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Pre- and post-flowering impacts of natural heatwaves on yield components in wheat

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ABSTRACT

Context: Wheat crops are highly sensitive to elevated temperatures and experience significant yield losses when short periods of heat occur at sensitive developmental phases.
Objective: This research aimed at quantifying wheat responses of grain yield and yield components to heat indicators in fluctuating field conditions.
Methods: The impacts of high temperature on yield and its components were assessed for 20–35 wheat lines in irrigated multi-environment trials over three years. Genotypes were cultivated using a novel photoperiod-extension method (PEM) adjacent to some conventional yield plots with different sowing dates. In the PEM, either single stems or plant quadrates were tagged at specific growth stages and hand-harvested at maturity, while conventional plots were mechanically harvested at maturity. The impact of heatwaves was estimated for events occurring at different developmental stages and for different temperature thresholds (26–35°C).
Results: The strongest correlation between heat and grain number was observed between 300 and 200°Cd before flowering for a threshold temperature of 28°C. For each hot hour (T > 28°C) during this period, wheat genotypes lost on average an extra 0.25 grain at the spike level, and 281 grains m^{-2} at the canopy level in conventional plots. For individual grain weight, correlations were statistically the closest for threshold temperatures above 32°C post-flowering. In the tested environments, grain number was most sensitive to heat between 300 and 200°Cd before flowering. Each post-flowering hour with T>32°C (between 0 and 500°Cd after flowering) reduced individual grain weight by an average of 0.26 mg at the spike level (PEM spike harvest) and grain yield by 2.44 g m^{-2} at the canopy level (conventional plot harvest). Impacts of heatwaves were clearest when measured at the organ level (i.e. spikes) and for material with synchronised phenology. In addition, results suggest that heat impacts can also be quantified more reliably using finer time units (i.e. hot hours rather than days).
Conclusions: In the studied well-watered conditions, natural heatwaves strongly impacted grain number for temperatures above 28°C and individual grain weight for temperatures above 32 °C. Reductions in grain number and individual grain weight were strongly associated with accumulated hot hours that occurred during 200–300°Cd before and 0–500°Cd after flowering, respectively.
Implications: The findings from this study will assist improvement for crop modelling in response to heatwaves, development of relevant phenotyping methods and selection of cultivars with better adaptation to warmer environments.

Abbreviations: T_{max}, average daily maximum temperature; T_{mean}, average daily mean temperature; T_{thresh}, threshold temperature; IGW, individual grain weight; PEM, photoperiod extension method; GN, grain number; TotGW, total grain weight; Sow1, time of sowing1; Sow2, time of sowing 2; GAT, Gatton; TOS, Tosari; WAR, Warwick; $Tair_h$,, hourly air temperature; VPD, vapour pressure deficit.

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1. Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop, contributing 20 % of the daily calorific and protein needs to the global food supply (Shiferaw et al., 2013). The establishment of wheat yield and yield components (grain number and individual grain weight) results from intricate and complex interactions between the crops and their environment (e.g. Slafer et al., 2023; Borrell et al., 2023; Vadez et al., 2024). Even in the absence of abiotic stress, grain number is highly dependent on radiation and temperature. In addition, individual grain weight depends on the number of grains set by the crop and other factors primarily determined pre flowering, such as stem water soluble carbohydrates (Ehdaie et al., 2006; Dreccer et al., 2014). Wheat is also highly sensitive to abiotic stresses, including heatwaves. For instance, a 5.6 % grain yield reduction has been estimated for each 1°C increase in the atmospheric mean temperatures (Lobell and Field, 2007). Similarly, controlled environment studies show a 3–5 % reduction in wheat grain yields for each 1°C rise in mean temperature > 15°C (Gibson and Paulsen, 1999). Wheat crops across many parts of the world already experience frequent high temperatures (e.g. > 34°C) during grain development (Asseng et al., 2011; Talukder et al., 2014) with significant grain yield losses (Hatfield et al., 2011). Modelling studies have reported a significant increase in the frequency of heatwaves over recent decades, particularly during the grain-filling period (Ababaei and Chenu, 2020), with further increases projected (Collins and Chenu, 2021; Field et al., 2014; Lobell et al., 2015). Therefore, quantifying the impact of natural heatwaves on crop yield is critical for developing management practices to sustain food production under changing climates (Collins and Chenu, 2021; Flohr et al., 2018; Zheng et al., 2012).

Grain yield losses in wheat are strongly influenced by the duration, intensity, and timing of the heatwaves (e.g. Djanaguiraman et al., 2014; Chenu and Oudin, 2019a). The reproductive and grain-filling phases of wheat are highly sensitive to heat stress (e.g. Girousse et al., 2018 and 2021). For example, a single hot day (30/20°C day/night temperature) during the pollen tetrad or meiosis stage significantly inhibits pollen viability and translates into reduced grain number (Saini and Aspinall, 1982). Hence, late-flowering tillers (secondary tillers) that experience heat during gametogenesis produce significantly fewer grains per spike than main tillers that were subjected to heat at flowering (Aiqing et al., 2018).

Individual grain weight (IGW) is vulnerable to both pre- and post-flowering heat events (Calderini et al., 1999; Ugarte et al., 2007). Final grain size in wheat is particularly sensitive to high temperatures during the early phases of grain development (0–10 days post-anthesis in Egli, 1998); 0–15 days after anthesis in Stone and Nicolas, (1995)). Although sustained high temperatures (30–38°C) from flowering to maturity can significantly limit wheat grain yield formation, the magnitude of the effect across reported experiments varied from 20 to 50 % (Wardlaw et al., 1989; Tewolde et al., 2006). In contrast, controlled environment studies indicated that a brief heatwave during the sensitive phase could significantly reduce the IGW of wheat (Talukder et al., 2014). For example, a single hot day (40/21°C day/-night) was found to induce a 14 % reduction in IGW (Stone and Nicolas, 1998), while in another study, 14 hot days (32/22°C) during early grain-filling reduced IGW by 44 % (Djanaguiraman et al., 2020). However, reports of such detailed information are mostly limited to controlled environments in parts due to difficulties in observing the impacts of brief heatwaves on plants grown in the field. In addition, results from controlled environments do not systematically translate in the field (e.g. Rebetzke et al., 2014; Bonada and Sadras, 2015) due for instance to heat effects on pots and roots. Furthermore, pot studies commonly focus on extreme stress, sometimes screening for survival, although the environments targeted by breeders are very different, with stress patterns varying in time and intensity (e.g. Chenu et al., 2013; Collins and Chenu, 2021, Ababaei and Chenu, 2020; Vadez et al., 2024). Heat response, and in particular threshold temperatures, have not been studied in detail, in naturally fluctuating field conditions and during specific developmental stages.

A limitation on screening for heat tolerance in field conditions is that observed heat response can be confounded by genotypes of different maturity types being impacted by heatwaves at different developmental stages. The literature discussed above suggests that the same heatwaves in one trial hence likely result in different impacts on yield and yield components for genotypes of different maturity types. To reduce this potentially confounding effect, a new method has been developed to bring genotypes of different maturity types to a similar developmental stage during the post-flowering period in field heat tolerance trials using photoperiod extension (Ullah et al., 2023).

This study aimed to (i) characterise the most heat-sensitive developmental stage(s) of wheat crops for grain yield components in natural field conditions, (ii) determine threshold temperature (intensity and duration) for these developmental stages and (iii) quantify the impact of heatwaves on grain yield components. Fully irrigated field experiments were conducted at three locations over three consecutive years, each with two sowing times. The impact of high temperature on grain yield components of wheat was quantified at spike and sub-plot (i.e. quadrats) levels with matched flowering using the photoperiod-extension method (PEM) developed by Ullah et al. (2023). These results were compared to those obtained from adjacent conventional yield plots with natural flowering, as has been more commonly used in previous studies (e.g. Thistlethwaite et al., 2020). The finding could assist in (i) improving modelling capability to simulate the performance of genotypes in a wide range of environments to better predict impact of heat stress in different scenarios, and (ii) developing a phenotyping method for relevant high-throughput screening of heat tolerance. Overall, we anticipate that results will assist in evaluating genotypes for improved adaptation to current and projected future environments.

2. Materials and methods

2.1. Planting materials

Wheat (*Triticum aestivum* L.) genotypes used in the study (Table S1, Supplementary) include commercially Australian cultivated cultivars such as Suntop, Spitfire, Gregory, Janz, Hartog, EGA Wylie, Corack, Yitpi, Mace and Scout. A set of CIMMYT genotypes described as heat tolerant under Australian environments was obtained from the University of Sydney (Thistlethwaite et al., 2020). The remaining wheat genotypes were selected from a multi-reference parent nested association mapping (MR-NAM) population developed for screening for heat and drought tolerance in wheat (Christopher et al., 2015, 2021; Richard, 2017; Fletcher, 2020). A total of 35 wheat genotypes were tested across trials. Out of these, 32 genotypes were gown at each trial, with the same genotypes planted in both the PEM and conventional plots at any given year, except for the PEM trial with quadrate harvest in 2020, when only 20 selected genotypes were used (Table S1, Supplementary). In conventional plot trials, 32 genotypes were used in each trial.

2.2. Field trials

Field trials were conducted over three consecutive years, from 2018 to 2020, at three locations across south-eastern Queensland, Australia, at The University of Queensland Research Farm, Gatton (27°34′50″S, 152°19′28″E), Queensland Department of Agriculture and Fisheries Hermitage Research Station, Warwick (28°12’40’’S, 152°06’06’’E) and at the Tosari Crop Research Farm, Tummaville (27°49’09"S 151°26’15"E). Between 20 and 32 wheat genotypes (from a total of 35 different genotypes) were tested under different growing environments (Table S1) using conventional plots and the newly developed PEM (Ullah et al., 2021, 2023). Each year, the trials were established in a randomised complete block design with two times of sowing and four replicates per genotype. To ensure a range of growing temperatures

across developmental stages, trials were sown at dates ranging from late May to early September (Table 1). Standard crop management practices were adopted for all trials, including weed, disease and pest control.

With the PEM, the genotypes were planted either in (i) single rows in 2018 and 2019 or (ii) small plots (1×5 m) in 2020 (Table 1). At one end of each row or plot, artificial supplemented lights were set up to extend the photoperiod to 20 h. LED lamps (CLA LT401, 9 W T40 LED LAMP, 3000 K 760LM) with a lumen efficiency ≥ 80 and a spacing of 0.8 m were installed above the seedlings at ~ 1 m at the ground level. As the light intensity diminished with the distance from the light source, plants closer to the light received more supplemental light than the plants further away. The light-intensity gradient induced a gradient in the flowering date along the test rows such that the photoperiod-extension effect was greatest and the flowering date earliest, nearer to the lights (Ullah et al., 2023). For all genotypes, individual spikes and/or quadrats (0.5 linear meter) of each row or plot were tagged at anthesis (Zadoks growth stage 65; Zadoks et al., 1974) on a single day by selecting spikes or sections of the row at different distances from the lights for genotypes of different maturity types. Spike data were collected from approximately 20 individual spikes of each genotype tagged at anthesis. The plants were later harvested at maturity. Grain samples were manually counted to estimate grain number per unit of area and IGW.

