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Supplementary Material

A high-throughput method for measuring critical thermal limits of leaves by chlorophyll imaging fluorescence

Pieter A. Arnold[A,C], *Verónica F. Briceño*[A], *Kelli M. Gowland*[A], *Alexandra A. Catling*[A], *León A. Bravo*[B] *and Adrienne B. Nicotra*[A]

[A]Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia.

[B]Department of Agronomical Sciences and Natural Resources, Faculty of Agropecuary and Forestry Sciences and Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Casilla 54D, Temuco, Chile.

[C]Corresponding author. Email: pieter.arnold@anu.edu.au

Table S1. A non-exhaustive sample of the variety of heating or cooling rates for temperature change used for measuring thermal tolerance limits of leaves from the literature

Cold tolerance limits	
Cooling rate	**Reference**
2 °C h^{-1}	Taschler and Neuner (2004); Neuner and Pramsohler (2006); Sierra-Almeida and Cavieres (2012)
3–4 °C h^{-1}	Robberecht and Junttila (1992)
5 °C h^{-1}	Buchner and Neuner (2009)
10 °C h^{-1}	Menon *et al.* (2015)
1800 °C h^{-1}	Pospíšil *et al.* (1998)
Heat tolerance limits	
Heating rate	**Reference**
30 °C h^{-1}	Frolec *et al.* (2008)
42 °C h^{-1}	Bilger *et al.* (1984)
60 °C h^{-1}	Schreiber and Berry (1977); Schreiber and Armond (1978); (Smillie 1979; Smillie and Nott 1979); Smillie and Gibbons (1981); Bilger *et al.* (1984); Braun *et al.* (2002); Knight and Ackerly (2002); Kim and Portis (2005); Neuner and Pramsohler (2006); Frolec *et al.* (2008); O'Sullivan *et al.* (2013); Tovuu *et al.* (2013); Buchner *et al.* (2015); O'Sullivan *et al.* (2017); Zhu *et al.* (2018)
90 °C h^{-1}	Macias (2011)
120 °C h^{-1}	Bilger *et al.* (1984); Ilík *et al.* (2003); Frolec *et al.* (2008)
180 °C h^{-1}	Frolec *et al.* (2008)
240 °C h^{-1}	Nauš *et al.* (1992)
300 °C h^{-1}	Tovuu *et al.* (2013)
600 °C h^{-1}	Tovuu *et al.* (2013)
648 °C h^{-1}	Tovuu *et al.* (2013)
1800 °C h^{-1}	Pospíšil *et al.* (1998)

Table S2. Mean ± standard error values for CT_{MIN}, CT_{MAX}, and F_V/F_M for each species and experimental condition (Experiment 2 heating and cooling rates shown for 6, 15, 30, and 60°C h^{-1}

Exp. 1 Dry surface	**Species**			
Trait	***W. ceracea***	***M. citrina***	***E. rubra***	***Q. phellos***
CT_{MIN}	–13.8 ± 0.4°C	–16.9 ± 0.37°C	–14.0 ± 0.3°C	NA
CT_{MAX}	43.2 ± 0.9°C	46.8 ± 0.8°C	NA	49.2 ± 0.5°C
F_V/F_M	0.81 ± 0.004	0.77 ± 0.009	0.74 ± 0.008	0.80 ± 0.007

Exp. 1 Wet surface	**Species**			
Trait	***W. ceracea***	***M. citrina***	***E. rubra***	***Q. phellos***
CT_{MIN}	–9.8 ± 0.6°C	–13.9 ± 0.4°C	–9.8 ± 0.8°C	NA
CT_{MAX}	41.6 ± 0.9°C	47.2 ± 0.2°C	NA	48.5 ± 0.5°C
F_V/F_M	0.80 ± 0.004	0.78 ± 0.008	0.74 ± 0.006	0.78 ± 0.013

