Yield and grain weight responses to post-anthesis increases in maximum temperature under field grown wheat as modified by nitrogen ~~availability~~supply

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Abstract

High-temperatures reduce yield of wheat and with global warming episodes of heat waves (only few days of high maximum temperatures) during grain filling will become more frequent. It has been recently reported that the magnitude of the yield penalties imposed by high temperatures under field conditions may interact with nitrogen (N) availability both in barley and maize. We determined, under field conditions, the penalties imposed by post-anthesis high-temperatures waves (increased maximum –but not minimum- temperatures during part of the grain filling period) on wheat yield under contrasting soil N ~~growing conditions~~supply during two consecutive years. The high temperature treatment was imposed for 10 d starting 10 d after anthesis by placing over the crops transparent polyethylene film (125 μm) mounted on wood structures of 1.5 m height above the ~~soil level~~ground. This high-temperature and the unheated controls were imposed on 5 modern and well adapted cultivars under contrasting N availabilities (376, 268 and 68 KgN ha^{-1}). Averaged across N conditions, high-temperature treatments reduced yield by *c.* 1.5 Mg ha^{-1} (a loss of *c.* 17%) even though the treatment was rather mild in terms of different average temperature during grain filling. The magnitude of the loss was consistently shaped by the N condition in which the treatment was imposed: yield penalty produced by high-temperature increased from less than 1 to 2.6 Mg ha^{-1} (which represent losses from 10 to 25%) in parallel with the increased N ~~availability~~supply. The penalties were related to both yield components (grain number and average grain weight) which also were more severely penalised under high than under low N ~~availability~~supply. As episodes of high-temperature waves will become more frequent in the future the tools used to establish the needs of N fertilisation should be revised as the rates maximising yield (or gross margin) might induce higher sensitivities to these episodes. Also simulation models used to upscale physiological

responses to regional or even global domains might need to be revised to include the effect of heat waves (which would be larger per ºC increase than what is estimated from experiments increasing temperature during the whole day and over longer periods) as well as the interaction with N ~~availability~~supply.

Key words: grain growth, high temperature, fertilisation, *Triticum aestivum*, yield components.

1. Introduction

There is an urgent need to increase global wheat yields. In the relatively near future the population will increase to at least 9 billion people (UN, 2015) and the per capita income growth will increase the individual demands simultaneously (Fischer et al., 2014). Projections suggest that a cereal production increase of at least 50% (Fischer et al., 2014), or even more (Hall and Richards, 2013; Ray et al., 2013) will be needed by 2050. And these remarkable increases must be achieved in the context of serious inconveniences: no major contributions may be expected in acreage cropped with cereals (Albajes et al., 2013) and thus future agricultural growth will be more reliant than ever on raising yields (Reynolds et al., 2012; Fischer et al., 2014) at a time when we expect changes in climate which will make crops more frequently exposed to ~~heat stress~~higher temperatures (Battisti and Naylor, 2009; Lobell et al., 2011; Cairns et al., 2013; Challinor et al., 2014). In this context, not only average global temperatures, but also heat waves are predicted to increase under future climate scenarios (Asseng et al., 2010; Rahmstorf and Coumou, 2011; Semenov and Shwery, 2011; Barlow et al., 2015). And this increase in frequency (and severity) of heat waves are expected in regions characterised to be relatively warm like the Mediterranean Basin (Sánchez et al., 2004) as well as in relatively cool regions (Semenov, 2007). In fact, Semenov and Shewry (2011) concluded that an increase in frequency and magnitude of heat stress around flowering in wheat will seriously increase the vulnerability of the crop (more so than drought).

Wheat plays a major role in food security (Reynolds et al., 2012) as it is the crop most widely grown worldwide, and is the main source of calories and proteins for the world population (Braun et al., 2010; Shewry and Hey, 2015). As wheat is grown minimising the likelihood of frost events during flowering, it is rather common that in most environments grain filling takes place with some likelihood of heat stress, and this likelihood will only increase during the next decades (IPCC, 2014). It has been repeatedly shown that exposure to higher temperatures does reduce yield of cereals (Prasad and Djanaguiraman, 2014; Prasad et al., 2006; Jagadish et al., 2007; Lobell and Field, 2007; Prasad et al., 2008; Hatfield et al., 2011; Asseng et al., 2015; García et al., 2015), and physiological factors behind the yield penalty imposed have been studied ~~since~~ for a long time ~~ago~~ (e.g. Slafer and Miralles, 1992; Jenner 1994; Wardlaw and Wrigley 1994; Calderini et al., 1999), including cases in which the effect of heat waves (only few days of high maximum temperatures) were considered (Jenner 1991; Stone and Nicolas 1994; 1995; Savin et al. 1996; Savin and Nicolas 1996; Wallwork et al. 1998; Passarella et al. 2002, 2005). It is therefore critical to identify breeding and/or management tools to mitigate post-anthesis heat effects on wheat yield (Prasad et al., 2017).

Management studies have almost only focused on the possibility of escaping (at least partly) the stress, reducing the likelihood of heat waves during grain filling, through advancing anthesis (with changes in sowing dates and cultivars). Although this could be possible in some cases, in most growing conditions anthesis is optimised when occurring immediately after the last frosts, and therefore other management tools, more directly related to the mitigation of the heat penalty should be identified. It has been recently reported that the magnitude of the effects of high temperatures on yield or its components may interact with nitrogen (N) availability both in barley and maize under field conditions (Passarella et al., 2008; Ordoñez et al., 2015). Regretfully, as far as we are aware, there ~~are no studies documenting~~is only one study analysing this interaction in the field for wheat, but with the high temperatures imposed throughout the whole growing season, and particularly over the winter (Liu et al., 2013); which means that high temperature was beneficial, rather than stressful, for the crop, at least during the winter period. There are, however, very few examples of studies under controlled conditions reporting that interaction in wheat may also exist. For instance, Altenbach et al. (2003) found that grain weight of plants exposed to heat stressed was decreased, but the magnitude of the effect was milder under low- than under high-N availability. Slightly later, Zahedi et al. (2004) and Dupont et al. (2006) confirmed that N status may modify the magnitude of the penalty imposed by heat stress on wheat grain weight. Earlier, Mitchell et al. (1993) had grown in chambers a wheat cultivar under a combination of two CO_2 concentrations two temperatures and two levels of N supply, and overall both CO_2 levels heat reduced yield more under high than under low N supply. But extrapolations from controlled conditions to field and upscaling from individual plants to crop canopies is completely uncertain (Passioura, 2010; Sadras and Richards, 2014), particularly for complex traits. Furthermore, most of the studies mentioned in controlled conditions (i) imposed relatively strong temperature treatments, and (ii) provided data for grain weight and quality but not for yield. Therefore even if the risky extrapolation from controlled conditions to field crops were assumed, there are virtually no results on yield penalties produced by a heat stress consisting of few days of higher-than-normal maximum temperatures (a sort of "heat waves"), which is more common in the real field conditions, considering inter-annual variations, than a very long period (e.g. the whole period from anthesis to maturity) of consistently higher temperatures.

We determined, for the first time under field conditions, the penalties imposed by post-anthesis high-temperatures waves (i.e. increased maximum –but not minimum- temperatures during part of the grain filling period) on wheat yield under contrasting soil N growing conditions in well adapted, modern wheat cultivars. The hypothesis is that a high soil N ~~availability~~supply would produce crops that are not only higher yielding but also more sensitive to high-temperature waves when they occur. The hypothesis is not only original but also agronomically relevant. It owes its novelty to the fact that it has never been tested in wheat grown under field conditions. The support for the hypothesis comes from what has been found in the very few examples on which high-temperature and N treatments were factorially combined in the field (for maize and barley). The relevance is because, should it be accepted, the tools we use to recommend N fertilisation rates might need to be revisited, as episodes of high-temperature waves will become more frequent in the near future.

2. Materials and methods

2.1 *Experimental setup and treatments*

Field experiments were carried out close to Bell-lloc d'Urgell (41.64°N, 0.79°E), Catalonia, North-East Spain, in real fields rented to farmers to install the experiments in the most realistic possible background conditions. All experiments were conducted under potential conditions, with the exception of the soil N level, they were fully irrigated to avoid any water stress and biotic interferences were avoided through controlling weeds, insects and diseases following usual practices. In all cases sowing dates (22/11/2012 for experiment 1, EXP1 and 12/11/2013, for experiment 2, EXP2) and rates (300-350 seeds m^{-2}) were optimal. Temperature data were obtained from a government meteorological station located close to the experimental field (agro-meteorological network of Catalonia, XEMA, Generalitat de Catalunya). In both experiments, plots were 1.2 m wide (6 rows 0.20 m apart) and 4 m long; and treatments were arranged in a randomized complete block design with three replications.

EXP1 (2012/13) was sown in a field with rather high soil N availability at sowing (176~~.3~~±43~~.4~~ KgN ha^{-1} in the first meter depth). We therefore did not impose a "low-N" treatment in this year and fertilised it with another 200 KgN ha^{-1}, "N1"), in order to have an environment with a likely excess of soil N ~~availability~~supply (a relatively widespread condition in many European agroecosystems).

