1 Effect of leaf temperature on estimating physiological traits of wheat leaves from 2 hyperspectral reflectance

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14

15 Abstract

16 A growing number of leaf traits can be predicted from hyperspectral reflectance data. These
17 include structural and compositional traits, such as leaf mass per area, nitrogen and
18 chlorophyll content, but also physiological traits such as Rubisco carboxylation activity,
19 electron transport rate and respiration rate. Since physiological traits vary with leaf
20 temperature, how does this impact on predictions made from reflectance measurements?
21 We investigated this with two wheat varieties, by repeatedly measuring each leaf through a
22 sequence of temperatures imposed by varying the air temperature in a growth room. The
23 function predicting Rubisco capacity normalised to 25 °C predicted the same value,
24 regardless of leaf temperatures ranging from 20 to 35°C. Leaf temperature affected none of
25 the predicted traits: $V_{\text{cmax}25}$, J , chlorophyll content, LMA, N content per unit leaf area or
26 $V_{\text{cmax}25}/N$. However, as others have derived models to predict Rubisco activity that includes
27 variation associated with leaf temperature, we discuss whether these functions may include
28 a temperature signal within the reflectance spectra.

29 Keywords: Leaf temperature, hyperspectral reflectance, Rubisco carboxylation activity,
30 electron transport rate, leaf dry mass per area, chlorophyll content, leaf nitrogen, *Triticum*
31 *aestivum*

32 Introduction

33 Plant breeders continually strive to improve crop yield. For cereals, there has been a
34 recognition that future increases could benefit from improving photosynthesis (Parry *et al.*,
35 2011; Reynolds *et al.*, 2009). Crop growth is not simply related to a measurement of
36 photosynthetic rate of a particular leaf under one condition. Instead, photosynthesis

integrated over a day with contributions from all the leaves in the canopy drives crop growth. Subsequent conversion into biomass and the partitioning into harvested grains determines yield. All these processes combined pose a major challenge on how to meaningfully measure photosynthesis with the goal of improving yield. However, there are a few examples that have compared historical sequences of cultivars and observed correlations between leaf photosynthetic rate and wheat yield (Beche *et al.*, 2014; Fischer *et al.*, 1998; Gaju *et al.*, 2016; Yao *et al.*, 2019). It has also been found that radiation use efficiency (above ground biomass produced per unit of intercepted photosynthetically active radiation) has been increasing over time with changing wheat varieties in both the UK (Shearman *et al.*, 2005) and Australia (Sadras *et al.*, 2012). Interestingly, both studies found the same rate of increase ($0.012 \text{ g MJ}^{-1} \text{ y}^{-1}$).

It is possible to survey photosynthetic properties between wheat genotypes (Driever *et al.*, 2014; Silva-Pérez *et al.*, 2019), but detailed phenotyping is time-consuming which limits the number of genotypes that can be sampled. A promising alternative is to predict photosynthetic traits from leaf reflectance spectra. Serbin *et al.* (2012) derived models predicting nitrogen concentration, leaf dry mass per unit area, maximum Rubisco carboxylase activity (V_{cmax}) and photosynthetic electron transport rate (J) from hyperspectral reflectance measured on leaves of *Populus tremuloides* and *P. deltoides*. Leaf temperature varied between 20 and 30 °C, depending on the glasshouse regime, which strongly influenced V_{cmax} . A single model was presented that applied to both species, regardless of leaf temperature. It was argued that this implied that the derived V_{cmax} was not being predicted indirectly from another trait such as nitrogen (Serbin *et al.*, 2012). The hyperspectral reflectance approach was also successfully used to predict V_{cmax} for *Glycine max* measured between 26 and 34°C (Ainsworth *et al.*, 2014) and *Nicotiana tabacum* (Meacham-Hensold *et al.*, 2019) and *Zea mays* (Yendrek *et al.*, 2017) measured at various temperatures in the field.

