Nocturnal stomatal conductance in wheat is growth-stage specific and shows genotypic variation

Lorna McAusland1, Kellie E. Smith1, Alexander Williams1, Gemma Molero2 and Erik H. Murchie1

1School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, UK; 2International Maize and Wheat Improvement Center (CIMMYT), Km. 45, Carretera Mexico, El Baran Texcoco, CP 56237, Mexico

Author for correspondence:
Lorna McAusland
Email: lorna.mcausland@nottingham.ac.uk

Received: 3 March 2021
Accepted: 9 June 2021

New Phytologist (2021) 232: 162–175
doi: 10.1111/nph.17563

Key words: growth stage, nocturnal conductance, stomata, Triticum aestivum, water-use efficiency.

Summary

- Nocturnal stomatal conductance (g_sn) represents a significant source of water loss, with implications for metabolism, thermal regulation and water-use efficiency. With increasing nocturnal temperatures due to climate change, it is vital to identify and understand variation in the magnitude and responses of g_sn in major crops.
- We assessed interspecific variation in g_sn and daytime stomatal conductance (g_d) in a wild relative and modern spring wheat genotype. To investigate interspecific variation, we grew six modern wheat genotypes and two landraces under well watered, simulated field conditions.
- For the diurnal data, higher g_sn in the wild relative was associated with significantly lower nocturnal respiration and higher daytime CO2 assimilation while both species exhibited declines in g_sn post-dusk and pre-dawn. Lifetime g_sn achieved rates of 5.7–18.9% of g_d. Magnitude of g_sn was genotype specific and positively correlated with g_d. g_sn and g_d were significantly higher on the adaxial surface. No relationship was determined between harvest characteristics, stomatal morphology and g_sn, while cuticular conductance was genotype specific. Finally, for the majority of genotypes, g_sn declined with age.
- Here we present the discovery that variation in g_sn occurs across developmental, morphological and temporal scales in nonstressed wheat, presenting opportunities for exploiting intrinsic variation under heat or water stressed conditions.

Moreover climate change is resulting in global nocturnal (minimum, T_min) temperatures rising up 1.5-fold faster than daytime temperatures (Peng et al., 2004; Sillmann et al., 2013; Screen, 2014; Davy et al., 2017) leading to significant yield losses for major crop species such as rice (Welch et al., 2010) and wheat (Lobell & Ortiz-Monasterio, 2007; García et al., 2016). For a recent review, see Moore et al. (2021). These increases in T_min are having a greater impact on yield than daily maximum temperatures (T_max; Cossani & Reynolds, 2013; Martre et al., 2017). However, little consideration has been given to g_sn at different stages of development. In wheat, most research has focussed on the impact of rising T_min on the reproductive growth stages (e.g. anthesis and grain filling), reporting between 4% and 10% yield losses for every 1°C increase in T_min for winter (Hein et al., 2019) and spring wheat (Lobell & Ortiz-Monasterio, 2007; García et al., 2016). Any increases in diurnal ambient temperature are usually accompanied by increases in water use and loss by crops. At night, plants experience often highly contrasting environmental conditions to those experienced in the day; including significant drops in temperature, high humidity and dew-fall and lowered wind speed (Fig. 1; Jones, 2013).

Accounting for up to 55% of daytime water loss (Caird et al., 2007; Schoppach et al., 2014), g_sn occurs in crop and noncrop species alike (Resco de Dios et al., 2019). At a larger scale, landscape models indicate that an increase in g_sn from 0% to 5%
could account for a reduction in available soil moisture of up to 50% in semiarid areas, emphasizing the vital but often overlooked role of $g_{sn}$ in large-scale water systems as many models assume $g_{sn}$ to equal 0 (Lombardozi et al., 2017). Although high $g_{sn}$ appears indicative of low water-use efficiency (Claverie et al., 2018; Schoppach et al., 2020), as yet, a single species-wide role of $g_{sn}$ has yet to be established (Caird et al., 2007; Fricke, 2019; Resco de Dios et al., 2019).

Roles for $g_{sn}$ have been proposed: positive correlations have been observed between $g_{sn}$ and the breakdown of starch (Easlon & Richards, 2009; dos Anjos et al., 2018) and a role in the maintenance of growth (Fricke, 2019); promoting continuous water flow from the roots, aiding nutrient uptake and distribution while maintaining the turgor required for expansion (Donovan et al., 2001; Snyder et al., 2003; Marks & Lechowicz, 2007). Conversely, high $g_{sn}$ has also been shown to reduce hydraulic redistribution, limiting transpiration the following day. This could culminate in reduced plant productivity if daily water-use efficiency ($W_{i}$) is low (Howard et al., 2009). Noted by Resco de Dios et al. (2016), $g_{sn}$ is often higher pre-dawn than post-dusk,

Fig. 1 A schematic summarizing the main factors influencing diurnal water movement. At night, low temperatures, low vapour pressure deficit (VPD) and wind speed culminate in low boundary layer conductance. Accordingly, high boundary layer resistance limits the rate of nocturnal transpiration ($T_{n}$), stomatal conductance ($g_{sn}$) and minimum leaf conductance ($g_{min}$). Under the cooler nocturnal temperatures, the canopy also experiences lower rates of respiration and less water loss from the soil. The soil is cooler and hydraulic redistribution may occur in the root zone. As dawn approaches, dew-fall and guttation can occur as water vapour in the air condenses on the cooler leaf surface and hydathodes expel concentrated solutions to a near water vapour saturated boundary layer. Foliar uptake of $H_2O$ occurs in some species, providing an alternative water supply to the aerial parts of the plant. As the sun rises, temperatures and wind speeds increase, leading to high VPD and low boundary layer resistance. These conditions promote high rates of soil evaporation, root water uptake, transpiration and stomatal conductance. Under these conditions, characteristics such as high leaf emissivity and glaucousness serve to deflect high intensity solar radiation, cooling the leaf to maintain low levels of photosynthesis, protect from photoinhibition and mediate evaporative cooling under increased temperature loads. Schematic modified from images created by Carvalho (2019) and Lobell (2017). Arrows indicate plant (green), soil (blue) and environment (grey)-centric processes.
leading to the conclusion that this behaviour also serves to max-
imise carbon acquisition at dawn when VPDs are low (Resco de
Dios et al., 2016; Schoppach et al., 2020). This antecedent re-
sponse highlights a potential role for \( g_{\text{an}} \) to coordinate daily
time exchange of \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) in response to either a circadian cue (e.g. dawn or dusk) or a consistent but short-term event, for example heatwaves, increased soil water availability or decreased availability of starch under low \( \text{CO}_2 \).

As \( g_{\text{an}} \) regulates the exchange of \( \text{CO}_2/\text{H}_2\text{O} \) during the day, \( g_{\text{an}} \) could be a potential mechanism for facilitating the uptake of \( \text{O}_2 \) and release of respiratory \( \text{CO}_2 \) at night. With up to 70% of daily net photosynthetic carbon fixation estimated to be re-released via respiration in the following evening (Atkin et al., 2005, 2007; Liang et al., 2013), \( g_{\text{an}} \) could play a part in facilitating nocturnal cellular expansion and repair (Daley & Phillips, 2006; Even et al., 2018; Fricke, 2019). Recently an optimisation model was proposed based upon trade-offs between leaf temperature, evaporative cooling and respiration (Wang et al., 2021).

While the number of publications into crop \( g_{\text{an}} \) is growing (Resco de Dios et al., 2019), most studies have focussed on the response of \( g_{\text{an}} \) in specific developmental periods, for wheat the reproductive growth stages (booting to anthesis). Clearly different growth stages in wheat represent substantial changes in above and below ground architecture, metabolic capacity and hydraulic conductance. Understanding the magnitude of \( g_{\text{an}} \) and whether it changes with phenology is vital in identifying a role for \( g_{\text{an}} \) but also in generating targets for improving heat and drought tolerance. We hypothesise that nocturnal water loss in the earlier growth stages could have important implications for establishment and relative growth rate when sensitivity to temperature changes is greater, due to the smaller size of the plants (Slafer & Rawson, 1995). We also hypothesise that \( g_{\text{an}} \) is higher in genotypes with intrinsically higher \( g_{\text{a}} \) and lower \( W_{\text{r}} \) a relationship that has not been fully established (Rawson & Clarke, 1988; Schoppach et al., 2014; Resco de Dios et al., 2019). A final omission in the assessment of \( g_{\text{an}} \) is the use of realistic and appropriate conditions for precise experimentation over diurnal periods. To overcome this we utilised state-of-art controlled environment technology to simulate realistic diurnal fluctuations in light and temperature. The objective of this study was first to investigate genotype-specific differences in \( g_{\text{an}} \), assessing the contribution of \( g_{\text{an}} \) for a modern \( T. \text{ aestivum} \) and wild relative, \( T. \text{ urartu} \). Eight wheat cultivars were then investigated for genotypic variation in \( g_{\text{an}} \) under simulated conditions of north-western Mexico, and whether these differences were growth-stage specific. \( g_{\text{amin}} \) was assessed for each cultivar at flag leaf to determine the contribution of \( g_{\text{amin}} \) to \( g_{\text{an}} \) for each genotype. Finally, variation in stomatal density, size and ratio were determined, along with harvest characteristics.