Conventional field plots were established adjacent to the PEM trials under similar management. At each location, these plots were sown on the same day as the PEM trials with two sowing dates and four replication plots per genotype, except in 2018, when only sowing 2 (Sow2) plots were established at Gatton. The plot size was 2×6 m in 2018 and 2020 and 1×6 m in 2019. All conventional plots were planted with a 25 cm row spacing and a population density of 130 plants m^{-2}. Conventional plots were harvested using a small plot machine harvester at maturity when grain moisture was approximately 11 %. Grain samples were manually counted to estimate grain number and IGW.

The combinations of sites, years, flowering dates and tagging events correspond to 20 different "environments", each providing a unique set of temperatures and timing of heatwaves with respect to the developmental stages of each genotype. These environments were defined using an identifier of the site (Gatton, GAT; Tosari, TOS; and Warwick, WAR); the year of the trial, the time of sowing (Sow1 or Sow2) and when applicable, the tagging event (T1, T2, T3; Table 1). All trials were grown under non-limiting fertiliser conditions. Irrigation was applied weekly or prior to any potential water stress, except at Tosari in 2019 (TOS19) where crops had only pre-flowering supplementary irrigation and experienced mild post-flowering water stress. Trials were irrigated with a boom irrigator (conventional plots) and wobbler sprinklers (PEM plots). Standard crop management practices, including weed, disease and pest control were adopted during the season across all the trials.

2.3. Weather data

Wheater data were collected from the site weather stations (Campbell Scientific). Light sensors (Apogee SP-110 pyranometers) were installed at 1.5 m height to measure light interception. HMP60 (Vaisala INTERCAP®) probes were used to measure the air temperature (Tair) and relative humidity (RH) at 1.5 m above the ground.

Thermal time was calculated in degree days (°Cd) using the following equation (Jamieson et al., 1995):

$$Thermal\ time = \sum_{h=1}^{24}(-0.0032 * Tair_h^3 + 0.1369 * Tair_h^2 + 0.3968 * Tair_h + 0.993) \quad (1)$$

where $Tair_h$ is the hourly air temperature.

Vapour pressure deficit (*VPD*) was calculated hourly during the daytime as in Alduchov and Eskridge (1996):

$$VPD = 0.61094\left(\frac{1-RH}{100}\right)^{17.625*Tair_h/(Tair_h+243.04)} \quad (2)$$

where $Tair_h$ is the hourly air temperature, and *RH* is the hourly air relative humidity.

2.4. Plant measurements

For each PEM trial and sowing date in 2018 and 2019, approximately 20 stems of each genotype were tagged at flowering (Zadoks decimal growth stage 65; Zadoks et al., 1974). The induced gradient in phenology along the rows allowed tagging of genotypes multiple times for plants in rows or plots from each sowing time. One-to-three cohorts of stems/plants were tagged at precisely matched flowering in rows or plots from each sowing time and trial. These sequentially tagged cohorts were named 'tagging 1' (T1), 'tagging 2' (T2) and 'tagging 3' (T3). The spikes from tagged stems were manually harvested at maturity and processed for grain yield components.

In addition, in each test row of all PEM trials in 2018 and Sow1 trials at Tosari in 2019 (TOS19-s1), a quadrate consisting of a 0.5 m section of the row that originally contained heads from tagging 1 was manually harvested to estimate yield and its components. In 2020, a ~ 0.5 m section of each plot (four rows) was tagged at flowering (Zadoks 65), and a quadrate of two central rows (0.5 m each) within the tagged region was manually harvested at crop maturity.

In all plots from the conventional method, phenology was measured with Zadoks scores (Table S2). Conventional plots of all trials were harvested using a small plot machine harvester at maturity when grain moisture was approximately 11 %. Grain samples were manually counted to calculate individual grain weight (IGW).

2.5. Statistical analysis

Data were analysed using the R programming language (R Core Team, 2018). Data were presented as the mean (all tested genotypes) of the replicated data for each genotype. Data were analysed separately for spikes tagged individually (at synchronised phenology for all genotypes using the PEM), quadrate harvests (sections of each row or plot of the PEM) and conventional plots (unsynchronised phenology of genotypes).

Heat indicators were defined as maximum daily temperature (T_{max}), mean temperature (T_{mean}) or number of days or hours above specific threshold temperatures (T_{thresh}). Threshold temperatures of 26°C, 28°C, 30°C, 32°C and 35°C were tested. Linear correlations were examined between heat indicators and grain yield components for different crop developmental periods (from 600°Cd before flowering to 500°Cd after flowering) for each 100°Cd. Heat-sensitive phases were identified, where grain yield components most strongly responded to the temperature thresholds.

3. Results

3.1. A wide range of heat environments were tested

In total, 20 environments were studied, with plants harvested in different years, locations, from different sowing dates and tagging dates each experiencing different heat patterns during development (Table 1; Fig. 1). Overall, a wide range of environments were tested resulting in grain yield varying from 208 to 513 g m^{-2} in the PEM quadrates with matched phenology, and from 61 to 478 g m^{-2} in conventional plots (Table 1).

On average, crops from the later sowing (Sow2) experienced more heat than from the first sowing (Sow1), both in terms of average daily maximum (T_{max}) and mean (T_{mean}) temperatures (Fig. S1, Table 1). The warmer conditions experienced by Sow2 (s2) crops compared with their respective Sow1 (s1) crops accelerated the crop phenology, shortening

Table 1
Trial characteristics, including the environment identifier*, the type of the trial (i.e. photoperiod-extension method (PEM) with tagging and harvesting of either single spikes or quadrates; and conventional plots), the sowing date, environmental factors, and crop characteristics. Environmental factors presented are mean (T_{mean}) and max (T_{max}) daily temperature, day-time vapour pressure deficit (VPD) well as the mean duration of the pre- and post-flowering periods ("Days to flow." and "Post flow. duration", respectively). The duration of days to flowering and post-flowering were calculated from sowing to flowering and flowering to maturity, respectively for each trial (average across all genotypes) for each harvest type. Also presented are the cumulative heat (days/hour) above 30°C during 0–500°Cd after flowering, and the heat environment type (HET) for the first tagging of trials with the PEM. Average grain yield is reported for the PEM quadrate harvest and the conventional plots (average across all genotypes). Other phenological stages in the conventional plot trials are presented in Table S2.

Environment*	Trial type	Sowing date	T_{mean} (°C)	T_{max} (°C)	Mean VPD (kPa)	Days to flow. (days)			Post-flow. duration (days)	Cum. hours > 30°C (0–500°Cd after flow.)	Cum. days > 30°C (0–500°Cd after flow.)	Yield (g m^{-2})		HET***
						Spike harvest	Quadrate harvest	Plot harvest				Quadrate harvest	Plot harvest	
GAT18-s1-T1	Spike & quadrate PEM	03/07/2018	17.2	25.3	0.8	73	73		42	3	2	513±45		HET1
GAT18-s1-T2	Spike PEM	03/07/2018	17.7	25.5	0.9	81			38	3	2			HET1
GAT18-s1-T3	Spike PEM	03/07/2018	18.0	25.6	0.9	86			37	3	2			HET1
GAT18-s2-T1	Spike & quadrate PEM, & plots	31/08/2018	21.0	28.5	1.1	53	55	60±7	39	65	12	398±38	219±42	HET2
GAT18-s2-T2	Spike PEM	31/08/2018	21.9	29.0	1.1	60			35	67	13			HET2
GAT19-s1-T1	Spike PEM & plots	09/07/2019	17.0	25.5	1.2	71		68±8	39	31	5		193±39	HET2
GAT19-s1-T2	Spike PEM	09/07/2019	17.3	26.0	1.2	78			36	46	9			HET2
GAT19-s2-T1	Spike PEM & plots	03/09/2019	20.3	31.0	1.8	59		63±6	35	129	20		99±28	HET3
GAT20-s1-T1	Quadrate PEM & plots	26/05/2020	16.5	24.4	0.9	79	77	77±8	47	0	0	317±34	251±32	HET1
GAT20-s2-T1	Quadrate PEM & plots	04/08/2020	20.0	29.0	1.2	65	62	69±8	37	45	14	241±28	161±28	HET2
TOS19-s1-T1 **	Spike & quadrate PEM, & plots	16/07/2019	18.1	27.2	1.1	79	76	82±8	36	45	12	262±35	272±32	HET2
TOS19-s1-T2 **	Spike PEM	16/07/2019	18.4	27.7	1.1	84			35	35	11			HET2
TOS19-s2-T1 **	Spike PEM & plots	06/09/2019	23.2	32.7	1.7	62		55±6	34	162	22		61±12	HET3
WAR18-s1-T1	Spike & quadrate PEM	16/07/2018	16.0	24.0	0.9	73	70		39	1	1	386±37		HET1
WAR18-s1-T2	Spike PEM	16/07/2018	16.7	24.5	0.9	79			37	7	2			HET1
WAR18-s2-T1	Spike & quadrate PEM	12/09/2018	20.3	27.8	1.2	52	49		37	47	10	208±22		HET2
WAR18-s2-T2	Spike PEM	12/09/2018	20.4	28.2	1.2	56			36	41	11			HET2
WAR20-s1-T1	Quadrate PEM & plots	08/06/2020	14.8	23.3	0.7	101	95	105±8	49	0	4	437±40	478±45	HET1
WAR20-s2-T1	Quadrate PEM & plots	12/08/2020	18.6	26.6	1.1	66	62	71±6	46	10	4	345±38	369±38	HET1
WAR20-s2-T2	Quadrate PEM	08/06/2020	18.8	27.0	1.1	72	71		42	9	3	350±33		HET1

* An identifier of the site denominates environments as Gatton (GAT), Tosari (TOS) or Warwick (WAR); the year of the trial, the time of sowing (s1, or s2,), and the tagging event (T1, T2, T3) when applicable.
**While all other trials were fully irrigated, TOS19-s1 and TOS19-s2 only had supplementary pre-flowering irrigation and experienced mild post-flowering water stress.
*** Heat environment type 1 (HET1) corresponds to environments with 0 days or hours of temperature above 32°C between 0 and 500°Cd after flowering, while heat environment type 2 (HET2) corresponds to 1–9 days or 1–49 hours of temperature > 32°C during the same period (Fig. S3). Heat environment type 3 (HET3) had more than 10 days or 50 hours of temperature > 32°C between 0 and 500°Cd after flowering (Fig. S3).
Additional abbreviations: T_{max}, average maximum daily temperature; T_{mean}, daily mean temperature

