Night-time warming in the field reduces nocturnal stomatal conductance and grain yield but does not alter daytime physiological responses

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Summary

- Global nocturnal temperatures are rising more rapidly than daytime temperatures and have a large effect on crop productivity. In particular, stomatal conductance at night ($g_{sn}$) is surprisingly poorly understood and has not been investigated despite constituting a significant proportion of overall canopy water loss.
- Here, we present the results of 3 yr of field data using 12 spring Triticum aestivum genotypes which were grown in NW Mexico and subjected to an artificial increase in night-time temperatures of 2°C.
- Under nocturnal heating, grain yields decreased (1.9% per 1°C) without significant changes in daytime leaf-level physiological responses. Under warmer nights, there were significant differences in the magnitude and decrease in $g_{sn}$ values of which were between 9 and 33% of daytime rates while respiration appeared to acclimate to higher temperatures. Decreases in grain yield were genotype-specific; genotypes categorised as heat tolerant demonstrated some of the greatest declines in yield in response to warmer nights.
- We conclude the essential components of nocturnal heat tolerance in wheat are uncoupled from resilience to daytime temperatures, raising fundamental questions for physiological breeding. Furthermore, this study discusses key physiological traits such as pollen viability, root depth and irrigation type may also play a role in genotype-specific nocturnal heat tolerance.

Introduction

Climate change is driving rapid increases in global ambient temperature, particularly at night where minimum ($T_{min}$) night-time temperatures are rising at 1.4× the rate of daytime temperatures (Davy et al., 2017; Sadok & Schoppach, 2019). While episodic daytime heatwaves are either chronic (long-term) or acute (short-term), high nocturnal temperatures often extend over long periods of crop cycles and encompass large geographical areas leading to negative impacts on phenology and yield. These negative impacts have been reported for major crop species like rice (Oryza spp. – Peng et al., 2004; Welch et al., 2010; Coast et al., 2014; Shi et al., 2016; Bahuguna et al., 2017; Bheemanahalli et al., 2021), barley (Hordeum vulgare, García et al., 2015, 2016), sorghum (Sorghum bicolor, Prasad et al., 2011), soybean (Glycine max, Lin et al., 2021), quinoa (Chenopodium quinoa – Lesjak et al., 2017) and cotton (Gossypium hirsutum, Loka et al., 2010).

This penalty is particularly evident in wheat, with Spring wheat yields reported to decline 3.2–8.4% for every 1°C increase in $T_{min}$ (Lobell et al., 2005; Lobell & Ortiz-Monasterio, 2007) or 4% decline for every 1°C increase over 14°C (Fischer, 1985). High nocturnal temperatures have been shown to decrease wheat seed set, grain number and grain yield between 13 and 43% (Narayanan et al., 2015). This fall in yields has been attributed to rapid, premature depletion of photoassimilates for growth due to increases in respiration (García et al., 2016), a shortening of the grain-filling period and/or reductions in pollen fertility (Narayanan et al., 2018) leading to fewer grains setting.

While daytime heat tolerance mechanisms have been studied extensively (for a review; Moore et al., 2021), nocturnal processes have received less attention, especially under field conditions. One of the consequences of rising nocturnal temperatures is the...
significant alteration of whole-plant water use, either through reduction in available water at the roots or through changes in stomatal behaviour via increased \([\text{CO}_2]\), changes in water potential or increased vapour pressure deficit (VPD). While daytime water loss via the stomata \(g_s\) – please refer to Box 1 for all abbreviations used in this manuscript) can be correlated with the exchange of \([\text{CO}_2]\) for photosynthetic carbon assimilation \(A\), recent work has shown that stomata open at night in wheat (McAusland et al., 2021). However, a definitive role for nocturnal conductance \(g_{\text{tn}}\) has remained elusive, especially in the context of nocturnal warming. Nocturnal conductance typically accounts for 5–18% of all daytime water loss in wheat (Caird et al., 2007; McAusland et al., 2021) and is growth stage and genotypic-specific. Nocturnal stomatal conductance has been correlated with the breakdown of starch (dos Anjos et al., 2018), maintenance of growth (Fricke, 2019), and as a potential mechanism for facilitating the uptake of \(\text{O}_2\) and, in some cases, the release of respiratory \([\text{CO}_2]\) (Daley & Phillips, 2006; Resco de Dios et al., 2016; Even et al., 2018; Fricke, 2019). To date, no study has dissected the response of wheat \(g_{\text{tn}}\) in the context of nocturnal heating in the field.

There is an urgency to study the responses of field-grown wheat under elevated ambient temperatures at night to determine the ‘real-world’ impact of nocturnal warming as climate change progresses (Kimball et al., 2008). Since the responses of photosynthesis and plant productivity have not widely been characterised under the context of elevated nocturnal temperatures, it is also imperative to characterise natural variation between genotypes to determine whether this response can be optimised in the future to cope with increased temperatures. First proposed by Harte & Shaw (1995), using an array of infrared heaters provides a means to artificially raise the surface temperature of canopy below without having to overcome changes in boundary layer, in effect a Temperature Free-Air Controlled Enhancement (T-FACE) set-up. In addition, using canopy temperature sensors, the system can be closely modulated using feedback loops to maintain specific nocturnal temperature thresholds as the field environment fluctuates (Nijs et al., 1996; Kimball, 2005). T-FACE experiments have previously been used to assess the effect of elevated temperature in \(C_3\) crops such as perennial ryegrass (\(Lolium perenne\)), white clover (\(Trifolium repens\)) and rice (\(Oryza sativa\); Nijs et al., 1996; Zhang et al., 2019; Wang et al., 2022).