**Materials and Methods**

**Diurnal glasshouse measurements**

Plants were grown and analysed at Sutton Bonington Campus, University of Nottingham during the period 7–15 November 2019. Five wild relative plants, *Triticum urartu* (accession no. 1010001) and five modern spring wheat cultivar, *T. aestivum* ‘Paragon’, were potted into soil (John Innes No. 2; J. Arthur Bowers, UK) and drip irrigated twice per day for 1 min with Hoagland’s solution. Glasshouse conditions were maintained at 25/18°C ± 2°C (day/night) under regular mildew, aphid and thrip control measures. Photosynthetic photon flux density (PPFD) was maintained to achieve 16 h of light using supplemental lighting (Son-T; Philips, Surrey, UK), applying up to 250 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) PPFD at plant height when ambient PPFD fell below 500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). During the measurement period, maximum PPFD at chamber height ranged between 143.7 and 484.0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

Using the flag leaf – decimal growth stage 39–40, as defined by the UK HGCA (AHDB) growth-stage guide, which is based on the Zadoks 100 point growth scale (Zadoks et al., 1974; AHDB, 2018) – the ambient response of \( A/R_{d} \) and \( g_{d}/g_{\text{an}} \) was monitored using an infrared gas analyser (IRGA) (LI-6800; Li-
Cor, Lincoln, NE, USA) fitted with a clear-topped chamber. Leaf areas for the modern genotype were between 4.8–6 cm² and 1.5–2.4 cm² for *T. urartu*. To enable true assessment of \( g_{d} \) and \( g_{\text{an}} \) to ambient conditions, a carboy bottle buffered the ambient air from the glasshouse to the IRGA. As such, \( \text{CO}_2 \) concentration was not controlled within the chamber. Measurements were taken every 5 min with a match every 30 min for 24 h. Due to the small signal-to-noise ratio of the *T. urartu* samples, measurements of \( R_{d} \) were discounted from the analysis and discussion.

One wild relative plant and one modern genotype were mea-
sured per 24 h period using two IRGAs. Key diurnal periods were compared between replicates; dusk (1 h before sunset: 21:00–22:00), dawn (1 h after sunrise: 06:00–07:00) and post dawn (4 h after sunrise: 10:00–11:00).

**Growth room conditions and plant material**

Six modern spring wheat cultivars were chosen: ‘Paragon’, ‘Cadena’, ‘Pavon76’, ‘Vorobey’, ‘Sokoll’ and ‘Borlaug100’. The latter three are common high yielding check cultivars grown in the Yaqui Valley (Obregon, Sonora, NW Mexico) as part of CIMMYT field trials. Two Watkins landraces were also selected with reported high (accession no. 468-W468) and low (accession no. 483-W483) intrinsic water-use efficiency. Seeds were strati-
fied for 3 d on damp filter paper at 5°C, sown into compost (Levingtons, M3, Everris, Ipswich, UK). After 2 wk, seedlings were potted into loam soil representative of the Centro Experi-
mental Norman E. Borlaug (CENEB) research station (Yaqui Valley, Sonora, Mexico; 27.370°N, 109.930°W). Plants were randomised and drip irrigated for 3 min 3x day⁻¹, increasing to 4 min at heading and 5 min at anthesis. When 80% of plants were at anthesis, a high nitrogen fertiliser was applied for the remainder of the growing period (5% OMEX Standard; OMEX Agriculture Ltd, Kings Lynn, UK).

To simulate Mexican monthly temperature fluctuations the average \( T_{\text{min}} \) and \( T_{\text{max}} \) ambient temperatures were calculated for the months December (sowing) to April (harvest) for 2014–2018 as obtained from a field weather station (2016–2018, CIMMYT,
Obregon, Sonora, Mexico) and a weather location located at Obregon airport (2014–2016, 18 km from the field station). These values were then applied to the model of Campbell & Norman (2012) to simulate the daily changes in temperature (Campbell & Norman, 2012; Fig. 2a) while humidity was maintained at 67.2% (±4.5%) with a mean VPD of 0.73 ± 0.35 kPa. PPFD was determined following a three-parameter Gaussian sigmoidal function (Fig. 2b), applying a maximum of 1000 μmol m⁻² s⁻¹ at solar noon (12:30 h) and providing a photoperiod of 16 h : 8 h, light : dark. This response was staggered to allow pre-dawn gas-exchange measurements to occur within normal working hours of 08:00 to 18:00. Carbon dioxide concentrations were also closely monitored (Fig. 2c). While mean ambient CO₂ slightly increased between December and March, the maximum mean increase was 4.8 ppm (1.1%) and reflected an increase in measurements. CO₂ then declined in March, the maximum mean increase was 4.8 ppm (1.1%) and reflected an increase in measurements. CO₂ then declined in February to April to concentrations similar to those observed at Obregon airport (2014–2016, 18 km from the field station). (a) The response of photosynthetic photon flux density (PPFD) was maintained throughout at a maximum of 1000 μmol m⁻² s⁻¹. Daily Tmin and Tmax were changed per month (b) for December (dark blue), January (light blue), February (pink), March (yellow) and April (red), according to average values established between 2014 and 2018 from weather stations on or close to a CIMMYT field site (Obregon, Sonora, Mexico). (c) CO₂ in the growth room was also monitored every 15 min throughout the growing period. Boxplots show the median (horizontal line), the quartiles (boxes). The whiskers represent 1.5-times the interquartile range above and below the 75th and 25th percentile respectively, with extreme values indicated as dots, measured during measurement periods. The mean [CO₂] for the month is indicated above each boxplot.

Cuticular or minimum conductance – g_min

Following the method of Sack et al. (2003), all measurements were made on fully expanded flag leaves, sampled from the second tiller 2 h post dawn. The excised ends were dipped in wax, photographed for area and weighed to 4 dp. These leaf sections were placed on coarse wire mesh shelving in a dark growth cabinet at 43.1 ± 1.4% RH and 25.9 ± 1.0°C for 1 h to close stomata. The mesh shelving allowed airflow to both sides of the leaf without damaging the leaf surface. Leaf sections were then weighed at intervals of 20 min over a period of 5 h and placed back on the mesh, alternating the sides that faced upwards for maximum contact with the circulating air. Cuticular transpiration was measured as the slope of water loss vs time; the slope of the decline from 2 to 4 h was used to estimate cuticular transpiration. The value of g_min was calculated as the rate of cuticular transpiration divided by the mole fraction gradient in water vapour from the leaf to air, assuming the leaf internal air to be fully saturated (Pearcy et al., 2000; Sack et al., 2003). Ambient
temperature and RH were measured at leaf height every minute (TinyTag, Gemini Data Loggers, Chichester, UK).

**Stomatal density, size and ratio**

Stomatal impressions of the flag leaf (GS39-40) adaxial and abaxial leaf surfaces were taken of the same area using clear nail varnish. The abaxial surface was identified as the surface with the most prominent midrib for each leaf. The impression was removed using clear tape onto a microscope slide. Stomata were counted from 10 fields of view at ×200 magnification per sample (total area: 1250 μm²). Stomatal density and measurements of pore length, guard cell width enabled the estimation of anatomical maximum stomatal conductance to water (g_{max} Parlane & Waggoner, 1970; Franks & Beerling, 2009). Measurements were obtained using IMAGEJ (Rasband, 1997–2018) software at a scale of 5.36 pixel μm⁻¹. Stomatal ratio was calculated as the number of stomata found on the adaxial to abaxial surfaces of the leaf.

