

Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit

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Supplementary Tables and Figures

Table S1: List of accession numbers, primer sets and probes used for qRT-PCR. Probe numbers are from the Human Universal Probe Library (Roche, Australia).

Gene	Accession #	Protein	Primer Sequences (5'-3')	Probe#
PEPC1	XM_002280533	XP_002280569	F: cgccagttattatcaagtggaa R: caagcacagagtgatctatagcaga	34
PEPC2	XM_002280806	XP_002280842	F: atgcccgacttgctgtagt R: aatagaggaaccaccgtagc	113
PEPC3	XM_002285405	XP_002285441	F: aatagaggaaccaccgtagc R: caagaaggtccttggtccag	25
PEPCK	XM_003635567	XP_003635615	F: cattgatctctcaaggagaaaag R: tcgaacaccacattttccaac	147
NAD-ME	XM_002266661	XP_002266697	F: gactgactttgtcaacaaaagatag R: gccatattaagcacaccaatcc	4
PPDK	XM_002278776	XP_002278812	F: tgggaaccatgattgagattc R: gaagaattctgctccttcg	164
Ankyrin	XM_002283462	XP_002283498	F: ggttatggcaggaaggagtg R: ggtgtcttgccatccatgtt	120
Ubiquitin	XM_002273532	XP_002273568	F: gtggccacagcaaccagt R: gcaacctccaatccagtcac	143

Table S2: Compounds detected using GC/MS (Experiment 3). Values highlighted in bold indicate significant differences between berries of control and heated vines. Values were considered significantly different when the log2 difference was <-0.5 or >0.5, and p ≤0.05 according to 2-tailed t-tests. Also available as an .xls spreadsheet.