Exp. 2 6°C h^{-1} rate	**Species**		
Trait	***W. ceracea***	***M. citrina***	***E. rubra***
CT_{MIN}	–14.1 ± 0.5°C	–15.2 ± 0.7°C	–11.7 ± 0.6°C
CT_{MAX}	45.8 ± 1.1°C	35.3 ± 1.1°C	46.7 ± 0.2°C
F_V/F_M	0.82 ± 0.005	0.73 ± 0.008	0.72 ± 0.005

Exp. 2 15°C h^{-1} rate	**Species**		
Trait	***W. ceracea***	***M. citrina***	***E. rubra***
CT_{MIN}	–14.9 ± 0.2°C	–14.2 ± 0.5°C	–11.6 ± 0.5°C
CT_{MAX}	42.8 ± 0.5°C	38.6 ± 1.0°C	44.0 ± 0.3°C
F_V/F_M	0.81 ± 0.004	0.74 ± 0.006	0.71 ± 0.006

Exp. 2 30°C h^{-1} rate	**Species**		
Trait	***W. ceracea***	***M. citrina***	***E. rubra***
CT_{MIN}	–12.7 ± 0.6°C	–12.5 ± 0.6°C	–12.2 ± 0.4°C
CT_{MAX}	41.6 ± 0.4°C	41.1 ± 0.5°C	44.0 ± 0.4°C
F_V/F_M	0.83 ± 0.006	0.73 ± 0.006	0.76 ± 0.007

Exp. 2 60°C h^{-1} rate	**Species**		
Trait	***W. ceracea***	***M. citrina***	***E. rubra***
CT_{MIN}	–13.8 ± 0.5°C	–16.9 ± 0.5°C	–14.0 ± 0.3°C
CT_{MAX}	43.5 ± 0.3°C	42.5 ± 0.9°C	45.3 ± 0.4°C
F_V/F_M	0.82 ± 0.006	0.74 ± 0.011	0.73 ± 0.007

Table S3. Full statistical reporting for all species and species-specific effects of wet *vs* dry filter paper surface on CT_{MIN} and CT_{MAX}

Response: CT_{MIN}		**All species**			***W. ceracea***			***M. citrina***			***E. rubra***	
Fixed effects	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***
Dry surface / *E. rubra* (intercept)	–18.36	–32.49 – –4.22	**0.011**	Intercept: –5.71	–50.92 – 39.49	0.800	Intercept: –20.36	–35.80 – –4.93	**0.012**	Intercept: –31.26	–54.11 – –8.41	**0.009**
Wet surface	3.81	2.77 – 4.85	**<0.001**	3.92	1.99 – 5.86	**<0.001**	2.98	1.31 – 4.65	**0.001**	3.99	2.34 – 5.63	**<0.001**
F_V/F_M	6.19	–12.89 – 25.27	0.521	–9.89	–64.94 – 45.15	0.719	4.72	–16.14 – 25.58	0.645	23.54	–7.53 – 54.60	0.132
M. citrina	–3.50	–4.92 – –2.07	**<0.001**	--	--	--	--	--	--	--	--	--
W. ceracea	–0.42	–2.36 - 1.51	0.664	--	--	--	--	--	--	--	--	--
R^2	0.464	--	--	0.288	--	--	0.374	--	--	0.527	--	--

Response: CT_{MAX}		**All species**			***W. ceracea***			***M. citrina***			***Q. phellos***	
Fixed effects	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***
Dry surface / *M. citrina* (intercept)	32.76	18.03 – 47.49	**<0.001**	Intercept: 6.90	–27.59 – 41.40	0.683	Intercept: 36.31	4.91 – 67.71	**0.025**	Intercept: 47.34	31.89 – 62.79	**<0.001**
Wet surface	–0.55	–1.63 – 0.54	0.317	–1.47	–3.93 – 1.00	0.232	0.32	–1.33 – 1.97	0.694	–0.63	–2.08 – 0.82	0.379
F_V/F_M	18.20	–0.12 – 36.52	0.052	46.02	2.33 – 89.71	**0.040**	13.16	–26.19 – 52.50	0.497	2.32	–17.03 – 21.68	0.806
Q. phellos	2.01	0.68 – 3.35	**0.004**	--	--	--	--	--	--	--	--	--
W. ceracea	–4.47	–5.80 – –3.14	**<0.001**	--	--	--	--	--	--	--	--	--
R^2	0.593	--	--	0.213	--	--	0.028	--	--	0.041	--	--