In order to be able to make useful comparisons of Rubisco capacity between plants which may differ in their leaf temperature during sampling, one needs to know both V_{cmax} and leaf temperature. Alternatively, one could use the temperature responses of the Rubisco enzyme kinetic parameters (Bernacchi *et al.*, 2003; Silva-Perez *et al.*, 2017) to convert gas exchange estimates of V_{cmax} to a common temperature, e.g. 25°C ($V_{\text{cmax}25}$), which are then used to build a model from reflectance data. This has been done for a group of 37 broadleaf tree species (Dechant *et al.*, 2017), wheat (Silva-Perez *et al.*, 2018) and 21 tropical tree species from Panama and Brazil (Wu *et al.*, 2019). Heckmann *et al.* (2017) also presented predictions of V_{cmax} for *Brassica*, *Moricandia* and *Z. mays* from reflectance spectra, but measured only at 25°C. While Dechant *et al.* (2017) normalised their gas exchange to 25°C, the reflectance spectra were collected at prevailing leaf temperatures. It is not known whether the prediction of $V_{\text{cmax}25}$ from leaf reflectance is insensitive to the temperature of the leaf during the reflectance measurement. We, therefore, set out to specifically assess whether leaf trait predictions from hyperspectral reflectance varied with leaf temperature by repeatedly measuring the same leaf sequentially through a range of temperatures in two wheat cultivars. We hypothesized that leaf temperature would not affect predicted values of leaf traits obtained using leaf hyperspectral reflectance.

80

81 **Materials and Methods**

82 *Plant material and growth conditions*

83 Expt 1 Two spring wheat genotypes (*Triticum aestivum* Kukri and Seri) were grown in a
84 naturally lit greenhouse (day/night temperatures set at 25/15 °C) at the Australian National
85 University in Canberra during Sep-Nov 2018. Three seeds were sown in well-drained 3.5-litre
86 pots filled with commercial potting mix, containing basal fertilizer Osmocote (Scotts). Pots
87 were laid out according to randomized block design with six replicates and blocks
88 representing the replications. After emergence, seedlings were thinned down to one plant
89 per pot. Plants were watered daily until the end of the experiment.

90 Temperature treatment was given in a controlled environmental chamber, with day/night
91 temperatures set at 25/15 °C and irradiance set to 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. All the
92 measurements were made seven days after anthesis. Plants were moved to the chamber
93 one day before the actual measurements so that plants could acclimatize to the chamber's
94 environmental conditions. The next day, measurements were made at a chamber
95 temperature of 15, 25, 35 and 15 °C, in the described sequence. After achieving the desired
96 chamber temperature, plants were acclimatized at least 1 hr before the measurements
97 were made.

98 Expt 2 Two spring wheat genotypes (Kukri and Seri) and one triticale (Hawkeye) were grown
99 in a greenhouse with temperature set to 20/15 °C (day/night). Seeds were sown on multiple
100 days in March 2018 and each genotype was sown separately in shallow tray with raising mix.
101 After germination, seedlings were transplanted into 5L pots filled commercial potting mix,
102 containing basal fertilizer Osmocote (Scotts). Five-six weeks after sowing, half of the plants
103 were transferred to an adjacent greenhouse room set at 32/20 °C, where they grew for one-
104 two more weeks before gas exchange measurements were made.

105 *Hyperspectral reflectance measurements (Expt 1)*

106 Hyperspectral reflectance spectra were measured with a FieldSpec®4 (Analytical Spectral
107 Devices, Boulder, CO, USA) full range spectroradiometer (350–2500 nm) attached to a leaf
108 clip (Analytical Spectral Devices, Boulder, CO, USA) with a fibre optic cable. Leaf clip had an
109 internal calibrated light source and two external panels i.e. a white panel to calibrate the
110 instrument and a black panel for taking measurements. A mask containing a black circular
111 gasket was also attached to leaf clip, which was used to reduce the leaf-clip aperture to an
112 oval area ($1.15 \times 1.4 \text{ cm} = 1.264 \text{ cm}^2$) suitable for a wheat leaf (Silva Perez ref). For each
113 temperature, one reflectance measurement was made at the same place of the flag leaf of
114 each plant by putting the leaf vertically to the leaf probe as explained elsewhere (Silva-Perez
115 *et al.*, 2018).