Compound	59 D.A.F. (0 days of heating)					63 D.A.F. (3 days of heating)					71 D.A.F. (11 days of heating)					90 D.A.F. (ripe fruit)								
	Control	S.D.	Heated	S.D.	log ₂ difference	t-test	Control	S.D.	Heated	S.D.	log ₂ difference	t-test	Control	S.D.	Heated	S.D.	log ₂ difference	t-test	Control	S.D.	Heated	S.D.	log ₂ difference	t-test
Valine	0.00551	0.00075	0.00674	0.00037	0.291	0.189	0.00295	0.00065	0.02219	0.00561	2.910	0.014	0.00423	0.00086	0.02190	0.00421	2.373	0.006	0.00271	0.00036	0.01063	0.00195	1.972	0.007
Ethanolamine	0.01943	0.00501	0.01218	0.00194	-0.574	0.226	0.00419	0.00040	0.00824	0.00213	0.974	0.111	0.00298	0.00116	0.00354	0.00028	0.249	0.133	0.00439	0.00197	0.00377	0.00023	-0.221	0.754
Leucine	0.00525	0.00140	0.00808	0.00083	0.622	0.133	0.00172	0.00018	0.01182	0.00278	2.777	0.011	0.00226	0.00044	0.01046	0.00283	2.213	0.029	0.00167	0.00031	0.00634	0.00172	1.929	0.036
Glycerol	0.00360	0.00045	0.00473	0.00037	0.391	0.102	0.00336	0.00025	0.00949	0.00038	0.556	0.013	0.00403	0.00017	0.00384	0.00048	-0.071	0.718	0.00421	0.00064	0.00361	0.00035	-2.222	0.441
Isoleucine	0.00303	0.00060	0.00422	0.00009	0.478	0.096	0.00185	0.00026	0.01280	0.00285	2.789	0.009	0.00219	0.00045	0.01100	0.00296	2.327	0.026	0.00085	0.00019	0.00485	0.00120	2.157	0.017
Proline	0.00291	0.00063	0.00512	0.00036	0.814	0.023	0.00731	0.00255	0.03091	0.01331	2.081	0.199	0.01172	0.00058	0.00760	0.01559	2.760	0.009	0.02869	0.00086	0.13391	0.02626	2.222	0.009
Glycine	0.00499	0.00105	0.00488	0.00040	-0.031	0.928	0.00247	0.00031	0.00312	0.00025	0.334	0.155	0.00155	0.00027	0.00266	0.00029	0.672	0.031	0.00149	0.00019	0.00366	0.00060	1.297	0.014
Succinic acid	0.00167	0.00027	0.00236	0.00040	0.400	0.263	0.00124	0.00012	0.00071	0.00021	-0.799	0.073	0.00057	0.00003	0.00044	0.00009	-0.364	0.216	0.00039	0.00013	0.00061	0.00007	0.654	0.189
Dihydroxypropanoic acid	0.00866	0.00161	0.01015	0.00049	0.229	0.409	0.00557	0.00083	0.00658	0.00051	0.241	0.341	0.00271	0.00039	0.00341	0.00040	1.331	0.259	0.00129	0.00022	0.00124	0.00035	-0.055	0.912
Fumaric acid	0.00052	0.00004	0.00040	0.00005	-0.369	0.115	0.00022	0.00005	0.00113	0.00086	2.371	0.332	0.00021	0.00002	0.00050	0.00000	0.280	0.012	0.00025	0.00006	0.00023	0.00003	-0.115	0.792
Serine	0.00463	0.00081	0.00716	0.00030	0.631	0.026	0.00339	0.00091	0.00692	0.00207	0.128	0.170	0.00355	0.00087	0.00739	0.00088	1.056	0.021	0.00173	0.00048	0.00429	0.00057	1.310	0.014
Piperidinecarboxylic acid	0.00083	0.00022	0.00018	0.00018	0.070	0.889	0.00035	0.00005	0.00332	0.00067	3.256	0.004	0.00039	0.00011	0.00617	0.00112	3.994	0.002	0.00041	0.00008	0.00364	0.00125	3.154	0.041
Threonine	0.00172	0.00038	0.00244	0.00039	-0.242	0.609	0.00124	0.00019	0.00149	0.00019	2.247	0.022	0.00208	0.00037	0.00059	0.00078	0.900	0.040	0.00170	0.00022	0.00127	0.00056	2.921	0.004
β-alanine	0.00063	0.00009	0.00072	0.00014	0.198	0.597	0.00071	0.00019	0.00172	0.00044	1.287	0.078	0.00075	0.00015	0.00177	0.00026	1.246	0.015	0.00064	0.00015	0.00151	0.00031	1.233	0.047
Malic acid	5.84767	0.83655	5.90177	0.18806	0.013	0.952	3.76015	0.38866	3.05117	0.53760	-0.309	0.297	2.44038	0.10028	1.45138	0.10834	-0.750	0.001	1.17503	0.05712	0.06178	0.06300	-0.764	0.001
Aspartic acid	0.00342	0.00042	0.00367	0.00024	0.104	0.616	0.00257	0.00073	0.00396	0.00061	0.624	0.195	0.00265	0.00054	0.00713	0.00129	1.427	0.018	0.00155	0.00023	0.00343	0.00043	1.142	0.008
γ-aminobutyric acid	0.00453	0.00124	0.00548	0.00087	0.273	0.555	0.00181	0.00026	0.00427	0.00074	1.237	0.020	0.00268	0.00061	0.00481	0.00024	0.844	0.018	0.00207	0.00037	0.00696	0.00174	1.751	0.033
Threonic acid	0.02314	0.00451	0.02850	0.00151	0.300	0.303	0.01164	0.00205	0.01273	0.00342	0.129	0.794	0.00733	0.00154	0.00569	0.00074	-0.365	0.375	0.00323	0.00030	0.00410	0.00097	0.343	0.426
