets (G3), with reductions of up to 25% relative to the healthy crop in this grain position. Considering both, healthy and diseased crops of control spikes, grain growth rate was higher in G1 and G2 grains (1.37 mg grain⁻¹ day⁻¹) than in G3 grains on central spikelets (1.13 mg grain⁻¹ day⁻¹) and the other grain positions placed on apical spikelets (1.08 mg grain⁻¹ day⁻¹). Except for grains from apical positions, leaf rust reduced grain growth rate without significant effects on grain filling duration (Table 1). Thus, variations in grain weight were linearly and positively associated with changes in grain growth rate ($r^2=0.72$; $P<0.001$) but not with the grain filling duration (data not show).

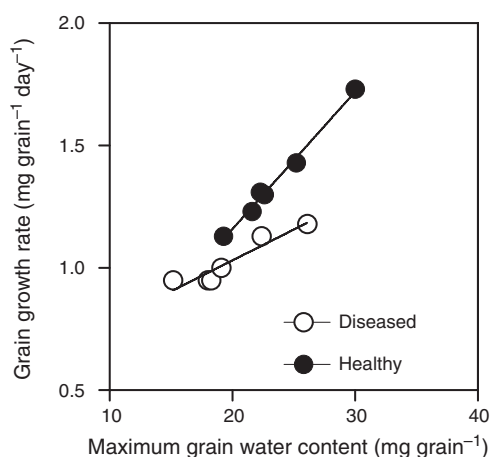
With the exception of G3, there were no significant differences between healthy and diseased plots in MGWC, indicating that late foliar diseases did not affect the potential size of the grains (Table 1). Moreover, grain growth rate was linearly and positively related with MGWC in both healthy ($r^2=0.99$; $P<0.001$) and diseased ($r^2=0.89$; $P<0.01$) crops (Fig. 3). Coincidentally, the area under the curve of the endosperm cell number dynamics in the grains (an estimator of grain size potential as it integrates successive cell counts) was similar between healthy and diseased crops, at least for G1 and G2 positions of central spikelets (Fig. 4). However, GGR was clearly different between healthy and diseased crops, even when both situations had almost the same range of MGWC (Fig. 3). The regression slope of the relationship between GGR and MGWC was significantly ($P<0.001$) lower in diseased than in healthy crops. Thus, GGR was always higher in healthy than in diseased crops, especially at higher values of MGWC.

During the first part of grain filling, WSC was stored in stems, reaching the maximum value ~15 days after anthesis (data not shown). Although the maximum levels of WSC were consistently lower in diseased than healthy crop, those differences were not statistically significant when compared with healthy crops (Fig. 5). During the last part of grain filling, WSC was remobilised from the stems to the growing grains in both healthy and diseased crops. However, the WSC content at physiological maturity was significantly reduced in diseased compared with healthy crops (Fig. 5). The level of assimilate remobilisation during grain filling was different between years (according to the level of grain setting, clearly higher in 2007 than in 2008) and between healthy and diseased crops (according to GLAD reduction level). Whereas in 2007 the remobilisation of reserves was ~76% and 100% (for healthy and diseased crops), in 2008 the remobilisation of reserves was ~22% and ~35% (for healthy and diseased crops, respectively). There were greater differences between years in the level of reserves unused per grain during grain filling. In 2007, there were no reserves remaining in stems during grain filling, whereas in 2008, grain weight of diseased crops could

Table 1. Grain weight, grain growth rate, grain filling duration and maximum grain water content (MGWC) for two spikelets position (apical and basal)

Percentage differences between control and trimmed treatments for diseased and healthy crops are showed in parentheses. Between fungicide and source–sink treatments for each year and spike position, means followed by the same letter are not significantly different at $P=0.05$

