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 a 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_{cmax25}, J, chlorophyll content, LMA, N content per unit leaf area or
26 V_{cmax25}/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

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

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

63   In order to be able to make useful comparisons of Rubisco capacity between plants
64   which may differ in their leaf temperature during sampling, one needs to know both V_{cmax}
65   and leaf temperature. Alternatively, one could use the temperature responses of the
66   Rubisco enzyme kinetic parameters (Bernacchi *et al.*, 2003; Silva-Perez *et al.*, 2017) to
67   convert gas exchange estimates of V_{cmax} to a common temperature, e.g. 25°C (V_{cmax25}),
68   which are then used to build a model from reflectance data. This has been done for a group
69   of 37 broadleaf tree species (Dechant *et al.*, 2017), wheat (Silva-Perez *et al.*, 2018) and 21
70   tropical tree species from Panama and Brazil (Wu *et al.*, 2019). Heckmann et al. (2017) also
71   presented predictions of V_{cmax} for *Brassica*, *Moricandia* and *Z. mays* from reflectance
72   spectra, but measured only at 25°C. While Dechant et al. (2017) normalised their gas
73   exchange to 25°C, the reflectance spectra were collected at prevailing leaf temperatures. It
74   is not known whether the prediction of V_{cmax25} from leaf reflectance is insensitive to the
75   temperature of the leaf during the reflectance measurement. We, therefore, set out to
76   specifically assess whether leaf trait predictions from hyperspectral reflectance varied with
77   leaf temperature by repeatedly measuring the same leaf sequentially through a range of
78   temperatures in two wheat cultivars. We hypothesized that leaf temperature would not
79   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 ^{o}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 ^{o}C and irradiance set to 500 μmol photons m^{-2} 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 ^{o}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 ^{o}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 ^{o}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 x 1.4 cm = 1.264 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, CO_2
133 response curves were measured in 21% O_2 using inlet CO_2 concentrations of 400, 50, 100,
134 150, 250, 400, 600, 800, 1000, 400 µmol mol^{-1}. Subsequently, the air was changed to 2% O_2
135 with a CO_2 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_{cmax}) was calculated from CO_2 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_{cmax25} 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_{cmax25} values were not significantly different from the initial values. By
150 contrast, V_{cmax} 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

158 mainly at 25°C but ranging up to 32°C. However, in contrast to V_{cmax}, J is less sensitive to leaf
159 temperature, increasing by 30% between 15 and 25°C and then plateauing (Fig. 2B). The
160 temperature responses of J were comparable to the previously published data from Silva-
161 Perez et al. (2017). Growth temperature shifted the temperature response. For plants
162 grown under hot conditions, J was less than that from plants grown under cool conditions at
163 15°C but greater at 35°C. We also measured J using a MultispeQ instrument following the
164 collection of leaf reflectance spectra at each temperature (denoted LET, Fig. 2A). This was
165 measured under a PPFD of 1000 μmol m^{-2} s^{-1}, similar to the irradiance in the growth cabinet.
166 As it was collected rapidly, it does not represent the steady state. However, it also indicated
167 that the rate of electron transport was similar between 20 and 30°C, then declined slightly
168 at 35°C.

169 Predicted values of chlorophyll content were insensitive to the leaf temperature
170 when reflectance spectra were measured (Fig. 3). A similar result was observed for
171 chlorophyll content estimated with the MultispeQ. The absolute values obtained with the
172 MultispeQ were about 20% greater than that predicted from reflectance. The chlorophyll
173 content values are predicted from reflectance using a model built on measurements using
174 SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd, Japan) whereas the MultispeQ uses
175 relative transmissions of red (650 nm) and infrared (940 nm) light. Additionally, MultispeQ
176 has two in built differences from Minolta SPAD; 1) MultispeQ takes a series of transmittance
177 measurements over a range of increasing light intensities, and 2) MultispeQ also averages
178 values over a larger leaf area (~ 1 cm^2) (Kuhlgert et al. 2016). Nevertheless, additional
179 calibration comparisons were not made as the focus was on temperature. Values predicted
180 for three other leaf traits, leaf dry mass per unit leaf area (LMA), nitrogen content and
181 Rubisco carboxylation capacity normalised to 25 °C per unit leaf nitrogen (V_{cmax25}/N), were
182 also independent of the leaf temperature when reflectance spectra were measured (Fig. 4).
183 A statistical comparison of the effects of temperature treatments on various measured and
184 predicted leaf traits is also provided separately (Supp. Table 1).