Glutamic acid	0.00217	0.00043	0.00232	0.00023	0.094	0.775	0.00136	0.00039	0.00282	0.00115	1.052	0.274	0.00149	0.00029	0.00455	0.00076	1.604	0.009	0.00114	0.00039	0.00323	0.00029	1.506	0.005
Tartaric acid	4.10258	0.47001	5.05466	0.22746	0.301	0.118	2.49530	0.16884	2.44492	0.64016	-0.029	0.942	1.56869	0.20779	1.83431	0.19157	0.266	0.384	1.16937	0.11965	1.09399	0.11200	-0.212	0.367
Phenylalanine	0.00184	0.00026	0.00189	0.00035	0.043	0.902	0.00114	0.00022	0.00453	0.00149	1.991	0.626	0.00112	0.00026	0.00244	0.00059	-1.119	0.887	0.00157	0.00033	0.00285	0.00123	0.858	0.354
Trihydroxypentanoic acid	0.00127	0.00020	0.00127	0.00022	-0.009	0.980	0.00077	0.00016	0.00154	0.00070	1.008	0.320	0.00079	0.00019	0.00069	0.00012	0.204	0.518	0.00050	0.00008	0.00063	0.00021	0.384	0.580
Glucosaminic acid 1	0.01058	0.00149	0.01871	0.00183	0.822	0.014	0.00599	0.00066	0.00577	0.00121	-0.055	0.877	0.00364	0.00020	0.00505	0.00053	0.471	0.047	0.00479	0.00104	0.00446	0.00071	-0.102	0.803
Glucosaminic acid 2	0.00548	0.00063	0.00680	0.00050	0.310	0.152	0.00502	0.00083	0.02416	0.01888	2.267	0.350	0.00486	0.00060	0.00490	0.00010	0.013	0.947	0.00584	0.00155	0.00969	0.00450	0.730	0.450
Putrescine	0.00110	0.00041	0.00299	0.00011	0.511	0.063	0.00143	0.00015	0.00386	0.00062	1.428	0.027	0.00135	0.00021	0.00500	0.00037	1.894	0.000	0.00093	0.00006	0.00182	0.00041	0.869	0.073
Citric acid	0.06354	0.00934	0.04442	0.00328	-0.516	0.102	0.02957	0.00413	0.02230	0.00773	-0.407	0.439	0.01827	0.00267	0.01457	0.00098	-0.327	0.240	0.00913	0.00090	0.00807	0.00094	-0.177	0.447
Quinic acid	0.00299	0.00068	0.00279	0.00033	-0.096	0.807	0.00219	0.00038	0.00278	0.00026	0.345	0.246	0.00193	0.00033	0.00207	0.00035	0.096	0.791	0.00174	0.00040	0.00170	0.00026	-0.037	0.929
Fructose 1	2.57005	0.80991	3.70153	0.36335	0.526	0.250	7.94272	0.70335	6.82930	0.97273	-0.191	0.452	9.32155	0.41205	10.25022	0.29627	0.137	0.117	12.62596	1.26666	13.80294	1.88011	0.129	0.617
Fructose 2	1.97170	0.60596	2.74923	0.24954	0.480	0.280	6.04415	0.60194	5.78966	0.57065	-0.062	0.769	6.72728	0.34046	8.44702	0.42094	0.139	0.203	10.68163	1.27530	11.43884	2.06685	0.099	0.766
Glucose 1	11.45127	1.50854	13.22281	0.51419	0.272	0.196	16.76545	0.99582	14.94693	2.88395	0.214	0.413	18.08993	0.73109	22.60245	0.72810	0.321	0.005	26.11457	3.55714	26.33348	5.30222	0.012	0.974
Glucose 2	2.06636	0.37026	2.75219	0.28548	0.413	0.193	4.52420	0.49536	4.45738	0.50934	-0.021	0.928	5.29248	0.48003	6.46737	0.33694	0.289	0.092	7.07578	0.38812	7.29243	0.72293	0.043	0.801
Glucuric / Galacturic acid	0.00049	0.00015	0.00037	0.00009	-0.401	0.523	0.00043	0.00010	0.00018	0.00018	0.558	0.361	0.00053	0.00008	0.00107	0.00014	1.022	0.015	0.00110	0.00030	0.00241	0.00062	1.137	0.105
Inositol	0.82730	0.07558	0.66077	0.03430	-0.324	0.002	0.58946	0.02323	0.80570	0.10235	0.451	0.085	0.48223	0.03715	0.92289	0.05394	0.936	0.001	0.52556	0.02239	0.76314	0.02987	0.538	0.001
Caffeic acid	0.01485	0.00184	0.01339	0.00091	-0.149	0.505	0.00763	0.00079	0.00640	0.00171	-0.254	0.538	0.00488	0.00056	0.00343	0.00056	-0.509	0.119	0.00371	0.00023	0.00226	0.00051	-0.715	0.041
Glucose-6-phosphate	0.00073	0.00018	0.00037	0.00005	-0.965	0.108	0.00020	0.00006	0.00045	0.00004	1.174	0.030	0.00028	0.00010	0.00022	0.00011	-0.346	0.706	0.00023	0.00002	0.00027	0.00009	0.204	0.727
Sucrose	1.28610	0.09631	1.32808	0.10027	0.047	0.768	1.95568	0.24915	2.24188	0.29962	0.197	0.413	2.60145	0.03337	3.07095	0.27788	0.239	0.144	3.17671	0.27685	4.11315	0.41381	0.373	0.109
n-tetradecanoic acid	0.03754	0.00568	0.02597	0.00203	-0.532	0.103	0.02243	0.00126	0.02375	0.00092	0.430	0.030	0.0											

