How does post-flowering heat impact grain growth and its determining processes in wheat?

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Running title

Impacts of post-flowering heat on grain growth in wheat

Highlight

Wheat yield is increasingly constrained by post-anthesis heat. Temperature response curves were established for grain growth and expansion. Different heat sensitivities were revealed among processes involved in grain elaboration.

Abstract

Wheat grain yield is anticipated to suffer from the increased temperatures expected under climate change. In particular, the effects of post-anthesis temperatures on grain growth and development must be better understood to improve crop models. Grain growth and development involve several processes and we hypothesized that some of the most important processes, *i.e.* grain dry matter and water accumulation, grain volume expansion and endosperm cell proliferation, will have different thermal sensitivity. To assess this, we established temperature response curves (TRC) of these processes for steady post-anthesis temperatures between 15°C and 36°C. From anthesis to maturity, grain dry mass, water mass, volume and endosperm cell number were monitored, whilst considering grain temperature. Different sensitivities to heat of these various processes were revealed. The rate of grain dry biomass accumulation increased linearly up to 25°C while the reciprocal of its duration linearly increased up to at least 32°C. By contrast, the growth rates of traits contributing to grain expansion, e.g. increase in grain volume and cell numbers, had higher optimum temperatures,

while the reciprocal of their durations were significantly lower. These TRC can contribute to improve current crop models, and allow to target specific mechanisms for genetic and genomic studies.

Keywords

Wheat grain, grain development, growth rate, growth duration, water accumulation, endosperm cell number, grain filling, temperature response curve, high temperature, thermal stress.

Introduction

Temperature is one of the main drivers of plant growth and development. In plant sciences, temperature response curves (TRC) also referred as temperature performance curves; Schulte *et al.,* 2011) are used to evaluate how natural variations in temperature (excluding sub- and supra-temperatures) affect the rates of processes such as enzyme activity (Hawker and Jenner, 1993), photosynthesis (both at the molecular level (Rubisco: Bernacchi *et al.*, 2001) and at the plant level (Nagai and Makino, 2009)) and organ growth (e.g. Parent *et al.*, 2010). In simulation studies, TRC are integrated into mechanistic models to account for the effects of temperature (e.g. Granier and Tardieu, 1998; Wang *et al.,* 2017).

TRC typically describe (i) the rate or (ii) the reciprocal of the duration of processes related to plant growth and development. The reciprocal of duration is a mean developmental rate that characterizes the percentage of achievement rate of the process under concern. TRC typically have an asymmetrical and left-shewed shape (Dowd *et al.*, 2015; Schulte *et al.*, 2011) but are commonly characterized by two linear relationships with three cardinal temperatures: a minimal temperature T_0 below which there is neither growth nor development; an optimal temperature T_{opt} at which rates reach a maximum; and a maximal temperature T_{max} at which growth and/or development are impeded by extreme temperatures (Monteith, 1984; Parent *et al.*, 2010). In general, TRC are established and cardinal temperatures estimated in growth chamber conditions (Poorter *et al.*, 2010) where the temperature is controlled as well as possible. Responses to temperature are often evaluated with measurements of local air temperature, though they should ideally be evaluated by the organ temperature (Bonhomme, 2000).

TRC are commonly used in plant or crop models that predict integrated traits such as grain yield (Porter and Semenov, 2005; Liu et al., 2020). Today, these predictions are of paramount importance as it is anticipated that production of crops such as wheat will be negatively affected by the increased frequency of high temperatures expected under climate change (; Zheng *et al.*, 2012; Rosenzweig *et al.*, 2013; Asseng *et al.,* 2015; Lobell *et al.*, 2015; Zheng *et al.*, 2016). However, while TRC are known to be genotype- and process-dependent (Slafer and Rawson, 1995a, b; Luo, 2011), research on the responses of wheat to temperature has mainly focused on (i) the duration of pre-flowering phenological phases (e.g. Slafer and Rawson, 1995a, b) and (ii) the rate and/or duration of germination, emergence and root elongation,

cell multiplication and/or tissues expansion in stems or leaves (e.g. Porter and Gawith, 1999; Granier *et al.*, 2002; Parent and Tardieu, 2012). Few studies deal with TRC on the growth and development of grains, despite their importance to the establishment of grain yield (Reynolds *et al.*, 2011). Due to recent and foreseen increase in high temperature episodes during grain filling (IPCC, 2019), and their impact on productivity (Ababaei and Chenu, 2020), it is crucial to consider and improve the description of the effects of high temperatures on grain filling (Porter and Semenov, 2005; Challinor *et al.*, 2014; Chenu *et al.*, 2017).

A few post-anthesis studies have established TRC on rate and duration of biomass accumulation during grain filling from wheat grown in fields (Angus *et al.*, 1981; Slafer and Savin, 1991) and controlled conditions (Sofield *et al.,* 1977; Chowdhury and Wardlaw, 1978). All these studies focused on a range of temperatures where the maximum daily temperature was below 30°C and where TRC can be considered as linear. In addition, these few studies on post-flowering have focused on biomass accumulation in grains over the whole grain filling period. The reduction of grain dry biomass in response to high temperature has been related to a reduction in grain starch synthesis (Bhullar and Jenner, 1986; Spiertz *et al.*, 2006). Among the enzymes regulating starch synthesis, the soluble starch synthase (SSS) is the most sensitive one to heat stress (Jenner, 1994; Zahedi *et al.*, 2003) and TRC of SSS activity have been established (e.g. Keeling *et al.*,1993).

However post-anthesis temperatures are likely to impact grain growth and development differently depending upon the grain development phase and on the underlying processes involved (Slafer and Rawson, 1995a, b). Indeed, grain growth and development is generally divided into three phases during which different processes occur: a rapid cell proliferation phase called 'lag-phase', an effective filling phase and a maturation phase (Egli, 1998). Cell proliferation within the grain endosperm begins just after fertilization. During this first phase, water is rapidly accumulated inside the grain, contributing most to the volumic growth of the grain. At the end of the lag-phase, the grain length is set (Lizana *et al.*, 2010; Nadaud *et al.*, 2010), the maximum number of endosperm cells is attained, and correlates positively with the grain filling capacity (Brocklehurst, 1977). The second phase consists of the accumulation of assimilates into the endosperm cells. Water mass during this phase is maintained constant and dry matter content increases linearly over time (Egli, 1998). Finally, maturation of the grain begins when the final dry biomass and lateral dimensions of the grain are set. During

the maturation phase, grains desiccate while the polymerization of storage proteins and assembly with starch within the endosperm cells contribute to the final grain quality. If TRC have already been established for one of the main processes related to grain dry biomass accumulation (SSS synthesis), no such curves are available for the main processes related with grain expansion (cell proliferation and water accumulation).

We hypothesized that the different processes involved in the successive phases of grain filling may have different temperature responses, and that this may contribute to the response of final grain dry biomass. Therefore, we established TRC for some of the main processes involved in final grain size and biomass, namely grain dry biomass and water accumulation, grain volume expansion, and endosperm cell proliferation. This included (i) parameterizing cardinal temperatures for response curve of studied processes, (ii) testing the range of temperatures where linear responses to temperature could be considered, and (iii) evaluating whether the processes involved in the constitution of the final grain mass shared a common sensitivity to temperature. TRC were established based on results of five experiments where constant grain temperature (from 15°C to 36°C) was applied during the grain filling period, for a unique genotype, and on grains at a fixed position on the spike. Here we show that the different processes do have different temperature responses. These TRC can contribute to bring more robustness into the calculation of post-anthesis thermal time in crop models, and allow to target specific mechanisms for genetic and genomic studies and finally for plant breeding.

Material and methods

Plant material and growth conditions

Seeds of spring wheat (*Triticum aestivum* L.), genotype SxB049 (Pinto *et al.*, 2010 and provided by CIMMYT) were sown in 50 mL pots and placed in a greenhouse. At the three-leaf stage, the plants were transplanted by pairs into PVC tubes (inner diameter 7.5 cm; length 50 cm) filled with compost enriched with 2.5 kg m^{-3} of fertiliser 9:12:16 (N:P:K), and iron (Fe). The PVC tubes were arranged into two contiguous containers to form a homogeneous canopy surrounded by green and perforated screens to reduce edge effects. All plants were cultivated at a 16h

156 photoperiod, and watered daily in excess with a 0.5 strength Hoagland nutrient solution to
157 maintain the soil water potential above -0.5 MPa.
158 Both containers were first located in a “control” growth chamber (set at 19°C) during the
159 vegetative phase. A few days before anthesis, when the entire spikes had emerged from the
160 sheath, the main stem of each plant was labelled. Their number of spikelets was counted, and
161 only spikes with similar number of spikelets were retained. Anthesis was recorded as the date
162 when anthers of the basal florets of the middle spikelets of the main stem appeared. Each PVC
163 tube from one of the containers was transferred in the “treatment” growth chamber two days
164 after the averaged anthesis date of its two plants, in order to avoid or limit the impact of high
165 temperature on final grain number. If the time between the anthesis dates of the two plants
166 in the same tube was more than 2 days, only the second plant to reach anthesis was
167 considered for the study.
168 Five temperature treatments constant over 24h were applied: 36, 32, 29, 24 and 15°C in five
169 successive experiments conducted in the “treatment” chamber (Table 1). In each experiment
170 a control treatment at 19°C was conducted in the control chamber, so that there were 5
171 replicates of the control treatment at 19°C. In each growth chamber, the air temperature next
172 to the wheat spikes was measured using copper-constantan thermocouples placed under a
173 shield screen. Grain temperature was also measured using 0.2 mm copper-constantan
174 thermocouples inserted into basal grains of central spikelets of tillers (different than those
175 sampled for measurements). As the insertion of the thermocouples into the grain caused local
176 necrosis, thermocouples were moved to different grains every two days. In each chamber,
177 four thermocouples for measurement of air temperature and four thermocouples for
178 measurement of grain temperature were equally spaced into the plants. Air and grain
179 temperatures were recorded every 10s on a CR 1000 datalogger (Campbell Scientific Ltd,
180 Logan, UT, USA) and averaged over 15min. The mean daily temperature variation within the
181 growth chambers was less than 1.5°C. Plants received throughout the experiment a mean total
182 daily photosynthetic photon flux (PPF) of 250 ± 57 and 354 ± 81 µmol m^{-2} d^{-1} in “control” and
183 “treatment” growth chambers, respectively. Relative humidity varied between 66.6 and
184 79.0 % depending on experimented temperature leading to an average VPD of 1.7 kPa for the
185 36°C treatment, and 1.1 kPa or lower for all the other treatments. Environmental conditions

in each growth chamber and for each experiment are reported in Table 1 and the variations of air and grain temperatures in the five experiments are presented in Figure S1.