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

191 **Discussion**

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

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

210 *Parameters independent of temperature*

211 A leaf structural property that has been widely reported is leaf dry mass per unit leaf
212 area as it is easy to measure and relates to lifespan and other traits (Wright *et al.*, 2004).
213 Robust predictions of LMA can be made from hyperspectral reflectance data (Ecarnot *et al.*,
214 2013; Serbin *et al.*, 2012; Silva-Perez *et al.*, 2018). As LMA is a leaf property that would not
215 change in response to short term changes in temperature, models predicting LMA from
216 hyperspectral reflectance should also be insensitive to the temperature of the leaf during
217 measurement. This was found to be true for LMA (Fig. 4A) as well as for other leaf
218 constituents, chlorophyll (Fig. 3) and nitrogen (Fig. 4B).

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

228 *Parameters that vary with temperature*

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

240 with an r^2 of 0.91 (Fig. 6). It is unlikely that this equation has general application as it arose
241 from only two genotypes measured under one environment and it is known that the power
242 and generality of models predicting dark respiration (Coast *et al.*, 2019) and V_{cmax25} (Wu *et*
243 *al.*, 2019) improved as the diversity of calibration data increased. However, the point is that
244 leaf temperature apparently can be extracted from reflectance spectra which could explain
245 how models can predict reaction rates from reflectance.

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

268 **Conclusion**

269   Leaf temperature varying between 20 to 35°C during the measurement of leaf
270 reflectance did not affect predicted values of leaf traits (V_{cmax25}, chlorophyll and nitrogen
271 contents per unit area, LMA and V_{cmax}/N), for wheat. It was possible to extract leaf
272 temperature from reflectance spectra which may explain how models that predict rates that
273 vary with temperature (V_{cmax}, J, dark respiration) could arise. Models predicting traits that
274 vary with leaf temperature should be tested using multiple measurements from each leaf
275 covering a range of temperature. Reflectance appears to have the potential to predict leaf
276 temperature, but to construct a robust model would require calibration with a broader set
277 of experiments.