In the next season (2013/14) we selected a field with relatively low N availability at sowing (68~~.4~~±12~~.1~~ Kg N ha^{-1} in the first meter depth) and carried out EXP2, under two contrasting N fertilisation management: unfertilised ("N0") and fertilised with 200 KgN ha^{-1} ("N1"). Therefore we created three levels of soil N ~~availability~~supply: a case with an excessive (though not rare) condition of 376 KgN ha^{-1} available (EXP1 - N1), a case with a clear deficiency with only 68 KgN ha^{-1} available (EXP2 - N0), and an intermediate situation with a high, though unlikely excessive, N ~~availability~~supply (268 KgN ha^{-1}; EXP2 - N1). All these values are ~~availability~~supply considering soil mineral N at sowing plus fertilised N, but actual availabilities values had been higher due to the contribution of mineralisation.

Within these three different N-~~availability~~supply conditions treatments were the factorial combination of five modern, well adapted, wheat cultivars and two high-temperature conditions, a control and a heated treatment during 10 days starting 10 days after anthesis. To impose these treatments we ~~label~~ assessed anthesis plot by plot and imposed the treatments based on the phenology of each individual plot in each of the growing seasons (Figure 1).

Cultivars chosen were Tribat, Nogal, Ingenio, Sensas, and Rodolfo. All of them are high-yielding, modern, well adapted hexaploid wheat cultivars. They are a selection of a slighter larger range of cultivars we grew in previous experiments (Elía et al., 2016) that were selected for best performance across different conditions of that study.

The post-anthesis heat treatment was imposed through enclosing the canopy area designated for the treatments with transparent polyethylene film (125 μm) mounted on wood structures of 1.5 m height above the soil level (with c. 0.5 m of each leg buried; as illustrated in Figure 2, top panels), but leaving the bottom 30 cm of the four sides of each structure open, in order to facilitate free gas exchange through that area. Temperature sensors (connected to dataloggers EM5b Decagon Devices) were regularly distributed in order to monitor air temperatures inside and outside the structures at the height of the spikes. These enclosing structures increased maximum temperature by less than 5ºC (averaging over the 10 days of treatment and across all cultivars x yielding condition) while minimum temperature was practically not altered (Figure 2, bottom panel). Thus, these treatments resulted in moderately high maximum temperatures during 10 days and treatments did not exceed ~~exaggerate~~ what can easily found in realistic field conditions. In fact the overall increase in average temperature was rather mild during the treatment imposition (only 2.27±0.18ºC, averaged across cultivars and growing conditions), and virtually negligible (less than 1ºC) over the whole grain filling period. In this context it can be trusted that any significant effect would have to be due to the heat wave, rather than to the increased mean temperatures. The interception of solar radiation by the polyethylene film was determined at noon of sunny days with a 1-m-long linear sensor of photosynthetically active radiation. The decrease of that radiation in these conditions (i.e. maximum levels of incoming radiation) produced by the structures with the polyethylene film mounted was *c.* 12%.

2.2 *Measurements and analyses*

Main developmental stages (seedling emergence, DC 12; jointing, DC 31; anthesis, DC 65; and maturity, DC 92) were recorded, according to the decimal code (DC) scale of Zadoks et al. (1974), when 50% of the plants in the plot reached the stage.

From anthesis onwards, one main shoot spike from each ~~treatment~~ plot was randomly harvested once weekly to study grain growth dynamics. In each sample, dry weight of these grains was measured after drying the samples 48 h at 80ºC. Grain filling dynamics were estimated by ~~bilinear~~ logistic regression of grain weight against days after anthesis (DAA) using the following equation: Y=~~A+B*X*(X<=C) +B*C*(X>C)~~W $(1+e^{B-C\ x})^{-1}$, where Y is the grain dry matter, ~~A~~ W is the maximum grain weight ~~at the initial point~~, B ~~is the rate of grain filling~~and C are parameters of the curve fitting, and x is ~~the~~ time from anthesis and ~~C is the time when physiological maturity is reached (final grain weight). Grain weight data of less than 10% of the final grain weight were excluded from the bilinear regression according to Loss et al. (1989). Lag phase was calculated based on the abscissa intercept from the bilinear regression and it was subtracted from the total period from anthesis to maturity to calculate the effective duration of grain filling~~the end of the grain filling period was estimated when 95% of W was reached.

Since the beginning of the heat treatment we made weekly determinations of senescence for four weeks. Senescence was estimated by two complementary variables. Firstly we visually estimated the percentage of flag leaves and spikes losing the green colour in three plants per experimental unit. Simultaneously, chlorophyll content was assessed with a hand-held chlorophyll meter (Minolta SPAD-502 chlorophyll meter) on the green parts of the flag leaf of each treatment averaging three lectures per individual sample.

At maturity sample of 1.0 m long was taken from a central row of each experimental unit. Plants were cut at the soil level and taken to the lab for processing. The spike was separated from the stem and leaves and threshed, after oven-dried all tissues for 48 h at 80ºC and yield components were determined. All tissues were milled and their N concentration determined by Kjeldahl. N uptake was estimated by the total biomass and its N concentration.

Data of all samples were subjected to analyses of variance and the relationships between variables were estimated by linear regression.

3. Results

3.1 Yield and yield penalties due to high-temperature

As the main driving force behind the different yielding conditions was the management of N availability, there was no surprise that N uptake was highly significantly affected by the yielding condition (Table 1). Crop N uptake at maturity ranged from *c.* 100 KgN ha^{-1} (EXP2 – N0) through 225 KgN ha^{-1} (EXP2 – N1) to almost 270 KgN ha^{-1} (EXP1 – N1). Naturally, crop yield was also very highly significantly affected by the growing condition: the N management determining the yielding condition of the experiment and the temperature treatments affected markedly yield (Table 1; Fig. 3, left panel). Importantly there was a significant yielding condition (*i.e.* N ~~availability~~supply) x temperature interaction (Table 1), implying that the response to the high-temperature treatment was markedly affected by the background N ~~availability~~supply. Averaging across all conditions genotypes (all modern well adapted wheat cultivars) did not significantly differ in yield, although there was a significant (albeit small considering the magnitude of this mean square) yielding condition x genotype interaction (Table 1). Disregarding that some cultivars responded more than others to N ~~availability~~supply (that is the origin of the mentioned significant interaction), the triple interaction was very small and not significant (Table 1); implying that the way the background N ~~availability~~supply ~~environment~~ modified ~~the~~ crop yield responses to high-temperature x yielding condition was similar for all cultivars.

Yield increased from 4.7±0.06 Mg ha^{-1} (EXP2 – N0) through 8.2±0.22 Mg ha^{-1} (EXP2 – N1) to 10.1±0.13 Mg ha^{-1} (EXP1 – N1) in parallel with the increased N ~~availability~~supply and uptake (Fig. 3, left panel). This difference in yield between the three N environments virtually paralleled the differences in grains per unit land area (10,538±321, 18,547±752, and 21,460±789 grains m^{-2}, respectively), as the average weight of the individual grains was far less responsive to the yielding conditions (44.7±1.2, 45.1±1.5, and 47.9±1.7 mg grain^{-1}, respectively). High-temperature treatments reduced yield in the three contrasting yielding conditions (Fig. 3, right panel). Considering the relatively mild magnitude of the treatments in terms of different average temperature during grain filling, the penalties imposed were remarkable, averaging across yielding conditions in which the high-temperature treatment was imposed a yield penalty of *c.* 1.5 Mg ha^{-1} (a loss equivalent to *c.* 17% of yield). However, the magnitude of the loss was consistently shaped by the yielding condition in which the treatment was imposed: yield penalty produced by high-temperature increased from ~~clearly~~ slightly less than 0.5~~1~~ Mg ha^{-1} (EXP2 – N0) through c. 1.5 Mg ha^{-1} (EXP2 – N1) to 2.6 Mg ha^{-1} (EXP1 – N1) in parallel with the increased N ~~availability~~supply and uptake (Fig. 3, right panel). Importantly, the different magnitude of the penalty was also evident if the penalty were determined in relative terms to the yield in the unheated controls within each yielding condition (i.e. we calculated the relative penalty for each N treatment separately). Thus, when the high-temperature treatment was imposed in the highest N ~~availability~~supply condition (EXP1 – N1) yield was reduced by 25.6±1.8% respect to the unheated yield of this yielding condition, whilst when the same treatment was imposed in the lowest N ~~availability~~supply condition (EXP2 – N0) yield was reduced by only 10.5±1.7% respect to the unheated yield of EXP2 – N0, and the relative reduction was intermediate (16.2±2.6%) in the environment with intermediate ~~availability~~supply of N (EXP2 – N1).

3.2 *Yield components*

Even though the high-temperature treatment was imposed during the effective period of grain filling, the penalty produced on yield was due to its effect on both yield components. And again, when considering the responses in absolute terms the magnitude of the penalty for both yield components was ~~inversely proportional~~higher under higher N ~~to the availability~~supply ~~of N~~ (Fig. 4). However, the interaction was clearer for grain weight than for grain number when considering the magnitude of the effect in relative terms to the unheated control in each case (Fig. 4).