Grain measurements

For each experiment and each temperature treatment (i.e. in both control and treatment growth chambers), sampling was conducted on 12 to 16 dates from anthesis to maturity. At each date and for each temperature, six tagged main spikes were cut and placed in plastic bags with a damp paper and transferred rapidly to the laboratory for measurements. For each spike, the two basal grains of the two middle spikelets (i.e. four grains) were sampled to measure fresh biomass and volume of individual grains. One grain from each spikelet was dried at 70°C for two days in order to measure dry biomass. Water mass was calculated as the difference between the fresh and dry biomass. The second grain from each spikelet was frozen, then dissected to isolate the endosperm that was prepared for cell count. The endosperm cell number was determined following the method described by Singh and Jenner (1982). At each sampling date and for each temperature treatment, the values of the traits measured on either two (for dry biomass and endosperm cell number) or four (fresh biomass and grain volume) grains of the same spike were averaged before doing further analysis on the six independent repetitions (i.e. six plants).

Analysis of trait dynamics over time and final value

The effects of high temperatures on grain volume, dry biomass and water mass at maturity, and on the endosperm cell number at around 300°Cd after anthesis were statistically tested followed by a Student-Newman-Keuls (SNK) test at the 5% level of significance for mean comparison.

Growth kinetics were studied for four traits (dry biomass, volume, water mass and endosperm cell number) describing mean grain growth for a range of temperatures. In a first step, classical candidate growth functions (Hunt, 1979; Ratkowsky, 1990) were fitted to the change over time of each studied trait for plants grown at 19°C (control) in each experiment with the nonlinear least squares *nls* procedure. The growth function describing most adequately the observations was chosen based on the homogeneity and values of residuals, biological coherence (e.g. shape, starting values, durations), and the Akaike information criterion (AIC).

When the data were heteroscedastic, non-homoscedastic variance structures were tested and compared (Robert *et al.*, 1999). For each trait, the chosen function (Table 2) was then used to fit the observations from all the temperature treatments (second step, see below).

In the second step, to account for variations observed across experiments, the chosen growth functions were fitted for each trait, except endosperm cell number, on the complete dataset with a nonlinear mixed model. Each function parameter was considered as the sum of a fixed effect dependant on the temperature treatment, and a random effect dependant on the experiment as to account for bias observed among controls of the different experiments. When an 'experiment' random term was negligible, it was removed from the model, and the resulting model was compared to the former with the Fisher statistic to ensure that it was not significantly different. These non-linear mixed models were fitted using the R package *nlme* (Pinheiro *et al.*, 2018), which optimises parameters based on optimisation-maximisation of the log-likelihood. Endosperm cell number, on the other hand, was too variable to include a random 'experiment' effect within each parameter, and thus, the adjustment included only temperature-dependant fixed parameters and was carried out with *nls (*Bates *et al.,* 2007).

Temperature response curve

Maximum values, average growth rates and durations of the studied processes were defined from the selected growth functions as described in Table 2. For each trait, two TRC were established by plotting (i) the growth rate and (ii) the reciprocal of the duration against the mean grain temperature (Table 1). Standard deviations were provided by the mixed models or calculated using the multivariate delta method (Cox, 2005).

A simplified Arrhenius-type function developed for responses to temperature and depending on minimum (T_{min}), optimum (T_{opt}) and maximal (T_{max}) temperatures (Wang *et al.,* 2017), was fitted on the TRC [Eq.1].

$$f(T) = a.f_{Wang17}(T) = a.\left(\frac{2(T-T_{min})^{\alpha}(T_{opt}-T_{min})^{\alpha}-(T-T_{min})^{2\alpha}}{(T_{opt}-T_{min})^{2\alpha}}\right)^{\beta} \quad \text{[Eq.1].}$$

with the α coefficient given by:

$$\alpha = \frac{ln2}{\ln\left(\frac{T_{max}-T_{min}}{T_{opt}-T_{min}}\right)}$$

For each trait, a coefficient *a* was added to normalise the function and obtain dimensionless values varying between 0 and 1. T_{opt}, T_{max} and *a* were estimated statically using *nls*, while T_{min} was set at 0°C, as commonly used in the literature for wheat (e.g. Slafer and Rawson, 1995b) and β was set at 1 as in Wang *et al.* (2017).

All statistical analyses were conducted with R 3.4.4 (R Core Team, 2018).

Results

High temperatures affect grain growth in various ways

Six steady temperature treatments (15, 19, 24, 29, 32, 36°C) were applied on plants during the whole grain growth duration. Different traits related to grain growth, namely the grain dry biomass, the grain volume, the grain water mass and the endosperm cell number were recorded from basal grains from central spikelets. Throughout the experiments, the target temperatures within the grains were consistent with the required temperatures (Fig. S1A). While the grain and air temperatures were highly correlated throughout the experiments (Fig. S1B), air temperature was significantly greater than grain temperature with a mean difference of 0.29°C (SD=0.42, T = 11.9, P < 0.0001, one tailed). This small difference is probably due to the growth cabinet conditions in which the instantaneous light flux on the spike is moderate.

As expected, high temperatures resulted in a strong significant reduction (P<0.001) of the final observed values of the different studied traits (Fig. 1). The maximal percentages of reduction on final values calculated between 15°C and 36°C were 81.0, 74.8, 91.5 and 52.9 % for grain dry biomass, grain volume, grain water mass and endosperm cell number, respectively.

The kinetics of the different traits were followed from 2 days after anthesis to maturity. For each trait, the growth curves had a similar pattern for the different temperature treatments (Fig. 2). Growth functions that best fitted the observations were chosen (Table 2) based on data from the control temperature (19°C) and were then applied to data from all temperature treatments. High correlations were found between observations and predicted values from the statistical model (Table 3) and the standardized residuals were evenly distributed, comforting the good adequation of the selected growth function at 19°C for all the

temperatures (Fig. S2). The adjusted growth functions are presented in Figure 3 for all traits and temperature treatments.

For all temperatures, grain dry biomass accumulation followed a classic sigmoid pattern (Fig. 2A and 3A). Final grain biomass began to be affected by high temperature for a threshold between 24 and 29°C (Fig. 1A). The dynamics of dry biomass accumulation was however different (not necessarily significantly) for all the temperatures tested (i.e. 15 to 36°C) with a greater impact observed for the highest temperatures.

Grain volume increased almost linearly up to a plateau under optimal temperatures (15°C; Fig. 2B and 3B). However, for most tested temperatures (19 to 36°C) and probably also for 15°C (although not apparent in Fig. 3B), grain volume increased up to a maximum before decreasing before the end of the grain filling period. This final volume (at maturity) decreased with increase in temperature up to 29°C, while the final volume from the 29, 32 and 36°C treatments were similar (Fig. 1B and 2B). The dynamics of the grain volume was also highly impacted by temperature, with the maximum volume being reached earlier and being lower for higher temperatures.

Similar trends were found for grain water mass, which increased up to a plateau before the end of biomass accumulation and then decreased during a dehydration phase up to a minimum content (Fig. 2C and 3C). The level of the water mass plateau decreased from 15 to 32°C and then increased substantially between 32 and 36°C, for which the plateau was close to that of the control (19°C) (Fig. 3C). The final water mass was similar for most treatments (from 24°C to 36°C, Fig. 1C and 2C) and may not have been reached in the 15°C treatment when the experiment ended. Note that the growth function used did not capture when water content stabilised to a final level (Fig. 3C).

Finally, for all temperatures up to 32°C, cell proliferation over time (Fig. 2D and 3D) increased at the beginning of grain development up to a maximum value, which was followed by a slight decrease and then a stabilization of the cell number defining the final cell number. Increased temperatures tended to accelerate early cell multiplication, reduce the duration of cell proliferation and ultimately result in a lower final cell number. Cell proliferation and cell number decrease was different for the extreme treatment at 36°C, for which cell proliferation occurred at a low rate and for a comparatively long period.