278 **Acknowledgements**

8

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 MulitispeQ (leaf temperature, LET, relative chlorophyll) or predicted from leaf hyperspectral reflectance (V_{cmax25}, J, chlorophyll content, LMA, nitrogen content, V_{cmax25}/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 ± 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 (μmol e⁻ m⁻² s⁻¹)	Relative Chlorophyll (SPAD units)	V_{cmax25} (μmol CO₂ s⁻¹ g⁻¹ (N))	J (μmol e⁻ m⁻² s⁻¹)	Chlorophyll content (SPAD units)	LMA (g m⁻²)	Nitrogen content (g m⁻²)	V_{cmax25}/N (μmol CO₂ s⁻¹ g⁻¹ (N))
Kukri	15 °C	19.6 + 0.4	142.9 ± 7.0	59.8 ± 0.7	162.5[a] ± 1.7	196.2[ab] ± 3.6	51.8[ab] ± 0.6	54.0 ± 1.2	2.81 ± 0.07	59.6[b] ± 1.2
	25 °C	26.2 + 0.2	145.2 ± 9.2	59.9 ± 1.0	154.2[b] ± 1.8	184.9[b] ± 3.7	52.6[a] ± 0.4	57.4 ± 0.8	2.90 ± 0.05	59.7[b] ± 0.9
	35 °C	33.4 + 0.3	127.0 ± 4.3	60.6 ± 0.6	161.3[a] ± 2.2	196.3[ab] ± 4.5	50.8[b] ± 0.4	56.3 ± 1.1	2.82 ± 0.06	64.1[a] ± 0.6
	15 °C	19.5 + 0.3	145.4 ± 8.1	59.2 ± 1.0	165.2[a] ± 2.6	203.8[a] ± 5.0	52.7[a] ± 0.3	55.7 ± 1.1	2.89 ± 0.05	60.6[b] ± 1.0
	LSD (5%)	n.s.	n.s.	n.s.	6.2**	12.5*	1.3*	n.s.	n.s.	2.8*
Seri	15 °C	19.7 + 0.3	144.2[a] ± 5.2	62.7 ± 0.8	173.9 ± 2.4	209.2 ± 4.0	53.7 ± 0.4	56.9[b] ± 0.7	3.05 ± 0.05	60.1[b] ± 0.8
	25 °C	27.3 + 0.1	148.6[a] ± 6.1	63.3 ± 0.8	169.5 ± 2.2	203.5 ± 3.7	53.8 ± 0.2	58.5[ab] ± 0.6	3.08 ± 0.05	61.0[b] ± 0.7
	35 °C	34.9 + 0.4	124.9[b] ± 3.2	64.5 ± 0.8	169.4 ± 2.2	203.7 ± 4.3	53.5 ± 0.4	60.4[a] ± 0.7	3.05 ± 0.05	65.7[a] ± 0.7
	15 °C	19.6 + 0.2	145.1[a] ± 6.5	62.8 ± 0.7	168.4 ± 2.2	208.5 ± 3.7	53.0 ± 0.3	57.9[b] ± 0.9	2.96 ± 0.04	61.5[b] ± 0.5
	LSD (5%)	15.9*	n.s.	n.s.	n.s.	n.s.	n.s.	2.1*	n.s.	2.04***

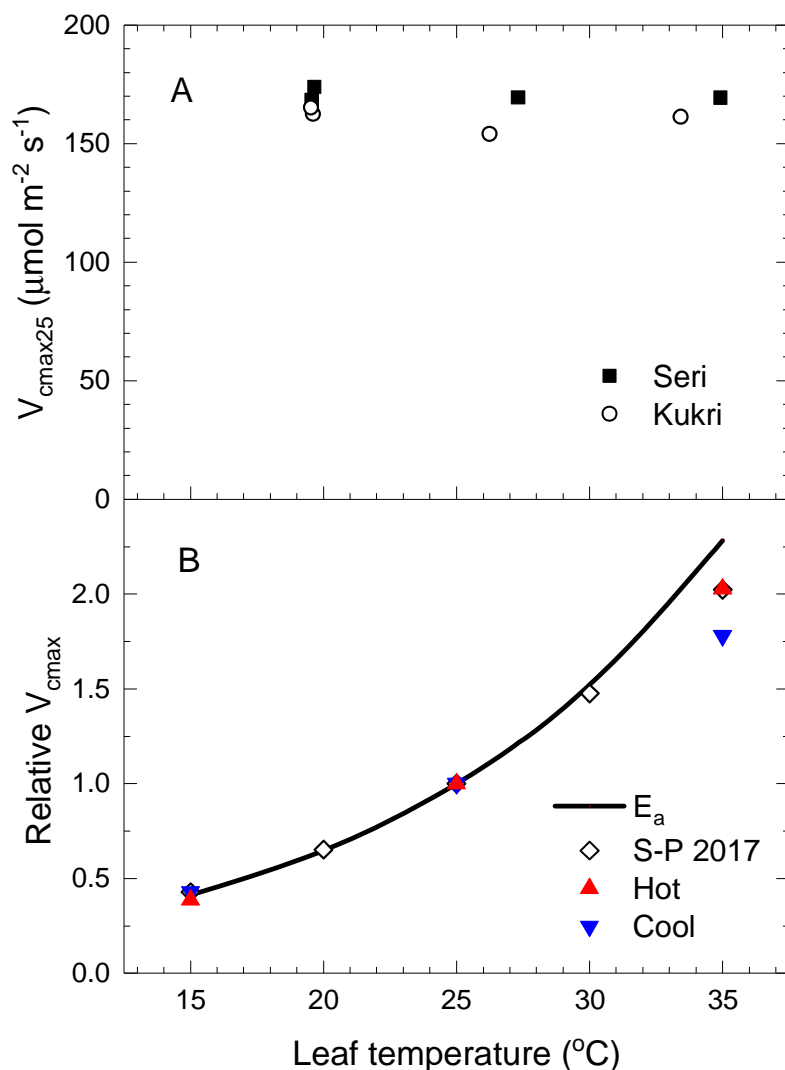*** = P <0.001, ** = P < 0.01, * = P < 0.05, n.s. = non-significant