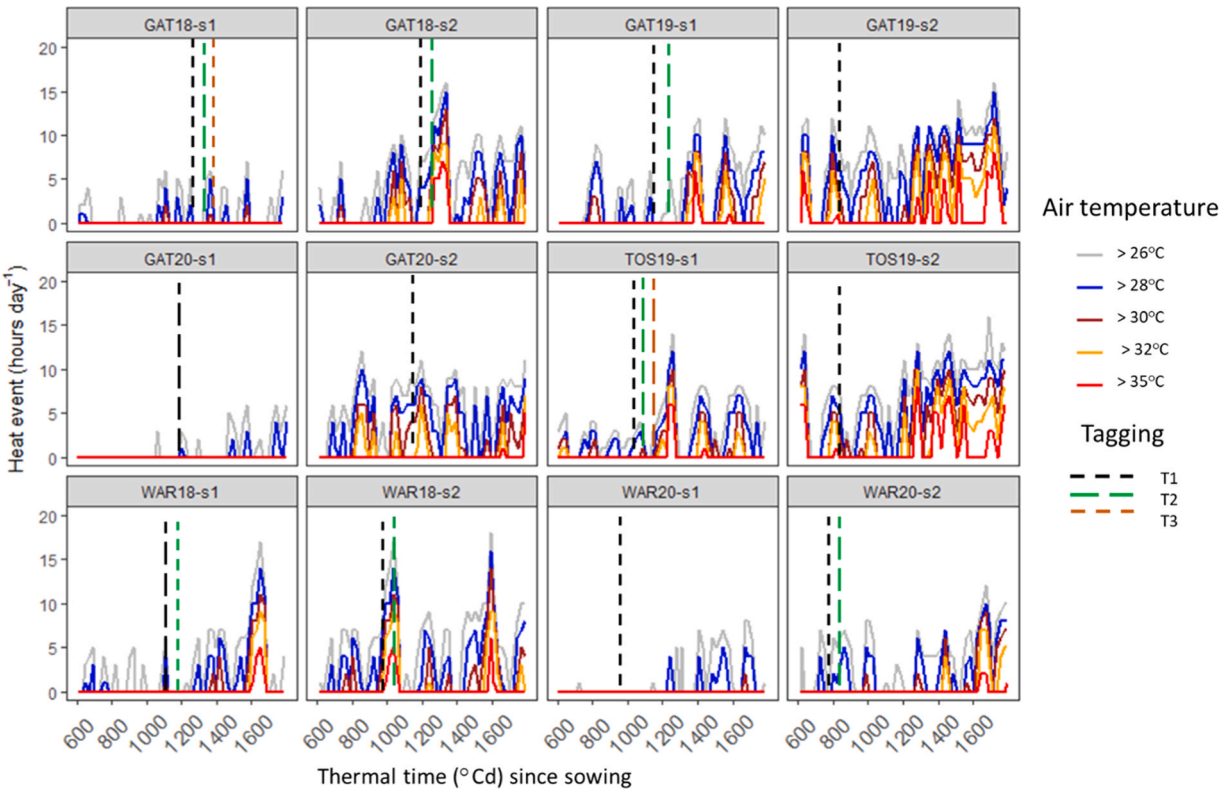

Fig. 1. Number of hours each day when air temperatures exceeded specific threshold temperatures (26 °C to 35 °C) is plotted against thermal time (°Cd) since sowing in each trial.

both the vegetative and grain-filling periods (Table 1 and S2). On average, late-sown crops reached tillering, stem elongation, jointing, booting, heading, anthesis and maturity 5.3, 6.8, 3.7, 2.7, 0.7 and 5.5 days, respectively, before the early-sown crops. However, phenological differences were greater when comparing different years and locations, e.g. days from sowing to anthesis ranged from 52 days for WAR18-s2-T1 to 101 days for WAR20-s1-T1.

The number of days or hours with temperature above threshold levels varied across environments, particularly with reference to flowering time. Wheat genotypes experienced relatively few pre-flowering heatwaves in the studied trials, with between 2 and 26 days (or 2 and 184 hours) with temperature > 26°C recorded pre-flowering across trials. GAT19-s2 and TOS19 (s1 and s2) and WAR20-s2-T1 were the only environments subjected to days or hours of temperatures > 32°C before flowering (Fig. S2). In contrast, a wide range of post-flowering (0–500°Cd) heat was observed, with 11–23 days or 30–250 hours above temperature above 26 °C, or with 1–21 days or 1–116 hours of temperature > 32°C (Fig. S3, Table 1). For simple

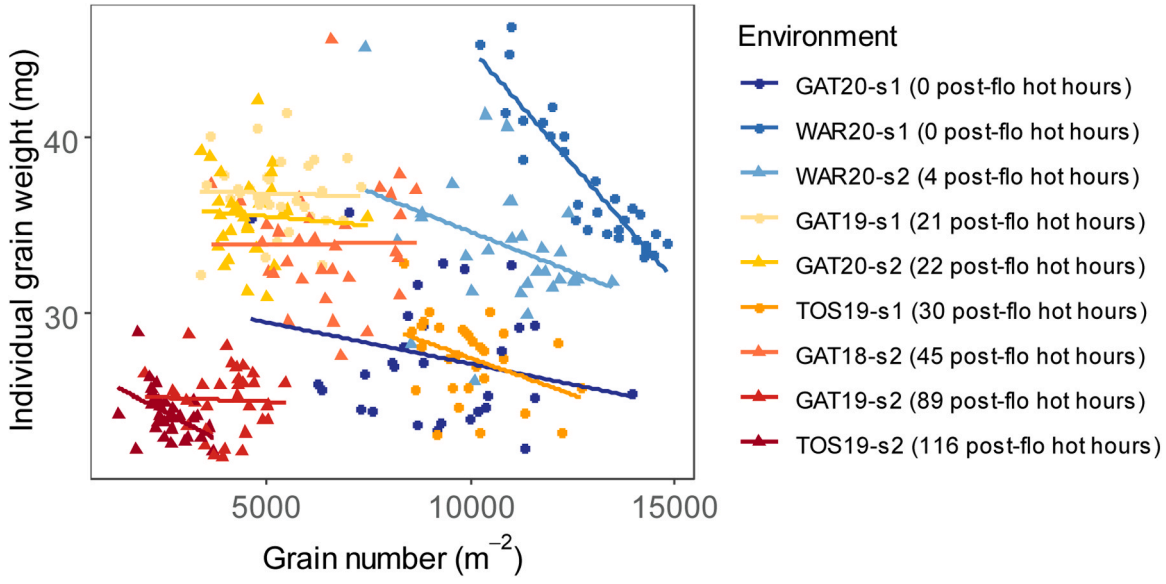

Fig. 2. Relationship between individual grain weight (IGW) and grain number in the studied conventional plot trials. Environments were coloured based on the number of hours with temperature > 32 °C that crops experienced between 0 and 500°Cd after flowering.

comparisons, we classified growing environments based on post-flowering heat into three heat environment types. Heat environment type 1 (HET1) corresponds to environments with 0 days or hours of temperature > 32°C between 0 and 500°Cd after flowering, while heat environment type 2 (HET2) corresponds to 1–9 days or 1–49 hours of temperature > 32°C during the same period (Fig. S3). Heat environment type 3 (HET3) had more than 10 days or 50 hours of temperature > 32°C between 0 and 500°Cd after flowering (Fig. S3).

Across environments, grain yield and components of the studied wheat genotypes responded strongly to heatwaves, with a sensitivity varying across developmental stages. As may be expected, individual grain weight (IGW) was negatively correlated to grain number when no or limited heatwaves occurred during the grain filling period, which was generally not the case when substantial post-flowering heatwaves affected the crops (Fig. 2). Reductions in grain number and IGW were strongly associated with pre-flowering and post-flowering temperatures, respectively (Figs. 3–6).

3.2. The impact of pre-flowering heat on grain number differed with heat environments

Despite fewer pre-flowering hot days or hours in the tested environments (Fig. S2), grain number responded clearly to heat indicators, particularly at the spike level and, to a lesser extent, at the plot level (Figs. 3, 4 and S4). Part of these responses would be due to the impact of increasing temperature on the shortening of the vegetative growth period (Table 1), and thus the reduced duration to accumulate resources (e.g. intercepted radiation) and to produce biomass pre flowering. The number of grains produced by studied genotypes also varied widely across the tested environments ranging from 26 to 36 grains spike^{-1} and from 2,500 to 12,500 grains m^{-2} at the plot level (Fig. 3). In this study, grain number was most closely related (r^2) to heatwaves during 200–300°Cd before flowering (Fig. 4 and S4), compared with any of the other 100°Cd intervals of development tested (i.e. from 400°Cd before flowering to 100°Cd after flowering, which corresponds to between 32 and 18 days before flowering to 8–12 days post-flowering depending on the studied environment; Fig. 4 and S4).

For each 1°C increase in T_{max} between 300 and 200°Cd before flowering, genotypes produced 0.74 fewer grains spike^{-1} (PEM spike harvest, $r^2 = 0.52$, Fig. 3a) and 677 fewer grains m^{-2} at plot level ($r^2 = 0.55$, Fig. 3i). With the PEM, grain number per spike responded relatively more strongly to T_{max}, and the number of hot hours than to T_{mean} (Fig. 3a-d). For each hot day (T_{max} > 28°C), between 300 and 200°Cd before flowering, wheat genotypes produced 4.4 % and 15 % fewer grains at the spike and plot levels, respectively (Fig. 3c, k). For each hot hour (> 28°C), grain number loss was estimated to be 0.75 % and 2.8 % in the PEM spikes and conventional plots, respectively (Fig. 3d, l). At the plot level, grain number response had a similar coefficient of determination for the different heat indicators, ranging between 0.55 and 0.69 (Fig. 3i-l). For the PEM quadrate, the grain number response to heat indicators was relatively weak, possibly due to the absence of extremely hot (HET3) environments (Fig. 3e-h).

3.3. The impact of post-flowering heat on individual grain weight and yield also varied between heat environments

In the tested conditions, wheat plants produced the largest grains when there were no days with temperature > 32°C, i.e. for Sow1 of 2018 and 2020 (HET1, Fig. 5 and S3). Under such relatively low-stress temperatures, IGW of 44.1, 41.2 and 32.9 mg was observed for the PEM spikes, PEM quadrat, and plot harvests, respectively.