Table S4. Full statistical reporting for effects of wet *vs* dry surface in combination with cooling rate on CT_{MIN}

Response: CT_{MIN}		**All species**			***W. ceracea***			***M. citrina***			***E. rubra***	
Fixed effects	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***
Dry surface / Rate = 15 °C h^{-1} / *E. rubra* (Intercept)	–11.49	–21.77 – –2.01	**0.019**	Intercept: –4.23	–43.83 – 35.37	0.832	Intercept: –12.39	–24.09 – –0.69	**0.038**	Intercept: –20.60	–32.47 – –8.74	**0.001**
Wet surface	4.72	3.45 – 5.99	**<0.001**	6.42	3.90 – 8.94	**<0.001**	4.03	2.10 – 5.95	**<0.001**	3.73	2.17 – 5.30	**<0.001**
Rate = 60 °C h^{-1}	–1.11	–2.14 – –0.08	**0.034**	1.17	–0.77 – 3.11	0.235	–2.69	–4.13 – –1.25	**<0.001**	–2.70	–4.15 – –1.26	**<0.001**
Wet surface × rate = 60 °C h^{-1}	–0.90	–2.53 – 0.73	0.279	–2.53	–5.74 – 0.68	0.121	–0.98	–3.42 – 1.45	0.420	0.34	–1.81 – 2.48	0.754
F_V/F_M	–0.55	–14.28 – 13.19	0.938	–13.12	–61.73 – 35.48	0.592	–2.44	–18.29 – 13.41	0.758	12.71	–3.99 – 29.40	0.133
M. citrina	–3.07	–4.11 – –2.02	**<0.001**	--	--	--	--	--	--	--	--	--
W. ceracea	–0.95	–2.50 - 0.59	0.225	--	--	--	--	--	--	--	--	--
Marginal R^2	0.458	--	--	0.387	--	--	0.538	--	--	0.552	--	--

Table S5. Full statistical reporting for all species and species-specific effects of variable cooling rate on CT_{MIN}

Response: CT_{MIN}		**All species**			***W. ceracea***			***M. citrina***			***E. rubra***	
Fixed effects	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***
Rate = 3 °C h^{-1} / *E. rubra* (Intercept)	–11.38	–17.01 – –5.75	**<0.001**	–40.89	–70.67 – –11.10	**0.008**	–16.82	–25.42 – –8.21	**<0.001**	–11.58	–19.21 – –3.96	**0.003**
Rate = 6 °C h^{-1}	–0.33	–1.14 – 0.48	0.422	0.62	–0.67 – 1.92	0.343	–1.81	–3.49 – –0.12	**0.036**	–0.15	–1.29 – 0.98	0.790
Rate = 15 °C h^{-1}	–0.32	–1.12 – 0.49	0.438	–0.12	–1.42 – 1.19	0.859	–0.80	–2.43 – 0.84	0.335	–0.10	–1.22 – 1.03	0.864
Rate = 30 °C h^{-1}	0.75	–0.05 – 1.55	0.065	1.67	0.37 – 2.97	**0.012**	0.91	–0.70 – 2.51	0.265	–0.74	–1.93 – 0.45	0.220
Rate = 60 °C h^{-1}	–1.34	–2.15 – –0.53	**0.001**	0.75	–0.47 – 1.97	0.225	–3.51	–5.19 – –1.82	**<0.001**	–2.47	–3.69 – –1.25	**<0.001**
Rate = 240 °C h^{-1}	–0.80	–1.60 – 0.01	0.052	0.70	–0.62 – 2.02	0.298	–1.74	–3.35 – –0.13	**0.035**	–1.90	–3.11 – –0.70	**0.002**
F_V/F_M	–0.89	–8.55 – 6.78	0.82	32.04	–4.23 – 68.32	0.083	4.66	–6.84 – 16.16	0.422	0.12	–10.42 – 10.66	0.982
M. citrina	–2.18	–2.76 – –1.60	**<0.001**	--	--	--	--	--	--	--	--	--
W. ceracea	–1.50	–2.36 – –0.63	**0.001**	--	--	--	--	--	--	--	--	--
Marginal R^2	0.230	--	--	0.126	--	--	0.332	--	--	0.220	--	--