**Biomass and yield**

Seeds and dry biomass were harvested c. 2.5–3 wk post anthesis. Ears were removed and counted for each plant. Three ears were weighed per plant and then all ears were threshed. The total seed weight and number was recorded. Harvest index was calculated using either an average over all the measurements or separated by growth stage. For visualisation, only significant (P < 0.05) pairwise Pearson correlations with significance are shown. The matri-matrix maximum stomatal conductance to water (g_{max}) was able to maintain significantly higher rates of g_{s} (P < 0.0001) for all three periods (Fig. 3b), enabling significantly (P < 0.0001) greater rates of carbon acquisition in these periods (Fig. S1). However, consistently high rates of g_{s} resulted in T. urartu demonstrating values of W_{2} 2.8-fold lower than that measured for T. aestivum during the day (Fig. 2d, 0.011 ± 0.004 mol CO_{2}/mmol H_{2}O m^{-2}s^{-1} and 0.029 ± 0.007 mol CO_{2}/mmol H_{2}O m^{-2}s^{-1}, respectively). At night, mean values of g_{s} were also 3.3-fold higher in T. urartu (Fig. 3c, 0.021 ± 0.014 mol m⁻² s⁻¹) compared with T. aestivum (0.006 ± 0.004 mol m⁻² s⁻¹).

The diurnal data were split into key periods to determine the impact of the magnitude of g_{s} and g_{sn} on the rates of photosynthesis and respiration respectively. During the day, mean responses of A and g_{s} were determined for 1 h after sunrise (‘dawn’), 4 h after dawn and 1 h before sunset (‘dusk’). T. urartu was able to maintain significantly higher rates of g_{s} (P < 0.0001) when compared with all other cultivars, with W468 demonstrating the highest rates of g_{s} (0.502 ± 0.004 mol m⁻² s⁻¹) and rates of CO_{2} assimilation (Supporting Information Fig. S1: 3.79 ± 1.14 μmol m⁻² s⁻¹), more than 4-fold and 1.5-fold higher than those observed for T. aestivum (0.098 ± 0.013 mol m⁻² s⁻¹ and 2.59 ± 0.49 μmol m⁻² s⁻¹, respectively). At night, mean values of g_{s} were also 3.3-fold higher in T. urartu (Fig. 3c, 0.021 ± 0.014 mol m⁻² s⁻¹) compared with T. aestivum (0.006 ± 0.004 mol m⁻² s⁻¹).

Nocturnal conductance in elite lines is genotype and growth-stage specific

Six modern spring wheat varieties and two Watkin’s Landraces were measured before and after dawn during six growth stages. Porometer measurements of g_{sn} at booting and anthesis determined that adaxial g_{sn} was significantly higher than abaxial g_{sn} in six of the eight genotypes measured for pre-dawn g_{sn} (Fig. S2a) and four of the eight post-dawn g_{s} (Fig. S2b). To our knowledge, this is the first report of this behaviour in wheat. However, to ascertain concurrent rates of g_{sn}/R_{d} or g_{s}/A, IRGA measurements were prioritised.

Combining gas-exchange data across all growth stages, significant differences were noted between the genotypes for both g_{sn}, g_{s} (Fig. S3a,b, P < 0.007), A and R_{d} (Fig. S3c,d; P < 0.02). In brief, Bolraug100 demonstrated significantly higher g_{sn} across all growth stages (P < 0.01; 0.063 ± 0.048 mol m⁻² s⁻¹) when compared with all other cultivars, with W468 demonstrating the lowest mean g_{sn} (0.023 ± 0.019 mol m⁻² s⁻¹). During the day, Pavon76 had the highest g_{s} (0.502 ± 0.176 mol m⁻² s⁻¹), which was only significantly higher than Vorobey and Paragon (P < 0.03). No significant differences were found between the individual genotypes for R_{d}, while Pavon76 achieved the highest

**Results**

*T. urartu* demonstrates significantly higher g_{sn} compared with *T. aestivum*

To ascertain whether variation in g_{s} and g_{sn} exists between two closely related species, diurnal gas-exchange measurements were made on the flag leaves of wild relative, *T. urartu*, and modern cultivar *T. aestivum* ‘Paragon’ (Fig. 3a). During the day, *T. urartu* demonstrated significantly (P < 0.05) higher mean g_{s} (Fig. 3b; 0.384 ± 0.097 mol m⁻² s⁻¹) and rates of CO_{2} assimilation (Supporting Information Fig. S1: 3.79 ± 1.14 μmol m⁻² s⁻¹), more than 4-fold and 1.5-fold higher than those observed for *T. aestivum* (0.098 ± 0.013 mol m⁻² s⁻¹ and 2.59 ± 0.49 μmol m⁻² s⁻¹, respectively). At night, mean values of g_{sn} were also 3.3-fold higher in *T. urartu* (Fig. 3c, 0.021 ± 0.014 mol m⁻² s⁻¹) compared with *T. aestivum* (0.006 ± 0.004 mol m⁻² s⁻¹).
Fig. 3 The diurnal responses of (a) stomatal conductance ($g_s$) in five biological replicates of modern genotype *Triticum aestivum* ‘Paragon’ and wild relative *T. urartu* 1010001 over 5 days – the red dotted line indicates the mean stomatal conductance for all measurements taken for each genotype. The response of the two genotypes was analysed at set periods during the day (b; see the Materials and Methods subsection Diurnal glasshouse measurements). *T. urartu* demonstrated significantly higher values of $g_s$ 1 h post sunrise (‘Dawn’), during the day (‘Daytime’) and 1 h before sunset (‘Dusk’). In addition, *T. urartu* also demonstrated significantly higher nocturnal conductance ($g_{sn}$) compared with the modern genotype during the period 1 h post-dusk and 1 h pre-dawn (‘Pre-dawn’). Measurements are individual data points ($n = 5$). Boxplots show the median (horizontal line), the quartiles (boxes). The whiskers represent 1.5-times the interquartile range above and below the 75th and 25th percentile respectively. A $t$-test was performed and asterisks indicate the following levels of significance: ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. When comparing (d) daytime CO$_2$ assimilation ($A$) and $g_s$ between genotypes, consistently higher $g_s$ led to low intrinsic water-use efficiency in the *T. urartu* (grey triangles) compared with Paragon (black circles). The dotted line on (b–d) indicates zero $g_s$ or $g_{sn}$. 

© 2021 The Authors
New Phytologist © 2021 New Phytologist Foundation

New Phytologist (2021) 232: 162–175
www.newphytologist.com
values of $A$ ($P < 0.05$) when compared with Paragon and the two Watkin’s lines.

When comparing the ratio of $R_d/A$, a significant negative correlation was observed ($R^2 = -0.48; P = 0.002$). Although this relationship was not found to be genotype specific ($P = 0.439$), there was a general trend observed between growth stage and $R_d/A$ ratio, with younger growth stages (<GS39, GS39) having higher values compared with older material (Heading and Anthesis; Fig. S4) however, no significant differences were observed between the growth stages ($P = 0.47$).

Separating the data by growth-stage highlights additional trends (Fig. 4). There was a general trend for decreasing conductance with age, both at night (Fig. 4a) and during the day (Fig. 4b), however this decrease was genotype specific. Vorobey demonstrated the greatest decrease in mean $g_{sn}$ and $g_i$ between <GS39 and anthesis (80.24% and 67.04% respectively), while the smallest decreases were observed for Pavon76 (31.32%) and Borlaug100 (21.13%).

Five of the eight cultivars indicated significant differences ($P < 0.05$) in $g_{sn}$ between growth stages (Figs 4, S5). Most significant comparisons were between <GS39 and heading. No significant differences were noted between <GS39 and GS39. For the post-dawn measurements, seven of the eight cultivars indicated significant ($P < 0.04$) differences in $g_i$ between growth stages. Growth stages <GS39 and GS39 also achieved values of $g_s$ which were significantly ($P < 0.04$) higher than booting, emergence and anthesis in the majority of cases. Borlaug100 was the only cultivar to show no significant difference in $g_i$ throughout <GS39 to anthesis ($P = 0.23$). In summary, the magnitude of $g_{sn}$ was genotype specific, accounting for between 5.7% ($\pm 2.1\%$) to 18.9% ($\pm 3.6\%$) of the $g_s$ values 4 h after dawn.

In general, genotypes with the greatest mean $g_{sn}$ saw the greatest percentage decreases in the proportion of $g_{sn}$ to $g_s$ as the plants aged, for example $g_{sn}$ in Borlaug100 fell from 16.9% of post-dawn $g_s$ at <GS39 to 9.6% $g_s$ at anthesis. Conversely, those plants in which mean lifetime $g_{sn}$ was low (<0.027 mol m$^{-2}$ s$^{-1}$), $g_{sn}$ made up a greater proportion of water loss as these plants reached anthesis; Paragon achieved one of the lowest mean $g_{sn}$ during its lifetime (0.03 mol m$^{-2}$ s$^{-1}$) – however, it demonstrated the second greatest decline in $g_s$ from <GS39 to anthesis (65%). Subsequently, $g_{sn}$ climbed from 9% of daily $g_s$ to 14.7%.