In order to identify the causes determining the penalty in grain growth we analysed the dynamics of grain growth from anthesis to maturity in main shoot spikes. Although grains from these spikes are normally a bit heavier than those of tiller spikes, it seems they represented well the whole population of grains in each case (Supplementary Fig. S1). There were differences between cultivars in their specific grain weights and in the response to high-temperature under each N-~~availability~~supply ~~environment~~ (Supplementary Fig. S2), but their differences were quantitative within each condition and not qualitative: all of them exhibit the same trend evidencing that the responses to high-temperature under contrasting N ~~availability~~supply conditions can be summarised with the average of all cultivars (Fig. 5). Alike when considering the average grain weight from all spikes of the canopy, the response of final grain weight of the average of all grains in the main shoot spikes also showed that exposure to high-temperatures, even when they were mild in terms of mean temperature increase, reduced grain weight and that the penalty increased with the increased ~~availability~~supply of N (Fig. 5). ~~The~~ When averaged across N supply conditions, the high-temperature treatment reduced slightly (*c.* 5%) both the duration and the rate of grain filling. But again there was a clear interaction with the N supply, and the magnitude of such effects was more pronounced under the highest N ~~availability~~supply condition (~~practically a week~~*c.* 4 d reduction in the phase and *c.* 10% reduction in rate), ~~almost~~ negligible under the lowest N ~~availability~~supply condition~~ (*c.* 2 days)~~, and intermediate at the yielding condition that had the intermediate ~~availability~~supply of N (~~*c.*4 days)~~ (Fig. 5; Table 2). ~~On the other hand~~Therefore, the expected effect of high-temperature of increasing the rate of growth (and then at least partially compensating for the reduction in duration of grain filling) was not observed ~~either~~ during the treatment imposition ~~nor~~ and was actually the opposite during the period after treatments were removed (~~please note that data-points are virtually overlapped in the curve until close to the end of grain filling for each case;~~ Fig. 5).

3.3 Senescence

High-temperature treatments only negligibly affected the pattern of senescence. Both the pace of loss of green parts of the leaf and the spike and the SPAD readings in the green parts of the flag leaf were very similar for the unheated controls and the high-temperature treatments within each of the N ~~availability~~supply conditions (Fig. 6).

4. Discussion

4.1 Experimental approach

We imposed high-temperatures in field plots through enclosing the canopy for the duration of the treatment ~~with transparent polyethylene. This has effectively produced the treatment sought after (~~producing a heat wave~~,~~ (where maximum temperatures increased but the minimum were unaltered) under field conditions through a "greenhouse effect"~~ meticulously described and quantified in Materials and Methods~~. However, the great advantage of modifying temperature under field conditions instead under controlled conditions (Passioura, 2010; Sadras and Richards, 2014) does not preclude secondary unwanted environmental alterations~~ (when there is no access to more sophisticated, devices such as free air temperature enrichment; Erbs et al., 2015)~~. In the context of this study the most relevant one may be that the polyethylene film did also reduce incident radiation ~~during the 10 days in which the treatment was imposed. That is why we did measure the interception of the film~~ (*c.* 12% at noon of sunny days~~, see Materials and Methods~~). ~~This means that considering radiation levels along the whole day and across all days of treatments (with different levels of cloudiness) the reduction in incoming radiations must have been much less.~~ This relatively small reduction in incoming radiation ~~on top of the canopy during the 10 days of the treatment~~ would have hardly reduced significantly the source-sink balance for grain filling. ~~One reason for this is because~~Firstly, the polyethylene film would have also changed the partitioning of incoming radiation between direct and diffuse, favouring the latter (Cabrera et al., 2009; Soar et al., 2009). As diffuse radiation increases radiation use efficiency (Sinclair et al., 1992) any small reduction in incoming radiation would have been compensated by a small increase in ~~potential~~ radiation use efficiency. Furthermore the reductions in grain size produced by the high-temperature treatment would have hardly be related to the small reduction in incoming radiation as there seemed unlikely that the source-sink ratio during grain filling would have been less favourable in the high-temperature treatments than in the controls (the argument for this statement is offered~~see~~ below in a specific discussion on the mechanism by which grain weight was responsive to high-temperature~~this issue~~).

Previous to this work, a field study by Liu et al. (2013) analysed soil N availability x high-temperature effects on wheat; but the heat treatment was very severe: it was imposed throughout the whole growing season and the intensity was such that even though the heaters were placed almost 2 m above the soil the temperature at 5 cm under the soil surface was 2°C higher than in the control. To the best of our knowledge, this is the first study carried out under field conditions manipulating maximum temperatures (i.e. affecting crop performance due to a heat wave period) without changing sowing date or locations (which change many other environmental variables and crop structure as well) testing wheat responses under contrasting N conditions.

Note that not only our study is unique in that it was conducted in the field with crop canopies, but also that the high-temperature treatments we imposed are similar to those actually expected to occur due to global warming in the forthcoming decades (Battisti and Naylor, 2009; IPCC, 2014): subtle when considering the average rise in temperature for the whole grain filling but characterised by an increase in extreme events (heat waves).

The general results of this study are commensurate with what is customarily found in the literature, with yield of irrigated wheat being (i) strongly positively responsive to the ~~availability~~supply of soil N (though with decreasing N use efficiencies), (ii) negatively affected by exposure to high-temperatures, and (iii) less affected by genotypic than by environmental factors (when genotypic differences were necessarily small as all materials were modern cultivars, high-yielding and well adapted ~~cultivars~~ to the region of the study). All these ~~general typical~~ responses to N and high-temperatures when considered as single factors offer a typical ~~more than reasonable~~ background condition in which to ~~interpret the results from~~ test~~ing~~ the hypothesis that the greater the N availability for crop growth the higher the magnitude of the penalty imposed by the occurrence of a high-temperature stress.

~~To the best of our knowledge, this is the first study carried out under field conditions manipulating maximum temperatures without changing sowing date or locations (which change many other environmental variables and crop structure as well) testing wheat responses under contrasting N conditions.~~ 4.2 *Yield penalty was increased by N supply*

~~Our results allow us to~~ We accepted the proposed hypothesis, as~~:~~ the magnitude of the yield penalty imposed by high temperature was positively affected by soil N availability. Penalties were naturally larger in absolute values (see Fig. 3 for our results), as yield was much higher in the high- than in the low-N growing conditions. However, the penalties estimated as a percentage of yield under unheated conditions of the same N treatment showed that the increase in magnitude of the damage produced by heat when increased N supply was real. Unfortunately, ~~as~~ we are not aware of any other field study of this nature, ~~it cannot be discarded that the conditions of our experiments might condition the response we found. Although, further studies are needed before the conclusions of this paper could be considered to represent a generally expected response, at least we know~~ that would allow us to state to what degree our results would be consistent with the literature, or otherwise. The unique other work under field conditions that we are aware of (Liu et al., 2013) cannot be compared straightforwardly as they heated the plots along day and night during the whole growing season and the heat was particularly relevant during winter in N China. Even with all the differences, they found that in three seasons in which heat produced a yield penalty, this was larger under high- than under low-N supply (averaging across the three seasons yield penalty under low N was 420 Kg ha^{-1}, equivalent to a 12.7% of the unheated control; while the penalty was 1,343 Kg ha^{-1}, equivalent to a 22.6% of the unheated control, under high N). Furthermore, similar results were observed in maize grown in the field: yield penalties imposed by high-temperature were magnified by the N supply (Ordóñez et al., 2015). There are also few studies of yield as affected by heat x N under controlled conditions ~~Benczе et al. (2007) also found that in the three cultivars they worked with the yield penalty was much stronger under high- (almost 40%, averaging across genotypes) than under low N (25%, averaging across genotypes). Penalties were naturally larger in absolute values (see Fig. 3 for our results), as yield was much higher in the high- than in the low-N growing conditions, but the relative penalties show the actual damage produced disregarding the magnitudes of the control. Furthermore, similar results were observed in maize grown in the field: yield penalties imposed by high-temperature were magnified by the N availability (Ordóñez et al., 2015).~~ , and the focus of these papers was more the effects on grain quality for which most of them reported penalties in grain weight but not in grain yield. Disregarding all the differences mentioned above, we put together the penalties from all these studies and those we measured (Fig. 7). Firstly, it seems obvious that the magnitude of the penalties was normally larger in the other studies than in the present work. This is because of the magnitude of the overall heat stress imposed was much stronger in the other studies (ranging the heat stress from 2.5 to 12°C higher than the control during the whole grain filling period or longer) than in our study (where mean daily temperature in the heat wave treatment was 2.2°C only through a third of the grain filling period, but imposed as a heat wave).

Notwithstanding the magnitude of the penalties and the type of high-temperature stress imposed, there was a remarkable agreement in that in general (averaging across different background conditions or genotypes in the studies including variation in these factors) the reduction derived from the same heat stress within each experiment was larger under high than under low N supply (Fig. 7). Averaging across all data-points, the reduction in grain weight under high N supply was 7.2±0.6 percentage points greater than under low N supply.

This finding is interesting academically: this interaction, that has not been explored much so far, suggests that there might be a sort of general acclimation to stresses. Therefore, a crop growing under N insufficiency would be better equipped to cope with another, rather different, abiotic stress (N is a resource and temperature a signal). But this finding is relevant in practical terms as well. It implies that well fertilised wheats may be more sensitive to high-temperature stresses, and therefore would require that the diagnosis of the needs of N-fertiliser may need to change to balance the unquestionable relevance of this management tool to maximise yields with the uncovered increase in sensitivity to heat waves it may be responsible for, particularly in the near future when the events of heat stress will become more frequent (Fischer, 2011). The need for fine-tuning current recommendations of N fertilisers would be even more critical if instead of yield (the trait we focused on) we regard the relevance of this N x high-temperature interaction for the gross margin of the crop (which would take into account the costs of the fertilisation as well).

