



Grain weight response to foliar diseases control in wheat (*Triticum aestivum* L.)

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ABSTRACT

Foliar diseases are the main biotic restriction reducing yield in wheat crops affecting both, grain number and/or grain weight, depending on developmental stage at which infection occurs (pre- or post-anthesis, respectively). Grain weight reductions due to foliar diseases were widely reported in the literature mostly associated with decreases on radiation interception during the grain filling period. However, different evidences in wheat showed variations on grain weight responses when fungicide was applied during the grain filling period, probably associated with the timing of fungicide application or with the amount of available resources per grain set when fungicides are applied. The present study was designed to determine the causes of grain weight reduction due to foliar diseases complex (including leaf rust, *Septoria* leaf blotch and tan spot) in wheat crops growing under contrasting agronomic and environmental conditions (i.e. different years, locations, cultivars and N supply). The experiments were carried out during 4 years under field conditions in different locations of Argentine and France. Five different commercial wheat cultivars were sown on early and late sowing dates; and two contrasting N availability and two fungicide treatments (protected and unprotected) were applied. Grain number was not affected by foliar diseases as they appeared after anthesis. Grain weight was strongly, poorly or not affected by foliar diseases and was not associated individually with both, the sink size and the source size. However, when the grain weight response due to fungicide application was plotted against the healthy area absorption per grain (HAA_C), a significant negative association ($r^2 = 0.81$; $p < 0.0001$) was found for the Argentine experiments. When the HAA_C was corrected by the grain weight potential (HAA_{CW}) all experiments conducted in Argentine and in France fit well to a common negative linear regression ($r^2 = 0.74$, $p < 0.0001$) for the relationship between grain weight variation and HAA_{CW} demonstrating that grain weight potential is an important feature to consider in diseases control programs. Foliar diseases forced the crop to use the accumulated reserved increasing the utilization rate of the water soluble carbohydrates (WSC_{UR}), depleting as a consequence the water soluble content at physiological maturity (WSC_{PM}) in all experiments. The association between WSC_{UR} and the healthy area absorption per grain corrected by grain weight of healthy crops (HAA_{CW}) suggest that foliar diseases in wheat cause source limitation, forcing to the crop to use the WSC reserve which could be insufficient to fill the grains previously formed.

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1. Introduction

Considering a simple model, grain yield can be described as the product of grain number and grain weight. However, in wheat, as in many other grain species, these yield components are generated during particular developmental stages. Grain number is mainly determined during the period immediately previous to anthesis (Fischer, 1985) when the spike and stem are competing for assimilates and the number of fertile tillers (Slafer and Rawson, 1994) and florets per spikelets are being established (Kirby, 1988). Grain weight is defined during the period from anthesis to physiologi-

cal maturity (PM) or even early (i.e. from booting to anthesis) as suggested by Calderini et al. (1999), as carpel size could be associated with the potential size of grains. The grain filling period can be divided in two different phases; (i) lag phase (LP) and (ii) active grain growth phase (AGGP) up to physiological maturity (i.e. the time when the grains reach the maximum dry weight). During the LP, endosperm cell division takes place defining the final number of endosperm cells per grain and thereby the potential grain weight (Brocklehurst, 1977; Sofield et al., 1977; Nicolas et al., 1985; Schnyder and Baum, 1992). The AGGP is characterized by a rapid grain biomass accumulation beginning in the LP and finishing in PM. Finally, from the end of the AGGP to harvest maturity, grain weight remains stable and the water content decrease until harvest time.

Foliar diseases are the main biotic restriction reducing yield in wheat crops affecting both, grain number and/or grain weight,

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Table 1

Summary of the different experiments presented in the present study. SD: sowing date, NT: nitrogen treatment, NF: nitrogen from fertilizer (kg ha^{-1}). N_0 and N_1 represent the low and high nitrogen application rates.

Years	Locations	Genotype	SD	NT	NF	Experimental code
2002	Arrecifes (A)	Baguette 10 (B10)	15 June	N_1	N_{120}	A2002
2004	Buenos Aires (BA)	K. Pegaso (KP)	29 June	N_0	N_0	BA2004
	Grignon (GR)	Soissons (SO)	20 October	N_1	N_{150}	G2004SO
		Isengard (IS)	20 October	N_0	N_{160}	G2004IS
2005	Buenos Aires (BA)	K. Pegaso (KP)	3 August	N_0	N_0	BA2005
	Pergamino (P)	Baguette 10 (B10)	24 June	N_1	N_{290}	P2005E
		K Don Enrique (KDE)	26 July	N_0	N_0	P2005L
2007	Buenos Aires (BA)	K. Pegaso (KP)	24 July	N_1	N_{115}	BA2007
				N_0	N_0	
				N_1	N_{250}	