116 Leaf reflectance spectra were processed according to Silva-Perez *et al.* (2018). A 'jump'
117 correction associated with a change in the detectors at 1000 and 1800 nm was applied
118 before the traits were predicted.

119 *MultispeQ measurements (Expt 1)*

120 Linear electron transport (LET) and relative chlorophyll content (SPAD units) measurements
121 were carried out using a handheld MultispeQ (Beta) device linked to the PhotosynQ
122 platform (www.photosynq.org) (Kuhlgert *et al.*, 2016). Relative chlorophyll content (SPAD
123 units) was estimated by measuring the transmittance of red (650 nm) and infrared (940 nm)
124 light. LET was estimated from the measurements of quantum yield of photosystem II (ϕII) via
125 pulse-amplitude modulation (PAM) fluorometry at photosynthetically active radiation (PAR)
126 of 1000 μmol photons m⁻² s⁻¹ (Kuhlgert *et al.*, 2016).

127 *Gas-exchange measurements (Expt 2)*

128 Gas exchange was measured on the most recently fully expanded leaves with a LI-6400XT
129 Portable Photosynthesis system (LI-COR Biosciences Inc., Lincoln, NE, USA) on plants placed
130 inside a controlled environment cabinet (Thermoline Science Model-TRIL/SL). The air flow
131 rate was 500 μmol s⁻¹ with a PPFD of 1800 μmol m⁻² s⁻¹ supplied by the LED light. Gas
132 exchange was measured at leaf temperatures of 15, 25 and 35°C. At each temperature, CO₂
133 response curves were measured in 21% O₂ using inlet CO₂ concentrations of 400, 50, 100,
134 150, 250, 400, 600, 800, 1000, 400 μmol mol⁻¹. Subsequently, the air was changed to 2% O₂
135 with a CO₂ concentration in the leaf chamber of 380 μmol mol⁻¹, the flow reduced to 200
136 μmol s⁻¹ and measurement continued for 60 minutes with concurrent sampling for carbon
137 isotope discrimination to determine mesophyll conductance (Evans and von Caemmerer,
138 2013). Maximum Rubisco carboxylase activity (*V_{max}*) was calculated from CO₂ response
139 curves using kinetic constants derived from wheat (Silva-Pérez *et al.*, 2017).

140 *Statistical analysis*

141 Data were subjected to analysis of variance using various packages in R (R coreR, 2013).
142 Means were compared for significant differences using Tukey's multiple comparison tests at
143 5% probability level.

144 **Results**

145 The consequence of using leaf reflectance spectra collected from leaves with varying
146 temperatures to predict leaf traits was investigated with two wheat varieties.

147 The predicted value of *V_{max25}* was not affected by the leaf temperature when
148 reflectance spectra were measured, for either cultivar (Fig. 1A). Upon returning the growth
149 cabinet to 15°C, the *V_{max25}* values were not significantly different from the initial values. By
150 contrast, *V_{max}* values derived from gas exchange increased fourfold between 15 and 35°C
151 (Fig. 1B). Values for wheat grown under cool or hot conditions showed a difference at 35°C,
152 with the cool grown plants falling further below the theoretical line (consistent with an *E_a* of
153 63kJ mol⁻¹) than plants from the hot treatment. The values for plants from the hot
154 treatment superimpose the previously published data from Silva-Perez et al. (2017).

155 Predicted values for the rate of electron transport, *J*, were also independent of the
156 leaf temperature when reflectance spectra were measured (Fig. 2A). In this case, the model
157 was built from data collected under a PPFD of 1800 μmol m⁻² s⁻¹ and leaf temperatures

mainly at 25°C but ranging up to 32°C. However, in contrast to V_{cmax} , J is less sensitive to leaf temperature, increasing by 30% between 15 and 25°C and then plateauing (Fig. 2B). The temperature responses of J were comparable to the previously published data from Silva-Perez et al. (2017). Growth temperature shifted the temperature response. For plants grown under hot conditions, J was less than that from plants grown under cool conditions at 15°C but greater at 35°C. We also measured J using a MultispeQ instrument following the collection of leaf reflectance spectra at each temperature (denoted LET, Fig. 2A). This was measured under a PPFD of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, similar to the irradiance in the growth cabinet. As it was collected rapidly, it does not represent the steady state. However, it also indicated that the rate of electron transport was similar between 20 and 30°C, then declined slightly at 35°C.