Significant positive relationships were noted between $A$ and $g_{sn}$ (Fig. 5a) and $g_s$ and $g_{sn}$ (Fig. 5b). A significant negative correlation was noted between $R_d$ and $g_{sn}$ (Fig. 5c) and between $R_d$ and $A$ (Fig. S4; $R^2_{adj} = -0.48$, $P = 0.002$) between the genotypes grown in the growth room. No significant relationship was determined between mean lifetime $A$ and $g_{sn}$ (Fig. S6a; $R^2_{adj} = 0.44$, $P = 0.27$), however a significant positive correlation was determined between $g_{sn}$ at GS39 ($R^2_{adj} = 0.71$, $P = 0.05$) and booting ($R^2_{adj} = 0.87$, $P = 0.014$) and $A$ achieved at anthesis (Fig. S6b).

This decrease in $g_i$ and $A$ over time was accompanied by significant increases in $W_i$ over time (Fig. S7). With the exception of Borlaug100 and W483, all genotypes demonstrated between a 51.0% (Pavon76) and 89.9% (Vorobey) increase in $W_i$ when comparing <GS39 and anthesis. While W468 and W483 were selected were due to high and low water-use efficiency in the field, there was no significant difference in $W_i$ between these genotypes ($P = 0.21$), nor was any single growth-stage $W_i$ was

![Fig. 4](https://example.com/fig4.png) The responses of (a) pre-dawn ($g_{sn}$) and (b) 4 h post-dawn ($g_s$) stomatal conductance in six modern spring wheat genotypes and two Watkins landraces (W468 and W483) from tillering (<GS39) to anthesis. The stomatal conductance data for each genotype is separated into five broad growth stages (see Gas-exchange measurements section for more details). Significant differences ($P < 0.05$) are indicated in the top right hand corner of each plot. Asterisks indicate the following levels of significance: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Data are the means with standard deviation ($n = 4$–6).
significantly different when comparing these two Watkins lines. However, there was a significant effect of growth stage within each of the other six genotypes measured ($P = 0.003$). Combined, $W_i$ was also not significantly different between the Watkins genotypes and the modern genotypes ($P = 0.782$).

Measurements of cuticular conductance ($g_{\text{min}}$) were performed at flag leaf (GS39-42). Measurements of $g_{\text{sn}}$ at flag leaf were on average 3.3-fold (Paragon) to 15-fold (Pavon76) higher than measurements of $g_{\text{min}}$ (Fig. 6a), suggesting that while $g_{\text{min}}$ does contribute to nocturnal water loss, stomata at this growth stage are actively open at night. $g_{\text{min}}$ was also significantly different between genotypes ($P = 0.003$) with cadenza reporting the greatest values ($0.0077 \pm 0.0017 \text{ mol m}^{-2} \text{s}^{-1}$) and Pavon76 the lowest ($0.0027 \pm 0.0009 \text{ mol m}^{-2} \text{s}^{-1}$).

Flag leaf stomatal density and ratio were also collected from all eight genotypes (Fig. 6b,c). Genotype-specific significant ($P < 0.05$) differences in stomatal density were only observed when comparing the abaxial (lower) leaf surfaces. In general, abaxial pores and guard cells were shorter (Fig. S8a) and thinner (Fig. S8b) with smaller estimated pore area (Fig. S8c). The smaller size and density of the abaxial guard cells culminated in smaller abaxial $g_{\text{max}}$ for the majority of genotypes (Fig. S8d). Significant differences were found between the genotypes for all four parameters ($P < 0.001$). While $A$ was found to positively...
correlate ($P = 0.01$, $R^2_{adj} = 0.82$ – Fig. S6a) with abaxial $g_{max}$ and height negatively correlated with abaxial $g_{max}$ ($P = 0.02$, $R^2_{adj} = 0.78$; Fig. S6a), there was no significant correlation ascertained between the stomatal measurements and $g_{sn}$ when culminating the data across the lifetime of the plants. In terms of growth-stage-specific comparisons, only adaxial $g_{max}$ and guard cell width was found to positively correlate with $g_{sn}$ at heading ($P = 0.05$, $R^2_{adj} = 0.71$; Fig. S6b) and flag leaf ($P = 0.05$, $R^2_{adj} = 0.71$; Fig. S6b) respectively.

For all genotypes, there were more stomata on the adaxial surface than on the abaxial surface resulting in stomatal ratios $> 1$, despite the greater $g_{sn}$ observed from the adaxial surface in most lines. These ratios were only significant for Pavon76, W468 and Sokoll.

A significant positive correlation was observed between stomatal density and seed number (Fig. S9; $R^2_{adj} = 0.71$, $P = 0.05$). Conversely, a negative significant correlation was observed between stomatal ratio and seed weight (Fig. S9; $R^2_{adj} = -0.74$, $P = 0.035$); plants with higher stomatal densities on the abaxial surface achieved greater numbers of heavier seed.

**Genotype-specific harvest traits**

On average, the Watkins genotypes had the fastest rates of development (see Fig. S9), exhibiting the number of tallest: 36.5 ± 7.5 cm compared with 20.8 ± 5.1 (Fig. S10b), W483 (41.9 ± 5.2 g) and Paragon (37.2 ± 2.2 g) produced the greatest total dry biomass while Borlaug100 produced the lowest (19.1 ± 1.4 g; Fig. S10e). Stem and leaf dry weight (DW) were consistent amongst all genotypes (34.6 ± 3.6% and 65.4 ± 3.6% of total DW respectively; Fig. S10d). Total DW was negatively correlated with harvest index ($R^2 = -0.89$, $P = 0.003$) and $g$ at heading ($R^2 = -0.84$, $P = 0.009$).

While the modern genotypes produced higher numbers of heavier seeds per plant, the Watkins genotypes produced greater numbers of smaller ears with smaller, less numerous seed content (Fig. S10f). The modern cultivars produced between 25 and 25 ears per plant, while the Watkins lines produced 25–45 (Fig. S10e). Seed weight was negatively correlated with stomatal ratio only ($R^2 = -0.74$, $P = 0.035$).

Harvest index (HI) was significantly different ($P < 0.0001$) between the genotypes; Borlaug100 had the highest HI, significantly (2.12 ± 0.07, $P < 0.05$) higher than the Watkins lines. Although the lowest HI values were observed in W483 (1.00 ± 0.05), these values were not significantly different to modern genotype, Paragon (1.15 ± 0.59). HI was negatively correlated (Fig. S6b) with height ($R^2 = -0.91$, $P = 0.001$) and total DW ($R^2_{adj} = -0.89$, $P = 0.003$). While the genotype with highest lifetime $g_{sn}$ also demonstrated the highest HI (Borlaug100), this relationship was not significant ($R^2_{adj} = 0.62$, $P = 0.09$).

**Discussion**

Nocturnal conductance still represents a biological enigma that has wide reaching implications for crop water use, especially under conditions of heat and drought. This study has determined genotypic variability in $g_{sn}$ for wheat lines grown under simulated environmental conditions of a north-western Mexican field (Fig. 2). These conditions – which are representative of 40% of wheat grown globally – are characterised by progressively warmer days and cool nights. Under these conditions, a significant positive correlation was observed between $g$ and $g_{sn}$ (Fig. 5b), with both measurements declining as the plants aged (Figs 3, 4). The magnitude of $g_{sn}$ was leaf surface and genotype dependent, declining as the plants aged. Cuticular conductance was also genotype specific (Fig. 6a) and significantly lower than $g_{sn}$.

### The diurnal response of $g_{sn}$

Evidence suggests that $g_{sn}$ has a circadian rhythm; characterised by a decrease in $g_{sn}$ after dusk and an increase in $g_{sn}$ pre-dawn (Caird et al., 2007; Resco de Dios et al., 2013a). This response has been documented for tree species, dicots (Resco de Dios et al., 2015, 2016) and for crops such as maize (Tamang & Sadok, 2018) and wheat (Schoppach et al., 2014; Claverie et al., 2016). Diurnal measurements provide key information on any antecedent behaviour of $g_{sn}$ and highlight any coordinated relationships with $g$, photosynthetic carbon uptake and respiratory carbon release.

In this study, high $g_{sn}$ in the wild relative (T. urartu) was positively correlated with high $g$ (Fig. 3), which was also reflected in the genotypes measured in growth room study (Fig. 5b) and has been reported in the literature (Cavender-Bares et al., 2007). The relationship between high $g$ and $g_{sn}$ has been linked to increases in specific leaf area (SLA; Tamang & Sadok, 2018) and relative growth rate (Resco de Dios et al., 2019). In general, the wild relatives of modern wheat tend to have higher SLA (McAusland et al., 2020) compared with their modern counterparts; supporting the observation that high $g$ and $g_{sn}$ maintain a resource-acquisitive strategy under well watered conditions (Cheng et al., 2016).