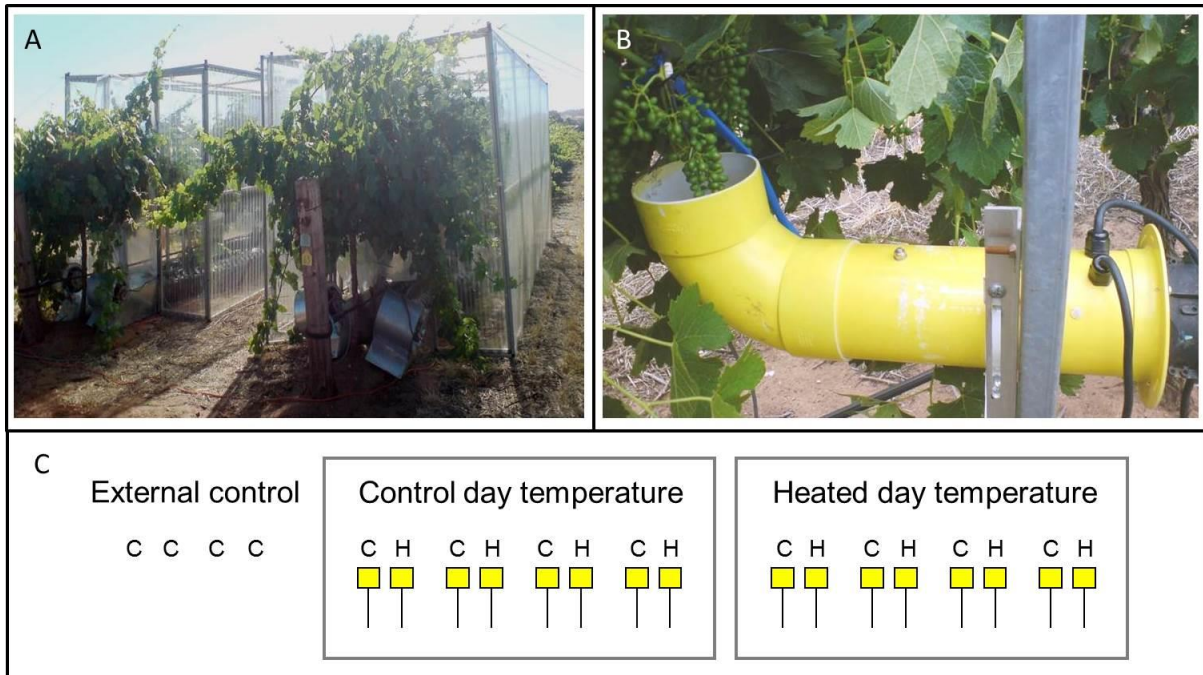


Figure S1: Experiment 2 equipment and design. (A) Closed-top chambers (Soar *et al.*, 2009) were used to modulate day temperature around whole vines and (B) fan heaters were used to modulate night temperature around individual bunches. (C) The two-factorial design comprising (i) two chambers, one set to simulate ambient day temperature (Control) and the other to elevate day temperature (Heated), and (ii) eight sets of fan heaters (yellow boxes) per chamber, four set to maintain ambient night temperature ‘C’ and four set to elevate night temperature ‘H’. External controls comprised vines in the absence of closed-top chambers and fan heaters. Bunches were tagged at 50% capfall and treatments were assembled at three berry phenological stages: pre-véraison (E-L 31), véraison (E-L 34) and ripening (E-L 36), for eleven days each.



