Net carbon exchange in grapevine canopies responds rapidly to timing and extent of regulated deficit irrigation

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Abstract. Whole-canopy net CO_2 exchange (NCE_C) was measured near key stages of fruit development in grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) that were managed under three approaches to regulated deficit irrigation (RDI): (1) standard practice (RDI_S), or weekly replacement of 60–70% of estimated evapotranspiration for well watered grapevines; (2) early additional deficit (RDI_E), or one-half of RDI_S applied between fruit set and the onset of ripening (veraison), followed by RDI_S; and (3) RDI_S followed by late additional deficit (RDI_L), or one-half of RDI_S applied between veraison and harvest. Summed between fruit set and harvest, nearly 40% less irrigation was applied to RDI_E vines and ~20% less to RDI_L vines than to those continuously under RDI_S. After ~5 weeks of additional deficit, NCE_C in RDI_E vines was 43–46% less per day than in RDI_S vines. After RDI_L vines had been under additional water deficit for ~3 weeks, NCE_C was ~33% less per day than in RDI_S vines. Instantaneous rates of NCE_C responded rapidly to irrigation delivery and elapsed time between irrigation sets. Concurrent single-leaf measurements (NCE_L) reflected the relative differences in NCE_C between irrigation treatments, and were linearly associated with NCE_C ($r^2 = 0.61$). Despite halving the water applied under commercial RDI, mid-day stomatal conductance values in RDI_E and RDI_L of ~50–125 mmol m^{-2} s^{-1} indicated that the additional deficit imposed only moderate water stress. There was no effect of additional deficit on yield or berry maturity.

Additional keywords: Cabernet Sauvignon, carbon assimilation, CO_2 fixation, drought, photosynthesis, *Vitis vinifera*, water stress.

Introduction

Regulated deficit irrigation (RDI) is a common management approach to wine grape production in many arid and semiarid regions, being used to restrain canopy growth and improve fruit quality. Soil water deficits imposed early in the growing season limit excessive shoot growth by inhibiting leaf appearance rate, leaf lamina expansion and internode elongation (Schultz and Matthews 1988; Lebon *et al.* 2006). Berry size and fruit composition, such as concentrations of phenolic compounds, can be influenced by the timing and extent of water deficit (Roby and Matthews 2004; Girona *et al.* 2009). There is growing evidence of direct effects of water deficit on berry metabolism (reviewed by Chaves *et al.* 2010; Pinheiro and Chaves 2011). Both the timing and extent of RDI-based water deficits are being investigated more widely to determine a balance between water conservation and wineries' fruit quality objectives without imposing deleterious consequences on vine productivity or longevity.

One implicit advantage of managing vines under RDI is water conservation. However, a potential disadvantage of RDI is that water deficits reduce rates of net carbon exchange (NCE). This response is attributed mainly to stomatal closure, with stomatal conductance (g_s) values reportedly in the range of 50–150 mmol H_2O m^{-2} s^{-1} for grapevines under moderate water stress (Flexas *et al.* 2002*a*). Most of the studies on water deficit and NCE have used single-leaf (~2–10 cm^2) measurements (NCE_L; de Souza *et al.* 2003; Medrano *et al.* 2003; Zsófi *et al.* 2009). Indeed, a preponderance of leaf-level work indicates stomatal regulation as the dominant mechanism (see reviews by Chaves *et al.* 2010; Lovisolo *et al.* 2010). Leaf-level systems effectively facilitate work on chlorophyll fluorescence, and on controlled light-, temperature- and CO_2-response curves. However, inconsistent correlations between leaf-level and whole-canopy measurements of NCE (NCE_C; Edson *et al.* 1993, 1995; Intrieri *et al.* 1997) suggest caution in making inferences about NCE_C from NCE_L (Poni *et al.* 2003, 2009). Rigorous measurements of NCE_C are

pertinent to developing 'best practices' for canopy management in commercial vineyards, and to estimating vineyard-scale carbon and water budgets for applications such as climate change modelling. There are NCE_C data from field-grown vines in relation to training systems and canopy management practices (Katerji *et al*. 1994; Intrieri *et al*. 1997, 1998; Petrie *et al*. 2003, 2009; Poni *et al*. 2003), and there is a substantial body of literature describing the consequences of water deficit on NCE_L, yield, and fruit quality. However, less is known about the timing and extent of RDI approaches in concert with NCE_C in mature field-grown vines.