Increased growth rate partly compensated shorter duration of grain growth under high temperatures

Fitting the growth functions allowed the estimation of the maximal value of each trait in each temperature treatment, as well as the growth rate and the duration of the growth processes (Fig. 4). Higher temperature accelerated the accumulation of biomass, the increase in volume and water accumulation, and cell proliferation in the grain up to a temperature threshold ranging from 24 to 32°C depending on the process considered (Fig. 4A, D, G, J). At the same time, the duration of these processes was reduced by higher temperatures (Fig. 4B, E, H, K). Overall, maximum estimated values were reduced by higher temperatures (Fig. 4C, F, I, L). Hence, estimated final dry biomass of grains from middle spikelets at the end of the grain filling decreased from 44 mg at 15°C to 6 mg at 36°C. Grain volume reached an estimated maximum from 67 mm^3 at 15°C to 40 mm^3 at 36°C, during the grain filling. Grains accumulated up to a maximum of 35 mg of water at 15°C, 24 mg of water at 32°C. Under the extreme treatment of 36°C, a surprisingly important water accumulation was observed, with a maximum of 31 mg per grain. The number of cells in the endosperm increased up to a greatest maximum of 90,000 at 15°C to a lowest maximum of 40,000 at 36°C.

Different temperature responses across traits

The temperature response of the growth rate and the reciprocal of the duration of each considered process were well fitted (R^2 in Table 4) by the same response function [Eq.1] (Fig. 5; Table 4). This function estimated optimal temperatures (T*opt*) between 25.0 and 29.3°C for the growth rates of the studied processes and between 29.9 and 45.0°C for the reciprocals of their durations. Maximal estimated temperatures (T*max*) varied between 37.2 and 43.7°C for growth rates and between 39.4 and 66.1°C for duration reciprocals. Note that for all the studied traits, the temperature responses could reasonably be deemed linear between 15°C and 30°C or a bit more in some cases (Fig. 5), thus allowing the use of the usual additive formalism of thermal time in these conditions.

The response of duration reciprocal for grain biomass accumulation is of particular interest to crop modellers and it can be used to deduce the formalism for thermal time during the grain filling period. The optimum temperature could not be properly estimated in this study and may be outside our experimental temperature range, but no clear change in the duration of

biomass accumulation was observed between 32°C and 36°C, and no treatment over 36°C was tested (Fig. 5B). This explains why the standard deviations for the estimated optimum and maximum temperatures are important compared to standard deviations estimated for the other traits (Table 4).

To enable comparisons between different physiological processes, the temperature responses of growth rates and of duration reciprocals of the four processes studied were normalized at 19°C (Fig. 6). The temperature responses of both growth rates and reciprocals of duration had similar values of optimum and maximum temperatures for grain volume, water mass and endosperm cell proliferation (Fig. 6A and 6B; Table 4). The lowest growth rate was found for biomass accumulation with an optimal temperature of 24.9°C and a maximal temperature of 38.2°C. Cardinal temperatures of the reciprocal of the duration for the biomass accumulation could not be estimated properly as treatment with higher temperatures would have been required.

Regardless of the trait, the amplitudes of the temperature responses were greater for reciprocals of duration (Fig. 6B) than for growth rates (Fig. 6A) with a normalisation at 19°C. For example, for biomass accumulation at the optimum temperature, reciprocals of duration were 2.7-fold than at 19°C (Fig. 6B), compared to < 1.2-fold greater for the growth rate (Fig. 6A). Variations of amplitude were also observed among traits. For instance, the reciprocal for the duration of endosperm cell proliferation was 1.9-fold greater than the one at 19°C (Fig. 6B) whereas for biomass accumulation it was 2.7-fold greater than the one at 19°C.

Discussion

High temperatures were applied from anthesis to maturity on whole plants. Thus, all processes at the whole plant level (e.g. leaf photosynthesis, senescence, global respiration, remobilization of stem reserves…) are likely to have been affected directly and thus may have impact grain growth indirectly. While the objective of the study is to investigate the sensitivity to high temperatures of grain growth and associated processes, the results will be discussed independently of the direct or indirect causal physiological sources of the response and focused on the grain scale.

The rate of grain biomass accumulation linearly increased up to 25°C while the reciprocal of its duration linearly increased up to at least 32°C

In our experimental conditions, when temperature increased from 15°C to 36°C, the final dry biomass of the grains continuously decreased (Fig. 1A, Fig. 4A). This result was expected and consistent with the literature (e.g. Sofield *et al*., 1977; Wardlaw *et al.,* 1980; Farooq *et al.,* 2011). The optimal temperature for the rate of grain filling was estimated to 24.9°C (Table 4), which is higher than those reported in the literature: 20.7°C (Porter and Gawith, 1999, who summarized the results from 7 studies), or between 18 and 22°C (Farooq *et al.*, 2011). The possible discrepancy between the literature and the present study may be related to (i) possible differences due to consideration of air or grain temperatures (but it is unlikely to fully explain such differences), (ii) the great heterogeneity of temperature treatments among the different studies, and especially the timing, duration and intensity (moderate vs heat shock) of applied high temperatures, or possibly (iii) genotypic differences, as genetic variability has been reported in wheat for cardinal temperatures of response curves for grain filling (Wardlaw *et al.,* 1989 ; Slafer and Rawson, 1995b;). In our experimental conditions, the higher optimum temperature response of grain filling may be inherent to the genotype SxB049, a warm-adapted genotype that has been shown to tolerate thermal and water stress (Pinto *et al.*, 2010). It is important to note however that in all these previous studies, the optimal temperature for grain filling was determined from the comparison between only 2 to 3 temperature treatments. To our knowledge, no meta-analysis, generalizing in a formal way across a number of independent experiments (such as in Poorter *et al.*, 2010) is available for the effect of temperature on wheat grain dry biomass accumulation.

The response of the grain final dry biomass to high temperature is the result of the response of both duration and rate of dry mass accumulation to heat. Between 15°C and 25°C, the duration of the dry biomass accumulation duration was shortened by increased temperatures while the rate of dry matter accumulation increased (Fig. 4; Table 4). This was however not enough to compensate the shortening of the grain filling period, and overall higher temperatures resulted in smaller final grain biomass. On the contrary, above 25°C, both the duration and the rate of grain dry biomass accumulation decreased. The duration of grain dry biomass actually decreased constantly between 15 and 32°C. This result is consistent with the literature (Sofield *et al.*, 1977; Jenner, 1994) showing that duration and rate of grain filling

display different types of response to variation in temperature in a range between 20 and 40°C. Moreover, the genotypic tolerance to high temperatures during grain filling has been associated with an increased rate of grain filling compensating the reduced duration of grain filling (Wardlaw and Moncur, 1995). This underlines the need to study specifically the response to elevated temperatures of both rate and duration of the grain filling.

The estimated optimal temperature for the grain growth rate was 24.9°C (Table 4). Sofield *et al.* (1977) suggest that the sensitivity to temperature of the grain filling rate could be influenced by the number of grains. In our experimental conditions, the grain number per spike was not modified by temperature as the plants were moved to the "treatment" chamber 2 days after anthesis when the effect of elevated temperatures on grain number per spike is null or very weak (Prasad *et al*., 2015). Unfortunately, for the duration of grain dry biomass accumulation, our data do not allow to determine with accuracy whether the grain filling duration still decreased or stagnated over 32°C. Moreover, note that our data do not allow the estimation of the base temperature reliably, as the minimum temperature tested was 15°C.

One of the aims of our study was to define the temperature range where the rate and the reciprocal of duration of grain dry matter accumulation increase linearly with the temperature. This is the most important assumption for the use of the linear "thermal time model" (Monteith, 1984) to determine the duration of the grain filling period. While there is a statistical uncertainty around the estimated optimum temperature and the response for a temperature above 32°C (Fig. 5B), our results clearly show that the response of grain dry biomass accumulation to temperature can be considered linear between 15 and 32°C (Fig. 5B).

Processes related to dry matter accumulation and expansion in the grain have different sensitivities to heat

Apart from accumulation of dry matter in the grain, other processes contribute to grain growth. These processes include cell proliferation in the endosperm that takes place during the early phase of grain development, and the accumulation of water which results in an increase in the grain volume via cell expansion.

Temperature increases resulted in a decrease in traits relative to cell proliferation and expansion growth, i.e. the maximum values of the volume, water quantity and number of cells

in the endosperm (Fig. 4A). Similar effects of temperature were previously observed for the maximum number of cells in the albumen (Commuri and Jones, 1999; Girousse *et al.*, 2018). In our experimental conditions, both the rate of increase and the reciprocal of duration for all the traits relative to cell proliferation and grain expansion had an optimal temperature around 30°C (from 27.8 to 31.9°C; Table 4; Fig. 5). Above this optimum temperature, the rate slowed down while the duration increased but allowing some compensation (Fig. 4). To our knowledge, this is the first time that TRC have been established for such processes. Overall, the rate of all the processes relative to cell proliferation and organ expansion had a similar sensitivity to temperature. The same was observed for the reciprocal of their duration, which responded similarly to temperature. Physiological processes presenting a common response to temperature have previously been found for other traits in various crops, including wheat (Parent *et al.*, 2010; Parent and Tardieu, 2012).

The temperature response of grain dry matter accumulation (growth rate and reciprocal of duration) adequately followed a modified Arrhenius function (Fig. 6A and 6B) as previously assumed for the reciprocal of duration of the post-anthesis development phase that finished when grains reach their final biomass (Fig. 4B of Wang *et al.*, 2017). In Wang *et al.* (2017), the temperature response for post-anthesis development was obtained with data from field experiments (Reynolds *et al.*, 1994; White *et al.*, 2011) and semi-controlled conditions in outdoor climate chambers (Triboi *et al.*, 2003). In these experiments, temperatures ranged between 12.5 and 31°C, which was not enough to properly assess the optimal temperature (*Topt*) that was estimated at 33°C (Fig. 7; Wang *et al.,* 2017). Data from the present study suggest that the optimal temperature for the reciprocal of duration of grain growth is likely to be above 33°C (Fig. 5A), at least for the genotype tested. But here too, treatments were not hot enough to allow a proper estimate of *Topt*.