IGW of tested genotypes responded strongly to post-flowering heat

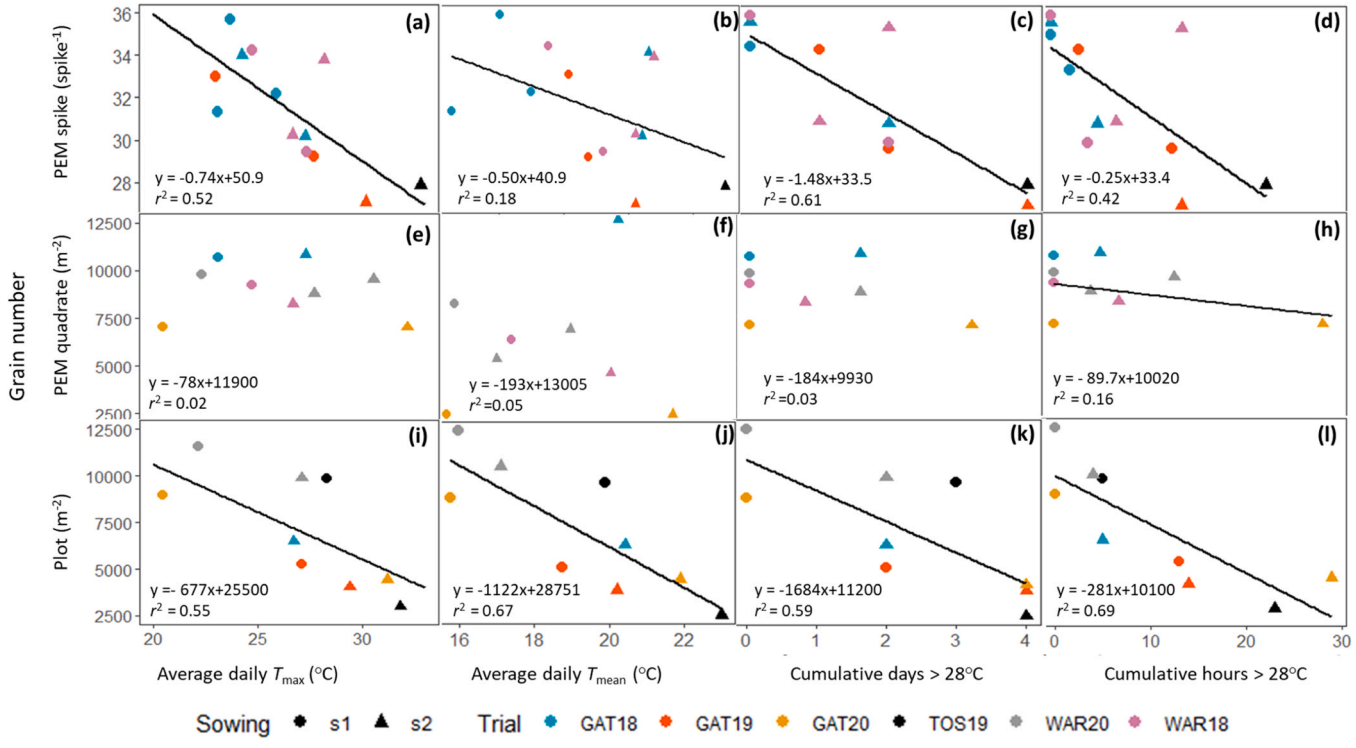

Fig. 3. Changes in grain number in response to pre-flowering (a, e, i) daily maximum temperature (T_{max}), (b, f, j) mean temperature (T_{mean})), cumulative (c, g, k) days and (d, h, l) hours above 28°C; all temperature indices were calculated between 300 and 200°Cd before flowering. Data correspond to the mean of 20 – 32 genotypes with four independent replicates in each environment. Spike data are collected from the individual spikes (~20 for each replicate) exposed to heat at synchronised developmental stages. Quadrate data were collected by harvesting 0.5 m linear meter of plants tagged at synchronised flowering in the PEM. Plot data were collected from the whole conventional plots of naturally flowering genotypes (stage not synchronised during heatwaves). Quadrate and plot data are presented per unit area (m^{-2}). Lines were plotted for regressions between mean grain number and temperature indicators that had a regression slope significantly different from zero ($P < 0.05$).

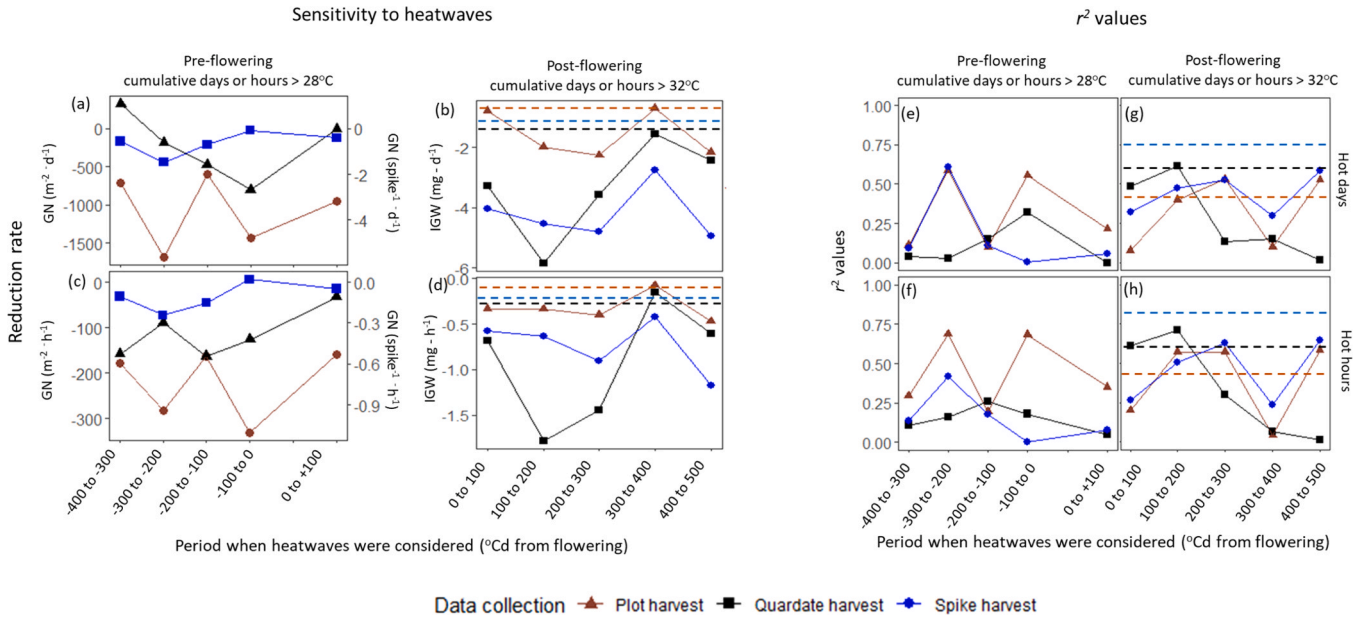

Fig. 4. Heat sensitivity of grain number (GN; a, c) and individual grain weight (IGW; b, d) in response to cumulative days (top graphs) or hours (bottom graphs) above the threshold temperature (28°C for GN; 32°C for IGW) for different pre- and post-flowering developmental phases (a to d), and their associated coefficients of determination (e to h). The slope (a-d) and coefficient of determination (e-h) for the linear response of GN (a, c) and IGW (b, d) to days (a, b) and hours (c, d) above threshold temperatures were derived from individual response curves computed for different periods, namely (i) for GN, 300–400°Cd, 200–300°Cd, 100–200°Cd, 0–100°Cd before flowering and 0–100°Cd post flowering, and (ii) for IGW, 0–100°Cd, 100–200°Cd, 200–300°Cd, 300–400°Cd and 400–500°Cd after flowering, as well as for the whole post-flowering period from 0 to 500°Cd (dashed horizontal line). Individual linear regressions are presented in Figs. S4 to S7. In (a, c), the right and left-hand Y-axis labels indicate values per unit area (m^{-2}) and per spike, respectively. Spike data were collected from the individual spikes (~20 for each replicate) exposed to heat at synchronised developmental stages. Quadrate data were collected by harvesting 0.5 m linear meter of plants tagged at synchronised flowering in the PEM. Plot data are collected from the whole conventional plots of naturally flowering genotypes.

(Fig. 4 and S6). In the environments tested, the correlations between IGW and post-flowering heat were globally the closest (i.e. highest r^2) when considering the whole 0–500°Cd after flowering rather than any specific 100°Cd-long post-flowering periods (Fig. 4 and S6). For this 0–500°Cd post-flowering period, IGW correlations with heat indices were strongest for the PEM spike harvests (r^2 = 0.53–0.80), followed by the PEM quadrate harvests (r^2 = 0.47–0.64) and the plot harvests (r^2 = 0.33–0.48; Fig. 5). Compared with averaged IGW under little if any heat stress (HET1), IGW was reduced by an average 3.3, 3.7 and 1.3 % for each hot day (> 32°C) during 0–500°Cd after flowering for PEM spike (r^2 = 0.75), PEM quadrate (r^2 = 0.62) and plot (r^2 = 0.48) harvests, respectively (Fig. 5c, g, k). Similarly, for each hot hour (> 32°C), IGW was reduced by 0.26, 0.3 and 0.09 mg for PEM spike, the PEM quadrate and the plot harvests, respectively (Fig. 5d, h, l).

For each 1°C increase in average daily T_{max} and T_{mean} during grain filling, the averaged IGW of the studied genotypes decreased by 2.58 and 2.74 mg, respectively, for PEM spikes (Fig. 5a, b), and by 2.11 mg and 2.39 mg for PEM quadrates (Fig. 5e, f).

However, for conventional plots, the correlations between IGW and heat indicators were not as close as for PEM (spike or quadrate harvests), suggesting that this method is less precise in estimating the IGW loss. For example, r^2 was less than 0.35 for the correlations between IGW of plot harvest and both T_{max} and T_{mean} (Fig. 5i, j), while r^2 was above 0.53 when looking at those correlations for spike or quadrate PEM harvests (Fig. 5a-d).

Heat response of total grain weight ('yield') was also strongly associated with the studied heat indicators, particularly for PEM spikes (Fig. 6). Each 1°C increase in either daily T_{max} or T_{mean} resulted in a 6.2 % reduction in total spike grain weight compared with the minimally-stressed HET1 plants (Fig. 6a, b). The yield of PEM quadrates (average of 391 g m^{-2}) was similar to the yield of conventional plots (366 g m^{-2}) in low-stress environments (HET1). However, yield responses to post-flowering temperatures were stronger in plots (r^2 = 0.39–0.65) than in PEM quadrates (r^2 = 0.17–0.24). This was partly due to quadrate not being taken in severe HET3 environments (GAT19-s2 and TOS19-s2). In conventional plots, each 1°C increase in average daily T_{max} or T_{mean} between 0 and 500°Cd post-flowering resulted in 27.8 or 24.7 g m^{-2} yield loss, respectively (Fig. 6i, j). Similarly, for each post-flowering hot day and hour with a temperature above 32°C, the yield was reduced by 14.7 and 2.44 g m^{-2}, respectively, in plots (Fig. 6k, l).