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 ± 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).
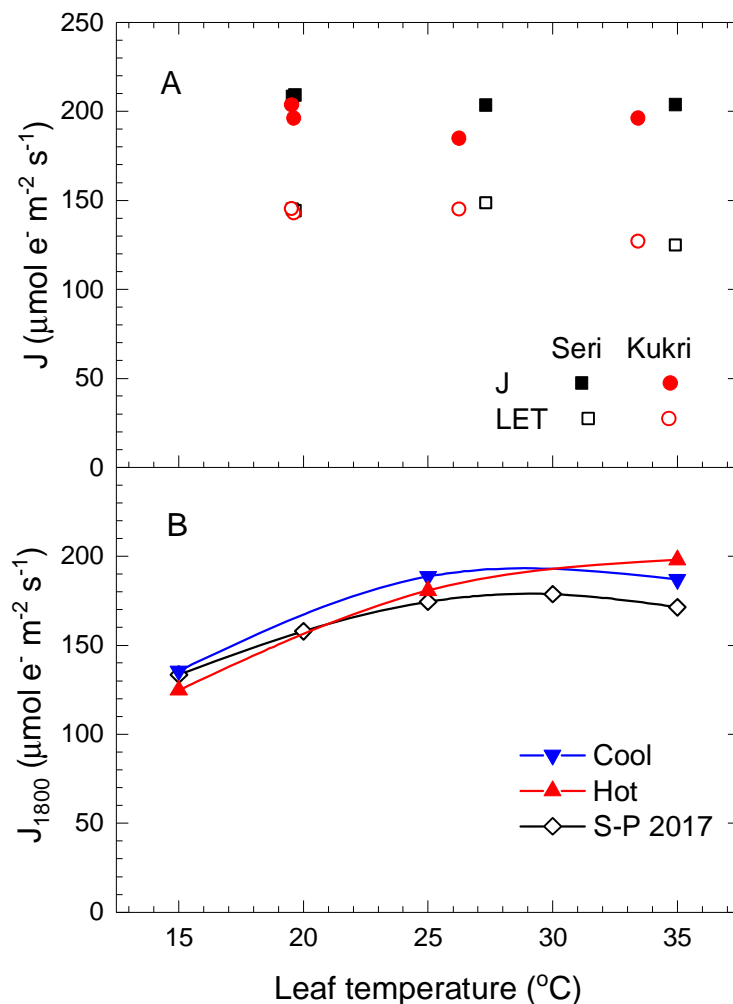
Fig. 2. Effects of leaf temperature on electron transport rate in wheat. A. Rate of electron transport (J) predicted from leaf reflectance under a PPFD of 1800 μmol m^{-2} s^{-1} (solid symbols) and linear electron transport (LET) measured using MultispeQ under a PPFD of 1000 μmol m^{-2} s^{-1} (open 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 ^{o}C, in a growth chamber (Expt 1). Symbols represent the mean ± 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 μmol m^{-2} s^{-1} from plants grown under cool (20/15 ^{o}C) or hot (32/20 ^{o}C) conditions (Expt 2), or from Silva Perez et al. (2017).
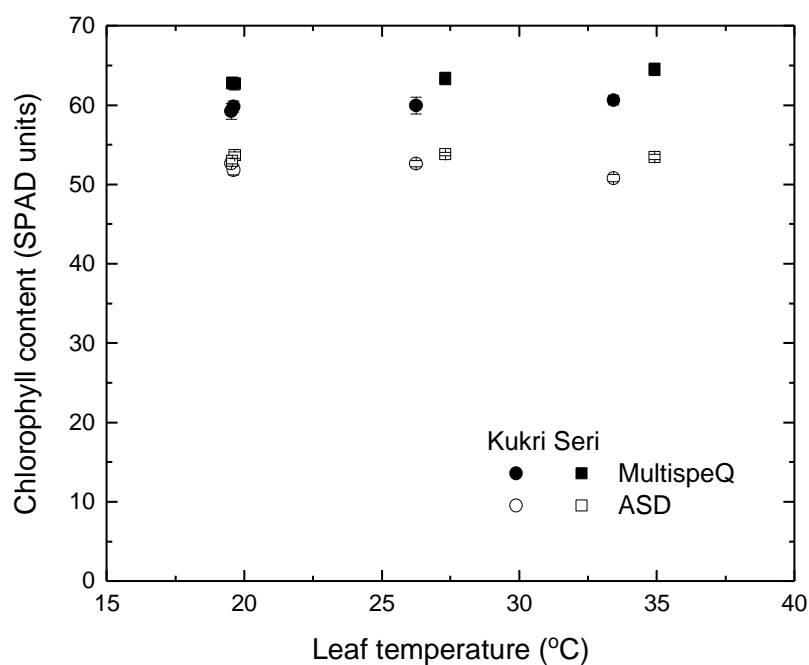