Despite significantly different magnitudes $g_{sn}$ for all time periods studied during the diurnal, both the wild relative and modern cultivar demonstrated a decline in $g_{sn}$ during the night from dusk to before dawn (Fig. 3c). This decrease in $g_{sn}$ contrasts with other species within the published literature, which suggests that stomata close in response to the onset of night and open before sunrise to maximally acquire carbon under low VPD conditions (Resco de Dios et al., 2016). The plants used in this study were not exposed to highly variable VPD and perhaps these results support the findings of those reported by Auchincloss et al. (2014) and Hassidim et al. (2017); with $A$ being more limited by biochemical processes than the rate of stomatal opening in the period after dawn. While slow stomatal closure at the onset of night could play a role in maintaining hydraulic processes, a decline in $g_{sn}$ pre-dawn may indicate a water-conservative approach to sunrise or a response to low temperature (Agurla et al., 2018).

There are significant, species-specific differences in the rate of stomatal closure and opening in response to light (McAusland et al., 2016, 2020). In species with dumbbell or graminaceous guard cells, there is a significant positive correlation with the...
magnitude of opening or closing and the maximum rate of response. There is also evidence that the higher the initial $g_{sn}$ the more rapid the opening when exposed to an increase in light (Wachendorf & Küppers, 2017). There may be a trade-off between the magnitude of stomatal opening pre-dawn (and subsequent water loss) and the rate of opening in response to dawn. In short, a plant that can rapidly open its stomata in response to dawn does not need to maintain high $g_{sn}$ before dawn. If pre-dawn $g_{sn}$ is negatively correlated with speed of stomatal opening, the magnitude of pre-dawn $g_{sn}$ may reflect prioritisation between water loss and early-morning carbon gain dependent on water availability. The long-term impact of this behaviour has yet to be explored and will depend on many in vivo and environmental factors; the speed of photosynthetic induction (and therefore the concentration of intercellular CO$_2$ - $C_i$ - and RuBisCo activation), the availability of starch (Kwak et al., 2017) and pre-dawn soil water availability due to competition or environmental stresses (Yu et al., 2019). Quantifying variation in response and magnitude of $g_{sn}$ in key nocturnal periods will be critical for screening crops for heat and drought tolerance.

The magnitude of $g_{sn}$ is growth-stage specific

While the magnitude of $g_s$ peaks at leaf maturity and declines towards leaf senescence (Field, 1987), less is known about intrinsic variation in $g_s$ throughout the lifecycle of wheat. From these data, we have determined that $g_s$ declines from vegetative stages towards anthesis for the majority of genotypes (Fig. 4b) – in line with rates of CO$_2$ assimilation (Fig. S6b) – and that $g_{sn}$ also follows this trend (Fig. 4a). For some genotypes, these declines were not significant, for example Borlaug100, Cadenza and W483. For Borlaug100, the genotype with the highest HI, both $g_{sn}$ and $g_s$ were not significantly different between the growth stages. In general, genotypes with the greatest mean lifetime $g_{sn}$ also saw the greatest percentage decreases in their contribution to daytime $g_s$.

While high $g_s$ can preclude low water-use efficiency, under well watered conditions high $g_s$ also promotes greater rates of carbon assimilation (Lawson & Blatt, 2014), biomass accumulation and light interception (Blum, 2009; Tricker et al., 2018). With strong positive correlations between $g_s$ and $g_{sn}$, it is possible that the magnitude of $g_{sn}$ reflects a method for fine tuning gas exchange across each 24 h period to optimise water uptake, growth and water loss during plant lifetime, a hypothesis that is in line with a recent model based on optimisation theory (Wang et al., 2021). While high $g_{sn}$ may improve yield under well watered conditions, it may also reduce heat tolerance under drought conditions by preventing complete plant rehydration before dawn (Kavanagh et al., 2007), especially if insensitive to heating (Rogiers & Clarke, 2013; Claverie et al., 2018). This suggests that although $g_{sn}$ has a role to play in the establishment and growth of the plant, there may be an optimum $g_{sn}$, one that facilitates the regulation of growth through water uptake/turgor maintenance while demonstrating some sensitivity to temperature and water availability. The plants in this study were maintained under well watered conditions, however declining $g_s$ and $g_{sn}$ after anthesis could reflect a switch in priorities from maintenance of growth to initiation of rapid senescence. More studies are needed to investigate whether these growth-stage specific changes are fine tuned to reflect whole plant priorities under stressed environmental conditions such as heat or drought.

There is a fine balance between photosynthesis, biomass production and respiration ($R_d$), with leaf-level $A$ strongly coupled to $R_d$ (Wang et al., 2020). In the growth room, nocturnal conductance was found to negatively correlate with respiration (Fig. 5c), with greater values of $g_{sn}$ accompanying smaller rates of CO$_2$ release in younger leaf material. However, the range of $R_d$ and $g_{sn}$ values are both very small, making it difficult to attribute a simple causal relationship between $g_{sn}$ and CO$_2$ release. $R_d$ is a key process driving growth processes at night (O’Leary et al., 2017) and is strongly influenced by temperature (Posch et al., 2019) although it has not been determined whether $g_{sn}$ substantially contributes to the nocturnal theromoregulation of leaves at high temperatures or is simply a mechanism to mediate the release of CO$_2$. Accumulation of CO$_2$ could inhibit the cytochrome respiration pathway (Resco de Dios et al., 2019) and in the absence of $g_{sn}$, $g_{min}$ is thought to be insufficient to alleviate increases of CO$_2$ (Even et al., 2018).

In the work presented here, plants were not exposed to heat stress. $R_d$ is typically more sensitive to changes in temperature than $A$ (Posch et al., 2019), therefore to further investigate the role between $g_{sn}$ and $R_d$, nocturnal heat and/or drought stress should be applied. Interestingly, while many studies have shown $g_{sn}$ decreasing under drought and heat (Rawson & Clarke, 1988; Cavender-Bares et al., 2007), $g_{sn}$ in wheat has been shown to increase, irrespective of water availability, under small increases in nocturnal VPD (Claverie et al., 2018). This perhaps unexpected response requires more experimentation and highlights that, although variation exists in the plants in this study, even greater variation in $g_{sn}$ may exist in response to different combinations of abiotic stress.

The magnitude of $g_{sn}$ is genotype specific

Significant differences in mean lifetime $g_{sn}$ were observed between the modern genotypes, accounting for between 5.7% and 18.9% of mean daytime $g_s$, which is consistent with the literature (Duursma et al., 2019) and supports the observation that there is variation to be exploited (Claverie et al., 2018) for drought and heat tolerance in wheat (Sadok & Jagadish, 2020). While the range of $g_{sn}$ was low compared with some studies, it should be noted that these plants still exhibited $g_{sn}$ under controlled, well watered conditions with no changes in water availability or VPD. In addition, under the well mixed conditions of the growth room, it is unlikely that the magnitude of $g_{sn}$ observed in this study reflected differences in boundary layer between the genotypes. In the field, increases in $T_{min}$ and wind speed would exacerbate any water losses at night through increasing the rate of evapotranspiration and reducing the boundary layer. Identifying variation in nocturnal water loss contributes to a growing body of work that nocturnal transpiration is underpinned by a wealth of genetic variation to be exploited for optimising crop water-use efficiency (Duursma et al., 2019; Resco de Dios et al., 2019).
While this variation has mostly been explored in woody species (Daley & Phillips, 2006; Phillips et al., 2010; Couipel-Ledru et al., 2016; Resco de Dios et al., 2016), interest is growing around wheat (Claverie et al., 2018; Schoppach et al., 2020). While the work here mostly focusses on pre-dawn responses, even greater variation in $g_{an}$ may also be observed at dusk (1 h after sunset) or integrating water losses throughout the night (Fig. 3b).