Figure S2: Temperatures during natural heatwaves and the monthly averages during South Australian summers from 1999-2014. Average (blue) and heatwave (red) temperatures for (A) maximum day, (B) minimum night and (C) diurnal range. (D) Difference between heatwave and average diurnal temperature ranges (dashed line indicates average difference of 3.4°C for the 15 year timeperiod). Data were recorded at the Nuriootpa Meteorological Station (ID 023373) by the Australian Bureau of Meteorology (BOM; <http://www.bom.gov.au/climate/data-services>). Heatwaves were defined as three or more consecutive days with a maximum temperature $\geq 35^{\circ}\text{C}$.

Average heatwave temperatures were calculated across the duration of the heatwave. Average temperatures were calculated for the corresponding months in which heatwaves occurred. Diurnal ranges were calculated as the average differences between maximum day temperature and minimum temperature from the previous night.

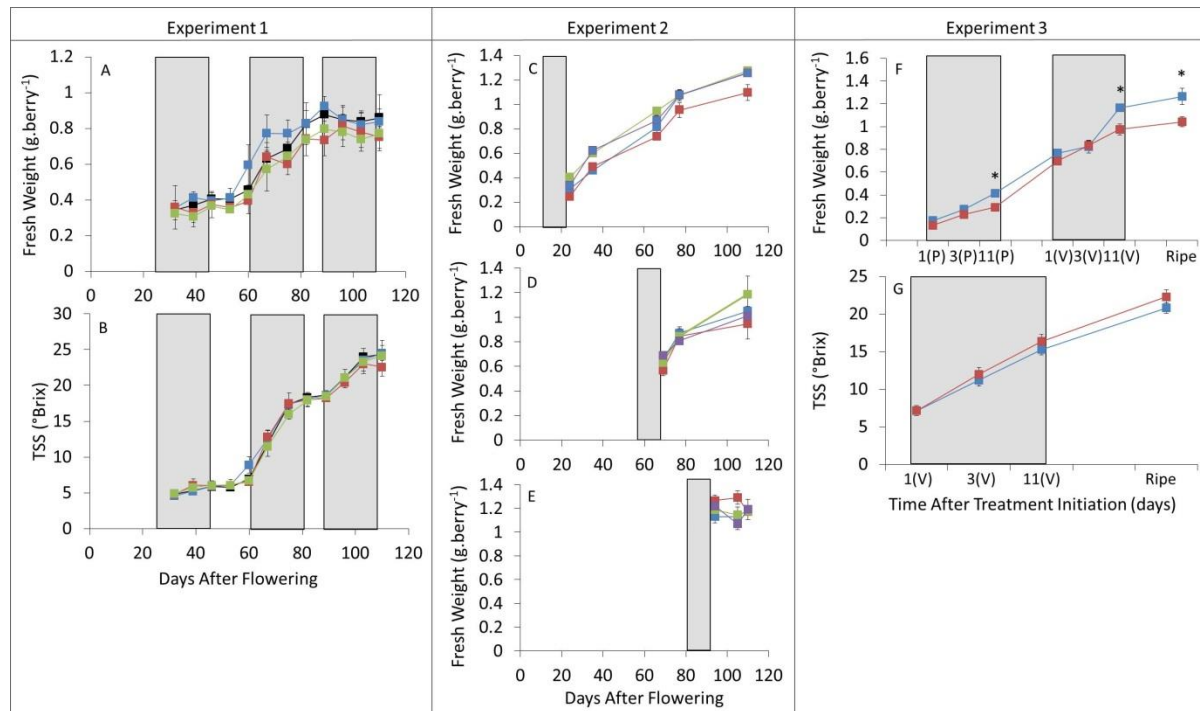


Figure S3: Effects of all elevated temperature treatments on berry fresh weight and TSS. (A) and (B) show berry fresh weight and TSS respectively, from control vines (black) and vines exposed to three-week warming treatments at pre-véraison (blue), véraison (red) and ripening (green) stages in the field (Experiment 1). ($n=3 \pm$ S.D.). (C), (D) and (E) show berry fresh weight for eleven-day heating treatments conducted at pre-véraison, véraison and ripening stages, respectively, in the field (Experiment 2), with vines exposed to control conditions (blue), or heating during the day-only (red), night-only (green), or both day and night (purple). ($n=4 \pm$ S.D.). (F) and (G) show berry fresh weight and TSS, respectively, for vines exposed to eleven-day control (blue) and heat (red) treatments in controlled-environment chambers (Experiment 3), plotted against the number of days after the initiation of pre-véraison [P] and véraison [V] treatments. ($n=8 \pm$ S.D.). *Significantly different from control (independent t-test, $p < 0.05$). Treatment periods for all experiments are highlighted in grey.