In our experimental conditions, the cardinal temperatures of the response of dry matter accumulation were substantially different from those of processes related to cell proliferation and grain expansion (Fig. 6; Table 4). The growth rate of traits contributing to the grain cell proliferation and expansion generally had a substantially higher optimum (between 27.8 and 29.3°C) and maximum (between 38.2 and 43.7°C) temperatures than the rate of dry biomass accumulation (optimal temperature of 25.0°C and maximum temperature of 37.2°C). While the optimum and maximum temperatures of the reciprocal of duration for dry matter

accumulation could not be estimated properly, they were much higher than for the variables related to cell proliferation and expansive growth (between 29.9 and 31.9°C; Fig. 5; Table 4). The contrast in temperature responses between traits relative to grain expansion and grain biomass accumulation is particularly well illustrated at 36°C where the grain volume and the accumulated water in the grain increased at rates only slightly lower than at 30-32°C even though accumulation of dry matter in the grain severely dropped to close to zero at 36°C (Fig. 5). One may assume that this difference comes from the temperature dependency of later processes related to the grain filling, and in particular starch synthesis. According to the model proposed by Pan *et al.* (2007; Eq[2]), the rate of grain starch accumulation results from the product of two components: (i) a developmental component that corresponds to a potential accumulation rate times a factor that depends on the post-anthesis thermal time and (ii) a direct effect of temperature on the biochemistry of starch accumulation. Following this framework, we assumed i) the developmental part to be reflected by the rate of volume expansion, which sets the capacity of the grain (in terms of both (a) the cytoplasmic volume defining possible sterical/mechanical constraints on grain filling and starch accumulation, and (b) the amount of cells and hence of glucose transport and starch synthesis machinery); and (ii) the biochemical part to be substantially driven by the main enzyme controlling starch synthesis (i.e. Soluble Starch Synthase) (Keeling *et al.*, 1993; Boehlein *et al.,* 2019), that is highly temperature dependent above 30°C (Keeling *et al.*, 1993; 1994). We propose that the rate of dry biomass accumulation ($\frac{d\,Mg}{dt}(t,T)$) over time (t) and in response to the temperature (T) depends on the developmental volume component ($\frac{d\,V}{dt}(t,T)$) and the biochemical components ($f(T)$) as follows:

$$\frac{d\,Mg}{dt}(t,T) = \frac{d\,V}{dt}(t,T) \times f(T) \qquad \text{[Eq. 2]}$$

The dependence to temperature of the developmental component is given by the relative temperature response curve established in Fig.6 and the dependence to temperature of the SSS relative activity is taken from Keeling *et al.* (1993; Fig.1, 120 min temperature treatment). The prediction of the model in [Eq. 2], is close to the experimental curve of the temperature response of the rate of grain dry biomass accumulation (R^2=0.76; Fig.8). While equation 2 does not correspond to a process-based model nor a mechanistic one, this phenomenological approach suggests that considering the temperature responses of the morphogenetic

processes in the early phase and the temperature response of the later grain filling processes may confer a robust estimate of grain biomass kinetic in response to fluctuating temperatures from outdoor conditions.

Differences in temperature response of different processes have been found in other studies. For instance, some morphological and physiological processes differ in their temperature responses in alfalfa and tall fescue (Zaka *et al.*, 2017). In wheat, Slafer and Rawson (1995a, b) found that the cardinal temperatures of the growth rates of different processes (e.g. leaf appearance, internode growth) tend to increase during the plant development. Our results revealed that within the same organ, the wheat grain, and during the same development phase (grain development), synchronized processes can have different temperature sensitivities. In particular, the process of dry matter accumulation and water accumulation in the grain appear to have different optimal temperatures for both their rate of accumulation and the reciprocal of their duration.

Challenges related to studies and simulations of heat stress impacts

In addition to timing and intensity, plant responses to temperature depend on i) the duration of exposure to high temperatures (Tashiro and Wardlaw, 1990; Stone and Nicolas, 1995; Prasad and Djanaguiramam, 2014; Chenu and Oudin, 2019), and as discussed before, ii) the physiological processes involved. Short-term exposures to high temperature, for example in the case of heat shock, can trigger metabolic/physiological changes at all spatial scales (molecular, cellular, tissue or organ) within a few hours (Wahid *et al.*, 2007; Wang *et al.*, 2011). If happening relatively early during the plant growth cycle, these modifications may enhance the plant ability to cope with higher temperature exposures at later stages, ability also known as acclimation (Wang *et al.*, 2011; Barlow *et al.*, 2015). Under our experimental conditions, temperature treatments were applied from 2 days after anthesis to the grain maturity, i.e. durations of exposition lasted between 2 and 9 weeks at 36 and 15°C, respectively. The TRC obtained were thus integrated responses to long-term high temperature, which can differ from short-term responses. In addition to metabolic or physiological modifications (such as photosynthesis, respiration, senescence…), such long-term exposures to high temperatures may also induce a degree of acclimation, which can occur within a few days (Sage and Kubien,

2007) and result in morphological or anatomical changes (Atkin *et al.*, 2006; Gorsuch *et al.*, 2010). Our data do not allow to assess such modifications. However, the durations of high temperature exposures were likely long enough to induce feedback between the various processes occurring in the different organs and tissues (including photosynthesis and respiration) and then to contribute to integrated responses (Atkin *et al.*, 2006). Although difficult to demonstrate, this effect of acclimation cannot be discarded; ignoring the acclimation potential of plants could lead to an overestimation of the responses to high temperature on the various developmental processes contributing to final grain biomass (Perdomo *et al.*, 2015).

Grains do not respond in the same way at high temperature as opposed to control temperature depending on their position on the spike (Tashiro and Wardlaw, 1990). The stability of the response curves should be checked as a function of the position of the grains on the ear. Here, only the basal grains taken from the central spikes of the spikes were studied. On a given spike, these grains are the largest (i.e. Bremner, 1972; Baillot *et al.*, 2018) and have higher sink forces than other grains on the same spike. The response curves established may thus vary with the position of the grains on the spike.

Another challenge when studying heat stress relates to simply measuring the temperature. Most studies record air temperature, rather than the organ (grain) temperatures (Jamieson *et al*., 1995; Bonhomme, 2000). Temperature responses presented in this study relate to grain temperature, even if small yet statistically significant differences (the slope of the regression between the two temperatures was equal to 0.96, with an intercept of 0.61°C) were observed between atmospheric and organ temperature in our well-watered and low-VPD conditions (Fig. S1B). However, this is unlikely to be the case in a large number of field conditions, especially during the grain filling period when heat and drought are the most frequent (Chenu *et al.*, 2013; Ababaei and Chenu, 2020).

Overall, this study provides for the first time response curves for temperatures between 15°C and 36°C established under identical growing conditions, with steady temperatures (Fig. 5; Table 4). Such results can be used to improve current crop models in regards to how temperature impacts the grain filling (Chenu *et al.,* 2017). Besides by defining a novel framework to model grain biomass accumulation based on the grain volume expansion and

biochemical grain filling processes, our findings can help to elaborate more robust models for grain dry biomass accumulation in particular in non-steady conditions. This can also assist mechanistic investigation and possible genetic selection. For instance, this focus should be not only on the biochemistry of grain filling but also on the morphogenetic processes that lead to volumic growth of the grain. Following this, more work is needed to fully understand and simulate the physiological processes and mechanisms involved. Moreover, the dependency of these processes on the timing, intensity and duration of the heat events and other influential environment factors (e.g. edaphic and atmospheric water deficits, atmospheric CO_2 content).

Supplementary data

Fig. S1. Relationship between air and grain temperatures

Fig. S2. Relevance of the adjusted growth functions (analysis of residuals)

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Tables

Table 1. Environmental conditions in each growth chamber. For control treatment (19°C), the values correspond to the average of the five successive experiments conducted in the same growth chamber. Average and standard deviations of air and grain temperatures, relative humidity (RH), vapour pressure deficit (VPD) during the day were calculated from anthesis to maturity.

Temperature setting (°C)	Air temperature (°C)	Grain temperature (°C)	Air RH (%)	Mean VPD (kPa)
15	15.5 ± 1.3	15.5 ± 1.1	76.3 ± 3.1	0.4 ± 0.1
19	19.4 ± 1.3	19.1 ± 1.9	77.2 ± 3.0	0.5 ± 0.1
24	24.8 ± 1.5	24.4 ± 1.5	79.0 ± 3.4	0.7 ± 0.1
29	29.4 ± 0.9	28.9 ± 0.9	77.5 ± 3.4	0.9 ± 0.1
32	31.9 ± 1.3	31.7 ± 1.2	76.5 ± 4.2	1.1 ± 0.2
36	36.1 ± 0.7	35.2 ± 0.9	66.6 ± 12.4	1.7 ± 0.3

Table 2. Characteristics of the growth functions used to fit observations from anthesis to maturity for each trait and each temperature. For each trait, the function and variance structure used to model the trait response to temperature are presented as well as the equations used to calculate the growth rate, duration and maximum value.