depending on developmental stage at which infection occurs (Madden and Nutter, 1995). Grain weight reductions due diseases infection were widely reported in the literature, mostly associated with decreases on leaf area duration reducing radiation interception during the grain filling period (Gooding et al., 2000; Dimmock and Gooding, 2002; Ruske et al., 2003; Robert et al., 2004). However, different evidences in wheat showed variations on grain weight responses when fungicide was applied during the grain filling period (Cornish et al., 1990; Gooding et al., 1994; Pepler et al., 2006). These variations could be associated with (i) the moment of fungicide application according to the grain biomass accumulation (Dimmock and Gooding, 2002) and/or (ii) the amount of available resources per grain set when fungicides are applied. Regarding the last point, different results were found in the literature. Kramer et al. (1980) hypothesized that non-tolerant cultivars (i.e. higher variations in grain weight due to foliar diseases) showed a relatively low source–sink ratio and, thereby, any loss in photosynthetic capacity cannot be compensated by reserves translocation determining grain weight reductions. However, Zilberstein et al. (1985) could not found an association between tolerant (i.e. null or lower variations in grain weight due to foliar diseases) and non-tolerant cultivars and the sink size.

During the grain filling period, the assimilate availability to fill the grain is provided by different sources as current photosynthesis by the healthy area absorption (HAA), and the water soluble carbohydrates (WSC) stored in stems that could be translocated to the grains (Ehdaie et al., 2008; Bingham et al., 2009). The availability of the WSC stored in the stems, depends on the growing conditions explored by the crop before anthesis, and the WSC mobilization to growing grains is mainly affected by: (i) sink size, (ii) environmental conditions and (iii) cultivars (Blum, 1998; Ehdaie et al., 2008). In those situations where foliar diseases decrease HAA during grain filling period, the importance of WSC stored in stems become particularly important, depending on the sink size previously established, to fill the formed grains (Gallagher et al., 1975; Zilberstein et al., 1985; Cornish et al., 1990; Gaunt and Wright, 1992). Probably, the differences found on grain weight reductions due to foliar diseases could be better explained by a simple model that incorporates the source–sink ratio in terms of HAA available per grain (HAA_C) and the amount and mobilization capacity of WSC stored in stems previous to anthesis.

The aim of this paper was to determine the effects of foliar diseases appearing during the grain filling period of wheat, growing under a wide range of environmental field conditions, on final grain weight. The HAA and WSC on stems were measured during the grain filling period to determine its relative contribution to grain filling on diseased wheat crops.

2. Materials and methods

2.1. Treatments and experimental design

Field experiments were carried out during 4 years (2002, 2004, 2005 and 2007) in 4 locations (three in the rolling pampas of Argentine and one in the northern of France). The particular combination of cultivars, sowing date and N fertilizer for each location and year are presented in Table 1. For details on experimental site and growing conditions see Serrago et al. (2009), as only a brief summary of inoculation method and the experimental design are given here.

In the experiments conducted at Buenos Aires (2004, 2005 and 2007), the diseases infection was promoted by artificial inoculation. Thus, the plots assigned to the unprotected treatment (UP) were sprayed with spores of *Puccinia triticina* (leaf rust) at different times during the crop cycle; at the onset of stem elongation (Z3.1) in 2004, at the onset of stem elongation and at middle of stem elongation (Z3.1 and Z3.7, respectively) in 2005, and at flag leaf emergence (Z3.9) during 2007 experiments. The inoculation was carried out by pulverization over the plots of spores and water suspension with surfactant (Tween 20®). After inoculation, plots (including healthy plots) were kept moisten by: (i) spraying using automatic sprinklers with water at several times a day during the following three days immediately after the inoculum application (2004) and (ii) by covering the plots with plastic tents during the following three nights (2005 and 2007) after inoculation. In the experiments carried out at Pergamino, Arrecifes and Grignon diseases were not artificially promoted as usually the infection appears naturally (normally leaf rust) from flag leaf emergence to physiological maturity as occurred in the present experiments. With the exception of experiments carried out at Grignon (France) and at Buenos Aires (Argentine) in 2007, which were sown under conventional tillage system, the rest of the experiments were sown under zero tillage. In all the experiments the healthy plots (protected plots-P-) were sprayed with fungicide ($750 \text{ cm}^3 \text{ ha}^{-1}$ of Tebuconazole) every 15 days from inoculation to the end of grain filling to prevent the foliar diseases infection.

The experiments at Grignon (France) and Pergamino, (Argentine), were arranged in a split plot design with three replicates per treatment. The arrangement of the treatments of the experiments carried out in Buenos Aires corresponded to a split plot design with three blocks (replicates). N treatments (N_0 , low N application and N_1 , high N application, see Table 1) corresponded to the main plots, and fungicide treatments (protected and unprotected treatment, P and UP, respectively) to the sub-plots. In A2002, the treatments consisted in fungicide application only (P and UP) using a completely randomized design with three replicates per treatment.

2.2. Measurements and analyses

2.2.1. Grain number and grain weight

At PM the central row in each plot (from 0.5 m to 1 m length, depending on experiment) was harvested to determine yield and its main numerical components (i.e. grain number and grain weight). Main stems and tillers were separated and both yield components were measured after oven-dried at 60 °C for 72 h.