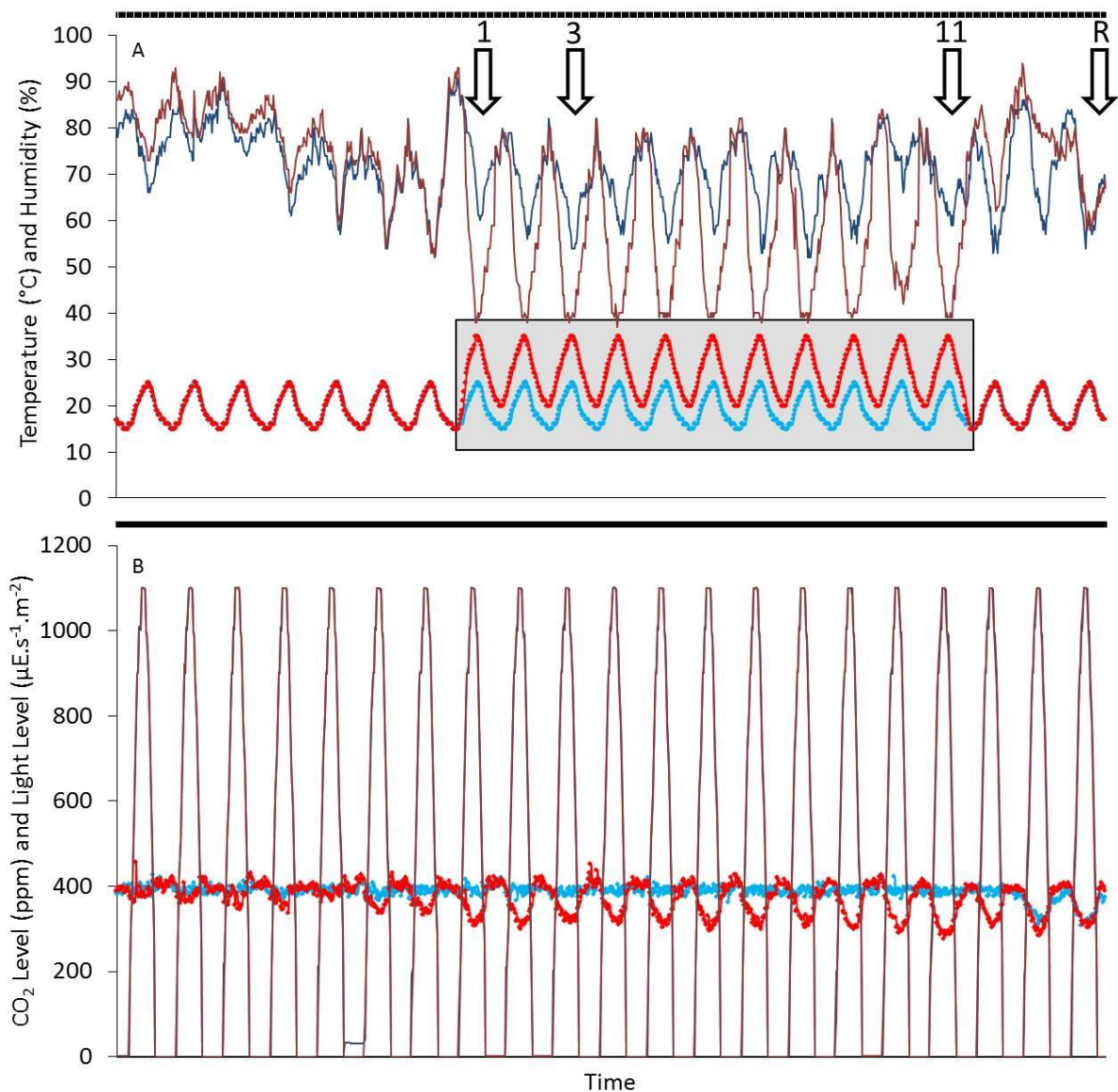


Figure S4: Controlled environment chamber growth conditions during véraison treatment for control and elevated temperature regimes (Experiment 3). Data from 30-minute intervals recorded within controlled-environment chambers containing potted Shiraz vines at The Plant Accelerator (The University of Adelaide, Waite campus). (A) Temperature (bright blue and red for control and heated chambers respectively) and humidity (dark blue and red for control and heated chambers respectively) and (B) CO₂ (bright blue and red) and light (dark blue and red) levels. Data were collected across the seven day equilibration period (both cabinets set to the control temperature regime, 25:15°C), eleven days of heating (one chamber set to elevated temperature regime, 35:20°C) and three days of recovery (both cabinets set to the control temperature