Trait	Type of function	Equation	Variance structure	Growth rate	Duration	Maximum value
Grain dry biomass	Gompertz[1]	$ae^{-e^{\frac{t-b}{c}}}$	Power	$\frac{a}{4c}$	$4c$	a
Grain volume	Gamma with constant[2]	$ae^{-ct}t^{b-1}+d$	Exponential	$\frac{ac}{b-1}(e^{1-b}\left(\frac{b-1}{c}\right)^{b-1}+d)$	$\frac{b-1}{c}$	$ae^{1-b}\left(\frac{b-1}{c}\right)^{b-1}+d$
Grain water mass	Segmented Linear function	$\begin{cases} ax, & 0 \le x \le b \\ ab, & b < x \le c \\ ab+d(x-c), & x > c \end{cases}$	Exponential	a	b	ab
Endosperm cell number	Gompertz with maxima[3]	$ae^{(b(x-c)-\frac{b}{d})(1-e^{-d(x-c)})}$	Exponential	$\frac{a}{c}$	c	a

[1] Winsor (1932); [2] Lebreton et al. (1982); [3] Werker (1997)

Table 3. Equation and coefficient of determination (R^2) for the fits between observations and predictions from non-linear mixed models for the four studied traits in all temperature treatments

Trait	Observed – predicted relationship	R^2
Grain dry biomass	-0.12 + 1.01x	0.92
Grain volume	0.25 + 0.99x	0.86
Grain water mass	0.75 + 0.99x	0.86
Endosperm cell number	0.04 + 0.99x	0.93

Table 4. Coefficient of determination (R^2), estimated optimum temperature (T*opt*), maximum temperature (T*max*) and normalisation coefficient *a* for the growth rate and the reciprocal of the duration for the studied traits. For a given trait, growth rate is expressed in unit per day and reciprocal of duration in day^{-1}. The estimated temperatures and the coefficient *a* are calculated with *nls* according to [Eq.1] and presented with their standard deviations.

	R^2		T*opt* (°C)		T*max* (°C)		*a* (same unit as rate or 1/duration)	
Trait (per grain)	**Growth rate**	**1/Duration**	**Growth rate**	**1/Duration**	**Growth rate**	**1/Duration**	**Growth rate**	**1/Duration**
Dry biomass accumulation	0.90	0.99	25.0 ± 1.0	45.0 ± 12.0	37.3 ± 0.9	66.1 ± 21.2	1.21 ± 0.07	0.070 ± 0.02
Volume increase	0.96	0.98	29.3 ± 0.4	31.9 ± 0.6	39.2 ± 0.9	39.9 ± 1.3	4.0 ± 0.2	0.094 ± 0.004
Water accumulation	0.74	0.76	28.9 ± 1.4	29.8 ± 1.1	43.7 ± 4.8	38.6 ± 2.1	3.5 ± 0.3	0.14 ± 0.02
Endosperm cell proliferation	0.56	0.85	27.8 ± 1.5	29.9 ± 0.9	38.2 ± 2.3	39.4 ± 2.0	9936 ± 1360	0.16 ± 0.01

1
2

Figure legends

Fig. 1. Effects of high post-anthesis temperature on final grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D). Each bar represents the mean ± standard deviation of final values (n=24 to 27 for grain volume, 10 to 17 for grain dry biomass, water mass and endosperm cell number); measurements were taken at grain maturity, except for endosperm cell number. For this trait, final values were obtained during the filling phase when cell number is set (between 300 and 400 °Cd after anthesis). For each trait, the different letters above vertical bars indicate significant differences between temperature treatments at a 5% level (SNK test).

Fig. 2. Impact of temperature on the normalized grain dry biomass (A), volume (B), water mass (C) and endosperm cell number (D) during grain development. Each point corresponds to the mean of measurements on 12 grains (2 grains and 6 spikes) for grain dry biomass and endosperm cell number, or on 24 grains (4 grains and 6 spikes) for grain water mass and volume. All control treatments (19°C) were averaged together. Within each experiment, data were normalized by the maximum mean value of the control (19°C) of the respective experiments.

Fig. 3. Growth functions fitted to observed values of grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D) over time after anthesis. Growth functions were selected on the base of growth curves obtained at 19°C (control temperature), and applied to other temperatures (Table 2) in a non-linear mixed model fitted on the whole dataset (i.e. all treatments at once).

Fig. 4. Temperature responses of growth rates, durations and maximal values for grain dry biomass (A to C), volume (D to F), water mass (G to I) and endosperm cell division (J to L). The values of these traits were estimated based parameters from the growth curves (Table 2) fitted with a non-linear mixed model. Lines in A, D, G and J represent the fit of the response function [Eq.1]. Error bars correspond to two times the standard deviation, on either side of the mean.

Fig. 5. Temperature response of growth rates and reciprocals of the duration for the grain dry biomass (A,B), cell volume (C,D), water mass (E,F), and endosperm cell number (G,H). Solid lines represent the fit of the response function [Eq.1]. Dashed lines (B, D, F, H) represent the linear regression fitted between 15°C to 32°C, for which the equation is given. Error bars correspond to two times the standard deviation, on either side of mean.

Fig. 6. Normalized temperature responses of growth rates (A) and reciprocal of growth durations (B) according to the response function [Eq.1] for the grain dry biomass accumulation, increase in volume, water accumulation and endosperm cell proliferation. Responses were normalized at 19°C. Solid lines indicate the range of temperatures for which observations were collected.

Fig. 7. Normalized temperature response of the reciprocal of duration of grain growth processes according to the response function [Eq.1]. Data concerning grain dry biomass accumulation, grain volume, water mass and endosperm cell numbers are those obtained from the experiments from this study. The post-anthesis development phase from Wang *et al.* (2017) corresponds to the duration between anthesis and when grains reach their final dry biomass. Response were normalized at 19°C. Solid lines indicate the range of temperatures for which observations were collected.

Fig. 8. Comparison between the normalized rate of grain dry-biomass accumulation estimated based on direct observations ([Eq.1]) or predicted from observed grain volumes and published temperature response for the relative activity of Starch Soluble Synthase enzyme ([Eq.2]). The comparison is presented in terms of (A) normalized temperature responses and (B) predicted values against experimental fitted values. Data concerning grain dry biomass accumulation used in [Eq.1] are those obtained from the experiments from this study. Predicted values from [Eq.2] derived from (i) data concerning grain volume increase obtained from the experiments of this study and (ii) the relative activity of the Starch Soluble Synthase enzyme, extracted

from Figure 1 (120 min temperature treatment) of Keeling *et al.* (1993) and normalized at 19°C.

Figures

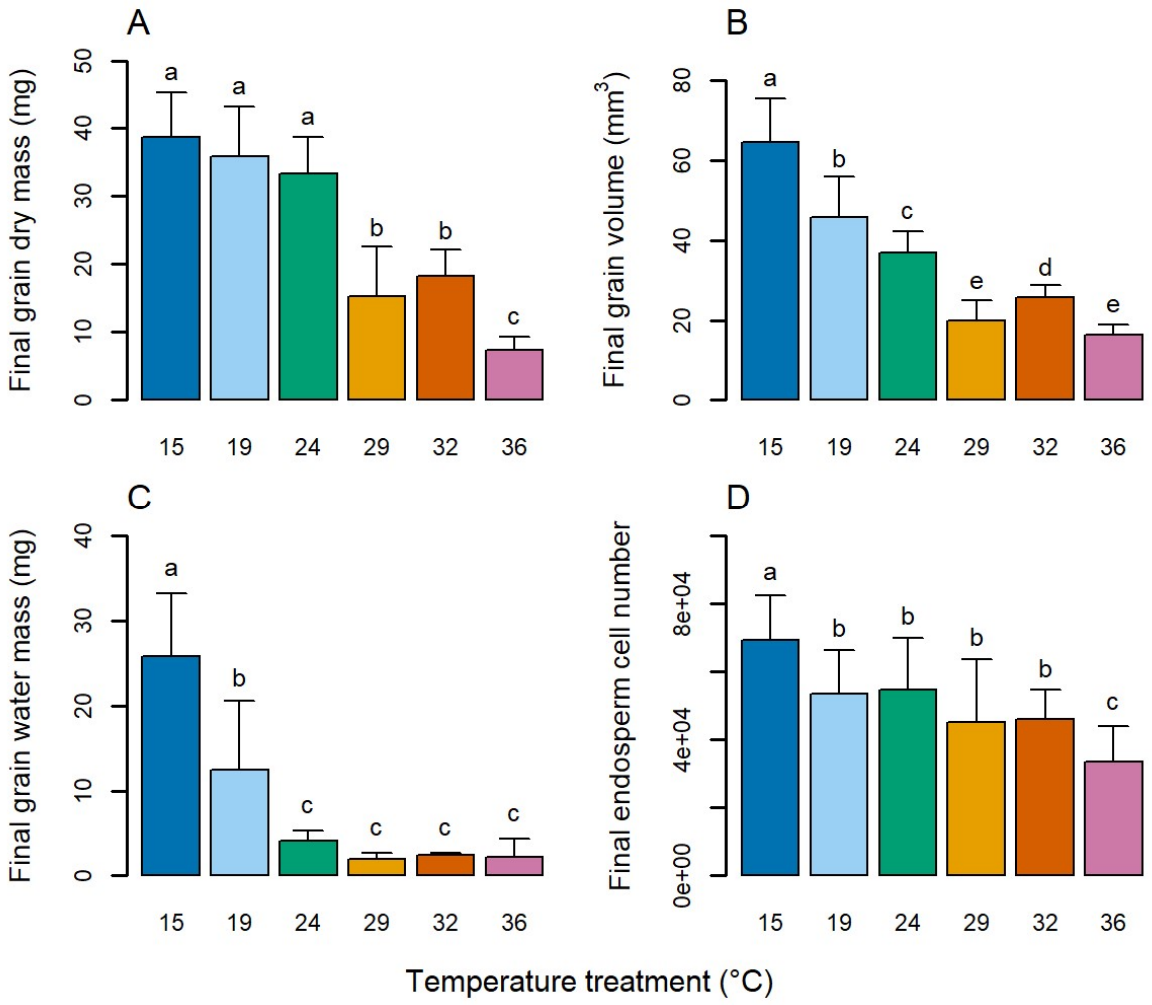


Fig. 1. Effects of high post-anthesis temperature on final grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D). Each bar represents the mean ± standard deviation of final values (n=24 to 27 for grain volume, 10 to 17 for grain dry biomass, water mass and endosperm cell number); measurements were taken at grain maturity, except for endosperm cell number. For this trait, final values were obtained during the filling phase when cell number is set (between 300 and 400 °Cd after anthesis). For each trait, the different letters above vertical bars indicate significant differences between temperature treatments at a 5% level (SNK test).