Here, 12 genotypes were selected based on previous evaluation of yield under heat tolerance and investigated for genotype-specific variation in \(g_{\text{tn}}\) at two growth stages under control and a nocturnal heat treatment of \(+2^\circ\text{C}\) above ambient. In this study, we aim to: characterise natural variation of photosynthetic traits, especially stomatal conductance both during the day and the night; assess the impact of nocturnal heat on stomatal conductance, respiration and leaf water potential; and evaluate the effect that elevated nocturnal temperatures have on overall yield and biomass production. We hypothesise that: \(g_{\text{tn}}\) will be genotype-specific and decline with nocturnal warming; this will be accompanied by a rise in the rate of respiration in response to night-time warming; daytime physiological measurements (e.g. leaf-level \([\text{CO}_2]\) assimilation) will demonstrate a decline under high night-time temperatures; and, finally, we hypothesise that heat-tolerant genotypes will also demonstrate greater yields under warmer nights when compared to genotypes with low heat tolerance.

### Materials and Methods

**Site, experimental treatments, irrigation regime and genotypes**

**Regular sowing** Three field experiments were carried out between 2020, 2021 and 2022, under fully irrigated yield potential conditions at the Norman E. Borlaug experimental station, Ciudad Obregon, Sonora, Mexico (27.33°N, 109.09°W). The soil composition is as described in Sayre et al. (1997). The experiment consisted of two biological replicates under both the control (yield potential) and heated set-up. Within each replicate, the 12 \(Triticum aestivum\) genotypes were sown in 0.5 × 3.2 m plots \((n=2)\). Three to five technical replicates (individual plants) were sampled within these plots. Each bed had a 24 cm gap between rows and 56 cm between beds. Herbicides, fungicides and pesticides were applied to minimise the impact of biotic stresses. Nitrogen fertiliser (100–200 units) was applied at the beginning of February each year. Irrigation was supplied throughout the growing period every 10–14 d using a drip-based system. The genotypes were selected on broad heat tolerance (Table 1).
Table 1 List of the genotypes investigated and their broad tolerance to heat.

<table>
<thead>
<tr>
<th>CIMMYT</th>
<th>Cross name</th>
<th>Type</th>
<th>Heat tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>GID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5893282</td>
<td>WEEBILL1</td>
<td>Bread</td>
<td>Low</td>
</tr>
<tr>
<td>3823821</td>
<td>PASTOR//HXL7573/2*BAU</td>
<td>Bread</td>
<td>Low</td>
</tr>
<tr>
<td>45180627</td>
<td>CMSS69Y040845-0Y-1B-131TLA-08-0Y-125B-0Y-0B</td>
<td>Bread</td>
<td>Low</td>
</tr>
<tr>
<td>7171118</td>
<td>SAUL or SAUAL/WH/PAIR/SAUAL/3/PBW343<em>2/KUKUNA2</em>2/FRTL/PIFED</td>
<td>Bread</td>
<td>Low</td>
</tr>
<tr>
<td>5077000</td>
<td>CIRNO C 2008 or CIRNO</td>
<td>Durum</td>
<td>Intermediate</td>
</tr>
<tr>
<td>7806808</td>
<td>BORLAUG100 F2014</td>
<td>Bread</td>
<td>Intermediate</td>
</tr>
<tr>
<td>2465</td>
<td>PAVON F 76 or Pavon76</td>
<td>Bread</td>
<td>High</td>
</tr>
<tr>
<td>3825355</td>
<td>SOKOLL</td>
<td>Bread</td>
<td>High</td>
</tr>
<tr>
<td>3855011</td>
<td>VOROBEY</td>
<td>Bread</td>
<td>High</td>
</tr>
<tr>
<td>7129721</td>
<td>SOKOLL/PUB94.15.1.12/WBL1</td>
<td>Bread</td>
<td>High</td>
</tr>
<tr>
<td>5865670</td>
<td>CMSS69Y040845-0Y-1B-93TLA-08-0Y-106B-0Y-0Y-0Y-0Y-0Y-0Y</td>
<td>Bread</td>
<td>High</td>
</tr>
<tr>
<td>6692380</td>
<td>PUB94 or PUB94.15.1.12/FRTL/5/</td>
<td>Bread</td>
<td>High</td>
</tr>
</tbody>
</table>

Phenology was scored using the Zadoks decimal scale (Zadoks et al., 1974), considering a phenophase when >50% of the population demonstrated the related characteristics. This study focused on two key developmental phases: booting (Z4.5) and heading (Z5.5). For the measurements of water-soluble carbohydrates, measurements were made at heading (Z5.5) and physiological maturity (Z8.7).

The genotypes selected for this study, including their genotype identification numbers (GIDs), cross names, type and their broad tolerance to heat based on previous evaluations utilising CIMMYT germplasm that has been sown over several field seasons under yield potential and heat stress conditions (M. P. Reynolds & G. Molero, unpublished).

Late sowing In 2021, data were collected from a late-sown trial at heading where all 12 genotypes were sown on 26 January 2021, 5 wk later than the regular sowing. Drip irrigation was applied every 10–15 d.

Nocturnal temperature treatment

Twenty-one heaters (FTE-1000 model, Comstock Park, MI, USA) were placed along the perimeter of the heat treatment blocks (Kimball, 2005) on a metal structure of 7.1 × 7.1 m surrounding each of the two heated blocks (Supporting Information Fig. S1) at 1.2 m above the canopy to maintain +2°C higher than the canopy temperature of the control blocks. Four infrared temperature sensors were placed in the corners of each of the four blocks (IRTS Apogee Instruments Inc., Logan, UT, USA) at an inclination of 45°. These sensors relayed temperature on the proportional integral derivative (PID) algorithm formulated in Kimball (2015), Kimball et al. (2015). Data were logged every 15 min (CR1000; Campbell Sci Inc., Logan, UT, USA).