2.2.2. Area under non green leaf area progress curve, healthy area absorption and water soluble carbohydrates

Total leaf area index (TLAI), green leaf area index (GLAI) and non green leaf area index (NGLAI) were calculated (for details see Serrago et al., 2009). The area under non green leaf area (AUNGLA) progress curve was considered as an estimation of the diseases presence on the respective treatments and plots. The AUNGLA was calculated according to Eq. (1):

$$\text{AUNGLA} = \sum_{i=1}^{n-1} \left[\frac{X_i + X_{i+1}}{2} \right] (t_{i+1} - t_i) \quad (1)$$

where X represent the percent of non green leaf area (NGLA) at time t .

The non green leaf area (NGLA) was calculated as the mean of the upper four leaves of the main stems for A2002, BA2004, BA2005 and BA2007 and as the mean of the leaves of the whole plant (main stems plus tillers) for G2004 and P2005. For each leaf layer the percentage of NGLA was estimated according to Eq. (2):

$$\text{NGLA}_i(\%) = \left(\frac{\text{NGLAI}_i}{\text{TLAI}_i} \right) \times 100 \quad (2)$$

when a particular leaf layer was absent in the plant, NGLA (%) of this layer was considered 100%.

HAA represents the total absorbed radiation by green leaf area and was estimated only for the grain filling period. To calculate the HAA, the radiation interception (RI) and the RI by healthy tissue (HRI) for each day were multiplied by the corresponding amount of solar photosynthetic active radiation and integrated over the time during the grain filling period to obtain the accumulated intercepted radiation (LAA – leaf area absorption) and accumulated absorbed radiation by healthy tissue (HAA – healthy area absorption), respectively. The RI was estimated through the TLAI according to the equation derived from Beer's Law (Monteith and Unsworth, 1990). The HRI was estimated as:

$$\text{HRI}_i = \text{RI} \times \left(\frac{\text{GLAI}_i}{\text{TLAI}_i} \right) \quad (3)$$

The WSC content was determined on the experiments carried out at G2004 and BA2007. For that, stems plus sheaths, leaf laminae and spikes were separated and oven-dried at 60 °C for 72 h. The determinations were made on stems plus sheaths, by sequential extractions in ethanol and water followed by determination using the anthrone method by Yemm and Willis (1954). The WSC dynamics were characterized by fitting the data to the following bi-linear models, Eq. (4):

$$\begin{aligned} y &= a + bx(x \leq c) \\ y &= a + bc + d(x - c)(x > c) \end{aligned} \quad (4)$$

The model considers WSC (y) in relation to the days after anthesis (x) as a bi-linear response. The parameters with biological interest are: (i) a , which represent the WSC content at anthesis (WSC_A), (ii) b , the WSC content storage rate (WSC_{SR}), (iii) c , the day after anthesis when the maximum WSC content was attained (Day_{MAX}) and (iv) d , WSC utilization rate (WSC_{UR}). The maximum WSC (WSC_{MAX}) and WSC content at the timing of PM (WSC_{PM}) were calculated following the Eq. (4).

PM was estimated by two different methods, (i) when constant grain dry weight was reached (A2002, G2004, BA2005 and BA2007) or (i) when constant above-ground biomass dry weight was reached (BA2004, P2005), in both cases following the dynamic of grain weight and/or above-ground biomass from anthesis to physiological maturity. Statistical differences among treatments were tested using standard analyses of variance with first level interactions. Statistically significant differences were then determined with Student Neuman–Keuls test. The overall error rate was $\alpha = 0.05$. In the text, the means were followed by standard deviation.

3. Results

3.1. Final grain weight and grain number

Foliar diseases (characterized by AUNGLA) mainly appeared in the crop, and their symptoms were visually detected, during the grain filling period (Fig. 1) as during the pre-anthesis period diseases symptoms appearance was extremely low or null (data not shown). In all experiments, and as was expected, the UP treatment always showed higher values of AUNGLA than the P treatment, specially in Buenos Aires experiments, while the lowest differences between P and UP treatments were found in P2005E (Fig. 1).

Experiments explored a wide range of variation on grain number among years and locations (Table 2). Although only in BA2005 the differences in grain number between N treatments were significant in statistical terms, the N fertilization increased the grain number per unit area ca. 18% (Table 2). The greater grain number per unit area was found in Grignon experiments (ca. 26,500 grains m^{-2}) and the lowest in A2002 (ca. 10,800 grains m^{-2}). With the exception of BA2005 N₁, where foliar diseases reduced grain number, no significant differences were found between fungicide treatments for this variable (Table 2). In average grain number (considering the whole data set) was $19,614 \pm 5272$ and $20,752 \pm 5094$ grains m^{-2} for UP and P treatments, respectively. The variations on grain weight between years, locations and N treatments were also high (ca. 21.0 to ca. 45.0 mg grain⁻¹, Table 2). Foliar diseases reduced grain weight in 12 of the 15 experimental situations explored in this study, however, only in 5 experimental situations grain weight was significantly reduced by foliar diseases (Table 2). In average, grain weight was 32.2 ± 5.2 and 35.3 ± 5.3 mg grain⁻¹ in UP and P treatments, respectively, and the range was from 20.9 to 39.3 mg grain⁻¹ for UP and from 26.4 to 44.9 mg grain⁻¹ for P. The higher reduction of grain weight due to foliar diseases were found on A2002 (ca. 21%), BA2005 N₁ (ca. 20%) and BA2007 N₁ (ca. 19%), while in the other experiments, grain weight was slightly or not affected by foliar diseases (e.g. BA2004, P2005 and BA2005 N₀). In some locations, there was an interaction between N and fungicide treatments as grain weight gains, due to fungicide application, were different depending on the N availability. Thus, fungicide application increased average grain weight 12.6 and 6.9% in N₁ and N₀ treatments, respectively.