4.3 *Physiology of the penalties*

The penalty imposed by high-temperature treatment on yield was due to its negative effect on both yield components, even when this stress started 10 d after anthesis. The reduction imposed by the high-temperature treatment on grain number implies that in this study the critical period for grain number determination extended beyond 10 d after anthesis; i.e. somehow longer than what is usually acknowledged in the literature (Slafer, 2003; Reynolds et al., 2012). The fact that heat imposed as late as 10 days after anthesis may induce a rather large degree of grain abortion has been evidenced by Hays et al. (2007). Grain abortion reduced dramatically fruiting efficiency (Slafer et al., 2015), which could have led to increases in potential grain size due to a possible trade-off between this efficiency and potential grain weight (Ferrante et al., 2015), putatively counterbalancing the negative effects of high-temperature on grain weight, should it be due to reductions in source strength (see below).

~~Expectedly from a large body of evidences in the literature, high temperature imposed during the first part of the effective grain filling period reduced final grain weight. In line with the hypothesis proposed for yield sensitivity to heat under contrasting N scenarios, yield components were also penalised by the same high-temperature treatment more under high than under low N availabilities. To the best of our knowledge, only few studies in wheat, under controlled conditions, tested this interaction, and the focus of these papers was more the effects on grain quality for which they reported penalties in grain weight but not in grain yield. We put together these penalties and those we measured under field conditions (Fig. 7). The magnitude of the penalties was normally larger in the other studies than in the present work, because of the magnitude of the overall heat stress imposed was also much stronger (ranging the heat stress from 2.5 to 12ºC higher than the control and imposed during the whole grain filling period, whilst in our study the overall difference was 2.2ºC only through a third of the grain filling period). Note that not only our study is unique in that it was conducted in the field with crop canopies, but also that the high-temperature treatments we imposed are similar to those actually expected to occur due to global warming in the next several decades (Battisti and Naylor, 2009; IPCC, 2014): subtle when considering the average rise in temperature for the whole grain filling but characterised by an increase in extreme events (heat waves). Notwithstanding the magnitude of the penalties, there seemed to be certain agreement in that the reduction derived from the same heat stress was larger under high than under low N availability. There were few exceptions but in general the pattern was significant: averaging across all data points, the reduction in grain weight under high N availability was 6.2±0.4 percentage points greater than under low N. Furthermore the parameters of the regression suggest a clear trend to increase the relevance of the N level in magnifying the sensitivity with increases magnitudes of stress.~~

~~Beyond the fact that the magnitude of the penalty was proportional to the availability of N, two elements of the results provided in this study are worth discussing: (i) the actual reduction in grain weight was higher than what would have been expected from the increase in mean temperature overall the anthesis-maturity period, (ii) the mechanism by which the stress affected grain weight.~~

Regarding the magnitude, it was highlighted that the penalty in our study was smaller than in the few other ones in which the heat was imposed under contrasting N conditions, because the other studies imposed a heat stress much stronger than what might occur in reality. But the magnitude of the penalty per unit of temperature increase was actually larger in our study (even more if considered the temperature increase during the whole grain filling period). Thus, if we included our data points in the relationship shown for controlled environments in Fig. 7, the coefficient of determination remains very high (R^2=0.87) but the intercept increases dramatically (from 1.67 to 5.11% percentage points greater under high than under low N) and the slopes reduces (from 1.14 to 1.04), and becomes close to a parallel to the dotted line. This implies that the heat waves might be more damaging than what would be expected from the increase in average temperature. In fact, the only study comparing directly high-temperature treatments with the same "heat load", compared against the unheated control, but from a "warmer grain filling" or from a high-temperature wave under controlled conditions showing that the penalty per °C increase was higher in the latter than in the former (Wardlaw et al., 2002). For instance, it has been estimated from simulation modelling that the global warming would penalise wheat yield by 6% for each °C of temperature increase during the growing season (e.g. Asseng et al., 2015; Zhao et al., 2016) or much less from direct field experimentation (Zhao et al., 2016), whilst we found a decrease ranging from 10 to 25% (from lowest to highest N availabilities) due to an increase in average temperature during the grain filling period of less than 1°C. Regarding grain filling in particular, grain weight reductions expected per °C increase in mean temperature after anthesis is normally below 7%, in well fertilised plants (Wardlaw and Wrigley 1994) while we observed a loss in the highest N availability level of almost 17% with a high-temperature treatment that resulted in an average increase of *c.* 1°C during grain filling. If extreme events (such as heat waves) will become more frequent in the future, we may need to revise the predictions of reductions in yield due to a particular average increase, and we may need to amend models used to predict the consequences of climate change to consider the magnitude of the penalties differently if they come from constant increases during the whole growing season or due to the more frequent occurrence of heat waves (as well as to take into account the level of N availability when quantifying the penalties).

Regarding the mechanism by which grain weight was responsive to high-temperature, the expected negative effect should be related to a shortening of grain filling partially compensated from a positive effect on the rate of grain filling (Fischer, 2011). In our study, we effectively found the reduction in duration of grain growth, but only when N supply was highest with unnoticeable effects of the heat wave treatment on the duration of the whole period of grain filling in intermediate and low N supply conditions. More surprisingly, not only the expected compensation from a faster rate of grain filling was not seen ~~(in some cases~~ but ~~there was~~ in fact a trend to ~~even~~ reduce the rate of grain filling due to the heat waves was consistently found, and the magnitude of this effect ~~in addition to the reduction in duration)~~was indeed affected by the N supply: the higher the N supply the larger the reduction in rate of grain growth. This may explain why the high-temperature waves resulted more damaging than an increase in temperature occurring during the whole day, or only increasing the minimum (night) temperatures (García et al., 2016), per unit of average temperature increased during the grain filling period. This is not fully surprising, although not studying the interaction with soil N availability, previous studies also showed that relatively brief periods of heat waves actually reduced not only the duration but also the rate of grain filling (Stone et al., 1995; Savin et al., 1996). In addition to the magnitude of the effect, it seemed that ~~it~~ the reduction in grain weight due to the exposure to the heat wave was due to a direct effect on the capacity of the grains to grow, disregarding any effect it might have had on the rest of the canopy. Different evidences support this statement. Firstly, the high-temperature stress did not affect senescence (neither the proportion of the flag leaves and spikes loosing green colour nor the chlorophyll content in the parts of the leaves remaining green) noticeably. This lack of effect on senescence was somewhat unexpected, as leaf senescence is well recognised to be sensitive to temperature. However, the way we imposed the treatments through a greenhouse effect, stratifies the temperature increase, which is maximum at the top of the enclosures and negligible at the bottom (that is opened to free air circulation). As we measured temperatures at the height of the spikes, the increase in temperature at the lower parts of the canopy would have been much lower. In addition, it may be also possible that the well-recognised effect of higher temperatures accelerating senescence might depend on the type of heat treatment. In other studies with increases in temperature for only few hours per day the expected effect on senescence was not noticeable either (e.g. Savin and Nicolas, 1996; García et al., 2016). The fact that the high-temperature treatment reduced grain number without reducing green area indicates that, if the treatment altered the source-sink ratio it would have increased it. Therefore, the only possible cause of the effect reported shall be a direct impairment produced by the treatment on the capacity of the grains to grow. This would be in line with the vast majority of the literature indicating sink-limitation dominates during grain filling in wheat (Fischer, 2011; Slafer et al., 2014), which may also explain why grain size is less plastic (and more heritable) than other yield components (Sadras and Slafer, 2012; Benincasa et al., 2017). The bases for such conclusion is that (i) grain size is largely unresponsive to moderate source-sink manipulations imposed after the lag-phase (e.g. Slafer and Savin, 1994; Borrás et al., 2004; Serrago et al., 2013), (ii) there seems that stem carbohydrate reserves -that are readily available to fill grains- are not fully used (Serrago et al., 2013), and (iii) photosynthesis seems down-regulated by lack of sink strength during the effective period of grain filling (Reynolds et al., 2005; Acreche and Slafer, 2009). These elements can be also seen when the crop is subjected to high temperatures during grain filling, not only in wheat (Slafer and Miralles, 1992; García et al., 2016) but also in maize (Ordóñez et al., 2015).

4.4 *Heat waves vs increasing temperature all day*

Beyond the fact that the magnitude of the penalty was proportional to the supply of N, it was surprising that the actual reduction in yield due to the heat wave was higher than what would have been expected from the increase in mean temperature for the same "heat load". It was highlighted that the penalty in our study was smaller than that in most other ones in which the heat was imposed under contrasting N conditions (Fig. 7), because the other studies imposed a heat stress much stronger. But the magnitude of the penalty per unit of temperature increase was actually much larger in our study. This implies that the heat waves might be more damaging than what would be expected from the increase in average temperature. In fact, the only study comparing directly high-temperature treatments with the same "heat load", compared against the unheated control, but from a "warmer grain filling" or from a high-temperature wave under controlled conditions showed that the penalty per °C increase was higher in the latter than in the former (Wardlaw et al., 2002).