Table S6. Full statistical reporting for all species and species-specific effects of variable heating rate on CT_{MAX}

Response: CT_{MAX}	All species			*W. ceracea*			*M. citrina*			*E. rubra*		
Fixed effects	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***
Rate = 60 °C h^{-1} / *E. rubra* (Intercept)	27.79	20.09 – 35.50	**<0.001**	Intercept: 14.87	–0.48 – 30.22	0.058	Intercept: 27.79	18.26 – 37.32	**<0.001**	Intercept: 41.75	31.47 – 52.03	**<0.001**
Rate = 6 °C h^{-1}	–0.68	–1.86 – 0.50	0.259	1.60	0.24 – 2.96	**0.021**	–7.71	–9.48 – –5.93	**<0.001**	1.38	–0.19 – 2.94	0.085
Rate = 15 °C h^{-1}	–2.43	–3.62 – –1.24	**<0.001**	–2.00	–3.47 – –0.52	**0.008**	–4.68	–6.43 – –2.94	**<0.001**	–1.40	–2.98 – 0.17	0.080
Rate = 30 °C h^{-1}	–1.74	–2.72 – –0.77	**0.001**	–2.11	–3.18 – –1.03	**<0.001**	–2.10	–3.66 – –0.54	**0.009**	–1.31	–2.61 – –0.01	**0.048**
Rate = 45 °C h^{-1}	–1.48	–2.44 – –0.51	**0.003**	–0.72	–1.80 – 0.36	0.188	–0.95	–2.53 – 0.62	0.232	–2.68	–3.96 – –1.40	**<0.001**
Rate = 120 °C h^{-1}	1.00	–0.06 – 2.07	0.065	1.78	0.62 – 2.95	**0.003**	2.24	0.48 – 3.99	**0.013**	–0.45	–1.95 – 1.05	0.554
Rate = 240 °C h^{-1}	2.03	0.95 – 3.12	**<0.001**	2.78	1.58 – 3.98	**<0.001**	3.76	1.86 – 5.67	**<0.001**	–0.13	–1.56 – 1.29	0.853
F_V/F_M	23.79	12.68 – 34.91	**<0.001**	38.48	17.91 – 59.05	**<0.001**	21.98	7.80 – 36.16	**0.003**	5.15	–9.68 – 19.99	0.492
M. citrina	–1.30	–1.96 – –0.63	**<0.001**	--	--	--	--	--	--	--	--	--
W. ceracea	–1.24	–2.02 - –0.45	**0.002**	--	--	--	--	--	--	--	--	--
Marginal R^2	0.429	--	--	0.619	--	--	0.863	--	--	0.319	--	--