Crop measurements

Water potential Leaf water potential (Ψ) was measured using a pressure chamber (Model 610; PMS instruments, Corvallis, OR, USA) following the method of Argentel-Martínez et al. (2019). Two or three flag leaves per plot were harvested and measured at predawn (Ψpd) and midday (Ψm).

Water-soluble carbohydrates To determine the concentration of water-soluble carbohydrates (WSCs), 12 fertile stalks (including leaves, leaf sheaths and heads, but not senesced plant material) were randomly sampled from each plot at heading and physiological maturity. The spikes were removed, and culms dried at 60–75°C until constant weight. Once dry, the leaf lamina and sheath were discarded. After measuring stem dry weight, samples were ground in a mill through a 0.5 mm screen and analysed using the Anthrone method (Yemm & Willis, 1954).

Flag leaf nitrogen and carbon content Three flag leaves were collected at heading in the 3 yr, oven-dried at 65°C for 48 h and ground to fine powder. Total nitrogen and carbon was estimated by flash combustion using an elemental analyser (Flash 2000; Thermo Scientific, Waltham, MA, USA).

Porometry Porometers (Table S1, Li-600; Li-Cor, Lincoln, NE, USA) were used to measure the response of gs and gso on the adaxial (top) and abaxial (bottom) flag leaf surfaces. Measurements were taken in 2021 and 2022 only. For 2020, this equipment was not available. For gs, measurements were taken across 3–4 clear, sunny days between 9:30 h–24:00 h and <7°C after irrigation. For gso measurements occurred 30 min after sunset (c. 19:00 h) for a period of 1–2 h before dewfall. Nocturnal measurements were made on the same days that diurnal measurements were conducted. Two to three measurements were taken for the adaxial and abaxial leaf surfaces of the same leaves. Four to six measurements were made per genotype per treatment (n = 2 biological replicates).

Gas exchange Measurements of A, gs, Rd and gso were taken using infrared gas analysers (LI-6400-XT; Li-Cor). For daytime measurements, survey-style measurements were conducted on flag leaves under 1800 μmol m−2 s−1 photosynthetic photon flux density (PPFD), using a 2 cm2 leaf chamber with a blue-red LED light source. Leaf temperature and VPD were maintained at 26°C (±1°C) and 1.2 kPa, respectively, while extracellular CO2 concentration (Ci) was maintained at 400 μmol mol−1. At night, leaf temperature was 16°C (±1°C), while leaf VPD and Ci were kept the same as during the day. Measurements were taken as soon as values stabilised (1–2 min). Two to three measurements were made per genotype per treatment (n = 2 biological replicates), at booting and heading across all 3 yr of measurement (2020–2022).

Yield components and final yield Grain yield (GY) was evaluated after genotypes had reached harvest maturity. Yield components were also measured: 1000-grain weight (TGW), harvest index (HI), final aboveground biomass (BM), grain number
(GN), spike number (SN), grain per spike (GPS), grain weight per spike (GWPS), grain-filling period (GFP), plant height (PH), grain yield production rate (GYPR = GY d⁻¹) and crop growth rate (CGR = BM d⁻¹). These evaluations follow the methodology of Pietragalla et al. (2012) and Pask et al. (2012). All traits related to yield and yield components were scaled on a per area basis for consistency.

Statistical analyses

Data were analysed utilising a mixed linear model, accounting for year (2020–2022; fixed), treatment (heat, control; fixed), genotype (fixed), replicate/block (random), stage (booting, heading; fixed) and time (only for WP: predawn, midday; fixed) as factors. When applicable, individual plot, row and/or column were included as random factors. For the porometer data, leaf surface was also included as fixed factor. For the late-sown material, overall parameter means were compared between the late-sown data and regular-sown heat and control plots. Mean discrimination between factors was determined utilising Tukey’s honest significant difference (HSD). Means were compared through the Tukey’s test at 95% of confidence. Visualisation and analysis of the data were performed in R (R Core Team, 2022).

Results

Field conditions

Maximum ($T_{\text{max}}$) and minimum air temperature ($T_{\text{min}}$) averaged across the control and night heated treatments varied significantly across years. The regular sowing of 2020 experienced a significantly lower $T_{\text{max}}$ while having a significantly higher $T_{\text{min}}$ compared with 2021 and 2022 and the late sowing treatment in 2021 (Fig. 1). The two subsequent field seasons did not vary significantly for $T_{\text{max}}$ or $T_{\text{min}}$. The late sowing treatment during the 2021 field cycle was significantly ($P<0.0001$) warmer for $T_{\text{max}}$ across all years (Fig. 1).

Conditions within T-FACE set-up and canopy temperature

The T-FACE system maintained the canopies of the heated blocks at 2°C above the temperature of the control blocks. An average of 87% of nights of the season across the 3 yr of experiments recorded temperatures of $>1.8°C$ (±10%) above the temperature of canopies in the control blocks, with the remaining 13% reporting $<2°C$ increases probably due to high wind (Tables S2, S3). VPD increased minimally in the heated plots (Fig. S2), an increase of 0.07 kPa between the heat and controls across the 3 yr (Table S3).

While the average $T_{\text{min}}$ of the nocturnally heated plots achieved 8.3°C for the measurement period in 2021, the late sowing plots achieved an average $T_{\text{min}}$ of 10.3°C during the nights between booting and heading (Fig. 1).