The objective of this experiment was to determine whether more severe water deficit (described hereafter as 'additional deficit') than a commercial standard RDI approach would be associated with lower NCE_C that would, in turn, adversely affect vine growth, yield, or variables that are commonly associated with fruit quality. Timing of the more severe deficit was investigated by imposing it independently during one of two main periods of fruit development: (1) shortly after fruit set to the onset of ripening (veraison); and (2) veraison to commercial maturity. A forthcoming paper will characterise canopy-level transpiration dynamics and vine water use associated with the RDI regimens described here.

Materials and methods

Field site

The NCE_C experiment was conducted during 2002 and 2003, which were years 4 and 5 of a 5 year study on RDI (Keller *et al*. 2008) in a commercial vineyard (4 ha) ~15 km west of Paterson, Wash., USA (45°53′N, 119°45′W, 125 m above sea level). Average annual rainfall is 155 mm and reference evapotranspiration (ET_o) is ~1050 mm (1994–2009; Washington State University AgWeatherNet (AWN; http://weather.wsu.edu, accessed 30 March 2011). The vineyard was located on a 14% south-facing slope on a uniformly deep (~1 m) Burbank loamy fine sand (sandy-skeletal, mixed mesic Xeric Torriorthents) with an estimated field capacity of 14.6% v/v and permanent wilting point of 7.1% v/v (http://websoilsurvey.nrcs.usda.gov, accessed 30 March 2011). The vineyard had been planted in 1992 to own-rooted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) in rows oriented north–south, with 2.7 m between rows and 1.8 m between vines for an average plant density of ~2000 ha^{-1}. Vines were trained to a bilateral cordon (permanent, horizontal extension of the trunk) at a height of ~1 m above ground. Shoots were loosely trained vertically between two foliage wires spaced 0.25 m apart at 0.2 m above the cordon. Vines were winter-pruned annually to two-bud spurs, yielding ~36–42 nodes per vine. All other horticultural practices were according to commercial convention for red wine grapes grown in the district, except that shoots were not thinned in 2001, 2002, or 2003 because low crop levels in the first 2 years of the RDI study were unacceptable to the commercial cooperator. Fertiliser and pest management interventions were applied uniformly across plots. Irrigation was delivered by drip through a single line per row using 1.8 L h^{-1}, pressure-compensated emitters spaced 1.2 m apart (three emitters for every two vines).

Meteorological variables were measured on-site, or where applicable, reference data were obtained from the Alderdale AWN station (~10 km west of site; 2002, 2003) and Paterson 'Station2' (~15 km east of site; long-term normals). Thermal time expressed as degree-days (DD, °C) was computed in daily increments using a lower threshold of 10°C and no upper threshold, summed from 1 April (day of year, DOY, 91) to 31 October (DOY 304) according to local convention for grape production. Global irradiance was measured on-site by a silicon pyranometer (LI-200S, Li-Cor, Lincoln, NE, USA) and incident photosynthetic photon flux density (PPFD) by a quantum sensor (LI-190S, Li-Cor). Reference air temperature (T_a) and relative humidity (RH) in the vineyard were measured at the height of the cordon and at a reference height ~2 m above the canopy, using combined temperature-RH probes (model HMP45, Vaisala, Helsinki, Finland) that were shielded and aspirated.