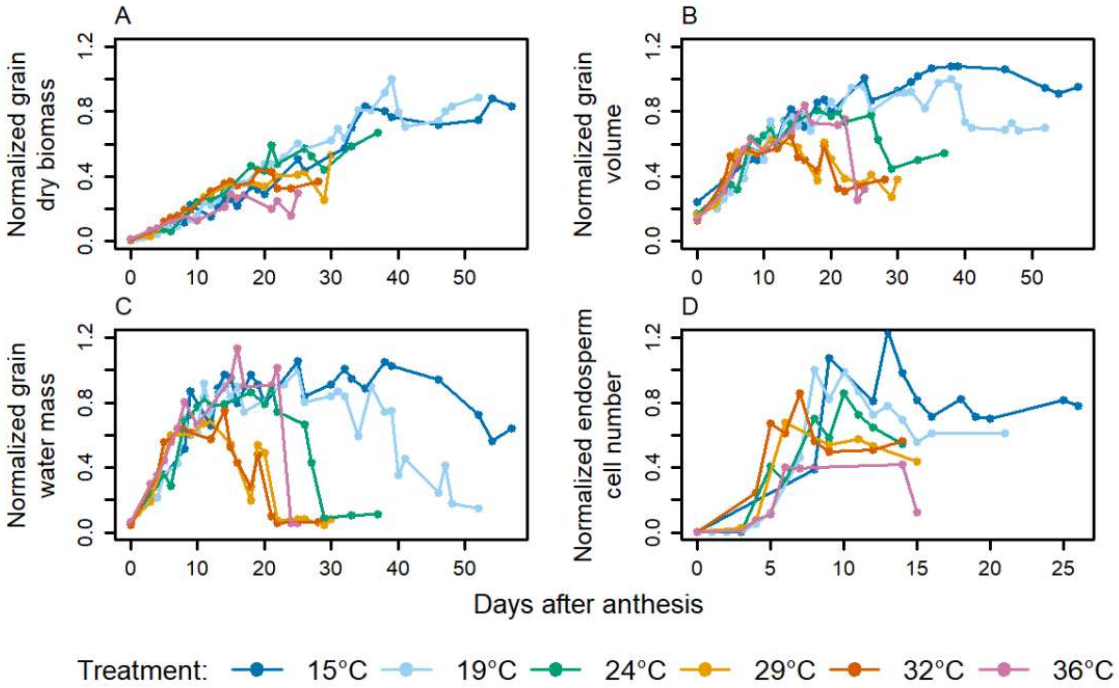


Fig. 2. Impact of temperature on the normalized grain dry biomass (A), volume (B), water mass (C) and endosperm cell number (D) during grain development. Each point corresponds to the mean of measurements on 12 grains (2 grains and 6 spikes) for grain dry biomass and endosperm cell number, or on 24 grains (4 grains and 6 spikes) for grain water mass and volume. All control treatments (19ºC) were averaged together. Within each experiment, data were normalized by the maximum mean value of the control (19°C) of the respective experiments.

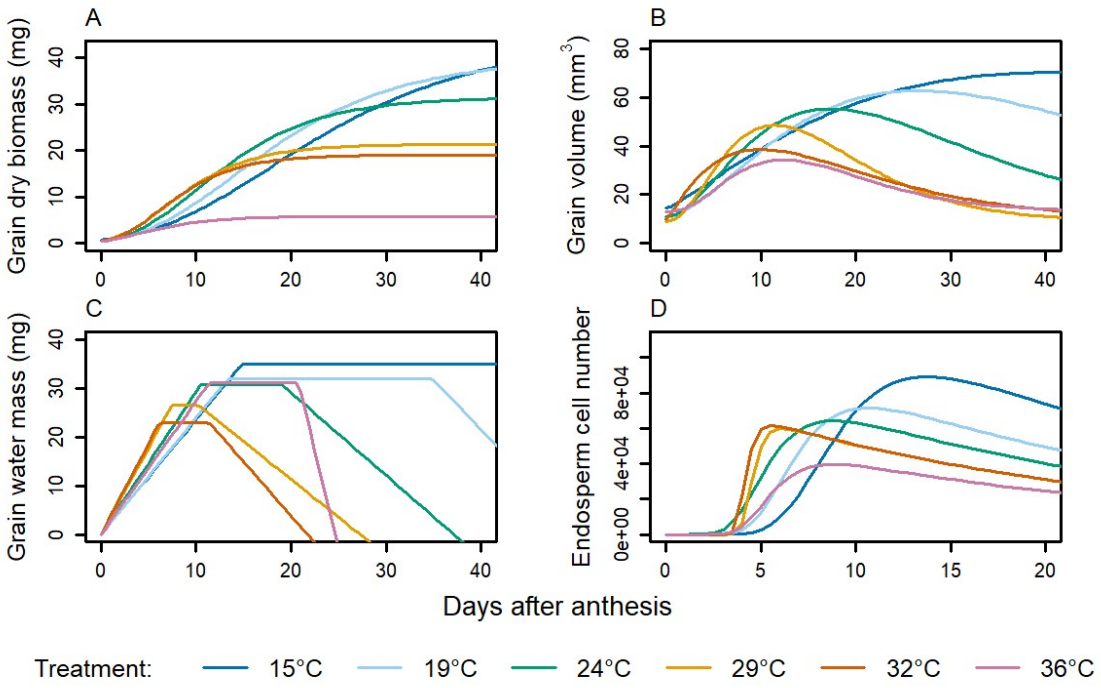
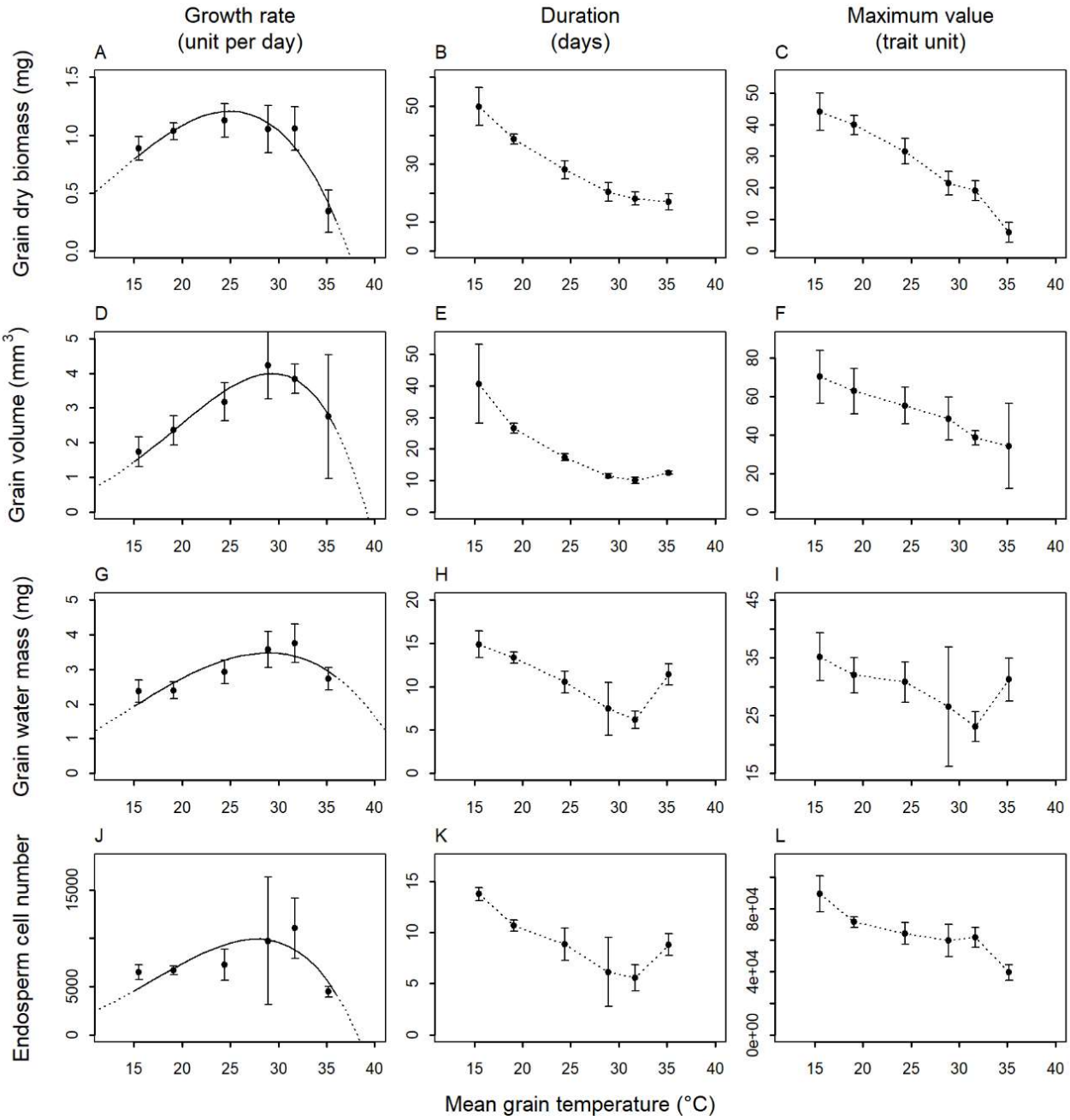


Fig. 3. Growth functions fitted to observed values of grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D) over time after anthesis. Growth functions were selected on the base of growth curves obtained at 19°C (control temperature), and applied to other temperatures (Table 2) in a non-linear mixed model fitted on the whole dataset (i.e. all treatments at once).