3.4. *Influence of temperature thresholds on the heat response of grain number*

Reductions in grain yield components (averaged across the studied genotypes) were calculated for each cumulative hot day or hour above different threshold temperatures (from 26 to 32°C) during different developmental periods to identify the threshold temperature and duration that allowed best estimation of heat impacts in the tested environments. The computed changes in yield components were derived from the individual response curves for different threshold temperatures from 26 to 35°C (Fig. 7a-f). The graph shows how the estimated reductions in yield components (for each hot day or hour) vary by increasing the threshold temperatures. The associated coefficients of determination (r^2) are also presented to reflect on the strength of responses to different threshold temperatures (Fig. 7g-l).

Irrespective of the harvest type, the impact of heat on the magnitude of the responses (negative slope) for grain number tended to progressively increase with the increasing temperature threshold, particularly for hot hours (Fig. 7a, b). However, the coefficient of determination (r^2) tended to either remain relatively unchanged or decline beyond 28°C (Fig. 7g, h). This is most likely because temperatures rarely exceeded 30°C early in the season (Fig. S2) and so there were fewer data points for these regressions. The results from the tested conditions suggest that the best estimation for grain number loss is around a threshold temperature of 28°C.

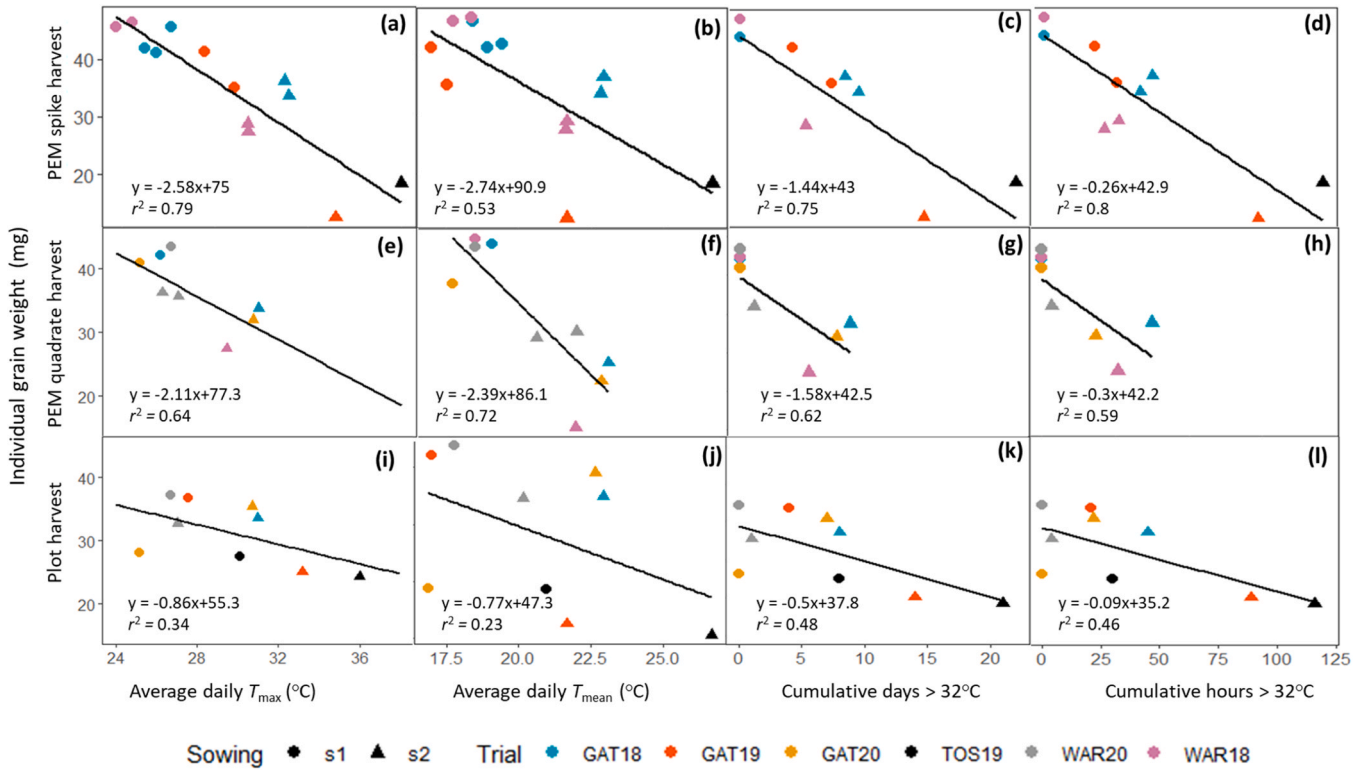

Fig. 5. Individual grain weight (IGW) of wheat genotypes in response to post-flowering (a, e, i) daily maximum temperature (T_{max}), (b, f, j) mean temperature (T_{mean}), cumulative (c, g, k) days and (d, h, l) hours above 32°C; all temperature indices were calculated between 0 and 500°Cd after flowering. Data correspond to the mean of 20 – 32 genotypes with four independent replicates in each environment. Spike data were collected from the individual spikes (~20 for each replicate) exposed to heat at synchronised developmental stages. Quadrate data were collected by harvesting 0.5 m linear meter of plants tagged at synchronised flowering in the PEM. Plot data were collected from the whole conventional plots of naturally flowering genotypes. Quadrate and plot data were presented per unit area (m^{-2}). Lines were plotted for regressions between mean grain number and temperature indicators that had a regression slope significantly different from zero ($P < 0.05$).

For conventional plots, the slope of grain number responded sharply to hot hours (more than doubling in absolute value) as the threshold temperature increased from 26 to 28°C, but it did not reduce further for 30 or 32°C (Fig. 7a). In contrast, the slope of the regression for grain number progressively decreased for the number of hot hours with the increasing threshold temperatures (Fig. 7b). For example, with each 1°C increase in threshold temperature from 26 to 32°C, wheat genotypes lost an additional 57 grains m^{-2} on average in conventional plots (Fig. 7b). This suggests that using hot hours rather than hot days for estimating grain number loss was more responsive, particularly at the plot level during this developmental period.

For grain number per spike collected on stems with synchronised development stages (PEM), the slope of grain number response to hot hours decreased mainly when the temperature threshold for hot hours was increased from 30 to 32°C (Fig. 7b). In other words, in the tested conditions, the grain number of PEM spikes was less responsive to increases in pre-flowering threshold temperatures below 30°C. However, coefficients of determination (r^2) were the strongest for low threshold temperatures of 28–30 °C (Fig. 7h) as higher temperatures occurred pre flowering in only a few environments (Fig. S2).

For PEM quadrates, loss in grain number progressively increased with the increasing threshold temperatures both for hot hours and hot days (Fig. 7e, f), but the coefficients of determination were low ($r^2 < 0.2$) (Fig. 7g, h), likely due to only a few heatwaves before flowering in the tested environments with PEM quadrates resulting in a limited number of data points for the regression curves (Fig. S2).

3.5. *Influence of the temperature thresholds on the heat response of grain weight*

Loss in IGW and total grain weight strongly responded to increasing post-flowering (0–500°Cd) threshold temperatures (Fig. 7i-l). Across the tested temperature thresholds for days and hours, these responses were strongest for PEM spikes, followed by PEM quadrates and plots (Fig. 7i, k). For instance, for temperature threshold for days, r^2 for IGW was maximum at 0.88 for PEM spikes, 0.62 for PEM quadrates and 0.36 for conventional plots (Fig. 7i); for total grain weight r^2 was maximum at 0.87 for PEM spike, 0.24 for PEM quadrate, and 0.64 for conventional plots (Fig. 7k). Further, for conventional plots, the response of IGW and total grain weight to temperature threshold became progressively stronger as r^2 increased from 28°C to 32°C, particularly for hot days and then slightly declined for 35°C (Fig. 7i-l). Hence, for the tested conditions in conventional plots, estimating heat impacts on IGW and grain yield appeared most precise when considering highly stressful conditions (i.e. high-temperature threshold of 32°C) that occur relatively frequently (Fig. S3). In contrast, it is less precise at temperatures such as 35 °C, which is relatively rare in tested environments. Additionally, the impact could typically be more precisely quantified using hot hours above threshold temperature rather than hot days (Fig. 7i-l, S3).

As expected, the heat impact per hot day or hour on either IGW or total grain weight generally increased when considering greater temperature thresholds (Fig. 7c-f). However, this was not always the case for non-stressful or less stressful conditions when considering daily data (Fig. 7c, e). In contrast, when considering hourly data (Fig. 7d, f) the impact of heatwaves on IGW and total grain weight (weight reduction $hour^{-1}$) progressively intensified as threshold temperature increased from 26 to 35 °C, except for the total grain weight of PEM quadrate