RDI regimens

All plots were irrigated to field capacity just after budbreak (early April). Thereafter, irrigation was withheld until shoots were ~1 m long and the rate of growth in main shoots was minimised. Shortly after fruit set, three RDI regimens were imposed, all of which were based on estimated crop evapotranspiration (ET_c) derived from ET_o (Allen *et al*. 1998) and a crop coefficient (K_c) for well watered Cabernet Sauvignon in eastern Washington (Evans *et al*. 1993). To meet the commercial 'standard' deficit practice (RDI_S), K_c was multiplied by 0.7, thus, RDI_S supplied 70% of estimated ET_c (1999–2002; 60% in 2003) on a weekly basis from shortly after fruit set until harvest. The 'early' (RDI_E) and 'late' (RDI_L) additional deficits further restricted irrigation during a portion of berry development such that 50% of the irrigation that was being applied to the RDI_S vines was delivered to these plots. In RDI_E, 35% (1999–2002; 30% in 2003) of ET_c was applied weekly from shortly after fruit set until veraison, after which vines were returned to RDI_S (60 or 70% of ET_c) until harvest. In RDI_L, vines were under RDI_S until veraison, then 35% of ET_c (1999–2002; 30% in 2003) was applied weekly between veraison and commercial maturity. The weekly irrigation allotment was delivered in two to five applications (sets). Actual water applied was estimated using the nominal flow rate of the drip emitters and the duration of water delivery as detected by pressure transducers in the drip line. For 4–5 weeks after harvest (1999–2002) vineyard staff irrigated all plots to replace 70% ET_c, then to field capacity in late October. In 2003, all plots were irrigated to field capacity immediately after harvest.

Volumetric soil water content (θ_v) was measured by vineyard staff using the neutron scattering method (HydroProbe 503 DR, Pacific Nuclear Corp., Martinez, CA, USA) at 0.15, 0.45, and 0.75-m depths. The average of these values was used to represent a 0.9 m deep soil unit for which the vineyard manager adjusted upward or downward the scheduled irrigation amount in response to deviations from the target θ_v (10% for RDI_S; 8.3% for additional deficit). Access tubes ($n = 3$ per plot) were installed in the vine row equidistant between drip emitters.

NCE measurement

In 2002 and 2003, rates of NCE_C were measured during five periods corresponding to key developmental stages in grape

berries: (1) fruit set (before initiation of the additional deficit); (2) pre-veraison (about the end of stage I of berry growth); (3) post-veraison (early in stage III of berry growth); (4) pre-harvest (just as the fruit approached commercial maturity); and (5) post-harvest (~2 weeks after removal of the fruit). Instantaneous rates (μmol CO_2 s^{-1}) and daily cumulative (g CO_2 d^{-1}) NCE_C were expressed per vine and per unit leaf area ($NCE_{C,LA}$). The NCE_C measurements were obtained using framed, open-top, flow-through chambers (~8 m^3 volume) that fully enclosed one vine each without modification of the canopy or trellis. Details of chamber design, operation, and calibration are provided elsewhere (Perez Peña and Tarara 2004). Air was exchanged at about two chamber volumes per minute. The T_a at the inlet and outlet of the chamber was measured by thermocouple (type T, 0.5 mm diameter, 24 AWG). To monitor the effect of the enclosure on T_a, shielded thermocouples also were suspended in the canopy at 1.6 m above ground in both enclosed and unenclosed vines.

Concentrations of CO_2 in air drawn from the chamber inlet and outlet were measured with an infrared gas analyser (IRGA; model CIRAS-DC, PP Systems, Haverhill, MA, USA) with a measurement range from 0 to 2000 μmol mol^{-1} and a precision of 0.2 μmol mol^{-1} at 300 μmol mol^{-1}. Instantaneous rates of NCE_C were calculated from the difference in [CO_2] between the air exiting and that entering the chamber, adjusted for the rate of air flow through the chamber. The IRGA was zeroed every 30 min and its calibration was checked after field runs using certified gas (359 and 305 ppm CO_2, Air Liquid, Houston, TX, USA) and a humidity calibrator (PP Systems). Six chambers operated simultaneously. A gas multiplexer (model GHU 161, ADC Bioscientific Ltd, Hoddesdon, UK) switched sample streams among chambers. Data were recorded at 2.5 s intervals and averaged every 2 min by datalogger (model CR7, Campbell Scientific, Logan, UT, USA) so that a mean was recorded for each chamber every 12 min. Measurements were collected continuously for 36–48 h, after which chambers were moved to a second set of replicate vines in each RDI regimen, the process was repeated, and again for a third set of replicate vines. Vines were paired randomly across rows so that on any one measurement day (d_m), the two vines were not along the same drip irrigation line. Replication was addressed by repeated-measurements across days within a developmental stage ($n = 6$ vines). The same 18 experimental vines were retained for both years.