Crop measurements

Phenology Pheno...
There were no genotype-specific significant differences either predawn ($P = 0.988; 2.5 \pm 0.3$ MPa) or at midday ($P = 0.978; 0.7 \pm 0.5$ MPa) for the 3 yr of experiments under control or heated conditions at either growth stage. $\Psi_{PD}$ was 2.9-fold higher in the late sowing ($1.8 \pm 0.35$ MPa) compared with the control plots from the regular sowing ($0.6 \pm 0.19$ MPa).

**Porometry – $g_{sn}$ and $g_s$ variability by leaf surface**  The increase of $g_s$ and $g_{sn}$ on the adaxial leaf surface was genotype-specific but independent of year or treatment ($P<0.0001$ for $g_s$; $P=0.0199$ for $g_{sn}$, Fig. S4). During the daytime, $g_s$ was 95% higher on the adaxial surface ($0.143 \pm 0.12$) compared with the abaxial ($0.073 \pm 0.06$ mol m$^{-2}$ s$^{-1}$). Similarly, $g_{sn}$ was 55% higher on the adaxial surface ($0.013 \pm 0.01$) compared with the abaxial.

Mean $g_{sn}$ for the adaxial leaf surface was only 11 and 13% higher than abaxial values at booting and heading, respectively ($P>0.05$). Mean $g_s$ and $g_{sn}$ for the adaxial leaf surface were also significantly higher in the heated and in the control blocks when compared to the abaxial measurements ($P<0.0001$, Table 2). The extent of the differences between leaf surfaces was highly dependent on treatment, showing that mean $g_s$ and $g_{sn}$ on the control blocks were 100% and 8% higher on the adaxial leaf surface compared with the abaxial measurements ($P<0.0001$ and $P=0.355$, respectively) and 85 and 26% higher on the heated blocks ($P<0.0001$ and $P=0.012$, respectively).

The increase of $g_s$ and $g_{sn}$ on the adaxial leaf surface was genotype-specific but independent of year or treatment ($P<0.0001$ for $g_s$; $P=0.0199$ for $g_{sn}$). During the daytime, $g_s$ was 95% higher on the adaxial surface ($0.143 \pm 0.12$) compared with the abaxial ($0.073 \pm 0.06$ mol m$^{-2}$ s$^{-1}$). Similarly, $g_{sn}$ was 55% higher on the adaxial surface ($0.013 \pm 0.01$) compared with the abaxial. For the late sowing, the magnitude of $g_s$ and $g_{sn}$ was also surface-specific ($P<0.0001$, $P=0.022$, respectively).

Nocturnal stomatal conductance ($g_{sn}$) as percentage of diurnal stomatal conductance ($g_s$) averaged across 2 yr of experiments (2021 and 2022) under field conditions. Means and standard deviations for $g_s$ and $g_{sn}$ under treatments, stages and leaf surfaces are shown. The $P$ value is indicated for the effect of (a) treatment or (b) leaf surface for each growth stage.

**Gas exchange data CO$_2$ assimilation – $A$.** Photosynthetic CO$_2$ assimilation was significantly ($P<0.0001$) higher in 2022 than in 2020 (+26.39%) and 2021 (+28.91%, Fig. S5). Nocturnal
<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Treatment</th>
<th>Mean $g_{an}$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>Mean $g_s$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>$g_{an}$ as a % of $g_s$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Booting</td>
<td>Control</td>
<td>0.048</td>
<td>0.291</td>
<td>16.8 ± 1.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Heat</td>
<td>0.026</td>
<td>0.287</td>
<td>8.8 ± 1.07</td>
<td></td>
</tr>
<tr>
<td>Heading</td>
<td>Control</td>
<td>0.063</td>
<td>0.231</td>
<td>32.7 ± 1.35</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Heat</td>
<td>0.037</td>
<td>0.261</td>
<td>15.8 ± 1.32</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Leaf surface</th>
<th>Mean $g_{an}$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>Mean $g_s$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>$g_{an}$ as a % of $g_s$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Booting</td>
<td>Adaxial</td>
<td>0.036</td>
<td>0.363</td>
<td>10.4 ± 1.07</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>0.032</td>
<td>0.214</td>
<td>15.1 ± 1.07</td>
<td></td>
</tr>
<tr>
<td>Heading</td>
<td>Adaxial</td>
<td>0.052</td>
<td>0.341</td>
<td>16.1 ± 1.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>0.046</td>
<td>0.151</td>
<td>32.3 ± 1.41</td>
<td></td>
</tr>
</tbody>
</table>

heating had a significant positive effect at booting in 2020 (+9.57%, $P=0.0062$), but not at heading or at any growth stage in 2021 or 2022. A declined with age, falling between 19.93% (2022 – heated plots) and −43.47% (2020 – heated plots) between booting and heading. No significant interaction was determined between growth stage and heat treatment for 2021 or 2022. No significant differences were determined between genotype and treatment at booting ($P>0.08$) or heading ($P>0.42$) for any year.

**Daytime stomatal conductance** – $g_s$. $g_s$ was significantly different between all years ($P<0.0001$, Fig. S8). The lowest values were observed in 2020 (0.025), while the highest values were measured in 2021 (0.057). Measurements in 2022 were between these 2 yr at 0.046. There was no significant effect of heating in 2021 ($P=0.127$); however, $g_s$ significantly declined between booting and heading ($P<0.0001$). In 2020 and 2022, $g_s$ significantly declined in response to heat ($P<0.0201$). There was no effect of growth stage in 2020 ($P=0.516$) or 2022 ($P=0.225$) and no interactions between growth stage and treatment for any of the measurement years ($P>0.216$). Although there were significant genotype-specific differences in 2020 and 2022 (Fig. S8, $P<0.003$, booting), there was no interaction between the individual genotypes and nocturnal heat. (Fig. 3, $P>0.163$).