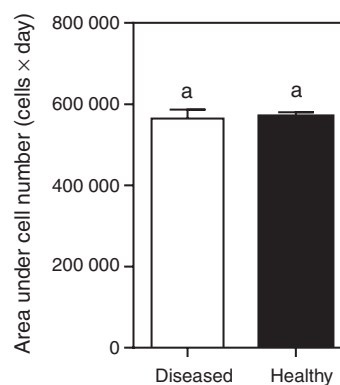
Spikelet position	Grain position	Source–sink	Fungicide	Grain weight (mg grain ^{−1})		Grain growth rate (mg grain ^{−1} day ^{−1})	Grain filling duration (days)	MGWC (mg)
2007								
Apical	G1, G2	Control	Diseased	25.0b		0.95b	26.2a	18.0a
			Healthy	32.3a		1.13ab	28.7a	19.3a
		Trimmed	Diseased	31.2a	(24.8)	1.21a	25.7a	20.0a
			Healthy	32.2a	(−0.3)	1.28a	25.6a	21.3a
Central	G1, G2	Control	Diseased	32.5b		1.13c	28.8a	22.4b
			Healthy	39.7a		1.43b	27.9a	25.2b
		Trimmed	Diseased	38.1a	(17.2)	1.41b	27.0a	24.9b
			Healthy	36.8a	(−5.6)	1.58a	23.2b	29.1a
	G3	Control	Diseased	26.0c		0.95b	27.4a	18.3b
			Healthy	35.1a		1.30a	27.2a	22.6a
		Trimmed	Diseased	31.2b	(20.0)	1.18ab	26.6a	19.5ab
			Healthy	32.5ab	(−7.4)	1.33a	24.4a	23.3a
2008								
Apical	G1, G2	Control	Diseased	26.7b		1.00c	26.8a	19.1c
			Healthy	30.7a		1.23bc	25.2ab	21.6bc
		Trimmed	Diseased	29.8a	(11.6)	1.56a	19.3c	25.0a
			Healthy	29.4a	(−4.2)	1.37ab	21.4bc	23.0ab
Central	G1, G2	Control	Diseased	28.4b		1.18b	24.0a	26.1a
			Healthy	36.3a		1.73a	21.0a	30.0a
		Trimmed	Diseased	35.8a	(26.0)	1.63a	22.4a	30.2a
			Healthy	34.5a	(−4.9)	1.35b	25.5a	26.2a
	G3	Control	Diseased	24.0c		0.95b	26.6a	15.2b
			Healthy	32.4ab		1.31ab	24.9a	22.3a
		Trimmed	Diseased	34.1a	(42.1)	1.71a	20.2a	22.2a
			Healthy	28.6bc	(−11.7)	1.38ab	21.6a	22.4a

**Fig. 3.** Relationship between grain growth rate and maximum grain water content for healthy and diseased crops.

have been increased ~13 mg if the total of reserves had been used to fill the grains.

Source–sink manipulation treatments

The reduction in grain number by trimming spikes increased grain weight in diseased but not in healthy crops (Table 1). Grain weight

**Fig. 4.** Area under the cell number curve for diseased and healthy. Cell number determination was made in G1 and G2 for central spikelets during the lag phase in the 2008 experiment. Capped lines indicate standard deviation of the mean.

of trimmed spikes in diseased crops increased ~20% relative to control spikes on both apical and central spikelets (G1 and G2). However, trimmed treatments showed a greater response in grains in distal positions of central spikelets. The grain weight in those positions (G3) increased ~30% compared with control spikes (Table 1), with the greatest responses during 2008 (~40%). Interestingly, grains of trimmed spikes in diseased crops reached

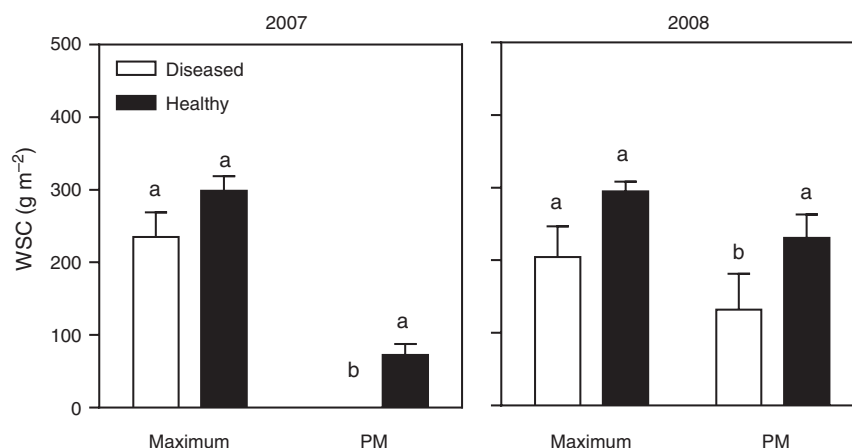


Fig. 5. Water-soluble carbohydrate (WSC) content in stems (an estimation of the reserve level) at the end of the accumulation phase (maximum) and at physiological maturity (PM). The determination was made in healthy and diseased crops during the 2007 and 2008 experiments. For each diseased–healthy pair, bars (means) with the same letter are not significantly at $P = 0.05$. Capped lines are standard deviation of the mean.

weights similar to those grains of healthy crops. In fact, no significant differences were found in weight of grains from trimmed spikes in diseased crops and healthy crops in all spikelet positions (Table 1).