Because of distance limitations for drawing gas samples, all experimental vines were within 60 m of the mobile laboratory that housed the IRGA, gas handling units, and data acquisition system. Because of variation in weather and in the days of the week on which irrigation was applied, two sets of analyses were conducted for NCE_C: (1) data were pooled across all d_m in a developmental stage ($n = 6$ vines); and (2) data were extracted for the 'optimal' measurement day in each run ($n = 2$ vines), which we defined as d_m with clear skies and the lowest likelihood of an RDI regimen being confounded by irrigation scheduling (i.e. the timing of multiple irrigation sets to apply the required amount of water). The data extracted for the optimal measurement day per run also were used to estimate potential maximum net CO_2 fixed by the canopy, which we calculated by integrating NCE_C over 24 h

and then applying linear interpolation between developmental stages.

Rates of NCE_L were measured concurrently with NCE_C at three developmental stages in 2002 (pre-veraison, post-veraison, pre-harvest) and at all stages at which NCE_C was measured in 2003. Repeated measurements (~0800–1600 hours) of NCE_L were recorded four times during the day (t_1 to t_4) in 2002 and six times per day (t_1 to t_6) in 2003, during each full day that NCE_C was recorded ($n = 9$ vines per developmental stage). On each vine designated for NCE_L estimation, four shoots ~1 m long (two per cordon) and bearing one fruit cluster each were tagged. Shoots were selected towards the exterior of the canopy to facilitate measurement on sunlit leaves, with shoots on the east aspect of the vine used for measurements recorded before solar noon and those on the west aspect of the vine used after solar noon. One fully expanded leaf that was located about 6–8 leaves from the shoot apex was selected. At each sample time during the day, a mean of two leaves per vine was retained. The NCE_L was measured with a portable photosynthesis system (model CIRAS-2, PP Systems) using a 2.5 cm^2 leaf cuvette (model PLC6(U)). Air flow through the cuvette was 200 mL min^{-1}. The NCE_L measurements were recorded under ambient irradiance.

Ancillary plant measurements

Leaf area per vine (LA_v) on each of the 18 NCE_C vines was estimated twice during 2002 (veraison and pre-harvest) and four times during 2003 (fruit set, veraison, pre-harvest, and post-harvest). In 2002, LA_v was estimated by a three-step process: (1) leaf width was regressed against leaf area measured by area meter (LI-3100, Li-Cor) for a sample of 200 leaves from vines near the 18 NCE_C experimental vines; (2) the widths of all leaves on a sample of shoots ($n = 8$) from each experimental vine were measured and individual leaf areas were computed from the regression equation; and (3) LA_v was calculated from the average leaf area per shoot. The estimation procedure was modified in 2003. At each developmental stage a linear regression model was fit between shoot length and leaf area per shoot ($n = 50$). Leaf area per NCE_C vine was estimated using measured shoot length (50% of shoots measured) and the length-to-area relationship. In 2002, LA_V from the pre-harvest sample was applied to post-harvest measurements of NCE_C. In both years, LA_V estimated at veraison was used for both the pre- and post-veraison measurement runs. Because LA_v was measured less frequently in 2002 than in 2003, where data were pooled across years, analysis of daily cumulative NCE_C was on a per vine basis.

Non-structural carbohydrate concentrations in leaf tissue were determined from leaf discs (6.3 mm diameter, two per leaf) collected concurrently with measurements of NCE_L, from the two leaves above and the two leaves below the one used for NCE_L. Leaf discs were excised between major veins with a modified commercial hole punch to which a 1.5 mL microtube had been attached. The microtubes were frozen immediately in liquid nitrogen and stored at $-80°C$ until analysis. Soluble sugars (glucose, fructose, and sucrose) and starch concentrations were determined using a sequential enzymatic degradation method as described by Hendrix (1993), with the following modifications. Briefly, frozen leaf tissue was first homogenised in the microtube

(45 s in a bead-beater (Mini-BeadBeater-8, Biospec Products, Bartlesville, OK, USA)). A glucose assay kit (GAHK20, Sigma-Aldrich, St Louis, MO, USA) was used. Absorbance was measured at 340 nm using a microplate reader (SpectraMax Plus384, Molecular Devices Corp., Sunnyvale, CA, USA). As per Hendrix (1993), the sum of glucose, fructose, and sucrose concentrations was expressed as total soluble sugars (SS; mg glucose equivalents per g fresh mass, FM). For starch determination (mg glucose equivalents per g FM), corn starch standards were digested and analysed concurrently with tissue samples. Total nonstructural carbohydrates were defined as the sum of SS and starch. For both leaf SS and starch concentrations, only data collected from d_m with clear skies that were preceded by a day with clear skies were used in the analysis ($n = 3$ per RDI regimen and sample time).