**Nocturnal observations.** No one genotype consistently achieved the highest $R_d$ or $g_{an}$ across growth stages and under control or heated conditions over all 3 yr of measurement. There was no trend between $R_d$ and magnitude of $g_{an}$ under control ($P=0.306$) or heated ($P=0.108$) conditions.

**The relationship between $g_s$ and $g_{an}$**. Irrespective of treatment or growth stage, there was a significant, weak positive correlation between $g_s$ and $g_{an}$ ($P=0.0139$ ($R_{adj}=0.035$ – Fig. S9)) when the data are separated by year, there is no trend between the magnitude of $g_s$ and $g_{an}$ irrespective of treatment (Fig. S10).

Finally, $g_{an}$ as a proportion of $g_s$ was calculated (Table 3). In general, $g_{an}$ made up a greater proportion of daytime $g_s$ in the control plots and at heading. Heating led to a decrease in this proportion for both growth stages measured; however, this decrease was much greater at booting. There was a significant effect of heating ($P=0.0239$) and growth stage ($P<0.0001$) but no interaction between them ($P=0.7536$).

Mean nocturnal conductance ($g_{an}$) as a percentage of daytime stomatal conductance ($g_s$) at booting or heating under control or nocturnally heated plots over the 3 yr. Data are the means ($n=6$, biological replicates, with standard deviation).
Comparing the response of regular and late-sown material. While no significant difference \((P = 0.77)\) was determined between the regular-sown plants, the late-sown plants demonstrated significantly lower \(A\) (Fig. S11a) compared with the control \((59.0\%)\) and heated plots \((60.4\%)\). Similarly, \(g_s\) in the late-sown plants exhibiting \(76.6\) and \(75.2\%\) declines compared with regular-sown control (Fig. S11b, \(P < 0.0001\)) and nocturnally heated \((P < 0.0001)\) plots.

There was no significant difference between the regular sowing treatments for \(g_s\) \((P = 0.76)\). \(R_d\) was highest in the late-sown plants (Fig. S11c), significantly higher than the plants under nocturnal heating \((P < 0.0001)\) but not significantly different to the regular-sown plants under control conditions. Finally, late sowing had a significant \((P < 0.0001, \text{Fig. S11d})\), negative impact on rates of \(g_{sn}\). Values were \(71.4\) and \(68.0\%\) lower than those observed in the regular-sown control or nocturnally heated plots, respectively.

### Water-soluble carbohydrates, carbon and nitrogen content

Water-soluble carbohydrates (WSC – Fig. 4) consistently decreased in all plots from heading to maturity. In 2020, the average WSC at heading was significantly lower \((c. \, 7\% \text{ to } 14\%, \, P < 0.05)\) than in 2021 and 2022. Conversely, at maturity, the average WSC in 2020 was highest (although not significantly for treated plots; \(P > 0.05\)). The nocturnal heat treatment resulted in higher concentration of WSC compared with control plants at heading in 2020 \((4.4\% \text{ higher}, \, P < 0.05)\). A similar trend \((P > 0.05)\) was observed during heading in 2022. WSC between treatments were not significant at maturity in any of the years.

Nocturnal heating consistently decreased the carbon content in the 3 yr \((C \text{ – } P < 0.001 \text{ – } \text{Fig. 5a})\); in 2021, the carbon content \((41.9\%)\) was significantly higher \((P < 0.001)\) than in 2020 \((41.4\%)\) and 2022 \((41.2\%)\). The average carbon content in the heated and control plots was \(41.2\) and \(41.8\%,\) respectively. There were no genotypic differences \((P > 0.05)\).

Significant differences were determined for flag leaf nitrogen content \((N \text{ – } \text{Fig. 5b, } P = 0.049)\) between the control \((4.17 \pm 0.44\%)\) and the nocturnally heated plots \((4.3 \pm 0.50\%).\)
There was no significant interaction between measurement year and treatment and no significant effect of genotype ($P > 0.05$).

The C:N ratio was significantly ($P = 0.00549$ – Fig. 5c) higher in the control plots (10.1) compared with the nocturnally heated plants (9.7). Irrespective of treatment, plants measured in 2022 (10.6) had significantly higher ratios than those observed in 2020 (9.7) and 2021 (9.5, $P < 0.0001$). No significant interactions were determined between measurement year and treatment ($P > 0.05$). No significant genotypic effects were found ($P > 0.05$).

**Yield and yield component trends**

Combining the data across years, significant differences were found among genotypes for yield and yield component traits (Figs 6, S12, S13). The genotypes with the highest average yields across years in control conditions (Fig. 6a) were GID-5077000 (576 g m$^{-2}$) and GID-7806808 (566.3 g m$^{-2}$), while the highest under nocturnal heat were GID-5077000 (557.8 g m$^{-2}$) and GID-7171118 (550.16 g m$^{-2}$). The lowest yielding genotypes under control conditions were GID-2465 (438.6 g m$^{-2}$) and GID-3855011 (488.6 g m$^{-2}$), while under heat treatment it was GID-2465 (373 g m$^{-2}$) and GID-7129721 (457.3 g m$^{-2}$). Interestingly, GID-2465, GID-3855011 and GID-7129721 are all considered high heat tolerance genotypes, while GID-5077000 and GID-7806808 are intermediate and GID-7171118 is considered a low heat tolerance genotype (Table 1).