Predicted values of chlorophyll content were insensitive to the leaf temperature when reflectance spectra were measured (Fig. 3). A similar result was observed for chlorophyll content estimated with the MultispeQ. The absolute values obtained with the MultispeQ were about 20% greater than that predicted from reflectance. The chlorophyll content values are predicted from reflectance using a model built on measurements using SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd, Japan) whereas the MultispeQ uses relative transmissions of red (650 nm) and infrared (940 nm) light. Additionally, MultispeQ has two in built differences from Minolta SPAD; 1) MultispeQ takes a series of transmittance measurements over a range of increasing light intensities, and 2) MultispeQ also averages values over a larger leaf area ($\sim 1 \text{ cm}^2$) (Kuhlgert et al. 2016). Nevertheless, additional calibration comparisons were not made as the focus was on temperature. Values predicted for three other leaf traits, leaf dry mass per unit leaf area (LMA), nitrogen content and Rubisco carboxylation capacity normalised to 25 °C per unit leaf nitrogen ($V_{\text{cmax}25}/N$), were also independent of the leaf temperature when reflectance spectra were measured (Fig. 4). A statistical comparison of the effects of temperature treatments on various measured and predicted leaf traits is also provided separately (Supp. Table 1).

The spectral response of correlations between single wavelength reflectance values and leaf temperature is shown superimposed on the reflectance spectrum (Fig. 5). Clear correlations were observed on the long wavelength shoulders of the two water absorption bands with peaks at 1531 and 2038nm and a third peak in the red edge at 720nm. By sequentially adding weighted reflectance values from single wavelengths, we were able to predict leaf temperature remarkably well with just four wavelengths (Fig. 6).

Discussion

Leaf hyperspectral reflectance is an optical signal that can provide information remotely and rapidly. With appropriate calibration data obtained from other methods, predictive models can be built for a range of leaf traits. The method has potential for use as a high-throughput tool for phenotyping photosynthetic traits at the leaf and canopy scale. While one might expect leaf reflectance to enable prediction of the amount of a substance e.g. leaf dry mass or nitrogen per unit area, it is harder to understand how physiological processes such as rates of reactions could contribute to reflectance. Thus, with Rubisco

being such a major constituent of leaf protein, a reflectance model could arise from a signal associated with leaf protein or nitrogen, as argued by Dechant et al. (2017). If this was the case, then changing leaf temperature would alter Rubisco activity but not Rubisco content. However, Serbin et al. (2012) successfully included variation in leaf temperature to derive a model predicting Rubisco activity, demonstrating that the model was not fundamentally associated with another constituent such as nitrogen. In contrast to gas exchange measurements, where leaf temperature is measured directly to enable the calculation of stomatal conductance, leaf temperature is not generally measured directly during the collection of hyperspectral reflectance. Consequently, it is necessary to consider how leaf temperature affects the estimation of physiological traits, such as V_{cmax} and J , using leaf hyperspectral reflectance.

Parameters independent of temperature

A leaf structural property that has been widely reported is leaf dry mass per unit leaf area as it is easy to measure and relates to lifespan and other traits (Wright *et al.*, 2004). Robust predictions of LMA can be made from hyperspectral reflectance data (Ecarnot *et al.*, 2013; Serbin *et al.*, 2012; Silva-Perez *et al.*, 2018). As LMA is a leaf property that would not change in response to short term changes in temperature, models predicting LMA from hyperspectral reflectance should also be insensitive to the temperature of the leaf during measurement. This was found to be true for LMA (Fig. 4A) as well as for other leaf constituents, chlorophyll (Fig. 3) and nitrogen (Fig. 4B).