Grain weight increases due to fungicide application, expressed in relative terms respect to the UP treatment, was not individually associated with the sink size of UP (expressed by the grain number) nor with the source size of UP (expressed by the HAA) (Fig. 2). In this sense, experiments with the same sink or source size explored a wide range of grain weight increases due to fungicide application (Fig. 2). However, when the grain weight response due to fungicide application was plotted against the HAA_G, a significant negative association ($r^2 = 0.81$; $p < 0.0001$) was found (Fig. 3a). Thus, the higher the amount of source available per grain set, the lower the grain weight response to fungicide application. Conversely, when each grain that was set into the crop had not enough source to be filled there were responses to fungicide

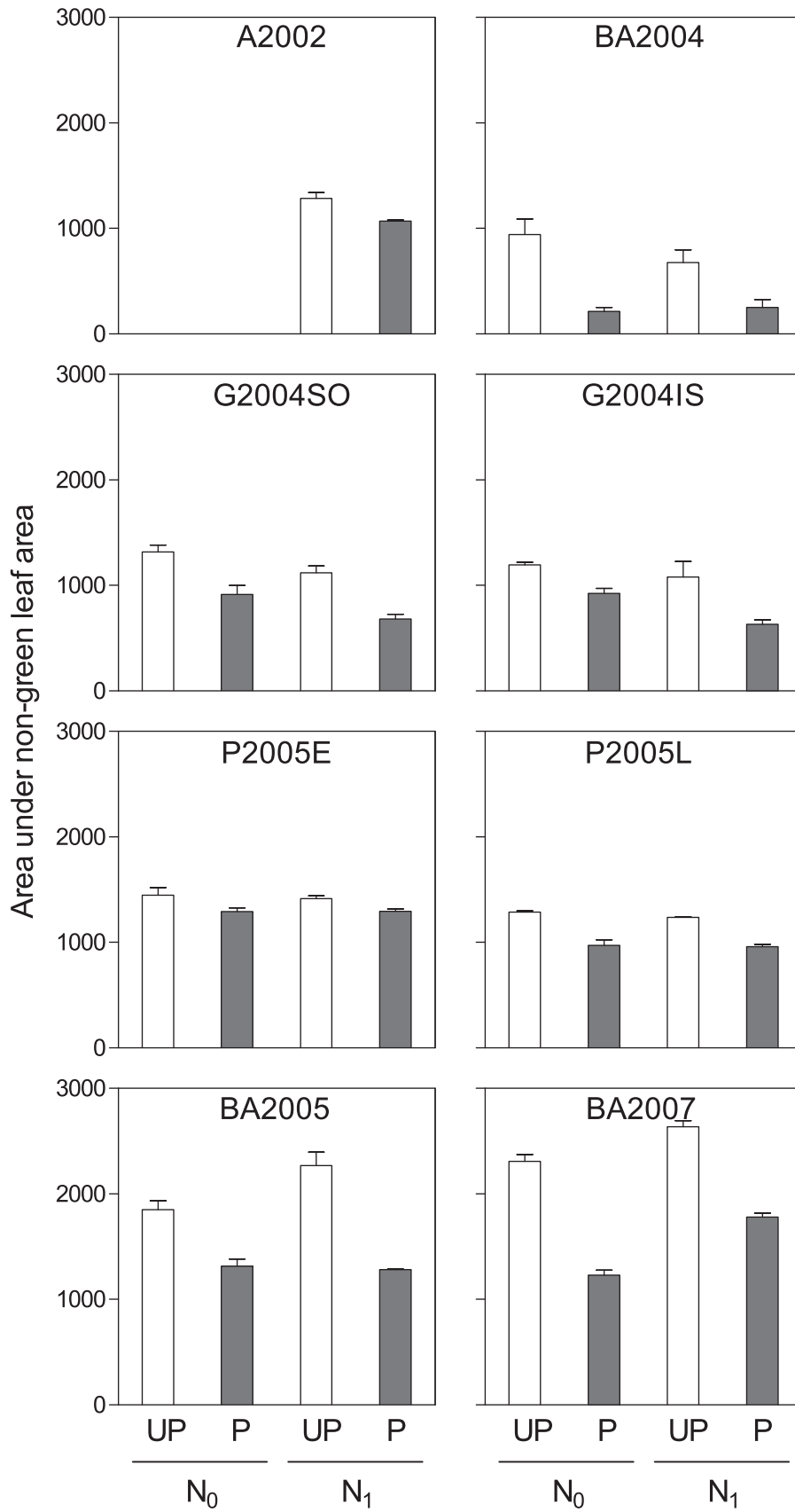


Fig. 1. Area under non green leaf area calculated during the grain filling period for all experiments evaluated. UP and P represent the unprotected and the protected treatment, respectively. N₀ and N₁ represent the low and high N treatment, respectively.