regime). The heat treatment period is highlighted in grey. Arrows indicate sample collection points and the number of days from treatment initiation.

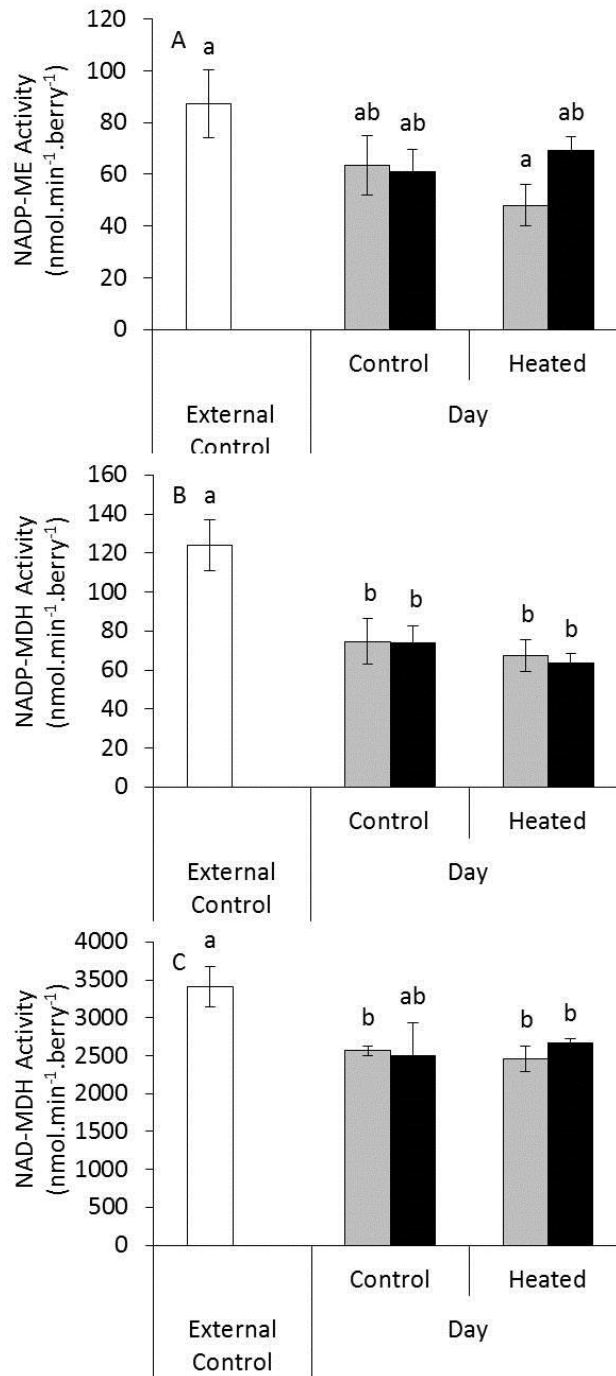


Figure S5: Activities of enzymes directly involved in malate metabolism following an eleven-day elevated temperature treatment at véraison (Experiment 2). Effects on (A) NADP-dependent malic enzyme activity, (B) NADP-dependent malate dehydrogenase activity and (C) NAD-dependent malate dehydrogenase activity. Control and heated day treatments are indicated on the x-axes, with different shades representing external controls (white), control nights (grey) and heated nights (black). ($n=4 \pm$ S.E.M.). Columns sharing a letter are not significantly different (two-way ANOVA with Tukey's multiple comparisons test; independent t-tests for comparison with external control, $p < 0.05$).