These genotype-specific differences extended to $g_{min}$ at flag leaf (Fig. 6a), with a >3.8-fold difference between the lowest and highest values, $g_{min}$ did not correlate with $g_{an}$ but contributed between 6.6% and 30.1% of the value of $g_{an}$. This high degree of intraspecific variation suggests that $g_{min}$ should always be assessed with $g_{an}$ and, as we have shown with $g_{an}$, could be growth-stage specific in wheat (Duursma et al., 2019). It was also interesting to note that the magnitude of $g_{an}$ is perhaps leaf-surface specific (Fig. S2a), with up to 1.2–1.3-fold greater rates of nocturnal water loss from the adaxial surface ($0.125 \pm 0.04 \text{ mol m}^{-2} \text{s}^{-1}$) compared with the abaxial surface ($0.051 \pm 0.01 \text{ mol m}^{-2} \text{s}^{-1}$) despite no consistency with morphology. This trend was also observed for post-dawn measurements, with adaxial $g_{s}$ consistently higher than abaxial $g_{s}$ (Fig. S2b). While morphological amphistomaty is thought to provide a gas-exchange advantage, shortening the pathways of CO$_2$ and H$_2$O exchange and promoting rapid growth (Drake et al., 2019), it is unclear why the stomatal aperture on the two surfaces of the leaf would respond differently at night. This behaviour could reflect more nocturnal-specific fine tuning of diurnal requirements for gas exchange.

Finally, HI was also genotype specific, demonstrating a significant positive correlation with $g_{s}$. With a positive strong correlation between $g_{an}$ and $g_{s}$, these results suggest that although any selection against high $g_{an}$ may improve drought tolerance or water-use efficiency (Sadok & Jagadish, 2020), there may also be a yield penalty, perhaps through limiting growth rates or dawn carbon acquisition (Resco de Dios et al., 2016), 2019). For this study, variation in $g_{an}$ under well watered conditions did not show strong correlations with individual harvest characteristics such as biomass, height and grain weight (Fig. S9). However, variation in $g_{an}$ and $g_{an}$ may be an indicator of tolerance under water or high temperature stress, with those genotypes able to maintain higher $g_{s}$ and $g_{an}$ also maintaining cooler canopies and greater yields (Fischer et al., 1998; Lu et al., 1998; Fischer & Edmeades, 2010). This would provide a tractable means of selection for improved genotypes, but using low $g_{an}$ and/or low $g_{s}$ under nonstressful conditions.

The relationship between $g_{smin}$, $g_{min}$ and stomatal characteristics

Cuticular conductance varies between species and is dependent on the growth environment (Sack et al., 2003); decreasing under drought and low humidity (Kerstiens, 1996). $g_{min}$ has also been shown as the primary source of water loss from expanding oak leaves (Kane et al., 2020), therefore it is conceivable that while $g_{min}$ was only up to 15-fold lower than $g_{an}$ at the flag leaf, it could also vary with age; contributing considerably more water loss in young wheat leaf material.

There are very few data available to link stomatal anatomical characteristics with nocturnal behaviours, with most published literature reporting changes in density and size due to large-scale environmental factors, for example lower densities under warmer temperatures (Rogiers & Clarke, 2013) and higher densities under high VPD (Claverie et al., 2016). While we found significant differences in stomatal density and ratio at flag leaf between the genotypes studied (Fig. 6b,c) these values did not correlate with $g_{an}$ at flag leaf. Although stomatal size also varied between the genotypes, only adaxial guard cell width positively correlated with flag leaf $g_{an}$ (Figs S6b, S8).

In amphistomatous species grown under well watered conditions, high adaxial to abaxial (AD : AB) densities have been linked to improving parallel CO$_2$ diffusion in the leaf, increasing mesophyll conductance and leading to higher rates of carbon acquisition (Drake et al., 2019; Pathare et al., 2020). Although one of the three genotypes to demonstrate significant AD : AB density also had the highest lifetime CO$_2$ assimilation (Pavon76; Fig. S3), there are too few genotypes in this study to conclusively link this trait with improved carbon acquisition. It is possible that the occurrence of high AD : AB densities may be more common under the optimum conditions presented here. The common observation of lower AD : AB densities generally reflects a need to conserve water by increasing AB densities in the more frequently shaded areas of the plant (Mott et al., 1982), in this way, high AD : AB ratios would not be advantageous under heat or water stress.

Variation in stomatal density is generally accompanied by changes in other anatomical characteristics such as vein density (Claverie et al., 2016) and root epidermal thickness (and aquaporin density) to optimise plant vascularisation in line with water demand. While root physiology are not covered in this work, roots are intrinsically linked to water uptake and the hydraulic status (Wasson et al., 2012) of the aboveground plant; with deeper roots leading to cooler canopies and greater yields (Li et al., 2019). As noted by Claverie et al. (2018), wheat grown under high nocturnal VPD demonstrates significantly higher $g_{an}$ and significantly greater root biomass than plants grown under low VPD, therefore it would not be unsurprising if a link arose between root growth, water availability and $g_{an}$ for these genotypes. Further analyses of wheat genotypes under water and heat stress conditions need to be conducted to determine whether $g_{an}$ plays a role in drought or heat tolerance and how $g_{an}$ interacts with other nocturnal traits to sustain or improve yields.

Conclusions

Here we describe genetic, developmental and morphology-dependent variation in nocturnal stomatal conductance in wheat together for the first time. Our results demonstrate that even under the well watered conditions used, different growth stages in wheat represent substantial variation in leaf-level nocturnal conductance and therefore contribute to aboveground water loss. Not only should this variation be taken into account for modelling crop water-use efficiency and nocturnal water flux, it
should also be considered when selecting for water-use efficiency and for heat- and drought-tolerant genotypes for an increasingly warm climate. To do this, knowledge of trade-offs of high or low \( g_{\text{so}} \), with other whole plant processes and with yield under a range of growing environments is required. The use of programmable controlled growth environments to impose realistic thermal regimes – which can uncouple confounding environmental variables – is a powerful tool in dissecting the role of \( g_{\text{so}} \) in plants.

Acknowledgements

This project was funded by a UK–Mexico grant from the Newton Fund and the Biotechnology and Biological Sciences Research Council (grant no. BB/S012834/1). KES is funded by the Nottingham BBSRC Doctoral Training Programme. This is a Nottingham Futurefoods Beacon tagged project. We thank Prof. Anthony Hall and Dr Rachel Rusholme Pilcher (Earlham Institute) for suggestions on the Watkins lines, Dr Elizabete Carmo-Silva and Dr Alejandro Perdomo-Lopez (Lancaster University) and the Nottingham BBSRC Wheat Research Centre (University of Nottingham) for the generous provision of seeds and glasshouse plants. We are also grateful to the reviewers for their useful and productive feedback on this manuscript.

Author contributions

LM, GM and EHM conceived the work and planned the experiment. LM, KES and AW collected the data, LM performed the data analysis. LM and EHM wrote the manuscript.

ORCID

Lorna McAusland https://orcid.org/0000-0002-5908-1939
Gemma Molero https://orcid.org/0000-0002-6431-7563
Erik H. Murchie https://orcid.org/0000-0002-7465-845X
Kellie E. Smith https://orcid.org/0000-0002-8569-9142

Data availability

The data supporting the findings of this study are available from the corresponding author, (Lorna McAusland), upon request.

References

Agurla S, Gahir S, Munemasa S, Murata Y, Raghavendra AS. 2018. Survival strategies in extreme cold and desiccation. In: Iwaya-Inoue M, Sakurai M, Uemura M, eds. Advances in experimental medicine and biology. Singapore City: Springer, 215–232.

AHDB. 2018. Cereal growth stages and benchmarks. Warwickshire, UK: Agriculture and Horticulture Development Board. [WWW document] URL https://bit.ly/32Nu7Tx [accessed 23 July 2020].

dos Anjos L, Pandey PK, Moraes TA, Feil R, Lunn JE, Stitt M. 2018. Feedback regulation by trehalose 6-phosphate slows down starch mobilization below the rate that would exhaust starch reserves at dawn in Arabidopsis leaves. Plant Direct 2: e00078.

Atkin OK, Bruhn D, Hurry VM, Tjoelker MG. 2005. Evans review no. 2: The hot and the cold: unravelling the variable response of plant respiration to temperature. Functional Plant Biology 32: 87–105.

Atkin O, Scheuwer I, Pons T. 2007. Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. New Phytologist 174: 367–380.

Auchincloss L, Easton HM, Levine D, Donovan L, Richards JH. 2014. Pre-dawn stomatal opening does not substantially enhance early-morning photosynthesis in Helianthus annuus. Plant, Cell & Environment 37: 1364–1370.

Barbour MM, Buckley TN. 2007. The stomatal response to evaporative demand persists at night in Ricinus communis plants with high nocturnal conductance. Plant, Cell & Environment 30: 711–721.

Blum A. 2009. Effective use of water (EUE) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Research 112: 119–123.

Caird MA, Richards JH, Donovan LA. 2007. Night-time stomatal conductance and transpiration in C3 and C4 plants. Plant Physiology 143: 4–10.