Fig. 3. Effects of leaf temperature on estimated chlorophyll content (SPAD units) in wheat. Seven days after anthesis, two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 ^{o}C, in a growth chamber (Expt 1). Symbols represent the mean ± SE of six different 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.
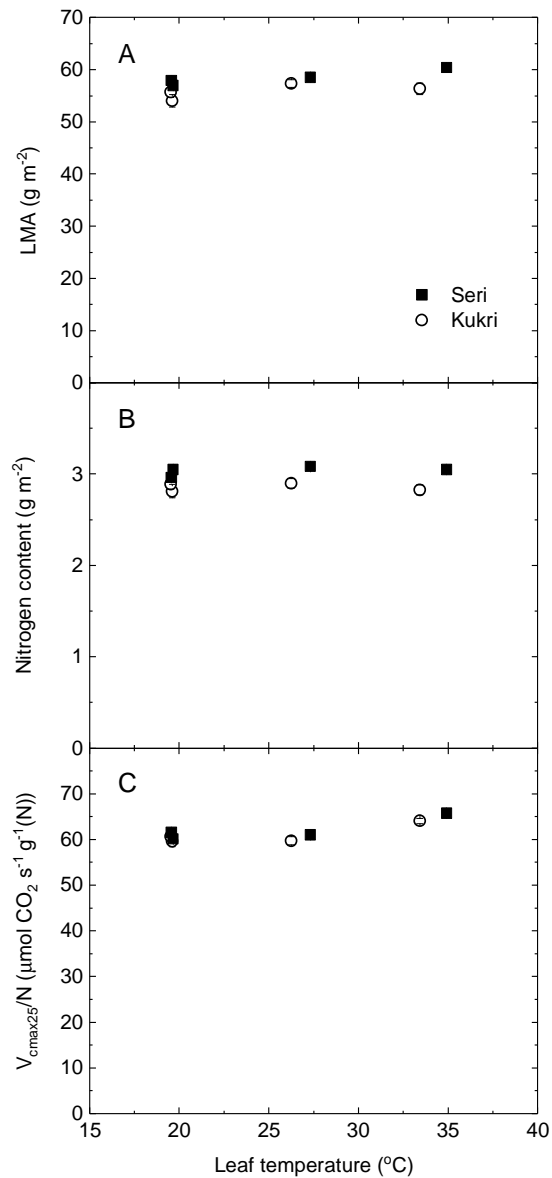
Fig. 4. Effects of leaf temperature on parameters predicted from leaf hyperspectral reflectance in wheat. A. Leaf dry mass per unit leaf area (LMA). B. Nitrogen content per unit leaf area. C. Rubisco carboxylation capacity normalised to 25 °C per unit leaf nitrogen (V_{cmax25}/N). Seven days after anthesis, 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 ± SE of six different leaves from six different plants.
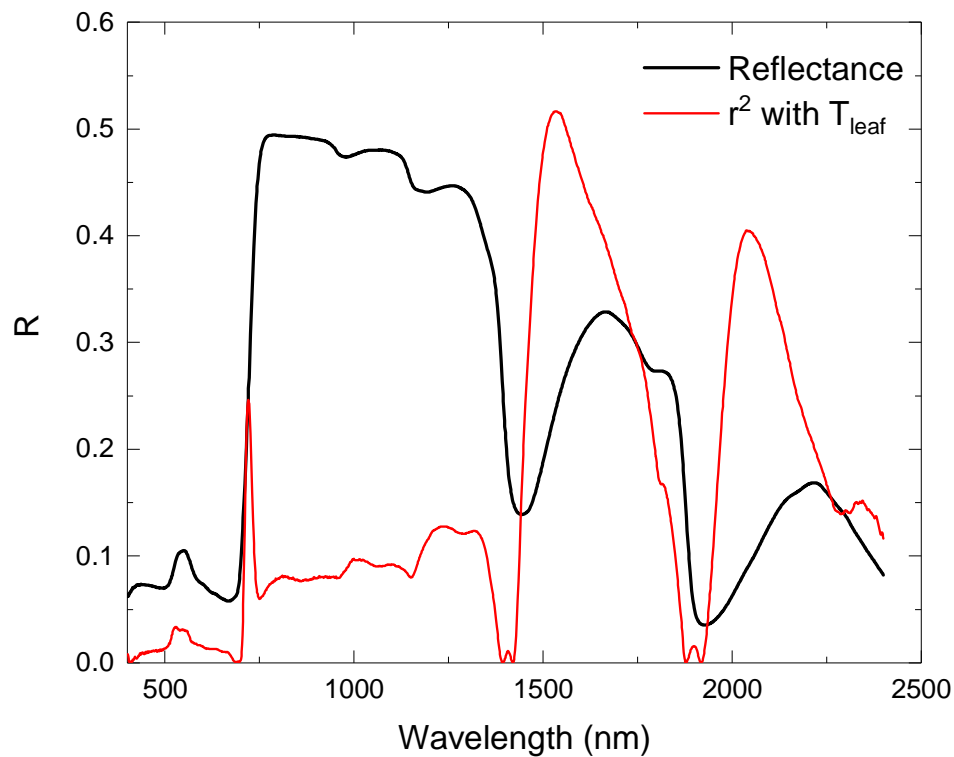