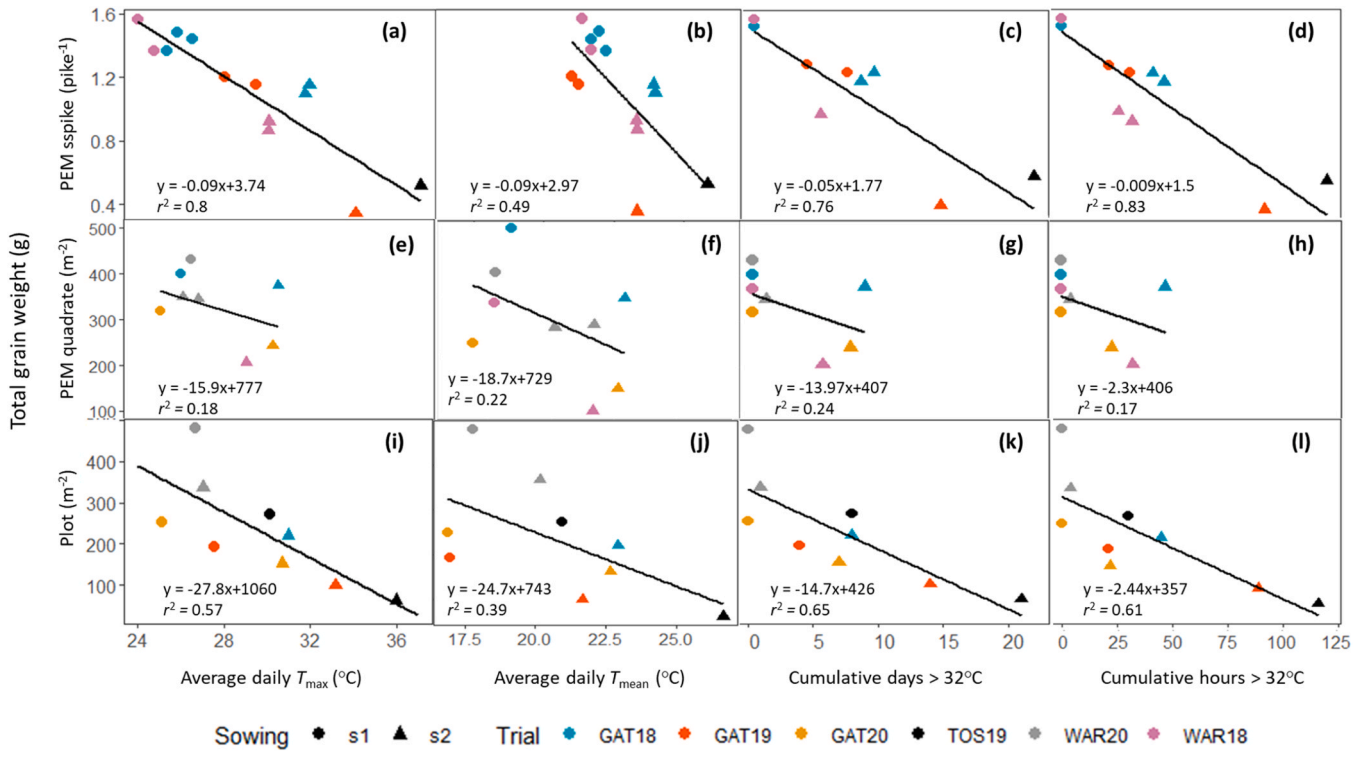

Fig. 6. Total grain weight ('yield') of wheat genotypes response to post-flowering (a, e, i) daily maximum temperature (T_{max}), (b, f, j) mean temperature (T_{mean}), cumulative (c, g, k) days and (d, h, l) hours above 32°C; all temperature indices were calculated between 0 and 500°Cd after flowering. Data correspond to the mean of 20 – 32 genotypes with four independent replicates in each environment. Spike data were collected from the individual spikes (~20 for each replicate) exposed to heat at synchronised developmental stages. Quadrate data were collected by harvesting 0.5 m linear meter of plants tagged at synchronised flowering in the PEM. Plot data were collected from the whole conventional plots of naturally flowering genotypes. Quadrate and plot data were presented per unit area (m^{-2}). Lines were plotted for regressions between mean grain number and temperature indicators that had a regression slope significantly different from zero ($P < 0.05$).

under 35°C. On average, for each 1°C increase in hourly temperature threshold (from 26 to 35 °C), wheat genotypes experienced an additional reduction in IGW of 0.04 mg per hot hour for both PEM spikes and quadrates (Fig. 7d) and a yield reduction of 0.32 g m^{-2} h^{-1} for conventional plots (Fig. 7f).

3.6. Impact of the timing of post-flowering heat

The impact of post-flowering heatwaves on grain weight was further investigated by exploring how the timing of the first heat event affects IGW (Figs. 4, 8 and 9). While in the studied environments, IGW was globally more closely related (r^2) to the whole 0–500°Cd period than any specific 100°Cd intervals of this period (Fig. 4), this was not the case for the sensibility to heat (slope of the relationship) at the different tested post-flowering periods (Fig. 4, S5, S6).

Based on the data presented above, and as previously suggested by Collins et al. (2000), a heat event was defined as at least a 4 h cumulative period of temperatures above 32°C in order to identify the first heat event. In the tested conditions, IGW was not or only little affected when intense heat only started occurring during the late period of grain development, e.g. 400 or 500°Cd after flowering. However, IGW responded strongly to the timing of the first heat event when it occurred early after flowering (Fig. 8).

For PEM spikes and quadrates, wheat genotypes were compared at synchronised development stages. The timing of the first heat shock affected genotypes at a similar stage relative to flowering in the PEM, but not in conventional field plots. Before late grain filling in HET1–2 environments, for each 100°Cd delay (~5 days) in the start of extreme heat shocks (4 h > 32 °C), the tested genotypes produced on average 2.4 mg ($r^2 = 0.91$) and 1.4 mg ($r^2 = 0.8$) larger grains at the spike and quadrate levels in the PEM, respectively (Fig. 8a, b). By contrast, no clear response to the timing of the first heat event was observed for IGW in conventional plots (Fig. 8c), possibly because of phenological variation across the tested genotypes when natural heat events occurred.

The importance of the timing of heatwaves on grain filling is evident when comparing GAT18-s2 and WAR19-s2 (Fig. 9a-c). The plants in GAT18-s2 produced bigger grains than WAR18-s2 despite being subjected to more hot hours (T > 28°C) during the grain filling period (0–500°Cd after flowering) (Fig. 9a, c). By contrast, when focusing solely on the first 100°Cd post-flowering, the plants responded similarly to the cumulative hot hours in both trials (Fig. 9b). Crops in WAR18-s2 received twice as many hours above 28°C compared to GAT18-s2 crops; and they experienced significantly more reduction in IGW at maturity, despite receiving overall fewer post-flowering hot hours (76 h in WAR18-s2 compared with 108 h in GAT18-s2 between 0 and 500°Cd after flowering; Fig. 9). This example illustrates the complexity of working with fluctuating natural environments, while it also highlights that heat events occurring during the early grain development are more impactful than those occurring later.

4. Discussion

Wheat is generally more sensitive to heat during the reproductive and grain-filling periods than during the vegetative growth phase (Farooq et al., 2011, Fernie et al., 2022). While previous studies suggest a significant rise in the frequency of heatwaves during wheat grain filling (e.g. Ababaei and Chenu, 2020), protecting crops from heat injury at those stages becomes critical. To better understand how the intensity, duration and timing of heat stress affect wheat in field conditions, we conducted multi-environment field trials and assessed for a relatively wide range of environments how yield and its components correlate to heat factors calculated at different developmental phases and for

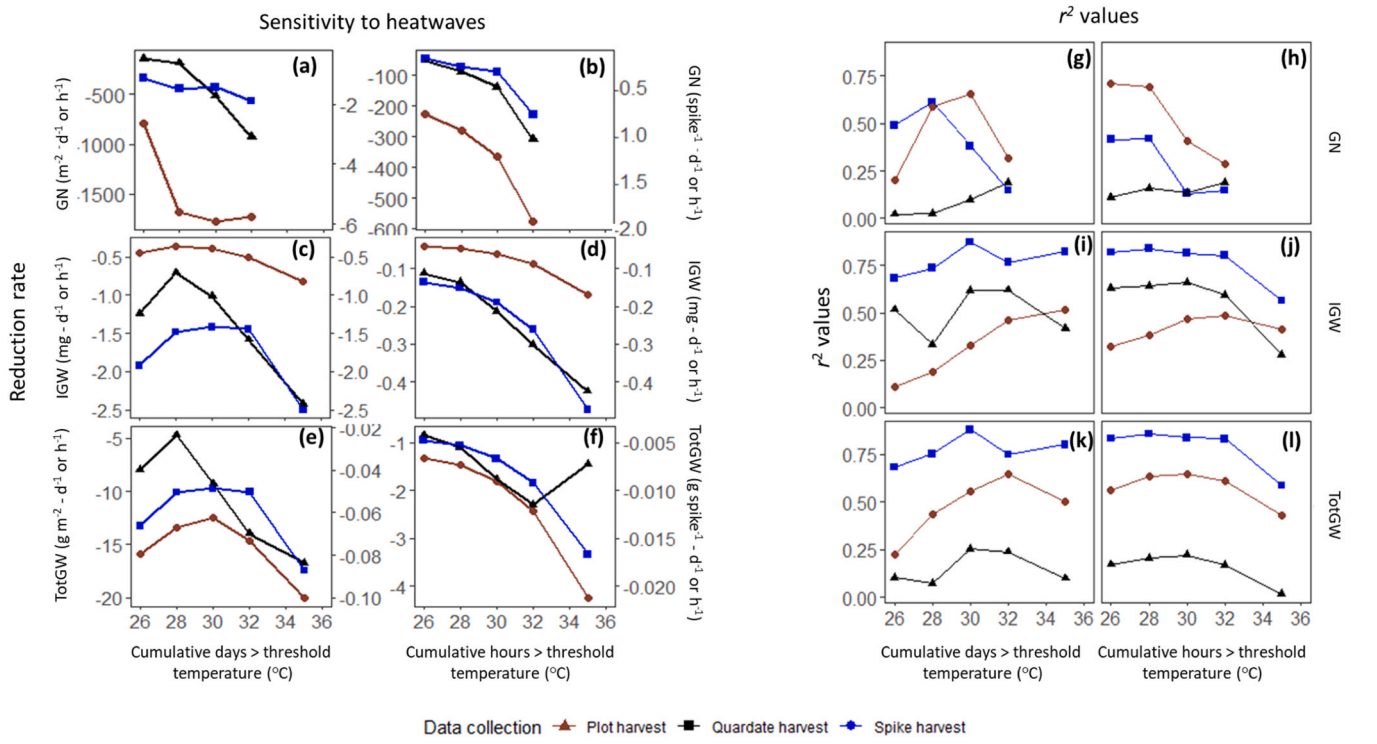

Fig. 7. Reduction rate in grain yield and its components in response to increasing heat intensity (a to f), and their associated coefficients of determination (g to l). The slope (a-f) and coefficient of determination (g-l) for changes in grain number (GN; a, b), individual grain weight (IGW; c, d) and total grain weight (TotGW; e, f) in response to days (a, c, e) and hours (b, d, f) above threshold temperatures were derived from the individual response curves of different threshold temperatures from 26 to 32°C to quantify the reduction for each additional hot day or hour above threshold temperatures on average for 20–32 genotypes with four independent replicates. Some individual linear regressions are presented in Figs. 3, 5, and 6. The strange increase in the total grain weight loss found for the hourly increase in threshold temperatures from 32 to 35°C in the plot trial (f) resulted from highly variable genotypic response (Fig. 4e) due to variation in the grain filling duration. In (a-f), the right and left-hand Y-axis labels indicate values per unit area (m^{-2}) and per spike, respectively. Spike data were collected from the individual spikes (~20 for each replicate) exposed to heat at synchronised developmental stages. Quadrate data were collected by harvesting 0.5 m linear meter of plants tagged at synchronised flowering in the PEM. Plot data are collected from the whole conventional plots of naturally flowering genotypes.