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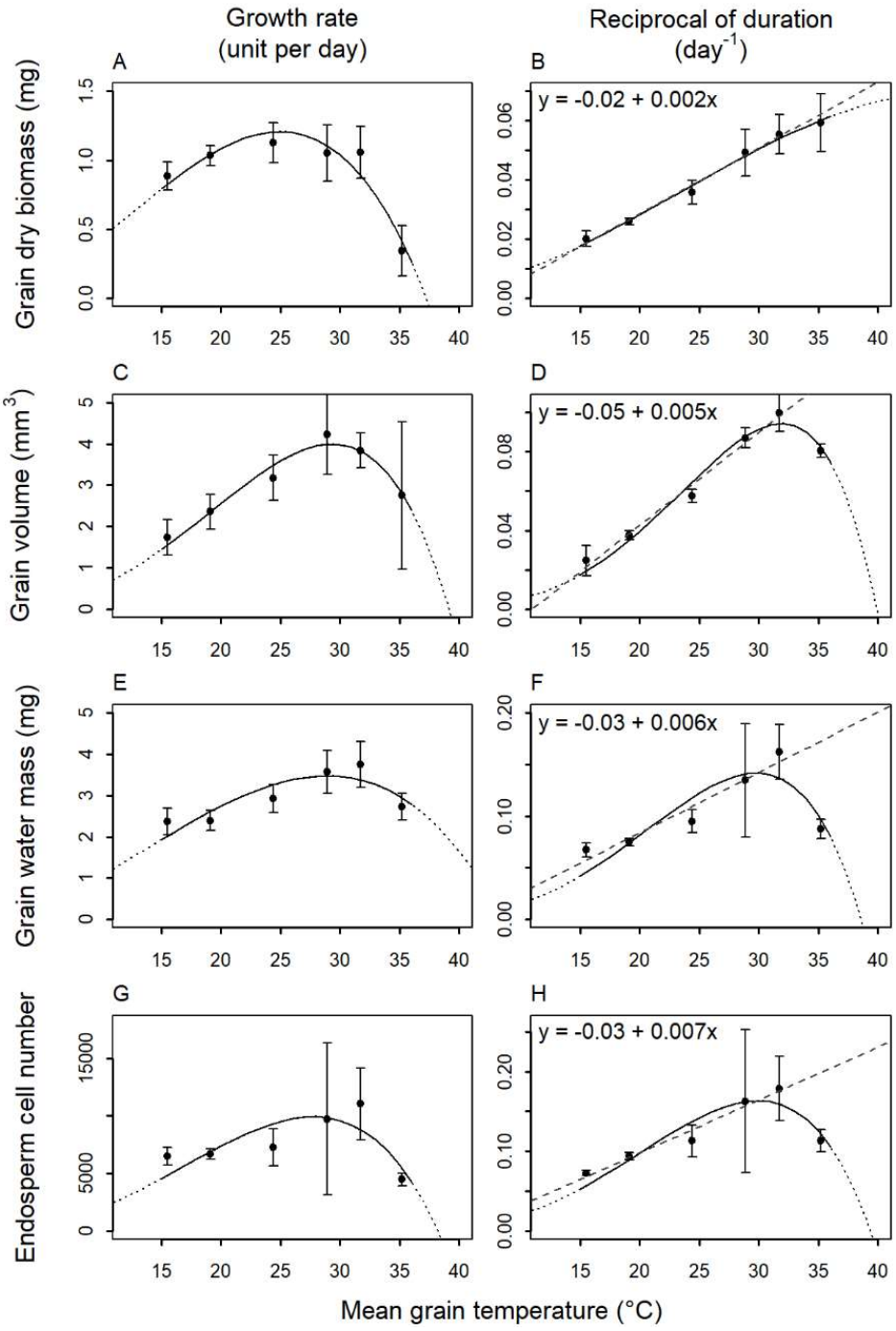

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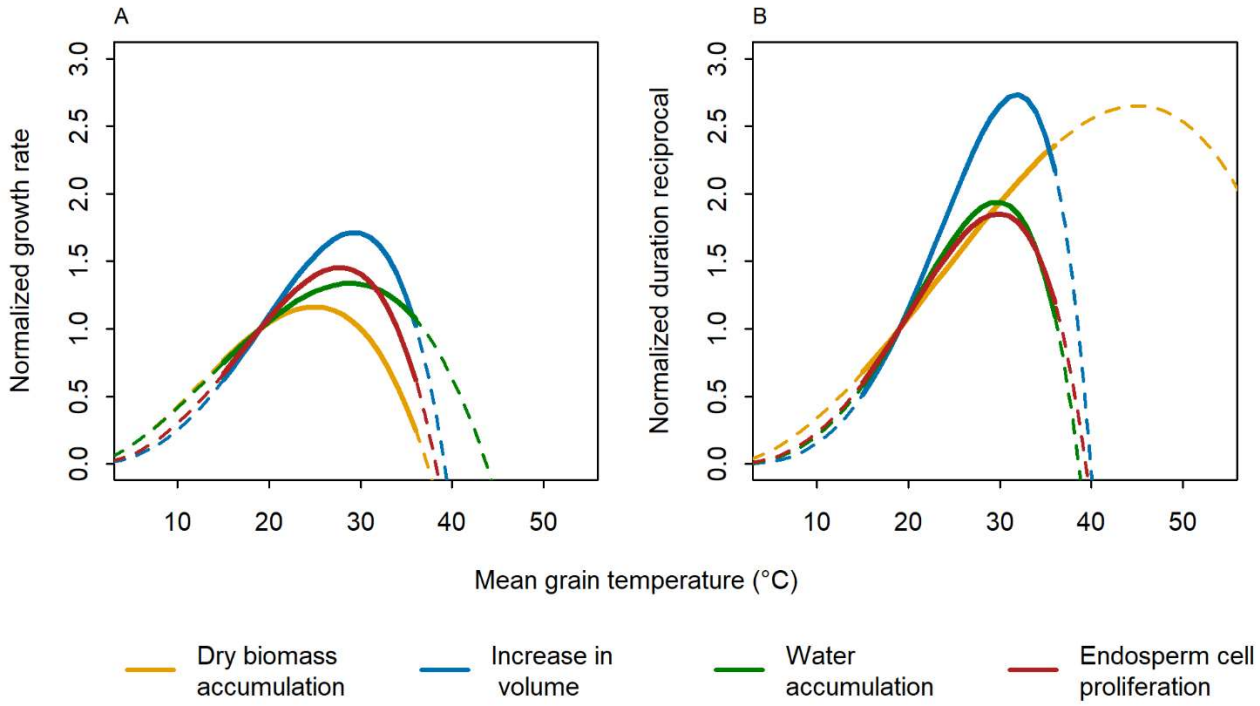

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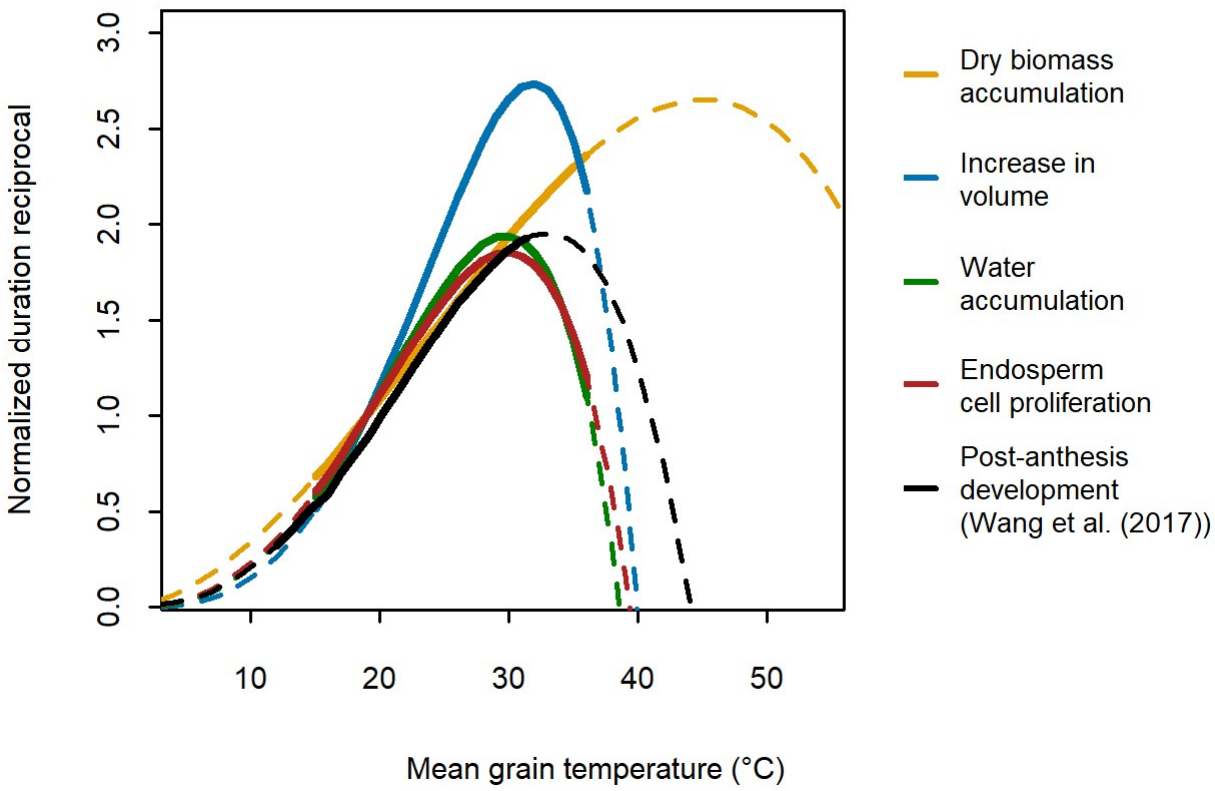