Table 2
Grain number and grain weight for all experiments evaluated. UP and P represent the unprotected and protected treatments, respectively.

Experiment code	Nitrogen	Grain number (m ⁻²)		Grain weight (mg)	
		UP	P	UP	P
A2002	N ₁	9605 a	12,019 a	20.9 a	26.4 b
BA2004	N ₀	16,982 a	19,039 a	34.5 a	34.2 a
	N ₁	18,931 a	20,588 a	34.0 a	33.7 a
G2004SO	N ₀	27,045 a	26,129 a	35.6 b	39.4 ab
	N ₁	26,561 a	25,604 a	37.6 ab	42.6 a
G2004IS	N ₀	25,292 a	25,481 a	39.3 a	44.9 a
	N ₁	27,107 a	28,289 a	39.2 a	44.1 a
P2005E	N ₀	14,511 a	14,413 a	32.8 a	34.9 a
	N ₁	15,089 a	15,717 a	31.4 a	33.2 a
P2005L	N ₀	17,528 a	20,672 a	28.7 c	29.9 ab
	N ₁	18,990 a	21,277 a	29.3 bc	30.6 a
BA2005	N ₀	14,307 c	13,969 c	34.3 a	33.8 ab
	N ₁	22,438 b	26,369 a	25.0 c	31.2 b
BA2007	N ₀	18,880 a	18,734 a	32.2 b	36.7 b
	N ₁	20,944 a	22,978 a	27.9 c	34.3 ab

Different letters indicate significant differences ($p < 0.05$) for the interaction nitrogen \times fungicide treatments within each experiment.

application (Fig. 3a). It is important to highlight, as was evident in Fig. 3(a), that the data obtained in France experiments did not fit well to the consistent negative relationship found for the Argentinean conditions, and were excluded from the general negative relationship found between grain weight response and HAA_G. However, when the source available per grain set (HAA_G) was corrected by the grain weights of the P treatment (HAA_{GW}) the whole data, including the experiments carried out in France, was fitted by a common linear regression ($r^2 = 0.74$, $p < 0.0001$; Fig. 3b). It is important to note that when this analysis was made taking account only the cases where the grain weight decreases by diseases were significant in statistical terms, the lineal relationship between the grain weight response and HAA_G or HAA_{GW} was also significant in statistical terms ($r^2 = 0.91$, $p < 0.05$ for HAA_G and $r^2 = 0.94$, $p < 0.01$ for HAA_{GW}). Additionally, the slopes of the relationship between the relative changes in grain weight and HAA_G or HAA_{GW}, considering only the points that showed significant differences between UP and P and the whole data set, did not show significant differences ($p > 0.1$) when the slopes test were performed.

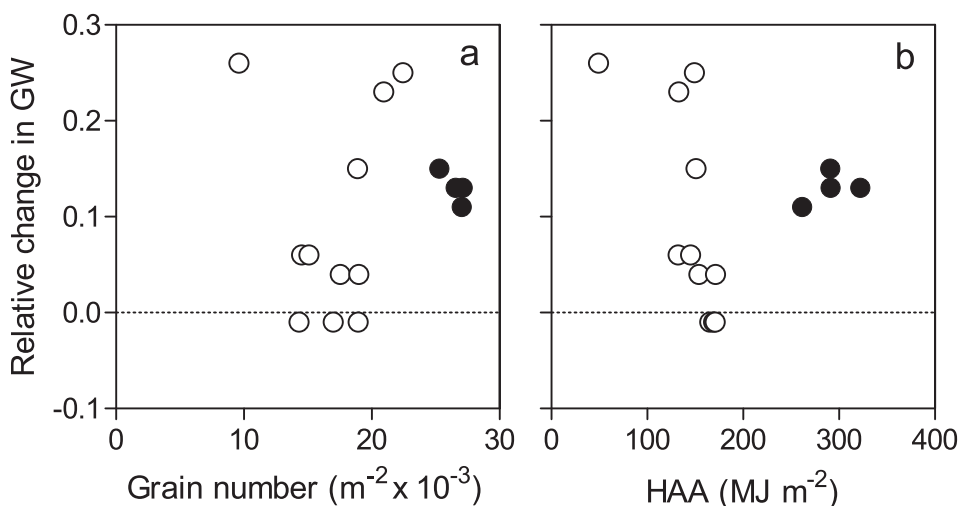


Fig. 2. Relationship between the relative change in grain weight due to fungicide application and (a) grain number and (b) healthy area absorption (HAA), of diseased crops. Empty and solid symbols represent the values corresponding to Argentina and France experiments, respectively.