Photosynthesis is a process involving many constituents, but has been successfully modelled in C_3 plants by considering the properties of Rubisco (Farquhar *et al.*, 1980). Knowing the amount of Rubisco per unit leaf area, its properties and a few assumptions, it is possible to predict photosynthetic responses to irradiance, atmospheric CO_2 and temperature. The amount of Rubisco is unlikely to vary significantly during short term changes in leaf temperature, but the carboxylase activity is strongly temperature dependent (Badger and Collatz, 1977; Bernacchi *et al.*, 2001; Sharwood *et al.*, 2016). Therefore, models using reflectance to predict Rubisco content or activity normalised to a fixed temperature should be independent of leaf temperature. Indeed, this was what we observed (Fig. 1A).

Parameters that vary with temperature

Enzyme activities vary with temperature which can be described with the Arrhenius equation through the energy of activation term E_a . V_{cmax} is the product of Rubisco content and catalytic rate. It seems possible that models predicting V_{cmax} from reflectance (Ainsworth *et al.*, 2014; Serbin *et al.*, 2012) may contain two components, one that is independent of temperature (representing Rubisco protein content) and another that varies with leaf temperature. Immediately prior to placing the leaf into the clip for measuring leaf reflectance, Ainsworth et al. (2014) measured leaf temperature with an infrared thermometer, but they only report the relationship between predicted V_{cmax} and leaf temperature. We, therefore, looked at our reflectance spectra collected at different leaf temperatures to see if we could predict leaf temperature. Predicted leaf temperature, using reflectance of just four wavelengths, clearly correlated with measured leaf temperature,

with an r^2 of 0.91 (Fig. 6). It is unlikely that this equation has general application as it arose from only two genotypes measured under one environment and it is known that the power and generality of models predicting dark respiration (Coast *et al.*, 2019) and $V_{\text{cmax}25}$ (Wu *et al.*, 2019) improved as the diversity of calibration data increased. However, the point is that leaf temperature apparently can be extracted from reflectance spectra which could explain how models can predict reaction rates from reflectance.

The Arrhenius equation predicts an exponential increase in rate with increasing temperature, whereas models calculating parameters from reflectance sum linear weightings of each reflectance at each wavelength and would, therefore, have linear responses to temperature. The difference between an exponential and a linear relationship may not be very noticeable over a narrow temperature range. In the case of V_{cmax} , values derived from gas exchange deviated below the Arrhenius function at 35°C (Fig. 1B), such that a linear function would fit the data well between 15 and 35°C. There are also indications that a single value for E_a may not be appropriate across the temperature range from 10 to 40°C. Sharwood *et al.* (2016) found it necessary to use lower values for E_a at leaf temperatures above 25°C. The rate of electron transport, J , also varies with temperature (Bernacchi *et al.*, 2003; June *et al.*, 2004; Medlyn *et al.*, 2002) but reaches a maximum around 30°C before decreasing again. As a result, the change in J between 20 and 35°C is less pronounced than for V_{cmax} . It is therefore uncertain whether models predicting J from reflectance (Dechant *et al.*, 2017; Serbin *et al.*, 2012; Silva-Perez *et al.*, 2018) would contain components that vary with temperature. In the case of J for wheat (Silva-Perez *et al.*, 2018), the reflectance model was built with data that varied little in leaf temperature and the predicted values of J were found to be unaffected by leaf temperature when reflectance was measured (Fig. 2A). However, given that J did not vary greatly over this temperature range (Fig. 2), this may not be a very rigorous test. By contrast, as Serbin *et al.* (2012) deliberately used variation in leaf temperature to generate a broader spread in J to build their reflectance model – testing their function with multiple spectra obtained from a leaf measured over a range of temperatures could be informative.

Conclusion

Leaf temperature varying between 20 to 35°C during the measurement of leaf reflectance did not affect predicted values of leaf traits ($V_{\text{cmax}25}$, chlorophyll and nitrogen contents per unit area, LMA and V_{cmax}/N), for wheat. It was possible to extract leaf temperature from reflectance spectra which may explain how models that predict rates that vary with temperature (V_{cmax} , J , dark respiration) could arise. Models predicting traits that vary with leaf temperature should be tested using multiple measurements from each leaf covering a range of temperature. Reflectance appears to have the potential to predict leaf temperature, but to construct a robust model would require calibration with a broader set of experiments.