As it is expected an increase in frequency of in heat waves (see above), it has been estimated from simulation modelling that the global warming would penalise wheat yield by 6% for each °C of temperature increase during the growing season (e.g. Asseng et al., 2015; Zhao et al., 2016) or much less from direct field experimentation (Zhao et al., 2016), whilst we found a decrease ranging from 10 to 25% (from lowest to highest N availabilities) due to an increase in average temperature during the grain filling period of less than 1°C (but in the form of heat wave of 10 d). Therefore, we may need to revise the predictions of reductions in yield due to a particular average increase, and we may need to amend models used to predict the consequences of climate change to consider the magnitude of the penalties differently if they come from constant increases during the whole growing season or due to the more frequent occurrence of heat waves (as well as to take into account the level of N supply when quantifying the penalties).

5. Conclusion

We conclude, from field experiments with treatments representing fairly what can be expected to occur more frequently in the near future in realistic field situations, that that sensitivity of yield and its components to high-temperature was increased by N fertilisation. Therefore the tools used to diagnose the needs of N fertilisation should take into account this interaction, as episodes of high-temperature waves will become more frequent in the near future and the rate maximising yield (or gross margin) might induce higher sensitivities to these episodes. The effect of increasing maximum temperatures for several days (a heat wave) was more damaging than what it would be expected from the increase in average temperature over~~all~~ the entire grain filling period. Quantitative extrapolations from controlled experiments increasing temperature during very long period to realistic field conditions where high-temperatures occur more frequently as heat waves shall be made with extreme care and realising that they likely underestimate the effect, should the same increase in average temperature be the consequence of a heat wave. Simulation models used to scale up the physiological responses of crops to regional or even global levels should be amended to consider the abovementioned different in magnitude of effects from heat waves and from constantly higher temperatures, as well as to take into consideration the level of N nutrition to estimate the expected penalty produced by a heat wave.

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Figures captions

Figure 1. Maximum (solid line) and minimum (dotted line) environmental temperatures from anthesis onwards for each cultivar in each experiment.

Figure 2. Top panels: pictures with a partial view of one of the field experiments at the time when the high-temperature treatments were imposed (left) and a closer view to one of the structures with Dr. Elía working inside (right). Bottom panel: temperatures during the course of the day during the period of treatment imposition for the unheated control (blue, bottom lines) and the heated treatments (red, upper lines). Temperatures were determined hourly in control and heated experimental units for each cultivar in each yielding condition (and data shown is the average across cultivars and yielding conditions.

Figure 3. Yield (left panel) and yield penalty imposed by the high-temperature treatment (right panel) in each of the three growing conditions ordered by decreasing order of N ~~availability~~supply (EXP1 – N1; EXP2 – N1; EXP2 – N0). Actual values of N ~~availability~~supply (as mineral N in soil at sowing plus N fertilisation) and N uptake in the above-ground biomass at maturity are shown below each growing condition. Each bar is the average of the five modern and well adapted cultivars grown in each experiment. The segment in each bar stands for the standard error of the means. All data are dry matter.

Figure 4. Penalty imposed by the high-temperature treatment on yield components: number of grains per unit land area (left panel) and average grain dry weight (right panel) in each of the three growing conditions ordered by decreasing order of N ~~availability~~supply (EXP1 – N1; EXP2 – N1; EXP2 – N0). Actual values of N ~~availability~~supply (as mineral N in soil at sowing plus N fertilisation) and N uptake in the above-ground biomass at maturity are shown below each growing condition. Each bar is the average of the five modern and well adapted cultivars grown in each experiment. The segment in each bar stands for the standard error of the means. Figures on top of each bar represent the penalty in percentage of the value under unheated conditions (and its standard error).

Figure 5. Dynamics of grain growth from anthesis to maturity. Data were taken from samples of main shoot spikes taken at weekly intervals for the unheated controls (circles) and high-temperature treatments (triangles) in each of the three N ~~availability~~supply conditions (top panel highest, bottom panel lowest) in which the high-temperature treatments were imposed (the period of high-temperature treatment is highlighted with a grey background). Each data-point is the average dry weight of all grains of a spike from each of the three replicates and across all cultivars. Lines fitted with a logistic regression (see Table 2).

Figure 6. Time course of either (i) loss of green colour from leaves and spikes (left column of panels) or (ii) SPAD measurements made in the green parts of the flag leaves (right column of panels). Both traits were determined weekly during four weeks from the onset of the treatments for the unheated controls (circles) and high-temperature treatments (triangles) in each of the three N ~~availability~~supply conditions (top panels highest, bottom panels lowest) in which the high-temperature treatments were imposed. Each data-point is the average of all grains of a spike from each of the three replicates and across all cultivars.

Figure 7. Relative reduction in either average grain weight (GW, only when treatments were applied after anthesis and yield data were not reported in the paper) or in grain yield (GY), in response to high-temperature imposed under high- vs under low-N conditions. Data from other studies are the average across cultivars or conditions when more than one was reported; and when different temperatures were used the data included in the figure is that of the two extreme thermal regimes). Datum from this paper (circle) refers to the comparison of the two extreme N supplies. Data from other papers include plants grown under controlled conditions (triangles) were extracted from Dawson and Wardlaw (1984), Mitchell et al. (1993), Altenbach et al. (2003), Zahedi et al. (2004), Dupont et al. (2006), Bencze et al. (2007) and Koga et al. (2015), and from the only other field study of which we are aware of (square), in with high-temperature increased all day and during the whole growing season (Liu et al., 2013). In all cases the data-points represent the loss (GW or GY in the unheated control minus under high-temperature treatment) as a percentage of the unheated control. Dotted line represents the 1:1 ratio (Y=X). ~~Relative reduction in average grain weight (GW) in response to high-temperature stress imposed under high- vs under low-N conditions. Data from this paper (circles) as well as from controlled conditions (triangles). Data from controlled conditions were extracted from Dawson and Wardlaw (1984), Altenbach et al. (2003), Zahedi et al. (2004), Dupont et al. (2006), Bencze et al. (2007) and Koga et al. (2015). In all cases the data-points represent the loss (GW in the unheated control minus under high-temperature stress) as a percentage of the unheated control. In the two studies that included different genotypes (2 cultivars in Koga et al., 2015; 3 cultivars in Bencze et al., 2007; in all others there was only 1 genotype), data represent the average across them. Solid line was fitted by linear regression of the data-points corresponding to experiments carried out under controlled conditions with high-temperature treatment imposed during the whole day and along the entire grain filling period. Dotted grey line represents the 1:1 ratio (Y=X).~~

Figure S1. Average grain weight determined as the average of all grains sampled at maturity against the same variable estimated for the grains of the main shoot spike as a parameter of the dynamics of grain growth from anthesis to maturity for the unheated controls (circles) and high-temperature treatments (triangles) in each of the three yielding conditions in which the high-temperature treatments were imposed. Each data point is the average of the five cultivars.

Figure S2. Dynamics of grain growth from anthesis to maturity. Data were taken from samples of main shoot spikes taken at weekly intervals for the unheated controls (circles) and high-temperature treatments (triangles) for each cultivar in each of the three yielding conditions in which the high-temperature treatments were imposed. Each data-point is the average of all grains of a spike from each of the three replicates.

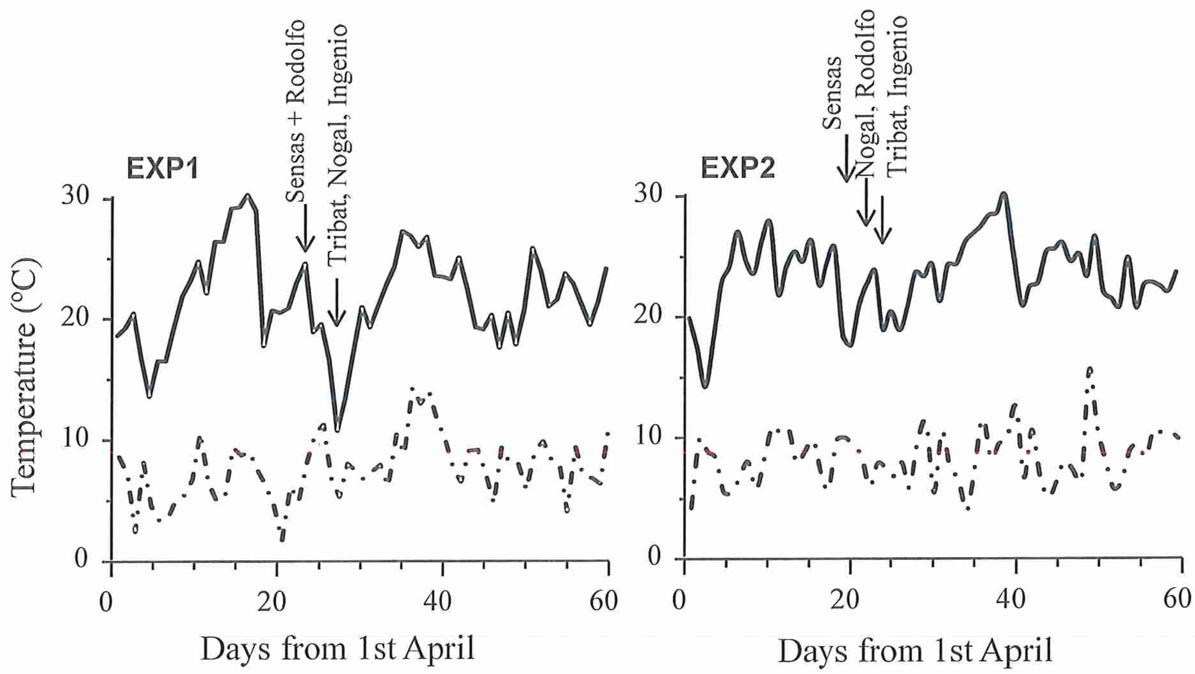

Figure 1. Maximum (solid line) and minimum (dotted line) environmental temperatures from anthesis onwards for each cultivar in each experiment.