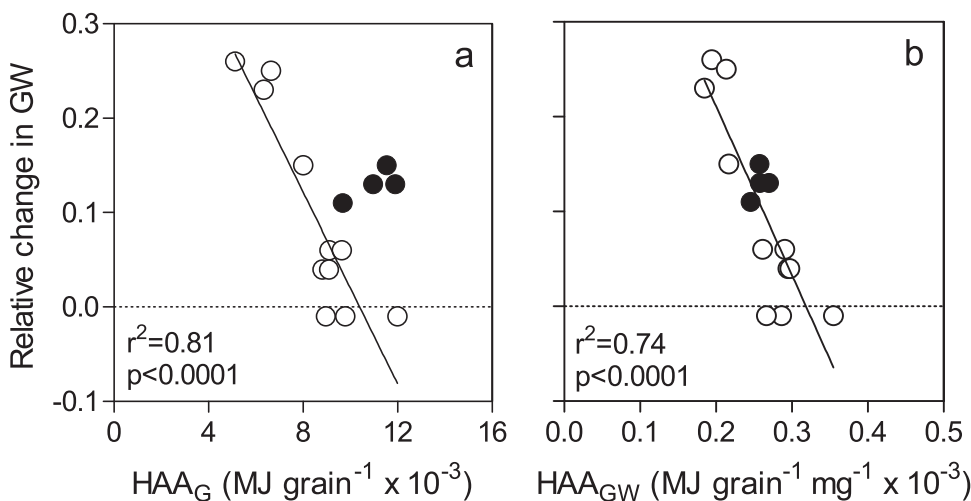


Fig. 3. Relationship between the relative change in grain weight due to fungicide application and (a) healthy area absorption per grain (HAA_G) and (b) healthy area absorption per grain corrected by grain weight potential (HAA_{GW}) of diseased crops. Empty and solid symbols represent the values corresponding to Argentina and France experiments, respectively. The regression lines adjust the values corresponding to Argentine experiments (a) and all data set (b).

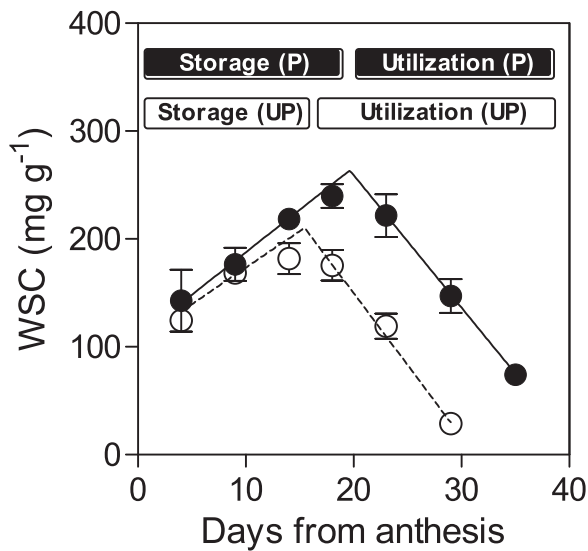


Fig. 4. Water soluble carbohydrates (WSC) dynamic during the grain filling period for BA2007 N₁ experiment. Solid and empty symbols represent the protected (P) and unprotected (UP) treatments, respectively.

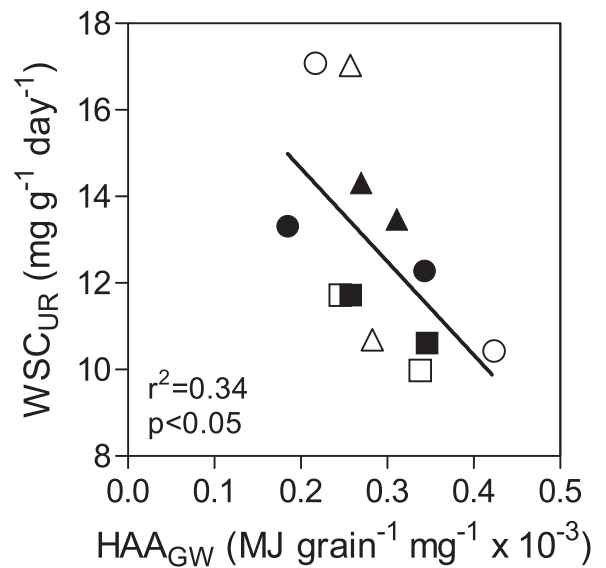


Fig. 5. Water soluble carbohydrates utilization rates (WSC_{UR}) related to the healthy area absorption per grain corrected by grain weight potential (HAA_{GW}) during grain filling period. Circles, squares and triangles represent BA2007, G2004SO and G2004IS, respectively. Open and solid symbols represent the N₀ and N₁ treatments, respectively.

3.2. Water soluble carbohydrates

The WSC dynamic (Fig. 4) showed two different phases: (i) an initial phase of WSC accumulation (storage phase) that represents the increase in WSC content from anthesis (WSC_A) to the maximum value of WSC content (WSC_{MAX}) determined by WSC storage rate (WSC_{SR}) and (ii) a second phase where the WSC are consumed (utilization phase), representing the WSC mobilization from the storage organs into the plant to growing grains, from WSC_{MAX} to WSC content at PM (WSC_{PM}). WSC_{MAX} was decreased by foliar diseases only on BA2007 (ca. 17%) and no changes were found on the Grignon experiments. In the same way, the storage phase parameters (i.e. WSC_A and WSC_{SR}) were mostly not affected by diseases and non significant differences were found between P and UP treatments (Table 3).