Fig. 5. Reflectance spectrum from a wheat leaf measured at 27°C (black thick line) with the spectral response of the correlation coefficient between leaf temperature and leaf reflectance (red thin line) superimposed.
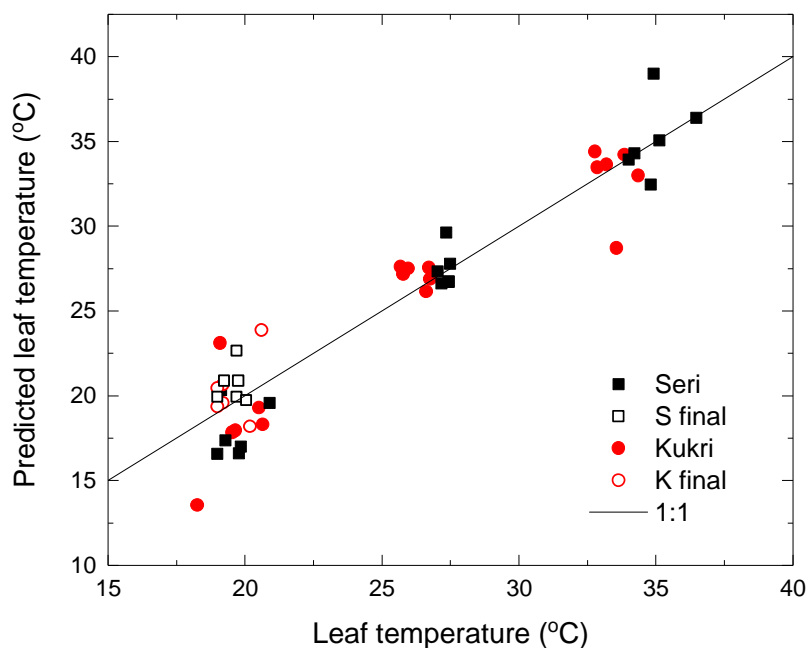
Fig. 6. Relationship between leaf temperature predicted from reflectance and measured using MultispeQ in wheat. Two wheat genotypes were exposed to a sequence of ambient air temperatures i.e. 15, 25, 35 and 15 °C which resulted in the leaf temperature increasing from 20 to 35°C (solid symbols) then decreased 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: $T_{leaf} = 1071.4\ R_{1531} - 945.65\ R_{1400} - 176.3\ R_{1507} + 196.8\ R_{697}$. The fitted regression equation (not shown) was y = 1.015x - 0.4, r^2 = 0.91.