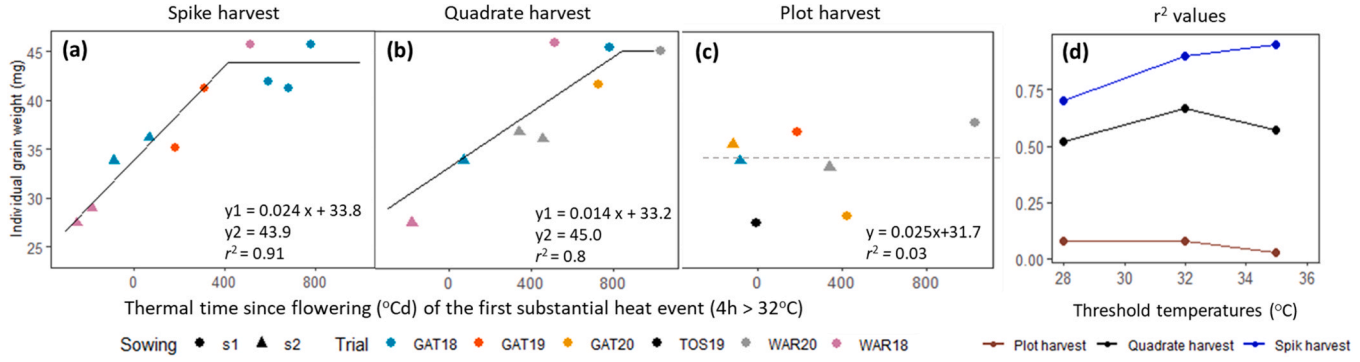

Fig. 8. Individual grain weight of wheat genotypes plotted against the timing relative to the flowering of the first heat event (4 cumulative hours > 32°C) for (a) spikes with synchronised phenology, (b) quadrates with synchronised phenology, and (c) conventional plots (without synchronised phenology), together with (d) associated coefficients of determination for different temperature thresholds (first heat event being defined as 4 hours > T_{thres}). Spike data were collected from individual spikes (~20 for each replicate) exposed to heat at synchronised developmental stages in the PEM. Quadrate data were collected by harvesting 0.5 m linear meter of plants tagged at synchronised flowering in the PEM. Plot data were collected from the whole conventional plots of naturally flowering genotypes. Data are the mean of 20 – 32 genotypes with four independent replicates. Slope and r^2 values are presented for trials from HET1 and HET2 only.

different temperature thresholds.

4.1. Heat impact on grain number and grain weight is highly stage sensitive

In wheat, the critical period for grain number determination occurs between −20 and +10 days around flowering (e.g. Fischer, 1985). In the present study, the impact of heatwaves was tested for every 100°Cd-interval period between −400 and +100°Cd around flowering (Fig. 4 and S4-S5). In the tested environments, grain number was most strongly correlated with high temperatures (> 28°C) between 200 and 300°Cd (~10–15 days) before flowering (Fig. 4). This period corresponds to pollen meiosis – the most stress-sensitive phase of pollen development (Dolferus et al., 2013; Masoomi-Aladizgeh et al., 2021). Damage to microspores during this phase is irreversible, translating into grain number loss at maturity, irrespective of temperature later during

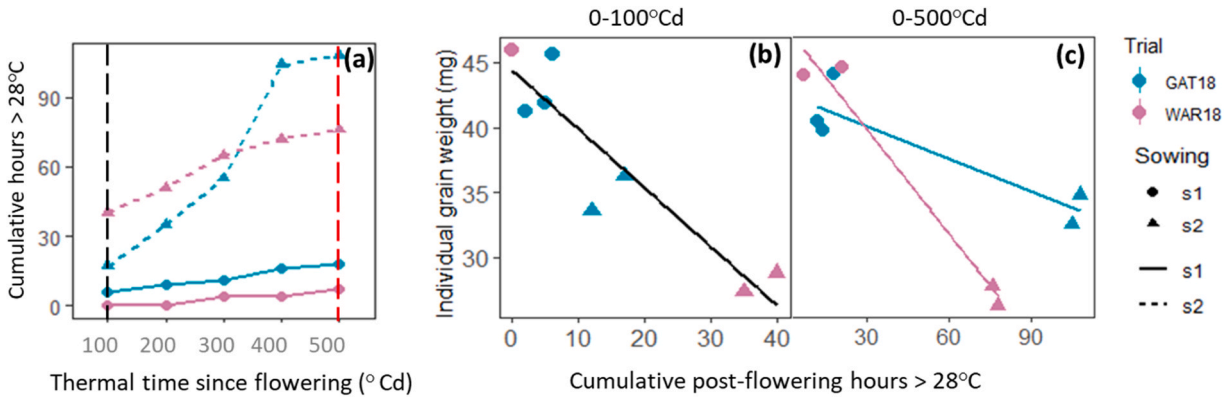

Fig. 9. Illustration of the importance of the timing of heat events on individual grain weight (IGW; b-c) in two trials with contrasting patterns of heat events (a). (a) Cumulative hours > 28°C for different post-flowering periods (0–100, 0–200, 0–300, 0–400 and 0–500°Cd after flowering) for the first tagging of each of two 2018 trials. The number of hours > 28°C during the first 100°Cd after flowering (black dashed vertical line in (a)) highlights warmer conditions in early grain filling in WAR18-s2 compared to GAT18-s2, while this environment was cooler towards the end of the grain filling. Changes in IGW in response to cumulative hours > 28°C were consistent across environments during 0–100°Cd after flowering (b). However, IGW was greater in GAT18-s2 than WAR18-s2 despite warmer average grain-filling temperature (0–500°Cd after flowering; c). This was due to warmer temperatures in GAT18-s2 occurring late in the grain filling period (400 to 500°Cd), when IGW was no longer highly sensitive to heat. In (b) and (c), IGW is presented for all taggings of 2018 trials. Data were collected from individual spikes (~20 for each replicate), and averaged for all 32 studied genotypes and replicates.

development (Dolferus, 2014; Ji et al., 2010). While field-based studies have mainly focused on the impact of heatwaves in response to the whole pre-flowering period rather than at specific pre-flowering developmental stages (Thistlethwaite et al., 2020; Talukder et al., 2014), controlled environment studies also linked wheat grain set with high temperatures during pollen meiosis (10–15 days before flowering) or around anthesis (Prasad and Djanaguiraman, 2014; Saini and Aspinall, 1982). However, the heat impact on pollen viability has previously been studied only *in-vitro* (Impe et al., 2020), in controlled environments under extremely high temperatures (i.e. 35 °C, Prasad and Djanaguiraman, 2014). For example, three hot days (35/22 °C day/night) during meiosis reduced the pollen viability of wheat genotypes by 57 % (Bokshi et al., 2021) and grain number per spike by 9 % (Thistlethwaite et al., 2020). Interestingly, no significant correlation was observed between grain number and the number of hot hours (> 28 °C) between 100 °Cd before flowering and 100°Cd after flowering for PEM harvests. This suggests that final grain number is relatively more susceptible to mild heat (~28 °C) during the early pollen developmental phase (~300 to 200°Cd before flowering) than around anthesis (±100°Cd). Similar results were reported from a glasshouse experiment, where plants experienced a 7-d heatwave at different stages within most of the plant cycle (Chenu and Oudin, 2019a).

IGW of the tested genotypes responded quite closely to high temperatures from 0 to 500°Cd post-flowering (Figs. 4, 5 and S5). In PEM harvests, for each 1°C increase in post-flowering daily T_{max}, IGW was reduced by 2.6 mg, i.e. 6.1 % at the spike level (PEM spike harvest) and by 2.1 mg, i.e. 5 % at the canopy level (PEM quadrate harvest; Fig. 5a, e).

Studies in controlled environments have reported that IGW is most sensitive to post-flowering heat at the beginning of grain filling (e.g. Tashiro and Wardlaw, 1990; Chenu and Oudin, 2019a; Kino et al., 2020). However, similarly as for impact on grain number, previous field-based studies tend to report the impact of heatwaves on IGW for the total post-flowering duration rather than for a short period specific to a given development stage (Telfer et al., 2018; Thistlethwaite et al., 2015). In contrast, synchronised phenology under PEM and detailed tracking of heat events (timing and intensity) in the current study allowed precise quantification of heat-induced IGW loss in natural field conditions (Fig. 1). In the current study, the response of IGW to heatwaves was strongly influenced during early grain filling, as evidenced from a close correlation ($r^2 = 0.91$) between IGW and the timing of the first post-flowering extreme heat event (4 h > 32°C) for spikes with synchronised development (Fig. 8a). High temperature during early grain filling appeared to cause a maximum reduction in IGW, but grains to become progressively less sensitive to heat at later stages (e.g. > 400°Cd post-flowering; Fig. 9). This is in adequation with the findings of Stone and Nicolas (1995), who found lower sensitivity of wheat grains to heat during later phases of development (30 days after anthesis) than 15–20 days after anthesis. The grain development period can be divided into three different phases: (phase I) rapid cell proliferation, (phase II) grain filling, and (phase III) maturation (Ellis, 1999). Our study suggested that IGW of tested wheat genotypes is more sensitive to heatwaves during early phases of grain development (i.e. I and II) compared to the maturation phase (III). When we compared the impact of high temperature for the first 100°Cd after flowering (cell proliferation phase) and most of the grain filling duration (0–500°Cd after flowering) in two trials where both stresses occurred, the results suggested that intensity and duration of heat during phase I (~0–10 days after flowering), is a more critical determinant of final grain size than total post-flowering heat (Fig. 9). This suggests that high temperature during early grain development can irreversibly impair cellular division and enlargement (Girousse et al., 2021), impacting the final grain size irrespective of temperature at later stages (Fig. 9).

4.2. *Which heat indicators to characterise heat stress impacts?*

Crops responses to heatwaves are complex as they depend on the timing, intensity, duration of the stress as well as other factors such as acclimation, making them hard to model quantitatively. In field conditions, a multitude of other varying environmental factors also affect crops and potentially interact with heat responses, which greatly complicates the analysis of the results. For instance, variations in growing environments across sowing dates (and even within a sowing date, across groups of plants tagged at different dates in the PEM) likely affect yield components via their impact on traits such as LAI, radiation interception, and stem water-soluble carbohydrate accumulation (e.g. Ehdaie et al., 2006; Slafer et al., 2023). Despite this, responses of yield components to heatwaves in the current study were found strongly correlated to temperature indicators (Figs. 3–5 and 7). The response strongly depended on the temperature indicator (i.e. T_{max}, T_{mean} and cumulative days or hours > threshold temperatures for different periods) and on the biological level (i.e. spike or canopy level) considered. For example, the coefficient of determination (r^2) between grain number and different temperature indicators ranged from 0.18 to 0.61 for PEM

spikes (Fig. 3a-d and 7). This highlights the importance of selecting a suitable temperature indicator to quantify heatwaves impacts with greatest sensitivity and accuracy.

Heat-induced losses in grain number vary widely across published studies, likely due to differences in growing conditions, heat indicators/periods considered and genetic backgrounds. Even in a controlled environment, impacts of heatwaves on grain number may vary. For instance, three hot days (35 °C) around anthesis was reported to reduce grain number by 22 % (Thistlethwaite et al., 2015), while in another study, a single day hot day (T > 35°C) reduced grain number by 24 % (Talukder et al., 2014). In the current study, we used various heat indicators in an attempt to better understand how the estimated grain loss varies in field conditions. We found that grain number responded strongly to pre-flowering heat indicators both at the organ level (PEM spikes) and canopy level (conventional plots; Fig. 3i-l). For instance, in conventional plots, grain number was impacted by 7 % for each 1°C increase in daily T_{max} averaged between 200 and 300°Cd before flowering (Fig. 3i). Similarly, for each hot hour (> 28°C) between 200 and 300°Cd before flowering, grain number was estimated to decrease by 2.8 % in conventional plots (Fig. 3l). Our study also highlighted that the sensitivity of grain number to 'heat' also increases when considering increasing threshold temperature, in particular for the definition of cumulative 'hot' hours, e.g. there is a great loss in grain number per extra hour > 32°C than per extra hour > 26 °C (Fig. 7a, b).