Regarding the utilization phase, WSC_{PM} was reduced by foliar diseases (with exception of G2004IS N₁). These reductions were consequence of differences on WSC utilization rate (WSC_{UR}) as foliar diseases increased the WSC utilization during grain filling period (Table 3). Considering the whole data set, there was a negative linear relationship between WSC_{UR} and HAA_{GW} (Fig. 5). When the analysis was made for particular cultivars, differences between them were found. Cultivars Soissons and K. Pegaso showed similar trends, the reduction of HAA_{GW} due to foliar diseases increased the WSC_{UR} (Fig. 5). However, for the same level of HAA_{GW} K. Pegaso showed higher reserve mobilization rate to the grains than Soissons. Conversely the cultivar Isengrain showed a sharp increase of WSC_{UR} as HAA_{GW} decreased due to foliar diseases. On the other hand, the N treatment did

Table 3

Maximum water soluble carbohydrates content (WSC_{MAX}), day at maximum WSC content was attained (Day_{MAX}) and parameters describing: (i) the storage phase; WSC content at anthesis (WSC_A) and WSC storage rate (WSC_{SR}) and (ii) the utilization phase; WSC content at physiological maturity (WSC_{PM}) and WSC utilization rate (WSC_{UR}), measures on stems plus leaf sheaths. N and F represent the nitrogen and fungicide treatments and their interaction (N × F). UP and P represents the unprotected and protected treatment, respectively.

Experiment	Genotype	Nitrogen	WSC _{MAX} (mg g ⁻¹)		Day _{MAX} (day)		Storage phase				Utilization phase			
			UP		P		UP		P		UP		P	
			UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
G2004	Soisson	N ₀	346.0	341.1	12.9	15.5	251.0	240.3	8.6	7.1	61.9	100.7	11.7	10.0
		N ₁	338.2	352.6	13.8	14.7	234.4	252.0	7.9	7.2	38.3	59.1	11.7	10.6
		N	ns		ns		ns		ns		ns		ns	
		F	ns		ns		ns		ns		*		***	
		N × F	ns		ns		ns		ns		ns		ns	
G2004	Isengrain	N ₀	403.8	399.1	16.8	11.8	288.5	293.5	7.4	9.4	11.2	63.9	17.0	10.7
		N ₁	387.9	380.0	13.1	14.9	242.2	251.9	11.9	8.8	6.1	3.9	14.3	13.5
		N	ns		ns		***		ns		**		ns	
		F	ns		ns		ns		ns		ns		*	
		N × F	ns		ns		ns		*		ns		ns	
BA2007	K. Pegaso	N ₀	233.2	270.4	15.5	16.8	92.6	82.9	10.8	11.6	5.7	90.8	17.1	10.4
		N ₁	201.0	254.8	15.5	19.7	105.6	109.0	6.8	7.9	5.7	72.6	13.3	12.3
		N	ns		ns		*		ns		ns		ns	
		F	**		ns		ns		ns		ns		***	
		N × F	ns		ns		ns		ns		ns		ns	

Asterisks indicate the level of significance as: *(p < 0.1), **(p < 0.05), *** (p < 0.01), ns = no significant.

not modify the general association between WSC_{UR} and HAA_{GW} (Fig. 5).

4. Discussion

In all the experiments carried out in this study (with the exception of BA2005 N₁) foliar diseases appeared during the grain filling period not affecting the grain number per unit area, but reducing (depending on the environmental conditions) the grain weight. This reduction, in terms of tolerant (lower reductions on grain weight due to foliar diseases) or non-tolerant (higher reductions on grain weight due to foliar diseases) cultivars, as classified by Kramer et al. (1980), was suggested to be related to grain number set at anthesis. Kramer's hypothesis suggested that the tolerant, with a lower grain number than non-tolerant cultivars, had enough leaf area to maintain a high photosynthetic activity to fill the lower grain number that was previously formed in those cultivars. Supporting the speculation described by Kramer et al. (1980), different works published in the literature showed that the effects of foliar diseases on grain weight were related to the amount of absorbed radiation during the grain filling period (Cornish et al., 1990; Gooding et al., 2000; Robert et al., 2004). Conversely that was described above, Zilberstein et al. (1985) showed that there was a lack of correlation between grain weight responses and the grain number set by the crop, suggesting that the sink size cannot explain itself the variations observed in grain weight due to diseases during the grain filling period.