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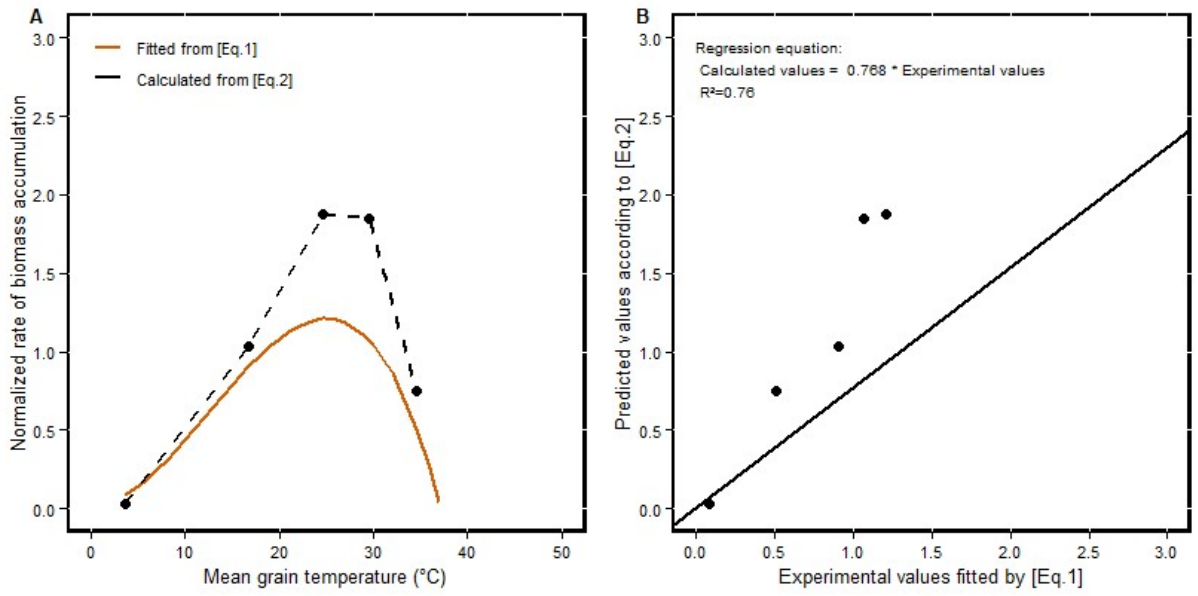

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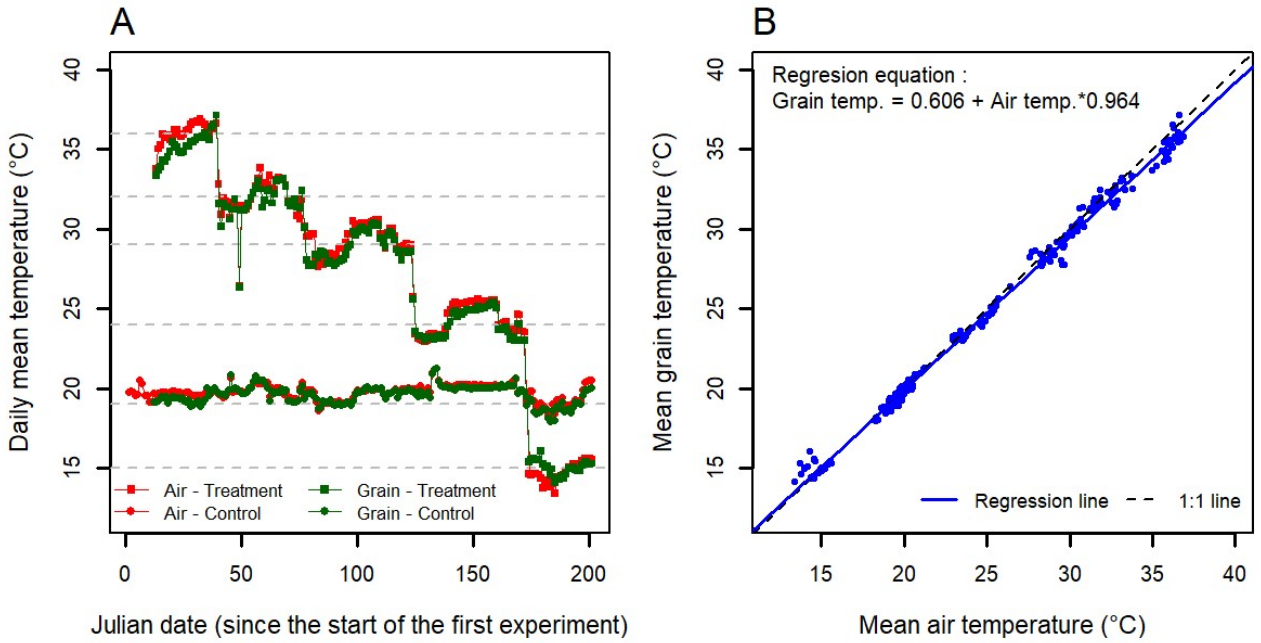

Fig. S1. Air and grain temperatures in the two growth chambers for the five successive experiments presented over time (A) and for grain temperature against air temperature (B). In (A), square symbol, control temperature; circle, temperature treatment; red, air temperature; green, grain temperature; the horizontal grey lines indicate the set temperatures for each experiment, i.e. 19°C for the control chamber, and alternatively 15, 24, 29, 32, and 36°C in the other chamber. In (B) daily mean grain temperature and air temperatures were considered for all the experimental data pooled together (B). Temperature was set to be constant over the whole days.

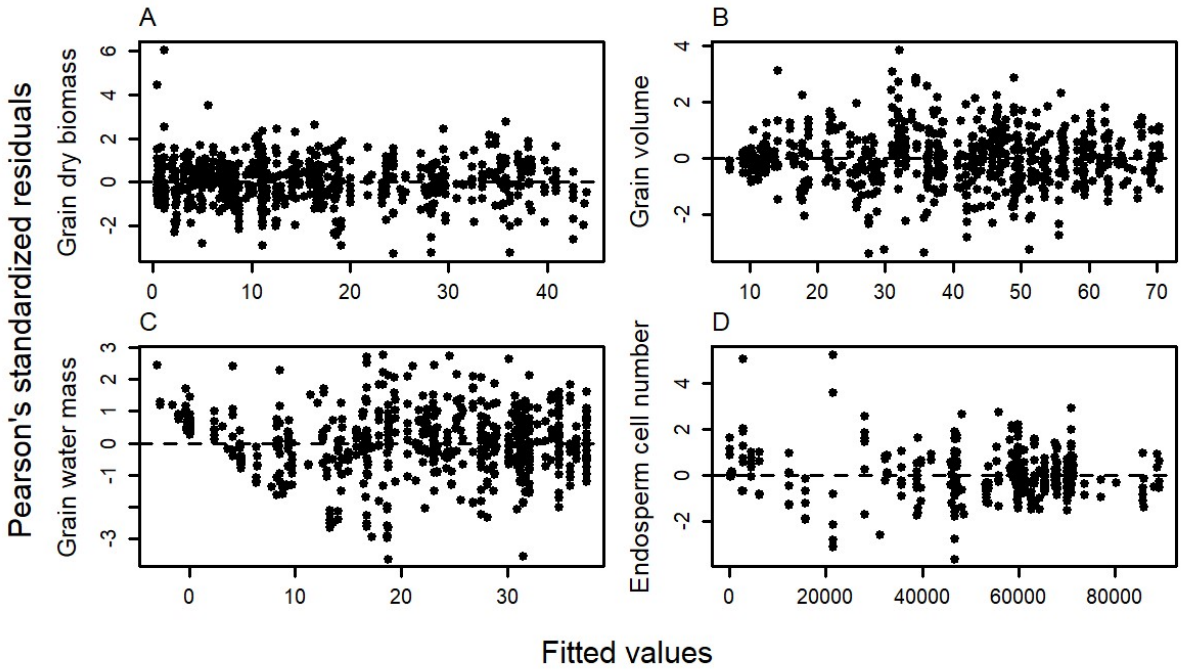

Fig. S2. Standardized residuals against fitted values of the adjusted growth functions describing the change over time in grain dry biomass (A), volume (B), water mass (C) and cell number (D). All temperature treatments were pooled. The data are presented in Figure 2. The growth functions are described in Table 2 and were fitted on the whole dataset using a non-linear mixed model, as described in the Material and Methods.