Campbell GS, Norman J. 2012. An introduction to environmental biophysics. New York, NY, USA: Springer Science & Business Media.

Carvalho D. 2019. Functional strategies in extreme cold and desiccation. In: Iwaya-Inoue M, Sakurai M, Uemura M, eds. Advances in experimental medicine and biology. Singapore: Springer, 215–225.

Carvalho D. 2019. Functional strategies in extreme cold and desiccation. In: Iwaya-Inoue M, Sakurai M, Uemura M, eds. Advances in experimental medicine and biology. Singapore: Springer, 215–225.

Cheng J, Chu P, Chen D, Bai Y. 2016. Functional correlations between specific leaf area and specific root length along a regional environmental gradient in Inner Mongolia grasslands. Functional Ecology 30: 985–997.

Claverie E, Meunier F, Javava M, Sadok W. 2018. Increased contribution of wheat nocturnal transpiration to daily water use under drought. Physiologia Plantarum 162: 290–300.

Claverie E, Schoppach R, Sadok W. 2016. Nighttime evaporative demand induces plasticity in leaf and root hydraulic traits. Physiologia Plantarum 158: 402–413.

Cossani CM, Reynolds MP. 2013. What physiological traits should we focus on in breeding for heat tolerance? In: Alderman PD, Quilligan E, Asseng S, Ed. Proceedings of the workshop on modelling wheat response to high temperature. Texcoco, Mexico: CIMMYT, 24.

Coupel-Ledru A, Lebon E, Christophe A, Gallo A, Gago P, Pantin F, Doligez A, Simonneau T. 2016. Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. Proceedings of the National Academy of Sciences, USA 113: 8963–8968.

Daley MJ, Phillips NG. 2006. Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. Tree Physiology 26: 411–419.

Davy R, Esau I, Chernokulsky A, Outen S, Zilininkievich S. 2017. Diurnal asymmetry to the observed global warming. International Journal of Climatology 37: 79–93.

Donovan L, Linton M, Richards J. 2001. Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. Oecologia 129: 328–335.

Drake PL, De Boer HJ, Schymanski SJ, Veneklaas EJ. 2019. Two sides to every leaf: water and CO\(_2\) transport in hypostomatous and amphistomatous leaves. New Phytologist 222: 1179–1187.

Duursma RA, Blackman CJ, Lopérè R, Martin-StPaul NK, Cochard H, Medlyn BE. 2019. On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. New Phytologist 221: 693–705.

Easton HM, Richards JH. 2009. Photosynthesis affects following night leaf conductance in Vicia faba. Plant, Cell & Environment 32: 58–63.

Even M, Sabo M, Meng D, Krezcisz T, Schreiber L, Fricke W. 2018. Night-time transpiration in barley (Hordeum vulgare) facilitates respiratory carbon dioxide release and is regulated during salt stress. Annals of Botany 122: 569–582.

Field CB. 1987. Leaf-age effects on stomatal conductance. In: Zeiger E, ed. Stomatal function. Palo Alto, CA, USA: Stanford University Press, 367.

Fischer R, Edmeades GO. 2010. Breeding and cereal yield progress. Crop Science 50: S-85–S-98.

Fischer R, Rees D, Sayre K, Lu Z-M, Condon A, Saavedra AL. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Science 38: 1467–1475.

Franks PJ, Beerling DJ. 2009. Maximum leaf conductance driven by CO\(_2\) effects on stomatal size and density over geologic time. Proceedings of the National Academy of Sciences, USA 106: 10343–10347.
174 Research

Fricke W. 2019. Night-time transpiration-favouring growth? Trends in Plant Science 24: 311–317.

Garcia GA, Serrago RA, Drecker MF, Miralles DJ. 2016. Post-anthesis warm nights reduce grain weight in field-grown wheat and barley. Field Crops Research 195: 50–59.

Hassidim M, Dakshin Y, Turjeman A, Hussien D, Shor E, Anidjar A, Goldberg K, Green RM. 2017. CIRCADIAN CLOCK ASSOCIATED1 (CCAI) and the circadian control of stomatal aperture. Plant Physiology 175: 1864–1877.

Hein NT, Wagner D, Bheemanahalli R, Šebda D, Bustamante C, Chiluwa A, Neilson ML, Jagadish SK. 2019. Integrating field-based heat tests and cyber-physical system technology to phenotype high night-time temperature impact on winter wheat. Plant Methods 15: 41.

Howard AR, Van Iersel MW, Richards JH, Donovan LA. 2009. Night-time transpiration can decrease hydraulic redistribution. Plant, Cell & Environment 32: 1060–1070.

Jones HG. 2013. Plants and microclimate: a quantititative approach to environmental plant physiology. Cambridge, UK: Cambridge University Press.

Kane CN, Jordan GJ, Jansen S, Mcdadam SA. 2020. A permeable cuticle, not open stomata, is the primary source of water loss from expanding leaves. Frontiers in Plant Science 11: 774.

Kavanagh KL, Pangle R, Schotzko AD. 2007. Nocturnal transpiration causing disequilibrium between soil and stem predawn water potential in mixed conifer forests of Idaho. Tree Physiology 31: 621–629.

Kerstiens G. 1996. Cucurbit water permeability and its physiological significance. Journal of Experimental Botany 47: 1813–1832.

Kwak MJ, Lee SH, Khaine I, Je SM, Lee TY, You HN, Lee HK, Jang JH, Kim I, Woo SY. 2017. Stomatal movements depend on intersections between external night light cue and internal signals activated by rhythmic starch turnover and abscisic acid (ABA) levels at dawn and dusk. Acta Physiologiae Plantarum 39: 162.

Lawson T, Blatt M. 2014. Stomatal size, speed and responsiveness impact on photosynthesis and water use efficiency. Plant Physiology 164: 1556–1570.

Lawson T, Matthews J. 2020. Guard cell metabolism and stomatal function. Annual Review of Plant Biology 71: 273–302.

Li X, Ingvardsen CH, Weiss M, Rebetzke GJ, Condon AG, James RA, Richards PW, eds. Environmental plant physiology. 2021. 621–629.

Li X, Ingvardsen CH, Weiss M, Rebetzke GJ, Condon AG, James RA, Richards PW, eds. Environmental plant physiology. 2021. 625–626.

Lobell DB, Ortiz-Monasterio JI, Woo SY. 2017. Annual Review of Plant Biology 70: 437–494.

Lobell DB, Ortiz-Monasterio JI. 2007. Impacts of day versus night temperatures on spring wheat yields. Agronomy Journal 99: 469–477.

Lobell G. 2017. Image of random root systems. Figs hare. doi: 10.6084/ m9.figshare.4684924.v2.

Lombardozzi DL, Zeppel MJ, Fisher RA, Tawfik A. 2017. Representing nighttime and minimum conductance in CLM4. 5: global hydrology and carbon sensitivity analysis using observational constraints. Geoscientific Model Development 10: 321–331.

Lu Z, Percy RG, Qualet CO, Zeiger E. 1998. Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. Journal of Experimental Botany 49(Special Issue): 453–460.

Marks CO, Lechowicz MJ. 2007. The ecological and functional correlates of nocturnal transpiration. Tree Physiology 27: 577–584.

Marre P, Reynolds MP, Asseng S, Ewert F, Alderman PD, Cammarano D, Maiorano A, Ruane AC, Aggarwal PK, Anothai J. 2017. The International Heat Stress Genotype Experiment for modeling wheat response to heat: field experiments and AgMIP-wheat multi-model simulations. Open Data Journal for Agricultural Research 3: 23–28.

Matyssek R, Günthardt-Goerg MS, Maurer S, Keller T. 1995. Nighttime exposure to ozone reduces whole-plant production in Beta vulgaris. Tree Physiology 15: 159–165.

McAusland L, Viallet-Chabrand S, Davey P, Baker NR, Brendel O, Lawson T. 2016. Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. New Phytologist 211: 1209–1220.

McAusland L, Viallet-Chabrand S, Jauregui I, Burridge A, Hubbard-Edwards S, Fryer MJ, King IP, King J, Pyke K, Edwards KJ et al. 2020. Variation in leaf photosynthetic traits across wheat wild relatives is accession-dependent not species-dependent. New Phytologist 228: 1767–1780.

Moore CE, Meacham-Hensold K, Lemonnier P, Slattery RA, Benjamin C, Bernacchi CJ, Lawson T, Cavanagh AP. 2021. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. Journal of Experimental Botany 72: 2823–2844.

Mott KA, Gibson AC, O’Leary JW. 1982. The adaptive significance of amphistomatic leaves. Plant, Cell & Environment 5: 455–460.