Mature fruit was harvested on DOY 262 in both years. The trigger for harvest was a soluble solids concentration of $\geq 24°$ Brix in a composite sample of fruit collected near the NCE_C experimental vines. Fruit was weighed and clusters were counted. About 200 berries were retained at random from each experimental vine to determine mean berry mass and estimate the number of berries per cluster. Fruit maturity indices also were determined from these samples: total soluble solids (by refractometry), pH and titratable acidity (TA, by acid titration), colour density and hue (by spectrophotometry at 420 and 520 nm), where colour density is $A_{420} + A_{520}$ (absorbance units per mL of juice) and colour hue is A_{420}/A_{520} (dimensionless).

During winter pruning (DOY 43; 2003, 2004), when the 1-year-old wood was trimmed to two-bud spurs, the mass of pruned canes for the 18 NCE_C vines was recorded in the field. Two common indices of grapevine crop load, leaf area : fruit mass (LA$_V$: fruit; $cm^2 g^{-1}$) and fruit mass : pruning mass (fruit : pruning; dimensionless) were computed. Cane pieces (2–5 cm long) were then collected from the basal ends of the excised tissue, i.e. above the second node from the base of the original shoot. The cane pieces were bagged, weighed in the laboratory, divided into 1 cm long segments, dried to constant mass at 60°C (~48 h), then ground to pass through a 0.08 mm mesh screen. Soluble sugars and starch were extracted and analysed by the same method as leaf tissue, using ~20 mg of the ground cane tissue per sample.

Statistical analyses

Data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variance using Brown-Forsythe. A general linear model procedure was used for analysis of variance. Means were compared by Tukey or Tukey–Kramer ($P \leq 0.05$) as appropriate. Where data were normally distributed, correlations between plant response variables were assessed using the Pearson product moment correlation coefficient (r). For linear regression analyses, data were transformed as needed to adjust for heterogeneous variances and for distributions that deviated from normal. All statistical analyses were performed using SAS (V. 8.2; SAS Institute, Cary, NC, USA). The NCE_C data collected at fruit set in 2002 were excluded from the analysis because of technical difficulties with the whole-canopy system. Where NCE_C values differed significantly between years, these instances are noted in the text but are not presented graphically.

Results

Meteorological summary

Cumulative DD were below the long-term average (1702 DD; 1994–2009) in 2002 and above average in 2003 (Table 1). In both years, daily maximum air temperature exceeded 40°C only briefly during July (DOY 192, 193, 194 (2002); DOY 211 (2003)). Budbreak, anthesis, and veraison occurred about one week earlier in 2003 than in 2002. During fruit development, 2003 was warmer than 2002. Cumulative ET_o between budbreak and leaf fall was similar in both years, and nearly identical to the long-term mean. Annual rainfall during the study was 28% (2002) and 7% (2003) below the long-term mean, and was consistent with the seasonal pattern of

Table 1.   Developmental stages, summary meteorological variables and irrigation applied to mature Cabernet Sauvignon grapevines under three regimens of regulated deficit irrigation (RDI): RDI$_S$ (industry standard RDI); RDI$_E$ (early additional deficit); and RDI$_L$ (late additional deficit)

Abbreviations: DD, cumulative thermal time expressed as degree days above 10°C base temperature; ET_o, evapotranspiration for a grass reference crop, calculated from the Penman–Monteith equation (Allen *et al.* 2004)