Significant differences were determined between treatments for grain yield (GY), harvest index (HI) and biomass (BM; Fig. 6a,c,d) across combined year data. GY, BM, grain number (GN)N and crop growth rate (CGR) overall were significantly lower ($P < 0.05$) in the nocturnal heat treatment (Figs 6a,d, S12, S13). While GY varied between years, while BM was consistently lower across all 3 yr. On average, GY and BM were 3.83 and 5.75% lower, respectively, in the heat treatment relative to the
control plots. HI was significantly higher under nocturnal heat conditions (+2.66%) relative to the control. However, some key yield component traits, such as TGW, were not significantly impacted by the heat treatment (Fig. 6b).

A significant year effect was seen for all evaluated yield and yield component parameters; however, interactions were not detected between year and genotype for GY, TGW, GN, SN, GPS, GWPS, GFP and GYPR. Interactions were found between year and treatment for GY, TGW, HI, PH, GWPS, GFR and GYPR. No differences were determined between years for biomass, grains per spike (GPS) and crop growth rate (Fig. S13). Finally, the only significant interaction found between year, treatment and genotype was for SN (Fig. S12).

Discussion

There is growing evidence that crop responses to rising nocturnal temperatures are a key, underexplored source of variation in determining climate resilience. Although seemingly less severe, nocturnal warming can last much longer than excessive daytime heat, exposing the crop to a chronic rather than acute stress (Cox et al., 2020; Sadok & Jagadish, 2020). Often overlooked as a period of plant inertia, night-time processes such as gs, nocturnal transpiration, respiration, fluctuating carbohydrate reserves, hydraulic redistribution and water potential are part of an adaptive and integral diurnal strategy to enable survival to both short- and long-term temperature stresses.

While traditional assessment of wheat heat tolerance, using techniques such as late-season sowing (Langridge & Reynolds, 2021), may offer a broad determination of yield under high temperatures, this study highlights that genotypes classified as traditionally heat tolerant were sensitive to small increases in night-time temperature even when no daytime stress was applied (Fig. 6). Furthermore, while nocturnal processes (gs, Rs, Ψ – Figs 3, S3, S7) responded to warmer nights, daytime measurements (A, gs – Figs S4–S6) were significantly less sensitive suggesting distinct night/day metabolic acclimation.

Over the 3 yr of treatments, and under well-irrigated conditions and artificially warmer nights, there was a cumulative negative effect on field grain yield; 3.83% overall – 1.9% per 1°C. At the leaf level, gs was genotype-specific and more negatively affected by age than warming night-time temperatures. As the plants aged, irrespective of treatment, rates of gs made up a greater proportion of total stomatal conductance as gs declined more than gsn (Table 2). Water potential mirrored the response of gs; increasing with age and in response to nocturnal heat (Fig. 2). Instead of an expected increase, Rs declined with age and in response to heat, indicative of rapid and sustained acclimation. As expected, late-sown plants experienced much more severe declines in A, gs and gsn, with Rs increasing (Fig. S11).

Using these responses and observations, we can contribute to the schematic proposed by Sadok & Jagadish (2020), drawing attention to key discrepancies in the response of wheat to
nocturnal warming, while also highlighting key areas for research focus (Fig. 7).

Stomatal responses and water availability

One of the core climactic changes associated with nocturnal warming is the decrease in available water, caused by increases in evapotranspiration, removing topsoil moisture and increasing canopy VPD. Using the T-FACE set-up, we observed minimal changes in canopy VPD with a 2°C increase in $T_{\text{min}}$ (Fig. S2) suggesting $g_{\text{sn}}$ (or $g_s$) was not responding to changes in humidity. The increase in $g_{\text{sn}}$ with age and the decline in leaf water potential in the heated plots predawn suggests a response to declining soil water availability. Less soil water uptake and higher $g_{\text{sn}}$ could indicate attempts to improve nocturnal redistribution in the warmer plots as the season progressed (Huang et al., 1991; Lombar-dozzi et al., 2017).

Stomatal responses measured in the field are strongly linked to water availability with the decline in water potential greatest in the heated plots predawn (Fig. 2). Increases in $g_{\text{sn}}$ have been linked to possible decreases in soil water availability, reducing available water at the roots produced by nocturnal hydraulic redistribution. A decrease in available topsoil water may be the indirect cause of genotype-specific differences in $g_{\text{sn}}$ (Saradadevi et al., 2015), with nocturnal heating having a greater detrimental impact on genotypes with wider, shallower rooting profiles and reducing the water available from heavy dewfall under control conditions. When compared to flood irrigation, drip-irrigated wheat produces a greater number of longer roots at the surface (Chen et al., 2021). The decrease in water potential both predawn and midday in the heated treatments (Fig. 2) suggests a decline in water availability in the nocturnally heated plots. While changes in daytime $g_s$ were minimal (Fig. S6), significant declines in $g_{\text{sn}}$ (Figs 3, S8) suggest a greater sensitivity to water availability than daytime $g_s$ (Gowing et al., 1990; Comstock, 2002). The lack of
change in $g_c$ also indicates changes in $g_n$ are not the result of the presence of the hormone, abscisic acid (ABA) which would likely affect daytime magnitudes of water loss both during the day (Fricker & Wilmer, 1996) and at night (Rawson & Clarke, 1988; Howard & Donovan, 2007). Finally, lower $g_n$ mirrored lower or indifferent leaf respiration rates (Fig. S7) and potential decrease in $O_2$ consumption, which can occur with warming nights (Coast et al., 2021; Posch et al., 2022a,b).