O’Leary BM, Lee CP, Atkin OK, Cheng R, Brown TB, Millar AH. 2017. Variation in leaf respiration rates at night correlates with carbohydrate and amino acid supply. Plant Physiology 174: 2261–2273.

Parlange J-Y, Waggoner PE. 1970. Stomatal dimensions and resistance to diffusion. Plant Physiology 46: 337–342.

Pathare VS, Koteyeva N, Cousins AB. 2020. Increased adaxial stomatal density is associated with greater mesophyll surface area exposed to intercellular air spaces and mesophyll conductance in diverse C4 grasses. New Phytologist 225: 169–182.

Peary RW, Schulze ED, Zimmermann R. 2000. Measurement of transpiration and leaf conductance. In: Parley RW, Ehleringer JR, Mooney HA, Rundel PW, eds. Plant physiological ecology. Dordrecht, the Netherlands: Springer, 137–160.

Peng S, Huang J, Sheehe JY, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG. 2004. Rice yields decline with higher night temperature from global warming. Proceedings of the National Academy of Sciences, USA 101: 9971–9975.

Phillips NG, Lewis JD, Logan BA, Tissue DT. 2010. Inter-and intra-specific variation in nocturnal water transport in Eucalyptus. Tree Physiology 30: 586–596.

Posch BC, Kariyasaawam BC, Bramley H, Coast O, Richards RA, Reynolds MP, Trethewan R, Atkin OK. 2019. Exploring high temperature responses of photosynthesis and respiration to improve heat tolerance in wheat. Journal of Experimental Botany 70: 5051–5069.

Raab WS. 1997–2018. ImageJ. Bethesda, MD, USA: U. S. National Institutes of Health. [WWW document] URL: https://imagej.nih.gov/ij/ [accessed 1 February 2020].

Rawson H, Clarke J. 1988. Nocturnal transpiration in wheat. Functional Plant Biology 15: 397–406.

Resco de Dios V, Chowdhury FI, Granda E, Yao Y, Tissue DT. 2019. Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. New Phytologist 223: 1696–1706.

Resco de Dios V, Diaz-Sierra R, Goudlen ML, Barton CV, Boer MM, Gessler A, Ferrio JP, Plautsch S, Tissue DT. 2013a. Woody clockworks: circadian regulation of night-time water use in Eucalyptus globulus. New Phytologist 200: 743–752.

Resco de Dios V, Loik ME, Smith R, Aspinwall MJ, Tissue DT. 2016. Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth. Plant, Cell & Environment 39: 3–11.

Resco de Dios V, Roy J, Ferrio JP, Alday JG, Landais D, Milcu A, Gessler A. 2015. Processes driving nocturnal transpiration and implications for estimating land evapotranspiration. Scientific Reports 5: 10975.

Resco de Dios V, Turnbull MH, Barbour MM, Onetdu J, Ghannoum O, Tissue DT. 2013b. Soil phsophorous and endogenous rhythms exert a larger impact than CO2 or temperature on nocturnal stomatal conductance in Eucalyptus tereticornis. Tree Physiology 33: 1206–1215.

Rogiers SY, Clarke SJ. 2013. Nocturnal and daytime stomatal conductance respond to root-zone temperature in ‘Shiraz’ grapevines. Annals of Botany 111: 433–444.

Sadok W, Jagadish SK. 2020. The hidden costs of nighttime warming on yields. Trends in Plant Science 25: 644–651.

Schoppach R, Claever E, Sadok W. 2014. Genotype-dependent influence of night-time vapour pressure deficit on night-time transpiration and daytime gas exchange in wheat. Functional Plant Biology 41: 963–971.

Schoppach R, Sinclair TR, Sadok W. 2020. Sleep tight and wake-up early: nocturnal transpiration traits to increase wheat drought tolerance in a Mediterranean environment. Functional Plant Biology 47: 1117–1127.
Screen JA. 2014. Arctic amplification decreases temperature variance in northern mid-to high-latitudes. *Nature Climate Change* 4: 577–582.

Sillmann J, Kharin VV, Zwiers F, Zhang X, Bronaugh D. 2013. Climate extremes indices in the CMIP5 multimodel ensemble: Part 2. Future climate projections. *Journal of Geophysical Research: Atmospheres* 118: 2473–2493.

Slafer G, Rawson H. 1995. Base and optimum temperatures vary with genotype and stage of development in wheat. *Plant, Cell & Environment* 18: 671–679.

Snyder K, Richards J, Donovan L. 2003. Night-time conductance in C3 and C4 species: do plants lose water at night? *Journal of Experimental Botany* 54: 861–865.

Tamang BG, Sadok W. 2018. Nightly business: links between daytime canopy conductance, nocturnal transpiration and its circadian control illuminate physiological trade-offs in maize. *Environmental and Experimental Botany* 148: 192–202.

Thomas J, Prasad P. 2003. Plants and the environment: global warming effects. In: Thomas B, Murray BG, Murphy DJ, eds. *Encyclopedia of applied plant sciences*. Oxford, UK: Academic Press, 2097.

Tricker PJ, ElHabti A, Schmidt J, Fleury D. 2018. The physiological and genetic basis of combined drought and heat tolerance in wheat. *Journal of Experimental Botany* 69: 3195–3210.

Wachendorf M, Kipppers M. 2017. The effect of initial stomatal opening on the dynamics of biochemical and overall photosynthetic induction. *Trees* 31: 981–995.

Wang H, Atkin OK, Keenan TF, Smith NG, Wright IJ, Bloomfield KJ, Katge J, Reich PB, Prentice IC. 2020. Acclimation of leaf respiration consistent with optimal photosynthetic capacity. *Global Change Biology* 26: 2573–2583.

Wang Y, Anderegg WR, Venturas MD, Trugen AT, Yu K, Frankenberger C. 2021. Optimization theory explains nighttime stomatal responses. *New Phytologist* 230: 1550–1561.

Wasson AP, Richards R, Chathrath R, Misra S, Prasad SS, Rebetzke G, Kirkegaard J, Christopher J, Watt M. 2012. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany* 63: 3485–3498.

Wei T, Simko V. 2016. corrplot: visualization of a correlation matrix. 0.84. [WWW document] URL https://cran.r-project.org/web/packages/corrplot/corrplot.pdf [accessed 30 January 2020].

Welch JR, Vincent JR, Aufhammer M, Moya PF, Dobermann A, Dawe D. 2010. Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proceedings of the National Academy of Sciences*, USA 107: 14562–14567.

Yu K, Goldsmith GR, Wang Y, Anderegg WR. 2019. Phylogenetic and biogeographic controls of plant nighttime stomatal conductance. *New Phytologist* 222: 1778–1788.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14: 415–421.

Zeppel MJ, Lewis JD, Chaszar B, Smith RA, Medlynn BE, Huxman TE, Tissue DT. 2012. Nocturnal stomatal conductance responses to rising [CO₂], temperature and drought. *New Phytologist* 193: 929–938.

**Supporting Information**

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

**Fig. S1** The diurnal responses of photosynthetic CO₂ assimilation (A) in five biological replicates of modern genotype *T. aestivum* ‘Paragon’ and wild relative *T. urartu* 1010001.

**Fig. S2** Leaf-surface-specific differences in gₘ pre-flag leaf emergence in eight modern *T. aestivum* genotypes.

**Fig. S3** The genotype-specific responses of stomatal conductance pre-dawn (gₘ), 4 h post dawn, nocturnal respiration (Rₙ) and CO₂ assimilation (A) in six modern spring wheat genotypes and two Watkins landraces.

**Fig. S4** The relationship between net respiratory CO₂ release (R₀) and net photosynthetic CO₂ assimilation (A) in six modern spring wheat genotypes and two Watkins landraces.

**Fig. S5** The growth-stage specific responses of pre-dawn respiration (R₀) and 4 h post-dawn rates of CO₂ assimilation (A) in six modern spring wheat genotypes and two Watkins landraces.

**Fig. S6** A correlation matrix of all characteristics measured either compared as a mean of the data across the lifespan of the plant or comparing individual growth stages.

**Fig. S7** The intrinsic water-use efficiency (Wₑ) of six modern genotypes and two Watkins landraces. Data are separated to reflect genotype and growth-stage specific differences.

**Fig. S8** The stomata dimensions of pore length, guard cell with, pore area and, incorporating stomatal density, an estimation of anatomical maximum conductance (gₘₕₕₕₕ) of six modern genotypes and two Watkins landrace genotypes.

**Fig. S9** A graphical representation of the phenology of six modern spring wheat cultivars and two Watkins landraces.

**Fig. S10** The harvest traits of six modern genotypes and two Watkins landrace genotypes.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.