The present study demonstrated that the relative change in grain weight between UP and P due to foliar diseases was not individually related to, (i) the sink (represented by grain number per unit area) nor to (ii) the source size (represented by HAA), suggesting that the responses in grain weight due to diseases is a more complex interaction between actual photosynthesis available per grain and the remobilization capacity according to the demand determined by the sink size. Thus, not only the amount of radiation available after anthesis should be considered, also HAA_C is important to quantify the responses to diseases control. As it was demonstrated in this study, the highest responses to grain weight, when foliar diseases were controlled during the grain filling period, were associated with the lowest HAA_C . Conversely, the lack of response on grain weight, despite of foliar diseases affecting the crops during grain filling period, was associated with higher values of HAA_C , even for a same genotype. Then, the genotype classification into "tolerant" and "non tolerant" should be take carefully. Considering the same genotype (as was used in the Buenos Aires experiments), the grain weight reduction due to foliar diseases ranged from ca. 0% (BA2004) to ca. 25% (BA2005 N₁), confirming the importance of the source–sink balance of the crop to estimate the response on grain weight due to foliar diseases control.

The differences in grain weight potential also affected the relationship between grain weight responses and HAA_C as was evidenced in the present study. In fact, it was not possible to fit to the same regression line data from Argentine and France experiments when grain weight responses were plotted against HAA_C since the grain weight responses were always greater in crops grown in France than those grown in Argentina for the same level of HAA_C . However, when HAA_C was amended by grain weight potential (i.e. grain weight of healthy wheat crops) a common fit between grain weight response and HAA_{GW} was obtained for both data set, suggesting that the greater responses in France were due to higher grain weight potential. Different authors (Foulkes et al., 2006; Bingham et al., 2009) suggested that those wheat cultivars that are source limited during grain filling period or those whose source and sink size are more closely balanced could show a higher response to

grain weight due to fungicide application. The results shown in this paper confirming these suggestions.

Based on different evidences in the literature it is possible to conclude that wheat crops are basically sink-limited or co-limited by source and sink (Jenner, 1979; Slafer and Savin, 1994; Borrás et al., 2004) as not important variations were found in grain weight when healthy wheat crops were artificially source–sink manipulated during grain filling period. However, a more detailed inspection of some of those papers (e.g. Borrás et al., 2004), show reductions in grain weight of ca. 25% due to defoliation during the grain filling period, in coincidence with the maximum reductions in grain weight registered in the present study when HAA_C was reduced by diseases (Fig. 3). The different reductions of grain weight associated with foliar diseases could be determined by the moment and the magnitude of the diseases that appeared during the grain filling period, determining: (i) a lack of source during LP, when the endosperm cell division occurs and the potential grain weight is defined (Sofield et al., 1977; Nicolas et al., 1985; Schnyder and Baum, 1992) and/or (ii) lack of source during the active grain growth phase (between LP and physiological maturity). The source limitation during LP could affect grain weight potential determining that subsequent modifications on source size do not affect the final weight of the grains which was in fact previously diminished. In this case, the grain weight reductions are linked with sink limitations more than with source limitations, because the sink size potential was already defined. On the other hand, grain weight reductions could be more linked with source limitations when diseases appear after the end of LP (i.e. between the begging of the active grain growth phase and physiological maturity). Although in the present study the grain weight potential was not directly determined, disease pressure increased from anthesis upwards being low the disease level during the LP and probably it is possible hypothesize that the potential size was not affected by diseases. Thereby, the main effect of diseases, at least in the present study, was associated with source restrictions during the active grain growth phase determining reductions in grain weight. Apparently, the lower HAA_C could not be compensated by mobilization of assimilates stored on the stems as was suggested by other studies (Gallagher et al., 1975; Zilberstein et al., 1985; Cornish et al., 1990; Gaunt and Wright, 1992).

In this study, WSC storage at anthesis was similar in both healthy and diseased crops due to the low level of diseases at this stage. However, as diseases progressed from anthesis to physiological maturity, WSC mobilization to growing grains was increased due to: (i) the increased demand of assimilates by the grains and (ii) the dramatically reductions of HAA by foliar diseases as grain filling period progressed. The reduced actual photosynthetic related to a reduced HAA promoted a diminished WSC_{PM} , mainly associated with higher WSC_{UR} . These results are in agreement with previous evidences that showed increases in reserve utilization between anthesis and physiological maturity, due to diseases appearance in the crop during grain filling period (Cornish et al., 1990; Gaunt and Wright, 1992; Bancal et al., 2007). In fact, the negative relationship between WSC_{UR} and HAA_{GW} , suggest that the lowest amount of source during the grain filling period (see Serrago et al., 2009) in the UP treatments probably induced a faster reserves utilization. In spite of the healthy crops had higher values of HAA during grain filling period than diseases crops, the values of WSC_{UR} were high (ranging from ca. 10 to 13 mg g⁻¹ day⁻¹), suggesting that the actual photosynthetic capacity of healthy canopy was not enough for providing the assimilates demanded by the crop, and thereby part of the accumulated reserved were used during the grain filling period.

Crops with (i) lower values of HAA (low source) during grain filling period and/or (ii) higher grain number per unit area (high sink) fixed during the critical period could become intolerant to diseases and have the risk of reducing the grain weight even when

the appearance of foliar diseases forces the crop to use the reserves (WSC) accumulated in vegetative organs. In the future, the release of new genotypes with higher yield potential will probably need to be linked to higher HAA_G for avoiding reductions of grain weight due to HAA reductions by diseases, insects, senescence, etc.

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