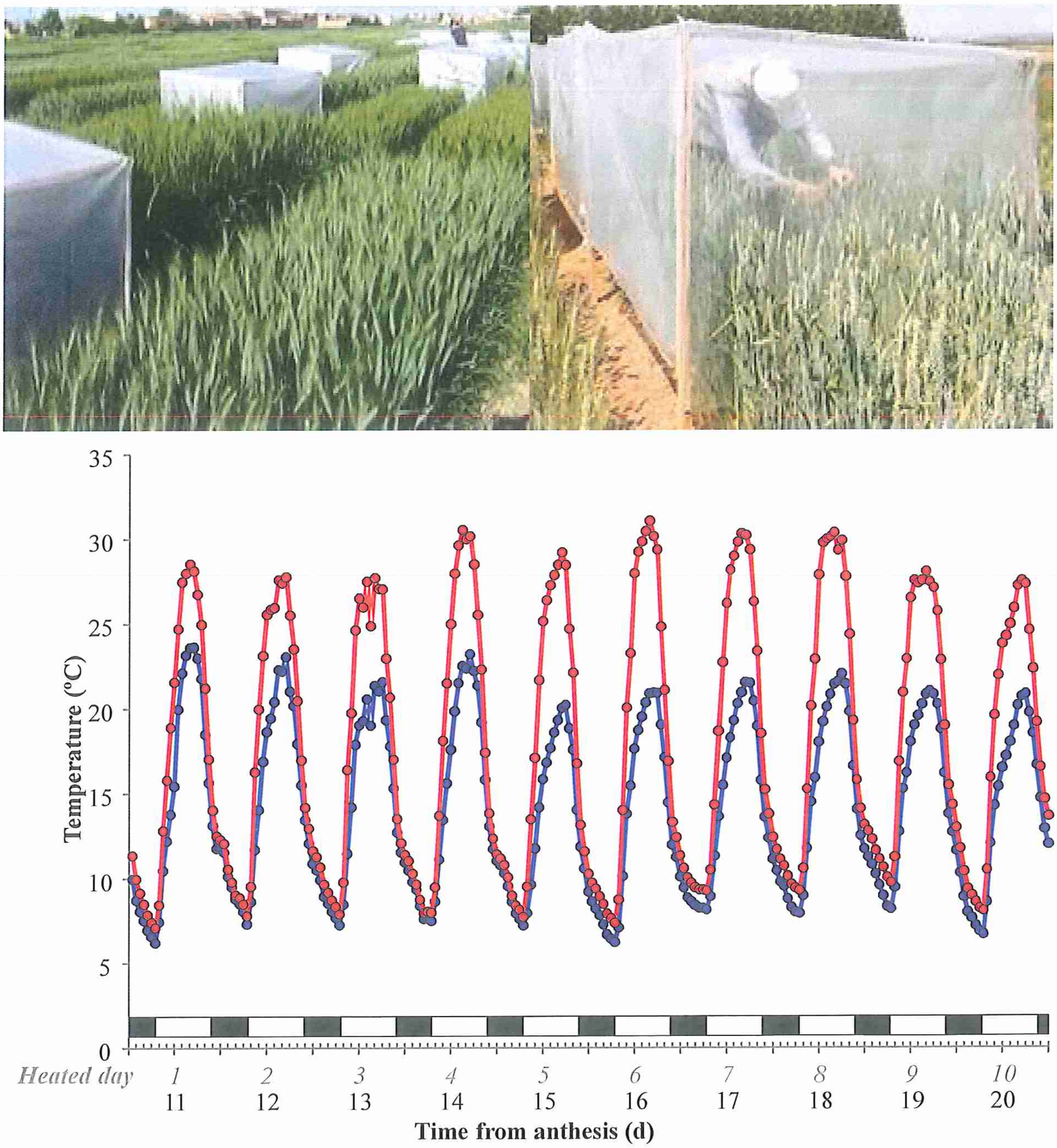

Figure 2. Top panels: pictures with a partial view of one of the field experiments at the time when the high-temperature treatments were imposed (left) and a closer view to one of the structures with Dr. Elía working inside (right). Bottom panel: temperatures during the course of the day during the period of treatment imposition for the unheated control (blue, bottom lines) and the heated treatments (red, upper lines). Temperatures were determined hourly in control and heated experimental units for each cultivar in each yielding condition (and data shown is the average across cultivars and yielding conditions.

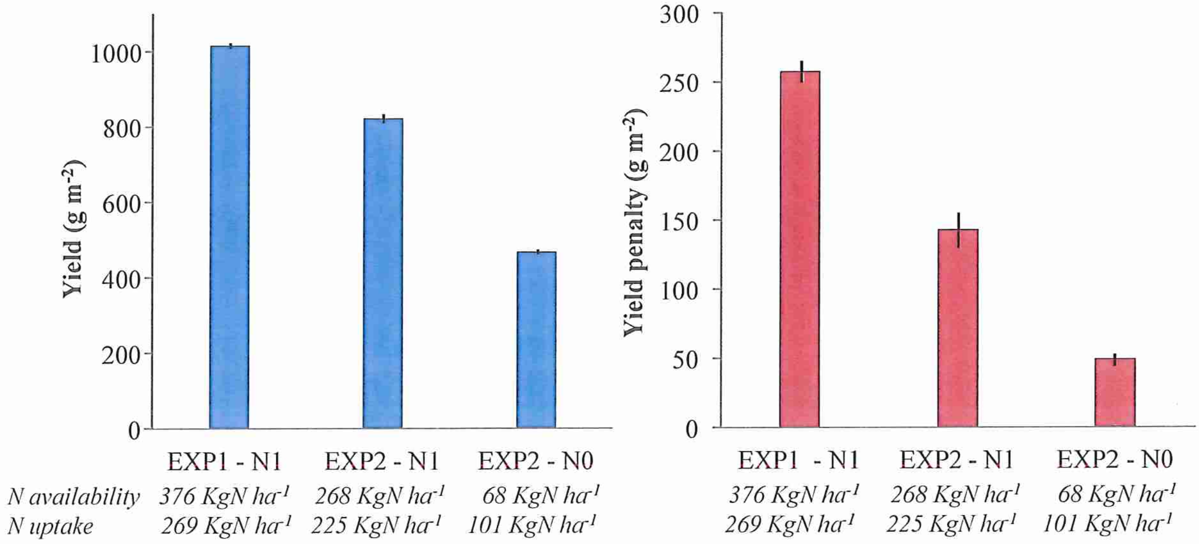

Figure 3. Yield (left panel) and yield penalty imposed by the high-temperature treatment (right panel) in each of the three growing conditions ordered by decreasing order of N availability (EXP1 – N1; EXP2 – N1; EXP2 – N0). Actual values of N availability (as mineral N in soil at sowing plus N fertilization) and N uptake in the above-ground biomass at maturity are shown below each growing condition. Each bar is the average of the five modern and well adapted cultivars grown in each experiment. The segment in each bar stands for the standard error of the means.

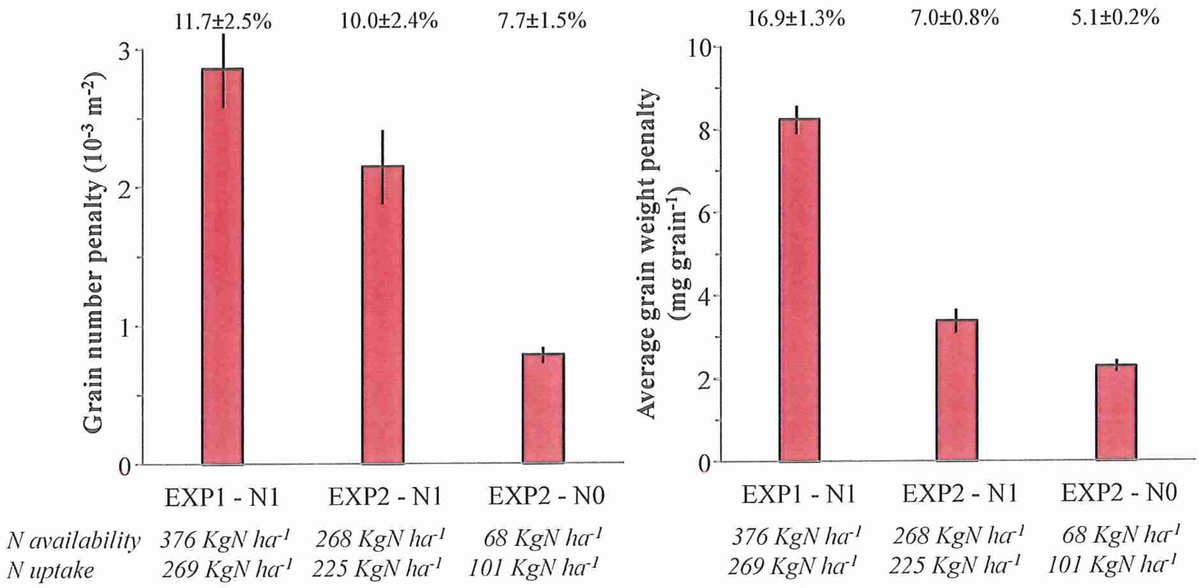

Figure 4. Penalty imposed by the high-temperature treatment on yield components: number of grains per unit land area (left panel) and average grain weight (right panel) in each of the three growing conditions ordered by decreasing order of N availability (EXP1 – N1; EXP2 – N1; EXP2 – N0). Actual values of N availability (as mineral N in soil at sowing plus N fertilization) and N uptake in the above-ground biomass at maturity are shown below each growing condition. Each bar is the average of the five modern and well adapted cultivars grown in each experiment. The segment in each bar stands for the standard error of the means. Figures on top of each bar represent the penalty in percentage of the value under unheated conditions (and its standard error).