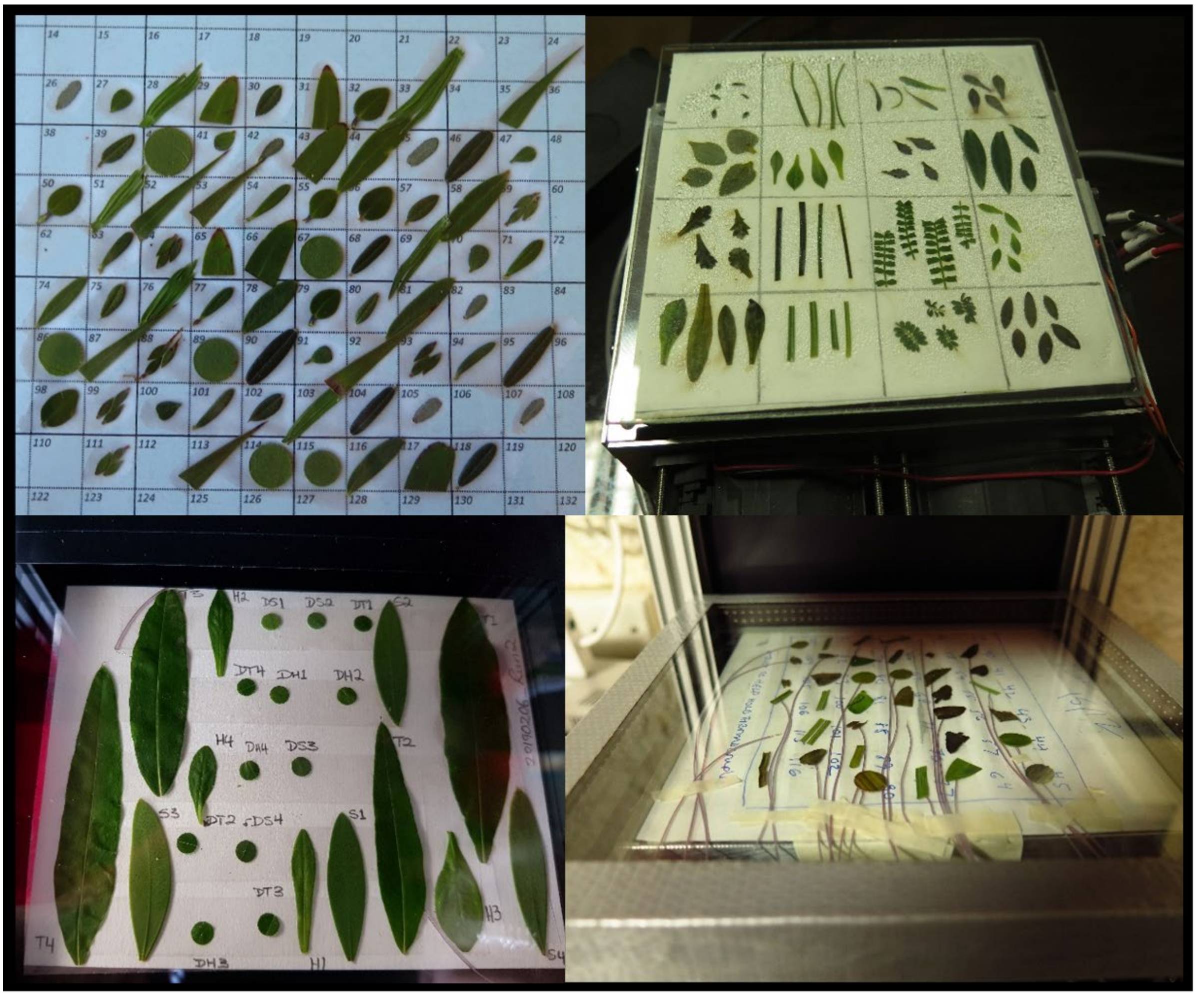

Fig. S1. Various experimental applications of the Peltier plate and chlorophyll fluorescence Maxi-Imaging-PAM system using whole leaves, leaf sections, and leaf discs of multiple species, and the potential application of type-T thermocouples for recording the temperature of individual samples. Images taken by Verónica F. Briceño and Pieter A. Arnold.