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284 **Supplementary material**

285 Table S1. Effects of leaf temperature on leaf physiological traits

286

Supplementary. Table 1. Effects of leaf temperature on leaf physiological traits measured using MultispeQ (leaf temperature, LET, relative chlorophyll) or predicted from leaf hyperspectral reflectance ($V_{\text{cmax}25}$, J, chlorophyll content, LMA, nitrogen content, $V_{\text{cmax}25}/N$) in leaves of two wheat genotypes exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 °C, in a growth chamber (Expt 1). Symbols represent the mean $\pm$ SE of six different leaves from six different plants. Sequential measurements were made on each leaf, seven days after anthesis.

Genotype	Ambient Air Temperature	Measured traits using MultispeQ			Predicted traits using leaf hyperspectral reflectance					
		Leaf Temperature (°C)	LET ($\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$)	Relative Chlorophyll (SPAD units)	$V_{\text{cmax}25}$ ($\mu\text{mol CO}_2 \text{s}^{-1} \text{g}^{-1} (\text{N})$)	J ($\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$)	Chlorophyll content (SPAD units)	LMA (g m^{-2})	Nitrogen content (g m^{-2})	$V_{\text{cmax}25}/N$ ($\mu\text{mol CO}_2 \text{s}^{-1} \text{g}^{-1} (\text{N})$)
Kukri	15 °C	19.6 $\pm$ 0.4	142.9 $\pm$ 7.0	59.8 $\pm$ 0.7	162.5 ^a $\pm$ 1.7	196.2 ^{ab} $\pm$ 3.6	51.8 ^{ab} $\pm$ 0.6	54.0 $\pm$ 1.2	2.81 $\pm$ 0.07	59.6 ^b $\pm$ 1.2
	25 °C	26.2 $\pm$ 0.2	145.2 $\pm$ 9.2	59.9 $\pm$ 1.0	154.2 ^b $\pm$ 1.8	184.9 ^b $\pm$ 3.7	52.6 ^a $\pm$ 0.4	57.4 $\pm$ 0.8	2.90 $\pm$ 0.05	59.7 ^b $\pm$ 0.9
	35 °C	33.4 $\pm$ 0.3	127.0 $\pm$ 4.3	60.6 $\pm$ 0.6	161.3 ^a $\pm$ 2.2	196.3 ^{ab} $\pm$ 4.5	50.8 ^b $\pm$ 0.4	56.3 $\pm$ 1.1	2.82 $\pm$ 0.06	64.1 ^a $\pm$ 0.6
	15 °C	19.5 $\pm$ 0.3	145.4 $\pm$ 8.1	59.2 $\pm$ 1.0	165.2 ^a $\pm$ 2.6	203.8 ^a $\pm$ 5.0	52.7 ^a $\pm$ 0.3	55.7 $\pm$ 1.1	2.89 $\pm$ 0.05	60.6 ^b $\pm$ 1.0
	LSD (5%)		n.s.	n.s.	6.2**	12.5*	1.3*	n.s.	n.s.	2.8*
Seri	15 °C	19.7 $\pm$ 0.3	144.2 ^a $\pm$ 5.2	62.7 $\pm$ 0.8	173.9 $\pm$ 2.4	209.2 $\pm$ 4.0	53.7 $\pm$ 0.4	56.9 ^b $\pm$ 0.7	3.05 $\pm$ 0.05	60.1 ^b $\pm$ 0.8
	25 °C	27.3 $\pm$ 0.1	148.6 ^a $\pm$ 6.1	63.3 $\pm$ 0.8	169.5 $\pm$ 2.2	203.5 $\pm$ 3.7	53.8 $\pm$ 0.2	58.5 ^{ab} $\pm$ 0.6	3.08 $\pm$ 0.05	61.0 ^b $\pm$ 0.7
	35 °C	34.9 $\pm$ 0.4	124.9 ^b $\pm$ 3.2	64.5 $\pm$ 0.8	169.4 $\pm$ 2.2	203.7 $\pm$ 4.3	53.5 $\pm$ 0.4	60.4 ^a $\pm$ 0.7	3.05 $\pm$ 0.05	65.7 ^a $\pm$ 0.7
	15 °C	19.6 $\pm$ 0.2	145.1 ^a $\pm$ 6.5	62.8 $\pm$ 0.7	168.4 $\pm$ 2.2	208.5 $\pm$ 3.7	53.0 $\pm$ 0.3	57.9 ^b $\pm$ 0.9	2.96 $\pm$ 0.04	61.5 ^b $\pm$ 0.5
	LSD (5%)		15.9*	n.s.	n.s.	n.s.	n.s.	2.1*	n.s.	2.04***