References

Ainsworth EA, Serbin SP, Skoneczka JA, Townsend PA. 2014. Using leaf optical properties to detect ozone effects on foliar biochemistry. *PHOTOSYNTHESIS RESEARCH* **119**, 65-76.

Badger M, Collatz GJ. 1977. Studies on the kinetic mechanism of ribulose-1,5-bisphosphate carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic parameters. *Carnegie Yearbook*, Vol. 76, 355-361.

Beche E, Benin G, da Silva CL, Munaro LB, Marchese JA. 2014. Genetic gain in yield and changes associated with physiological traits in Brazilian wheat during the 20th century. *European Journal of Agronomy* **61**, 49-59.

Bernacchi CJ, Pimentel C, Long SP. 2003. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant, Cell & Environment* **26**, 1419-1430.

Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell & Environment* **24**, 253-259.

Coast O, Shah S, Ivakov A, Gaju O, Wilson PB, Posch BC, Bryant CJ, Negrini ACA, Evans JR, Condon AG, Silva-Pérez V, Reynolds MP, Pogson BJ, Millar AH, Furbank RT, Atkin OK. 2019. Predicting dark respiration rates of wheat leaves from hyperspectral reflectance. *Plant, Cell & Environment* **42**, 2133-2150.

Dechant B, Cuntz M, Vohland M, Schulz E, Doktor D. 2017. Estimation of photosynthesis traits from leaf reflectance spectra: Correlation to nitrogen content as the dominant mechanism. *Remote Sensing of Environment* **196**, 279-292.

Driever SM, Lawson T, Andralojc PJ, Raines CA, Parry MAJ. 2014. Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. *JOURNAL OF EXPERIMENTAL BOTANY*.

Ecarnot M, Compan F, Roumet P. 2013. Assessing leaf nitrogen content and leaf mass per unit area of wheat in the field throughout plant cycle with a portable spectrometer. *Field Crops Research* **140**, 44-50.

Evans JR, von Caemmerer S. 2013. Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. *Plant Cell & Environment* **36**, 745-756.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *PLANTA* **149**, 78-90.

Fischer RA, Rees D, Sayre KD, Lu ZM, Condon AG, Saavedra AL. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Science* **38**, 1467-1475.

Gaju O, DeSilva J, Carvalho P, Hawkesford MJ, Griffiths S, Greenland A, Foulkes MJ. 2016. Leaf photosynthesis and associations with grain yield, biomass and nitrogen-use efficiency in landraces, synthetic-derived lines and cultivars in wheat. *Field Crops Research* **193**, 1-15.

Heckmann D, Schluter U, Weber APM. 2017. Machine learning techniques for predicting crop photosynthetic capacity from leaf reflectance spectra. *Molecular Plant* **10**, 878-890.

June T, Evans JR, Farquhar GD. 2004. A simple new equation for the reversible temperature dependence of photosynthetic electron transport: a study on soybean leaf. *FUNCTIONAL PLANT BIOLOGY* **31**, 275-283.