Year	Developmental stage	Dates	DD (°C)	Precipitation[A] (mm)	ET_o (mm)	Irrigation (mm) RDI$_S$	RDI$_E$	RDI$_L$
2002	Dormancy	November 8–April 25	–	114	–	–	–	–
	Budbreak-bloom	April 25–June 11	262	17	301	11	9	11
	Bloom-veraison	June 11–August 9	771	13	499	140	78	134
	Veraison-harvest	August 9–September 19	462	4	252	60	48	27
	Harvest-frost	September 19–October 25	128	4	104	46	41	42
	Growing season total	–	1623	38	1156	257	176	214
2003	Dormancy	October 25–April 15		170	–	–	–	–
	Budbreak-bloom	April 15–June 3	232	12	269	0	0	0
	Bloom-veraison	June 3–August 5	832	1	512	158	87	143
	Veraison-harvest	August 5–September 13	486	19	233	42	40	13
	Harvest-frost	September 13–October 21	284	5	148	48	45	48
	Growing season total	–	1834	37	1162	248	172	204

[A]Dormant season rainfall was summed between the dates of the first frost of the preceding year and budbreak of the current year.

precipitation in eastern Washington: 25% (2002) and 18% (2003) of the annual total fell between budbreak and leaf fall, with no rain detected between early June (pre-bloom) and early August (~veraison) in 2003.

Between fruit set and harvest, a total of 63% of the water applied to RDI_S was applied to RDI_E plots and 79% was applied to RDI_L plots. In both years, when the RDI_E vines were under the additional deficit, 55% of the cumulative amount of water applied to RDI_S was delivered to RDI_E plots, very close to the goal of 50% of the standard deficit. Water application was somewhat more variable for RDI_L vines: 45% (2002) and 31% (2003) of the cumulative amount for RDI_S was delivered when RDI_L was under the additional deficit. The θ_v reflected irrigation application (data not shown; refer to Schreiner *et al.* 2007, fig. 1) and treatment design. Occasional departures in θ_v from the target values occurred because of the inherent limitations in the *post-hoc* soil water balance approach, particularly under drip irrigation (Stevens and Douglas 1994).

NCE_C: diurnal patterns

Across treatments, LA_v ranged from ~6 to $10\,m^2$ between fruit set and harvest (Table 2). The LA_v was not different between measurement dates in 2002 ($P = 0.325$) but LA_v did differ by developmental stage in 2003 ($P = 0.001$). There were no significant interactions between RDI regimen and developmental stage in either year ($P = 0.378$ for 2002; $P = 0.586$ for 2003). Differences between treatments were related to final shoot numbers (Table 3) caused by variability in the co-operator's hand pruning. With data pooled across years, LA_v on RDI_E vines was similar to that of RDI_S vines ($P = 0.306$), which is not surprising because most shoot growth had occurred before imposition of the early additional deficit.

Table 2. Leaf area per vine (LA_V) by developmental stage for grapevines under three regimens of regulated deficit irrigation (RDI): RDI_S (industry standard RDI); RDI_E (early additional deficit); RDI_L (late additional deficit)

Values followed by different letters in the same row are significantly different at $P < 0.05$ by Tukey-Kramer; nc, data not collected

Year	Developmental stage	RDI regimen (m^2)		
		RDI_S	RDI_E	RDI_L
2002	Fruit set	nc	nc	nc
	Veraison	9.3ab	7.5b	10.7a
	Pre-harvest	10.1	8.4	10.6
	Post-harvest	nc	nc	nc
2003	Fruit set	8.8ab	8.2b	9.6a
	Veraison	8.4b	8.1b	9.7a
	Pre-harvest	7.0b	6.7b	8.7a
	Post-harvest	6.0b	6.2b	7.5a

The effect of the smaller deficit coefficient in 2003 ($0.6 \times K_c$) than in 2002 ($0.7 \times K_c$) was evident only later in the growing season; more senescence of basal leaves across all RDI regimens resulted in ~27% lower LA_V in 2003 than in 2002 ($P = 0.005$) at harvest.