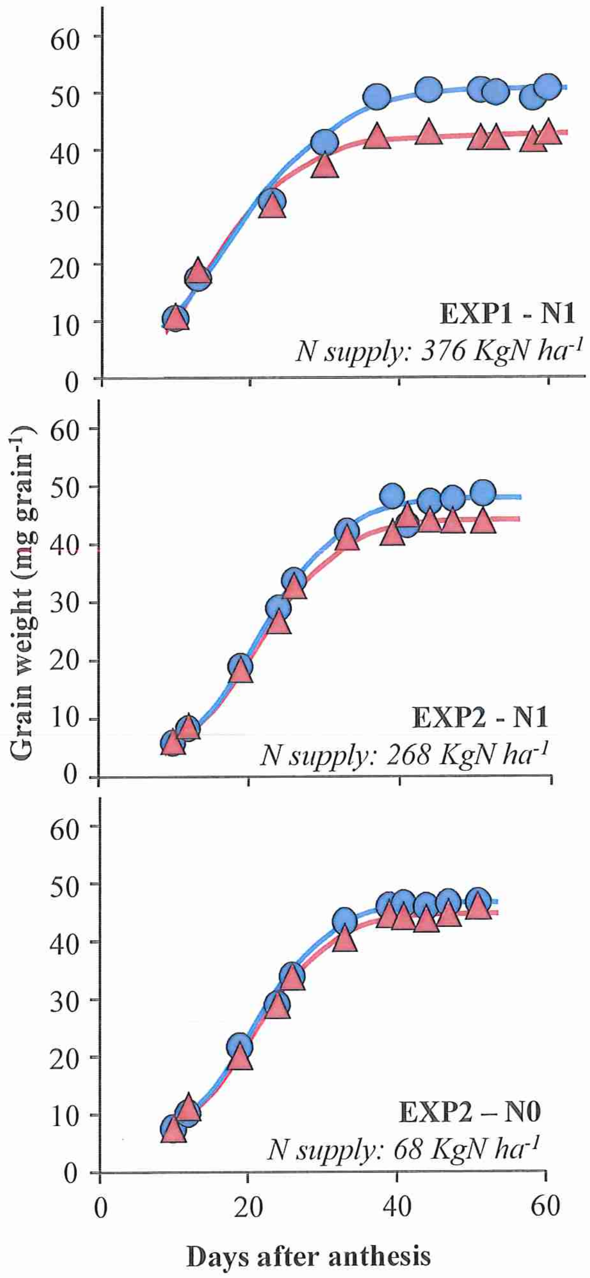

Figure 5. Dynamics of grain growth from anthesis to maturity. Data were taken from samples of main shoot spikes taken at weekly intervals for the unheated controls (circles) and high-temperature treatments (triangles) in each of the three N supply conditions (top panel highest, bottom panel lowest) in which the high-temperature treatments were imposed (the period of high-temperature treatment is highlighted with a grey background). Each data-point is the average of all grains of a spike from each of the three replicates and across all cultivars. Lines fitted with a logistic regression (see Table 2).

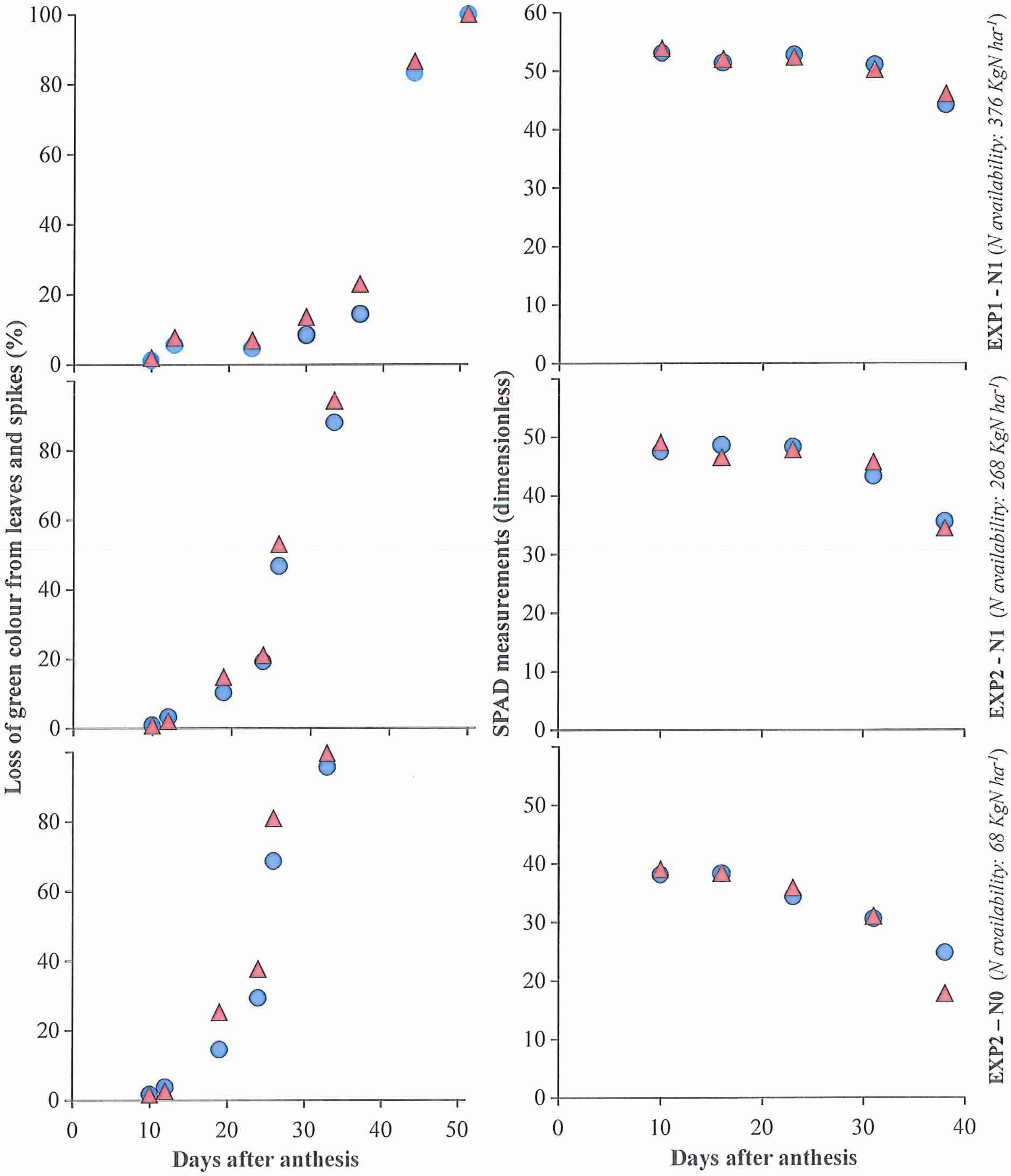

Figure 6. Time course of either (i) loss of green colour from leaves and spikes (left column of panels) or (ii) SPAD measurements made in the green parts of the flag leaves (right column of panels). Both traits were determined weekly during four weeks from the onset of the treatments for the unheated controls (circles) and high-temperature treatments (triangles) in each of the three N availability conditions (top panels highest, bottom panels lowest) in which the high-temperature treatments were imposed. Each data-point is the average of all grains of a spike from each of the three replicates and across all cultivars.

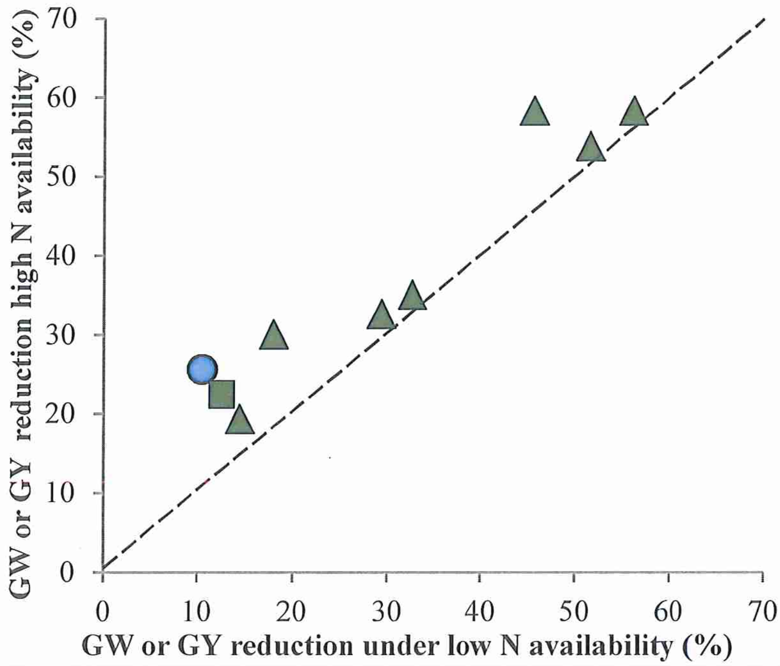

Figure 7. Relative reduction in average grain weight (GW, only when treatments were applied after anthesis and yield data were not reported in the paper), or in grain yield (GY), in response to high-temperature stress imposed under high- *vs* under low-N conditions. Data from other studies are the average across cultivars or conditions when more than one was reported. When different temperatures were used the data included in the figure represent the reduction produced considering the two extreme thermal regimes. Datum from this paper (circle) refers to the comparison of the two extreme N supplies. Data from other papers include studies under controlled conditions (triangles) extracted from Dawson and Wardlaw (1984), Mitchell et al. (1993), Altenbach et al. (2003), Zahedi et al. (2004), Dupont et al. (2006), Bencze et al. (2007) and Koga et al. (2015), and from the only other field study that we are aware of (square), in with temperature was increased during day and night over the whole growing season (Liu et al., 2013). In all cases the data-points represent the loss (GW or GY in the unheated control minus under high-temperature treatment) as a percentage of the unheated control. Dotted line represents the 1:1 ratio (Y=X).