Unlike growth room or glasshouse studies, the magnitude of $g_n$ and $g_c$ appear uncoupled in the field (Fig. S11) and in response to heat (Fig. S11). Similar to previous studies (McAusland et al., 2021), genotype-specific significant differences were determined in $g_n$ in the field (Fig. 3). The highest $g_n$ genotype, CIRNO, achieved similar values to those reported in growth room conditions, making up c. 9–19% of daytime rates of $g_c$, $g_n$ declined with both age and nocturnal heat; however, the reduction in daytime $g_c$ was more strongly influenced by age than nocturnal heating, enabling $g_n$ to make up a greater proportion of water loss as the plants matured (Table 3). It was also interesting to note that the magnitude of $g_n$ was leaf-surface-specific with the top of the leaf losing more water than the bottom at night (Table 2). This observation is supported by McAusland et al. (2021) and Wall et al. (2022), suggesting that the leaf surfaces can respond in both an independent manner but also coordinate to specific environmental stimulus (Wall et al., 2022). While Wall et al. focussed on responses to light, $g_n$ could be responding to other stimuli such as [CO$_2$] or [O$_2$] as a result of $R_d$. For this study, measurements were taken 30 min–1 h after sunset; it is possible greater variation of $g_n$ exists throughout the night and predawn (Resco de dios et al., 2016), although dewfall in the field prevented these measurements being collected. More work is needed to link magnitudes of surface-specific $g_c$ and $g_n$ to root water availability, uptake and transport under heat (Wang et al., 2021).

The role of respiration and acclimation in nocturnal heating

Rates of respiration are a major determinant in biomass accumulation, with up to 50% of carbon assimilated during the day being respired (Poomter et al., 1990). Typically, $R_d$ has been shown to increase in response to short periods of nocturnal warming (e.g. Impa et al., 2019); however, our results show that respiration was genotype-specific and declined in response to prolonged nocturnal heat (Fig. S7). In general, $R_d$ increases in response to increasing temperature (Atkin & Tjoelker, 2003), supporting increased maintenance and potentially diverting carbon away from growth. Our data suggest the genotypes acclimated to the prolonged nocturnal warming and declining with age. Recent work by Posch et al. (2022a,b) provides strong evidence that nocturnal warming is a stronger driver of respiratory acclimation than daytime maximum temperatures. While the authors noted no change in CO$_2$ release, oxygen consumption declined suggesting an increase in the respiratory quotient (Posch et al., 2022a,b). Interestingly, work by Bruhn et al. (2022) suggests that temperature accounts for less than half (48%) of the variation observed in $R_d$ and that $R_d$ declines through the night.

While genotype-specific differences in $R_d$ were only observed at booting in 2020, assessment at different times of night and determining the rate of acclimation, rather than measurements of $R_d$ at set phenological stages, may yield greater genetic variation between the genotypes (Bruhn et al., 2022).

In this study, $R_d$ did increase in response to the severe heat stress associated with late sowing; interestingly, the average $T_{min}$ of the regular-sown, nocturnally heated plots (11.7°C) was not dissimilar to the average $T_{min}$ of the late-sown plots (10.3°C). This suggests the higher daytime temperatures (and potentially light intensities) elicited a greater negative impact on $R_d$ than nocturnal temperatures alone.

Under high daytime temperatures, increases in $A$ can support higher daytime respiration. However, at night, respiration relies on stored assimilates. As observed for booting plants in 2020, this can result in increases in $A$ as a compensatory response to meet increased carbon demand (Turnbull et al., 2002; Fig. S5). There was a decline in leaf carbon content (Fig. 5a) for the regular-sown plants across the 3 yr of measurements, suggesting a negative impact of nocturnal heating on the carbon supply; however, in two (2020 and 2022) of the three measurement years, the percentage of water-soluble carbohydrates increased with nocturnal heating. Although not reflected in the gas exchange data, this suggests greater reserves of carbon at the stems and leaf sheaths to compensate for reduced photosynthates generated at the leaves.

Nocturnal heating reduced grain yield and biomass production

Previous T-FACE experiments have shown varied results relating to yield. For example, grain yield did not change significantly under combined, elevated day and night ambient temperature conditions in field-grown rice (Wang et al., 2022). In winter bread wheat and durum wheat (CIRNO), increasing ambient temperatures by 1.5°C and 2°C throughout the entire growing season reduced grain yield by 16.3–19.6% and 33%, respectively (Tian et al., 2014; Garatuza-Payan et al., 2018). A recent winter wheat study found that an increased nocturnal temperature by c. 1.4°C led to higher yields albeit in a single genotype (Fan et al., 2022).

Over 3 yr of measurements, we found that increasing nocturnal ambient temperature by c. 2°C significantly reduced yield and biomass production by 1.9% per 1°C increase in $T_{min}$ despite average $T_{min}$ being higher than the reported 14°C for decreasing yields in the literature (Fig. 1, Lobell & Ortiz-Monasterio, 2007). Overall, this was supported by decreases in leaf C:N ratio implying a reduced carbon availability, altered partitioning or increased N assimilation/mobilisation. Interestingly, water-soluble carbohydrates in the stems increased in the nocturnally heated plots at heading for 2020 and 2022 (Fig. 4), suggesting enhanced partition toward stem (Blum & Johnson, 1993); however, both control and heated WSC declined at maturity as the grain ripened to compensate for decreased photosynthetic contribution from the leaves due to age. Overall, there was no change in grain weight with increased nocturnal warming, suggesting the plants did not heavily rely on stored stem WSC to
maintain yields under high nocturnal temperatures under irrigation (Zhang et al., 2014; Ovenden et al., 2017).