Kuhlgert S, Austic G, Zegarac R, Osei-Bonsu I, Hoh D, Chilvers MI, Roth MG, Bi K, TerAvest D, Weebadde P, Kramer DM. 2016. MultispeQ Beta: a tool for large-scale plant phenotyping connected to the open PhotosynQ network. *Royal Society Open Science* **3**.

Meacham-Hensold K, Montes CM, Wu J, Guan KY, Fu P, Ainsworth EA, Pederson T, Moore CE, Brown KL, Raines C, Bernacchi CJ. 2019. High-throughput field phenotyping using hyperspectral reflectance and partial least squares regression (PLSR) reveals genetic modifications to photosynthetic capacity. *Remote Sensing of Environment* **231**.

Medlyn BE, Dreyer E, Ellsworth D, Forstreuter M, Harley PC, Kirschbaum MUF, Le Roux X, Montpied P, Strassemeyer J, Walcroft A, Wang K, Loustau D. 2002. Temperature response of

parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant, Cell & Environment* **25**, 1167-1179.

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu X-G, Price GD, Condon AG, Furbank RT. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *JOURNAL OF EXPERIMENTAL BOTANY* **62**, 453-467.

R. 2013. R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna, Austria.

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ. 2009. Raising yield potential in wheat. *JOURNAL OF EXPERIMENTAL BOTANY* **60**, 1899-1918.

Sadras VO, Lawson C, Montoro A. 2012. Photosynthetic traits in Australian wheat varieties released between 1958 and 2007. *Field Crops Research* **134**, 19-29.

Serbin SP, Dillaway DN, Kruger EL, Townsend PA. 2012. Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *JOURNAL OF EXPERIMENTAL BOTANY* **63**, 489-502.

Sharwood RE, Ghannoum O, Kapralov MV, Gunn LH, Whitney SM. 2016. Temperature responses of Rubisco from Paniceae grasses provide opportunities for improving C3 photosynthesis. *Nature Plants* **2**, 16186.

Shearman VJ, Sylvester-Bradley R, Scott RK, Foulkes MJ. 2005. Physiological processes associated with wheat yield progress in the UK. *Crop Science* **45**, 175-185.

Silva-Pérez V, De Faveri J, Molero G, Deery DM, Condon AG, Reynolds MP, Evans JR, Furbank RT. 2019. Genetic variation for photosynthetic capacity and efficiency in spring wheat. *JOURNAL OF EXPERIMENTAL BOTANY* **71**, 2299-2311.

Silva-Perez V, Furbank RT, Condon AG, Evans JR. 2017. Biochemical model of C-3 photosynthesis applied to wheat at different temperatures. *PLANT CELL AND ENVIRONMENT* **40**, 1552-1564.

Silva-Perez V, Molero G, Serbin SP, Condon AG, Reynolds MP, Furbank RT, Evans JR. 2018. Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. *JOURNAL OF EXPERIMENTAL BOTANY* **69**, 483-496.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* **428**, 821-827.

Wu J, Rogers A, Albert LP, Ely K, Prohaska N, Wolfe BT, Oliveira RC, Saleska SR, Serbin SP. 2019. Leaf reflectance spectroscopy captures variation in carboxylation capacity across species, canopy environment and leaf age in lowland moist tropical forests. *NEW PHYTOLOGIST* **224**, 663-674.

Yao YR, Lv LH, Zhang LH, Yao HP, Dong ZQ, Zhang JT, Ji JJ, Jia XL, Wang HJ. 2019. Genetic gains in grain yield and physiological traits of winter wheat in Hebei Province of China, from 1964 to 2007. *Field Crops Research* **239**, 114-123.

Yendrek CR, Tomaz T, Montes CM, Cao Y, Morse AM, Brown PJ, McIntyre LM, Leakey ADB, Ainsworth EA. 2017. High-throughput phenotyping of maize leaf physiological and biochemical traits using hyperspectral reflectance. *PLANT PHYSIOLOGY* **173**, 614-626.