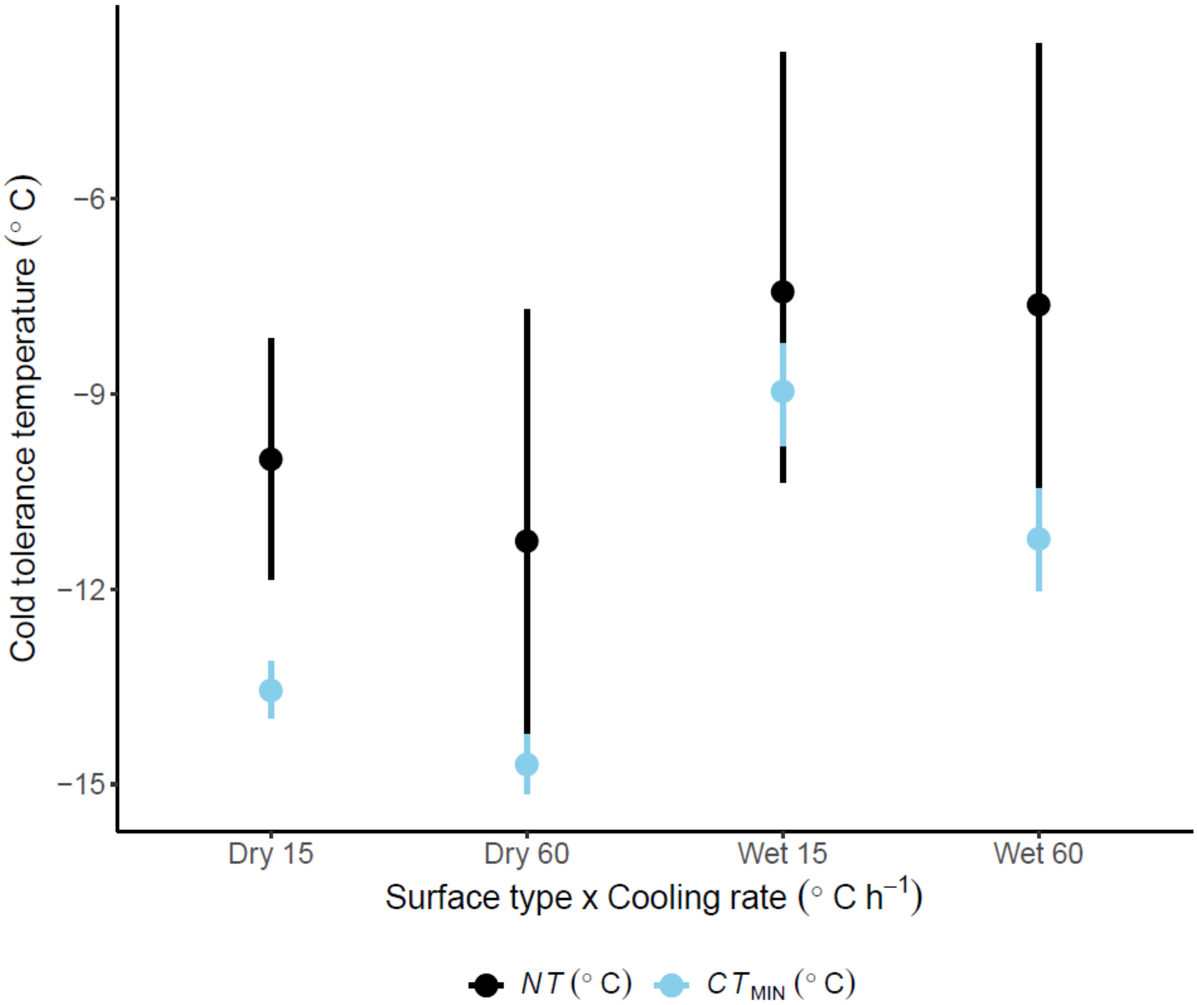

Fig. S2. The effect of varying cooling rate (°C h^{-1}) in combination with varying surfaces (dry *vs* wet filter paper) on the *NT* (black circles) and CT_{MIN} (light blue circles) estimates (°C). *NT* was measured only on a small, random subset of leaves using the two thermocouples attached to leaves from various species on the Peltier plate (total n = 17, therefore the 95% CIs are much larger than those of CT_{MIN}. For consistency in comparison, the CT_{MIN} values are also grouped across the three species.

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