Understanding response to increased temperature is complex, and impacts due to increased average temperature (thermal time) and acceleration of phenology were also observed in our study. In conventional plots, for each 1°C increase in T_{mean} during 300 to 200°Cd pre-flowering, the studied genotypes produced 10 % fewer grains in harvests with none or little stressed HET1 controls (Fig. 3j). This reduction is higher than reported by Fischer (1985), who observed that a 1°C increase in daily mean temperature during the spike growth period (~30 days prior to flowering) reduces grain number by 4 %, at least partly due to differences in growing conditions (e.g. average temperature varying between 14 and 22°C). Those findings are likely due to acceleration in phenology, which reduces the time crops have to accumulate resources (e.g. intercepted radiation) needed to produce biomass and grains. Using integrative factor, such as the photothermal quotient (Sadras and Dreccer, 2015), or combining the analysis with crop simulations (Chenu et al., 2017) could be helpful to clarify the direct impact of heatwaves on yield and its components.

In the current study, changes in grain number for spikes with synchronised flowering were more strongly associated with pre-flowering T_{max} than pre-flowering T_{mean}, suggesting an impact of heat stress (and not just a response to thermal time) on grain loss (Fig. 3a, b). This is in agreement with the report of Ferris et al. (1998), who found that grain number in wheat responded strongly to T_{max} but not to T_{mean} when the crop was exposed to heat during flowering. Similarly, Musa et al. (2021) also suggested T_{max} and the number of hot days as the best indicators of grain number loss in wheat genotypes under hot environments (T_{max} > 32 °C). However, in the absence of extreme heat, e.g. only a few days of > 32 °C, grain number may respond more strongly to T_{mean} (He et al., 2020, Ye et al., 2021) or the photothermal quotient. In conventional plots (without synchronised flowering), the response of grain number was relatively stable across all studied temperature factors (r^2 between 0.55 and 0.69; Fig. 3i-l).

IGW was also more strongly correlated to post-flowering T_{max} than T_{mean} (0–500°Cd after flowering), with r^2 (averaged across harvest types) of 0.59 and 0.44 for T_{max} and T_{mean}, respectively (Fig. 5). Compared with grain number, the impact of heatwaves on IGW was first estimated for a broad window (0–500°Cd after flowering; Fig. 5). For each 1°C increase in post-flowering T_{max} and T_{mean}, IGW was reduced by 2.58 and 2.74 mg (i.e. 6.1 % and 6.3 %), respectively, for spikes with synchronised development (Fig. 5a, b). A reduction of 2.80 mg in IGW was also previously reported for each 1°C increase in T_{mean} during the grain-filling phase of irrigated wheat by Wiegand and Cuellar (1981). As expected, the estimated impact of T_{max} on IGW was smaller and not as clear at the canopy level (i.e. for PEM quadrates and conventional plots) than at the organ level (i.e. PEM spikes), with e.g. 2.58 mg loss per degree in T_{max} for the PEM spike, compared to a 2.11 mg (5 %) loss for PEM quadrate (r^2 = 0.64), and 0.86 mg (2.5 %) loss for conventional plot (r^2 = 0.34).

In regard to yield, an average loss of 14.7 g m^{-2} (3.4 %; r^2 = 0.65) was found for each hot post-flowering day > 32°C in conventional plots (Fig. 6k), which is similar to 16.1 g m^{-2} for each hot day (> 30°C) between 100 and 600°Cd post-anthesis under field conditions (Telfer et al., 2018). Controlled-environment studies suggested a 23 % reduction in wheat grain yield after 4 post-flowering hot days > 35°C (Stone and Nicolas, 1994), which is slightly lower than our estimation, i.e. 4.6 % (r^2 = 0.5) for each hot day > 35°C compared to HET1 environments in conventional plots (Fig. 7e). Similarly, Thistlethwaite et al. (2020) reported a 40 g m^{-2} grain yield reduction for each 1°C increase in T_{mean} under irrigated field conditions. However, this value is substantially higher than our estimated grain yield loss of 24.7 g m^2 in conventional plots (Fig. 6j). Across the literature, a broad range of yield loss (3 – 18 %) has been reported for each 1 °C rise in post-flowering atmospheric temperature above the optimum in well-watered wheat crops (He et al., 2019; Mondal et al., 2013; Ullah, 2018). Such large variations are likely due to highly different growing environments, given how many environmental factors other than heat affect crops and also interact with crop response to heat (Slafer et al., 2023).

In the current study, IGW and total grain weight ('yield') loss were estimated more reliably with hours rather than days of cumulated heat, particularly for some of the low and high examined temperature thresholds (e.g. 26 and 35°C, Fig. 7c-f). Similarly, estimated responses were clearer at the organ than at the canopy level (as discussed above). Overall, finer 'spatio-temporal' characterisation of both heatwaves and plant traits allowed clearer estimations of heat responses. Characterisation considering differences in tiller ranking has also contributed to better explaining heat impacts in wheat (Chenu and Oudin, 2019b) and other studies have also focused on specific grains (e.g. basal grains of central spikelets; Girousse et al., 2021) to discard variations related to floret dynamic (Ferrante et al., 2013; 2020).

4.3. *Synchronised phenology across genotypes improved estimation of heat stress impact on individual grain weight*

The impact of heat stress was compared for (i) spikes at synchronised development stage, (ii) quadrates at synchronised development stage and (iii) conventional plots at a range of developmental stages (not synchronised). In this study, IGW was more reliably estimated with the synchronised phenology than in conventional plots, especially when considering individual spikes (i.e. organ level) that were at synchronised development stage. Coefficients of determination (r^2) from regressions between IGW and heat indicators (averaged across all tested indicators) were 0.72, 0.58 and 0.40 for PEM spikes, PEM quadrate and conventional plots, respectively (Fig. 5). For instance, for each 1°C increase in T_{max} or each hot day (> 32°C) during 0–500°Cd after flowering, IGW of the tested genotypes reduced by 6.1 % (T_{max}) or 3.3 % (T > 32°C) for PEM spike and 6.2 % (T_{max}) or 3.7 % (T > 32°C) for PEM quadrate and 2.5 % (T_{max}) or 1.3 % (T > 32°C) for conventional plots, respectively. For spike harvest, tillers of synchronised phenology experienced identical heat intensity during similar developmental stages, so that IGW responses to heat indicators were stronger, with both a greater estimated impact of heat and greater r^2, in PEM spikes than in PEM quadrates, and even more so compared to conventional plots (Figs. 5 and 7).

In conventional plots with unsynchronised flowering (due to differences in genotype maturity types and asynchronism between main stems, primary and secondary tillers), the tested genotypes/spikes experienced heatwaves at different developmental phases, which weakened IGW response to heat indicators (Figs. 5 and 7). Different responses of IGW from different tillers to heat stress around flowering

have already been confirmed under controlled environments (Aiqing et al., 2018), in particular, due to their asynchronism (Chenu and Oudin, 2019a). Highly development-phase-specific impact of heatwaves on IGW was recorded in the current study (Figs. 8 and 9). For spikes with synchronised phenology, IGW responded strongly ($r^2 = 0.95$) to the timing of the first heat event (4 hours > 35°C), but for conventional plots, no significant correlation was observed ($r^2 = 0.03$). This suggests that quantification of IGW loss in response to heatwaves could be achieved more reliably by focusing on the spikes (i.e. organ level) of synchronised phenology and, to a lesser extent, focusing on whole plants in quadrates (i.e. canopy level) of synchronised phenology (PEM quadrates; Fig. 8).

Further, total grain weight (spike^{-1} or m^{-2}) was reliably estimated from both spike and plot harvests (Fig. 6). Yet, relatively stronger correlations between heat indicators and grain weight were observed at spike than at plot level. For instance, for each 1°C increase in T_{max} during post-flowering 0–500°Cd, total grain weight was reduced by 2.4 % ($r^2 = 0.8$) and 2.6 % ($r^2 = 0.57$) for spikes and plots, respectively, compared with plants from optimum HET1 environments. Similarly, correlations for grain yield, with hot hours or hot days for spike harvest, were stronger than for the conventional plots across any tested temperature (Fig. 7k-l).

The current study suggests that heat impact on wheat yield can be most accurately estimated by focusing on specific developmental phases if possible (e.g. if natural heat events occurred during this period). For example, grain number responded strongly to heat indicators for a short period of ~ 5 days (200–300°Cd before flowering), presumably during the critical period of pollen meiosis (Dolferus et al., 2013; Masoomi-Aladizgeh et al., 2021). Although IGW correlated strongly to the heat indicator for a broader range time (0–500°Cd after flowering), grain weight loss was highly development-phase-specific with significantly more sensitivity to heatwaves during the early development phase (Fig. 9). Changes in yield components were also more accurately quantified using heat indicators such as T_{max} and the number of hot hours on wheat plants with synchronised phenology than T_{mean} and hot days.

The strongest relationships for heat responses of grain number were found for a threshold temperature of 28 °C from 300 to 200°Cd before flowering. For IGW, best-suited thresholds were ≥ 28 °C when considering cumulated hot hours, or thresholds were ≥ 32 °C for cumulated hot days.

5. Conclusion

The best estimations for yield-component response to heatwaves were achieved with spikes (i.e. organ level) or quadrates (i.e. canopy level) at synchronised development, rather than in conventional plots (i.e. genotypes without synchronised development). In the tested environments, the strongest relationships for responses of grain number were found for average daily maximum temperature and cumulative hours with the temperature above 28 °C during the period from 300–200°Cd before flowering. IGW was strongly correlated to maximum temperature as well as cumulative days or hours with temperature above 28 °C during grain filling (0–500°Cd after flowering) but appeared to be most sensitive during early grain filling (~0–100°Cd after flowering).

Results indicated that the impact of natural heatwaves on wheat grain yield components in the field could be best estimated with the selection of appropriate experimental techniques (i.e. synchronised development stages) and heat indicators (e.g. cumulative hot hours rather than cumulative hot days). Optimising field experimental techniques will be particularly important for screening the relative heat tolerance of genotypes in breeding programs.

CRediT authorship contribution statement

Brian Collins: Investigation. **Troy Frederiks:** Investigation, Methodology, Writing – review & editing. **John T Christopher:** Methodology, Writing – review & editing. **Najeeb Ullah:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Karine Chenu:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Najeeb Ullah reports financial support was provided by Queensland Government.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109489.

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