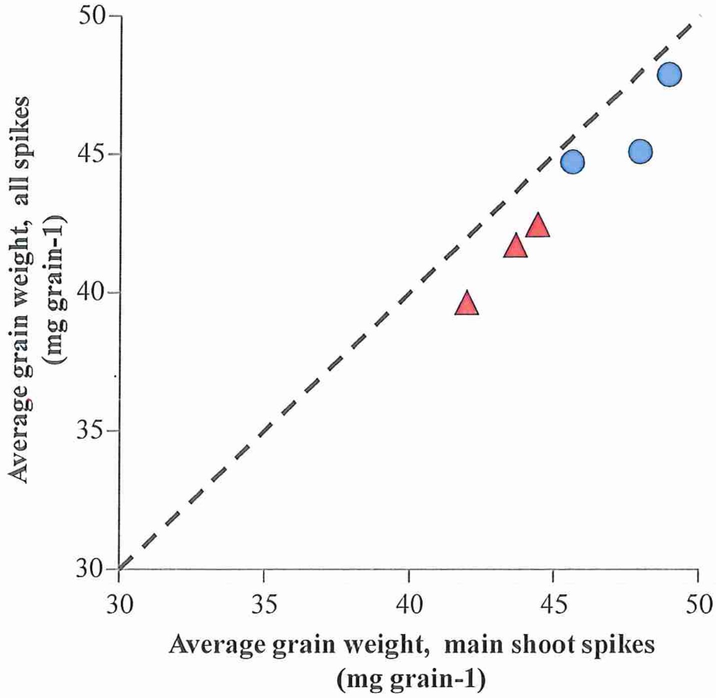

Figure S1. Average grain weight determined as the average of all grains sampled at maturity against the same variable estimated for the grains of the main shoot spike as a parameter of the dynamics of grain growth from anthesis to maturity for the unheated controls (circles) and high-temperature treatments (triangles) in each of the three yielding conditions in which the high-temperature treatments were imposed. Each data point is the average of the five cultivars.

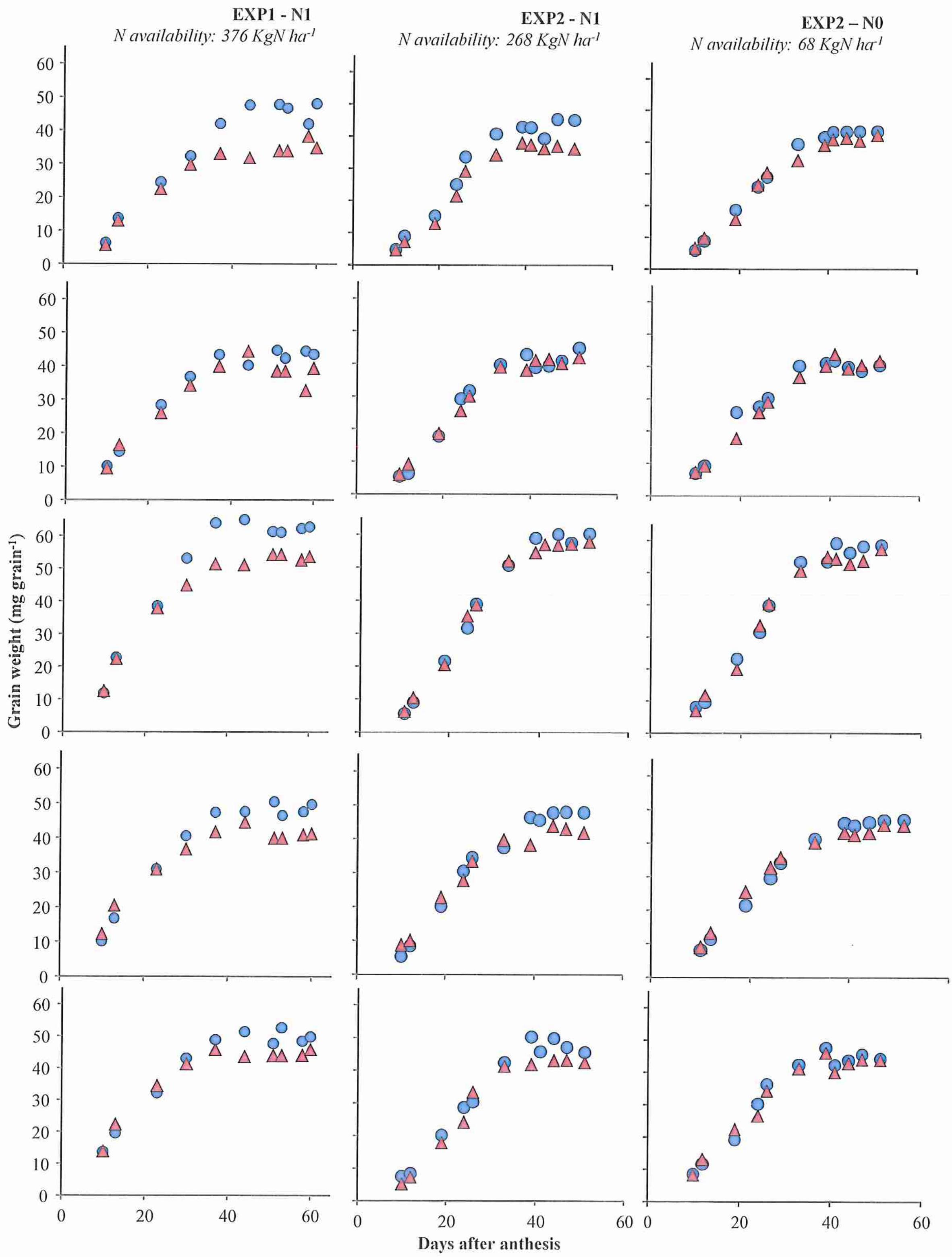

Figure S2. Dynamics of grain growth from anthesis to maturity. Data were taken from samples of main shoot spikes taken at regular intervals for the unheated controls (circles) and high-temperature treatments (triangles) for each cultivar (Tribat, Nogal, Ingenio, Sensas, and Rodolfo, from top to bottom, respectively) in each of the three yielding conditions (from highest- to lowest-N availability levels, from left to right, respectively) in which the high-temperature treatments were imposed. Each data-point is the average of all grains of a spike from each of the three replicates.

Table 1. Mean squares of the yield (g m^{-2}) and nitrogen uptake (gN m^{-2}). Yielding condition stated for three different nitrogen (N)-availability conditions treatments.

Source of variation	df	Yield (g m^{-2})	F-value	N uptake (gN m^{-2})	F-value
Yielding condition (YC)	2	1555308	108.35***	1924.02	161.82***
Genotype (G)	4	3837	0.27 ns	77.99	6.56***
Temperature (T)	1	502237	34.99***	216.80	18.23***
Block (B)	2	125889	8.77***	125.29	10.54***
YC x G	8	32849	2.29*	14.70	1.24 ns
YC x T	2	81686	5.69**	19.48	1.64 ns
G x T	4	19662	1.37 ns	17.34	1.46 ns
YC x G x T	8	9485	0.66 ns	12.33	1.04 ns
Error	58	14355		11.89	

Table 2. Grain growth rate (slope), grain filling duration and coefficient of determination of a logistic regression fitting the dynamics of grain growth (Fig. 5) for the unheated controls (UH) and high-temperature (HT) treatments in each of the three N availability conditions. Data shown are the parameters and their SEs.

Growing condition		N availability (KgN ha^{-1})	Grain growth rate* (mg d^{-1})	Grain filling duration** (d)	R^2
EXP1 - N1	UH	376	2.62±0.21	40.3±1.8	0.992***
	HT		2.35±0.23	36.1±1.9	0.988***
EXP2 - N1	UH	268	3.70±0.28	38.4±1.5	0.990***
	HT		3.62±0.22	37.9±1.2	0.993***
EXP3 - NO	UH	68	3.29±0.15	38.6±0.9	0.996***
	HT		3.12±0.15	39.1±1.0	0.995***

* Maximum rate, first derivate of the imnflexion point in the logistic curve

** The end of the phase was estimated when 95% of final grain weight was reached