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = non-significant

Figures

Fig. 1. Effects of leaf temperature on Rubisco activity in wheat. A. The maximum rate of carboxylation by Rubisco normalised to 25 °C (V_{cmax25}) predicted from leaf hyperspectral reflectance measurements at different leaf temperatures. Seven days after anthesis, two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 °C, in a growth chamber (Expt 1). Symbols represent the mean $\pm$ SE of six different leaves from six different plants. B. Temperature response of V_{cmax} normalised to 1 at 25 °C derived from gas exchange measurements (symbols) or modelled (E_a 63 kJ mol⁻¹). Data from plants grown under hot (20/15 °C) or cool (32/20 °C) conditions (Expt 2), or from Silva-Perez et al. (2017).

Fig. 2. Effects of leaf temperature on electron transport rate in wheat. A. Rate of electron transport (J) predicted from leaf reflectance under a PPFD of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (solid symbols) and linear electron transport (LET) measured using MultispeQ under a PPFD of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (open symbols) at each leaf temperature. Seven days after anthesis, two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 $^{\circ}\text{C}$, in a growth chamber (Expt 1). Symbols represent the mean $\pm$ SE of six different leaves from six different plants. . B. Rates of electron transport calculated from gas exchange measurements made under a PPFD of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ from plants grown under cool (20/15 $^{\circ}\text{C}$) or hot (32/20 $^{\circ}\text{C}$) conditions (Expt 2), or from Silva Perez et al. (2017).

Fig. 3. Effects of leaf temperature on estimated chlorophyll content (SPAD units) in wheat. Seven days after anthesis, two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 °C, in a growth chamber (Expt 1). Symbols represent the mean $\pm$ SE of six different leaves from six different plants. Leaf chlorophyll content was estimated using two different devices i.e. direct measurements with MultispeQ or predicted from leaf hyperspectral reflectance measurements made with an ASD FieldSpec Spectroradiometer.

Fig. 4. Effects of leaf temperature on parameters predicted from leaf hyperspectral reflectance in wheat. A. Leaf dry mass per unit leaf area (LMA). B. Nitrogen content per unit leaf area. C. Rubisco carboxylation capacity normalised to 25 °C per unit leaf nitrogen ($V_{\text{max}25}/N$). Seven days after anthesis, two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 °C, in a growth chamber (Expt 1). Symbols represent the mean $\pm$ SE of six different leaves from six different plants.

Fig. 5. Reflectance spectrum from a wheat leaf measured at 27°C (black thick line) with the spectral response of the correlation coefficient between leaf temperature and leaf reflectance (red thin line) superimposed.

Fig. 6. Relationship between leaf temperature predicted from reflectance and measured using MultispeQ in wheat. Two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 °C which resulted in the leaf temperature increasing from 20 to 35°C (solid symbols) then decreased back to 20°C (open symbols) (Expt 1). Measurements were made on six different leaves from six different plants. The predicted leaf temperature was calculated as: $T_{\text{leaf}} = 1071.4 R_{1531} - 945.65 R_{1400} - 176.3 R_{1507} + 196.8 R_{697}$. The fitted regression equation (not shown) was $y = 1.015x - 0.4$, $r^2 = 0.91$.

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