Discussion

Knowledge of crop physiology in terms of grain yield determination enhances the efficiency of crop management programs. As mentioned in the *Introduction*, understanding source and/or sink limitation in wheat crops could improve the design of foliar disease management programs, particularly for those diseases that appear during grain filling, such as leaf rust. Decisions about fungicide applications during grain filling would also be based on knowledge of crop physiology. Thus, different aspects related to the effect of foliar diseases on grain weight potential or grain weight reduction through effects on source size would be included in foliar disease control programs. For instance, if late foliar diseases affect grain weight potential, then subsequent efforts in the maintenance of green leaf area by fungicide applications will not affect the final weight of grains, as the potential size of the grains was early diminished by diseases. In this case, grain weight reductions are linked to sink limitations rather than to source limitations, because the sink-size potential is still defined. In these situations, fungicide applications to control late foliar diseases could have less relevance. In the present study, source–sink manipulation treatments in healthy and diseased crops were applied to test our assumption. We propose that if source limitation takes place during the active grain-growth phase, grain weight is affected by source limitation to growing grains. Conversely, if foliar diseases affect the assimilate supply during the lag phase, grain weight potential could be affected without effects on assimilation supply. Trimming treatments during grain filling did not affect grain weight in healthy crops. These results are in agreement with evidence in the literature that healthy wheat crops are generally

sink-limited (at least under non-stressed conditions) (Slafer and Savin 1994; Miralles and Slafer 1995; Borrás *et al.* 2004). On the other hand, trimming treatments significantly increased grain weight in diseased crops. The facts that (i) trimming treatments were made late during lag phase (7 days post-anthesis), and (ii) grain weight was similar between trimmed and control spikes on healthy crops, suggest that the source–sink treatment (i.e. trimmed spikes) did not affect grain weight potential; therefore, the differences in grain weight between trimmed and control spikes when the crop was affected by disease can be attributed to differences of grain growth that happened after setting of grain weight potential. Thus, these results support the hypothesis that foliar diseases could cause source limitation for grain filling beyond differences in grain weight potential when the crops are severely affected by late foliar diseases such as leaf rust.

The level of reserve remobilisation was clearly different between years. Considering healthy wheat crops, reserve remobilisation ranged from 75% (2007) to only ~20% (2008). Two relevant aspects can be highlighted in relation to the results of the present study: (i) the level of reserves remaining in the stems to sustain grain growth showed important variability (even when the same location, cultivar and crop management were considered); and (ii) there were healthy wheat crops with higher level of reserve remobilisation. The key point to explain this behaviour seems to be associated with the grain setting of the crop (as the number of grains per unit area was higher in 2007 than in 2008). Consequently, during 2007 the GLAD per grain was substantially lower ($10^{-3} \times 4.06 \text{ cm}^2 \text{ leaf cm}^{-2} \text{ soil day}$) than in 2008 ($10^{-3} \times 5.24 \text{ cm}^2 \text{ leaf cm}^{-2} \text{ soil day}$). On the other hand, late foliar diseases increased assimilate remobilisation from stem to grain growth during grain filling. Thus, the remobilisation increased from ~75% to 100% (2007) and from ~20% to ~35% (2008), highlighting the relevance of reserves to maintain grain growth in wheat crops (Borrás *et al.* 2004; Serrago *et al.* 2011). However, the capacity to use reserves in stressed conditions is a

complex process. The fact that some of the reserves were not utilised in diseased crops during 2008 raises issues not fully resolved for understanding the role of reserves during grain filling in stressed crops.

The general relationship between potential grain size, maximum grain water content and grain growth rate is widely known in wheat crops growing without restrictions during grain filling (Brocklehurst 1977; Sofield *et al.* 1977; Schnyder and Baum 1992). In wheat, variations in grain weight are generally linked to variations in GGR more than to changes in GFD (Miralles and Slafer 1995; Serrago *et al.* 2013), as in healthy crops, grain weight is not normally limited by source. However, as previously stated, diseased crops can be exposed to severe source restrictions to grain growth (Serrago *et al.* 2009; Carretero *et al.* 2010). The results reported in the present study are novel regarding cereals, because it was demonstrated that severe epidemics of late foliar diseases could reduce the grain growth rate without effects on grain weight potential. Additionally, the reductions in final grain weight were explained by changes in GGR and not by reduction in GFD (which, in fact, was similar between healthy and diseased crops).

However, when particular grain positions were analysed, e.g. those grains placed in distal positions (as G3 in central spikelets), the conclusions could be different. Foliar diseases reduced both final grain weight and MGWC in grains in distal positions (e.g. G3), indicating that grain weight potential of those particular grain positions could be affected. One hypothesis to explain the differences between grains placed in central and distal positions could be associated with the fact that not all wheat grains reach anthesis at the same moment, as the florets at distal positions (i.e. G3) usually reach anthesis few days later than those florets at central positions (i.e. G1 and G2). Thus, the restrictions to grain growth caused by leaf rust could affect the potential grain size in grains placed in distal positions because they occur earlier in grain development (e.g. during the carpel growth or during lag phase).

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