The response of harvest characteristics to nocturnal heat was genotype-specific, with some demonstrating increases in grain yield and yield component traits under elevated nocturnal temperature (Fig. 6). Despite decreases in overall GY and biomass production, HI significantly increased overall in the heat treatment relative to the control, perhaps the result of larger reductions of biomass relative to grain yield under heat conditions. Despite observing significant changes to overall yield and biomass, 1000-grain weight did not respond to the treatment. While nocturnal warming of the postanthesis has been implicated in reductions in grain weight (García et al., 2016), these data suggest that our reductions in yield are not due to changes in grain weight, but a decrease in the number of grains (Figs 6a,b, S12; Wang et al., 2020). Grain number (GN) is determined by duration of spike growth and fruiting efficiency or fertility index (Zhang et al., 2019; Langridge & Reynolds, 2021). In this study, nocturnal warming of c. 2°C was insufficient to significantly change the phenology of the plants under treatment (Table S2) suggesting a reduction in GN may be the result of reduced carbohydrate availability during spike development or spike fertility (Prasad et al., 2011; Draeger & Moore, 2017; Rieu et al., 2017; Narayanan et al., 2018).

Interestingly, genotypes that had high tolerance to heat were not always the highest yielding (and vice versa). For example, GID-2465 and GID-7127271 were classified as having high heat tolerance. However, both were among the lowest yielding genotypes under warmer nights. Previous heat tolerance was determined via exposure to long-term heat stress through late sowing where plants would be exposed to higher day- and night-time temperatures throughout development. However, the relative increase in daytime temperature far exceeds the increase in nighttime temperature. Although less severe by magnitude, nocturnal warming can last much longer than excessive daytime heat (Stilmann et al., 2013; Sadok & Jagadish, 2020), exposing the crop to a chronic rather than acute stress.

These results suggest that evaluation of heat performance largely based on $T_{\text{max}}$ which is experienced during the day, may not help inform performance under elevated $T_{\text{min}}$. Consequently, current high-temperature-tolerant genotypes may not be adapted to rising $T_{\text{min}}$ requiring a redefinition of metrics for heat tolerance - A greater understanding of the mechanism of yield – driven by $T_{\text{min}}$, $T_{\text{max}}$ and their interactions is needed. Future emphasis could be placed on understanding the mechanisms of individual wheat genotypes that had increases or no significant penalty to yield and yield components, such as GID-7171118 (Fig. 6). For example, processes such as the flexibility of PSII (Coast et al., 2022), the magnitude and ability of respiration to acclimate (Posch et al., 2019), pollen temperature sensitivity and the degree of acquired thermodurability from past increases in temperature (Wang et al., 2012; Djanaguiraman et al., 2013; Echer et al., 2014; Müller & Rieu, 2016) may all contribute to genotype-specific responses to rising $T_{\text{min}}$.

Conclusions and future considerations

There is growing awareness of the negative impact rising nocturnal temperatures has on crop yields; however, understanding the physiological responses to this phenomenon remains limited. In Fig. 7, we highlight how this study fits within our current understanding of nocturnal temperature stress. Genotype-specific differences in day- vs night-time heat tolerance strongly suggest assessment of nocturnal thermodurability should be considered when selecting ‘heat tolerant’ genotypes for future climates. We also acknowledge that high temperatures impact grain quality (Hein et al., 2022; Impa et al., 2019) as well as quantity.

While there is some evidence that nocturnal warming at key growth stages and under irrigated conditions could improve yields (Chen et al., 2014; Fan et al., 2022), it is more likely that a combination of stresses, accelerated by warmer nights, contributes to their decline; for example, decreased water availability, high VPD and combined high $T_{\text{max}}$. There is a lack of fieldwork investigating the combined impact of these nocturnal factors on leaf-level traits and how they interact to ultimately determine yield. Nocturnal conductance remains a genotype-specific, elusive trait night which plays a role in whole-plant water efficiency, while very little work has connected $g_{\text{an}}$ with root development and physiology under nocturnal heat, or the potential interactions with night-specific field traits such as dewfall – an important characteristic of nocturnal foliar uptake in trees but not commonly observed in crop species (Schreel & Steppe, 2020). With this in mind, we suggest future work should prioritise elucidating the variation in nocturnal heat tolerance and determination of the impact of diurnal water acquisition on the magnitude of $g_{\text{an}}$.

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Data availability
All data supporting the findings of this study are accessible and published online at www.figshare.com under the DOI: 10.6084/m9.figshare.23104817 and can be accessed using the following link; https://figshare.com/s/6a469e2f6258bfcc27df.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Photograph of the infrared heating set-up and arrangement of the field plots.

**Fig. S2** Vapour pressure deficit measured across all 3 yr in the regular-sown control.

**Fig. S3** Response of leaf water potential predawn and at midday between 2020 and 2022.

**Fig. S4** Response of $g_s$ and $g_{no}$ between 2020 and 2022 as measured using the porometer.

**Fig. S5** Response of photosynthetic assimilation to nocturnal heating.

**Fig. S6** Response of daytime stomatal conductance to nocturnal heating.

**Fig. S7** Response of nocturnal respiration to nocturnal heating.

**Fig. S8** Response of nocturnal stomatal conductance to nocturnal heating.

**Fig. S9** Comparing the responses of $g_s$ and $g_{no}$ under control and nocturnally heated conditions.

**Fig. S10** Correlating $g_s$ and $g_{no}$ at different growth stages and in response to nocturnal heat.

**Fig. S11** Comparing the control and nocturnally heated responses of $A$, $g_s$, $R_d$ and $g_{no}$ to measurements made on late-sown plants.

**Fig. S12** Distribution of grain number, spike number, grain per spike and grain weight per spike.

**Fig. S13** Distribution of plant height, grain-filling period, grain yield production rate and crop growth rate.

**Table S1** Settings used for the porometer measurements during the day and at night.

**Table S2** Efficiency of the heating system.

**Table S3** Meteorological data of the heat and control plots across the whole crop.

**Table S4** Days to booting, heading and maturity across years and treatments season by Stage × Time × Year.

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