Graphical and tabular presentation of NCE comprises complete datasets from 2003, wherein gas exchange and LA_V data were available at all developmental stages. In both years, on d_m with clear skies and no confounding irrigation timing, additional water deficits reduced daily maximum instantaneous rates of NCE_C ($P = 0.005$ for RDI_E; $P = 0.006$ for RDI_L) during the respective deficit periods. Instantaneous rates of NCE_C varied with irradiance, T_a, ET_o, and time elapsed from an

Table 3. Mean growth, yield, and fruit quality indices for grapevines under three regimens of regulated deficit irrigation (RDI): RDI_S (industry standard RDI); RDI_E (early additional deficit); RDI_L (late additional deficit)

The 'RDI regimen' columns represent data pooled across years; the 'Year' columns represent data pooled across RDI regimens. Abbreviations: LA_V, leaf area per vine; DM, dry mass; SS, total soluble sugars; TA, titratable acidity. Values followed by different letters within rows (RDI regimen) are significantly different at $P < 0.05$, by Tukey-Kramer. Levels of significance between years are indicated: $*P < 0.05$; $**P \leq 0.01$; $***P \leq 0.001$

Response variable	RDI regimen			Year		Significance
	RDI_S	RDI_E	RDI_L	2002	2003	
Yield (kg $vine^{-1}$)	3.9c	5.0b	5.5a	5.4	4.2	***
Shoots $vine^{-1}$	69b	70b	84a	62	87	***
Clusters $vine^{-1}$	93b	97ab	109a	93	109	**
Clusters $shoot^{-1}$	1.4	1.4	1.4	1.4	1.2	**
Average cluster mass (g)	41b	52a	51a	58	38	***
Berries $cluster^{-1}$	43b	55a	55a	59	41	***
Berry mass (g)	0.95	0.94	0.97	0.98	0.93	–
LA_V : fruit mass ($cm^2\,g^{-1}$)	23.3a	15.1b	18.9b	18.0	20.4	–
Dormant cane mass (kg $vine^{-1}$)	1.1a	0.8b	1.1a	1.12	0.93	***
Fruit : pruning mass (dimensionless)	3.3c	6.5a	4.8b	4.9	4.8	–
Dormant cane starch (mg g^{-1} DM)	100	105	105	107	99	–
Dormant cane SS (mg g^{-1} DM)	13	13	15	18	10	***
Berry soluble solids (°Brix)	26.4	26.0	26.1	25.6	26.8	***
Berry pH	3.7b	3.8a	3.7b	3.7	3.7	–
Berry TA (g tartaric acid L^{-1})	5.9ab	5.4b	6.4a	6.0	5.8	–
Colour density (absorbance units mL^{-1})	16.0	14.9	15.1	15.1	15.6	–
Colour hue (dimensionless)	0.20	0.20	0.20	0.20	0.20	–

irrigation set. In RDI_S vines, maximum instantaneous rates of NCE_C did not differ by developmental stage ($P = 0.9$).

Before initiation of the early additional deficit, instantaneous rates of $NCE_{C,LA}$ and their diurnal patterns were similar in all RDI regimens (Fig. 1; <DOY 183, 2003). The responsiveness of $NCE_{C,LA}$ to rapid dry-down and re-wetting in a soil of low water-holding capacity is evident in the first two d_m of the fruit set measurement run. From DOY 176 through DOY 178, a total of 15 mm irrigation was applied to all RDI regimens, uniformly across the three days. Daily maximum instantaneous rates of $NCE_{C,LA}$ approached ~9.5 μmol m^{-2} s^{-1} (Fig. 1a) and $NCE_{C,LA}$ generally followed the sinusoidal pattern of irradiance, indicating only a mild water deficit. By contrast, on

DOY 181 (Fig. 1b) instantaneous rates of $NCE_{C,\ LA}$ in all RDI regimens reflected a combined effect of high ET_o and no irrigation delivery over the previous 3 days. On DOY 181, from a morning maximum (~0800 hours) of ~8 μmol m^{-2} s^{-1}, $NCE_{C,LA}$ declined steadily to ~4 μmol m^{-2} s^{-1} after 1300 hours. Irrigation sets were applied on DOY 182 and 183, totaling 10 mm (RDI_S and RDI_L). However, only 4 mm were applied to RDI_E plots. By the afternoon of DOY 183 (Fig. 1c), instantaneous rates of $NCE_{C,LA}$ diverged among treatments. The diurnal pattern of $NCE_{C,LA}$ in RDI_E (DOY 183) mirrored that of all regimens under the restricted water supply of DOY 181, a result of the cooperator initiating the additional deficit on that day. Given an estimated plant available water of ~28 mm at our site and an
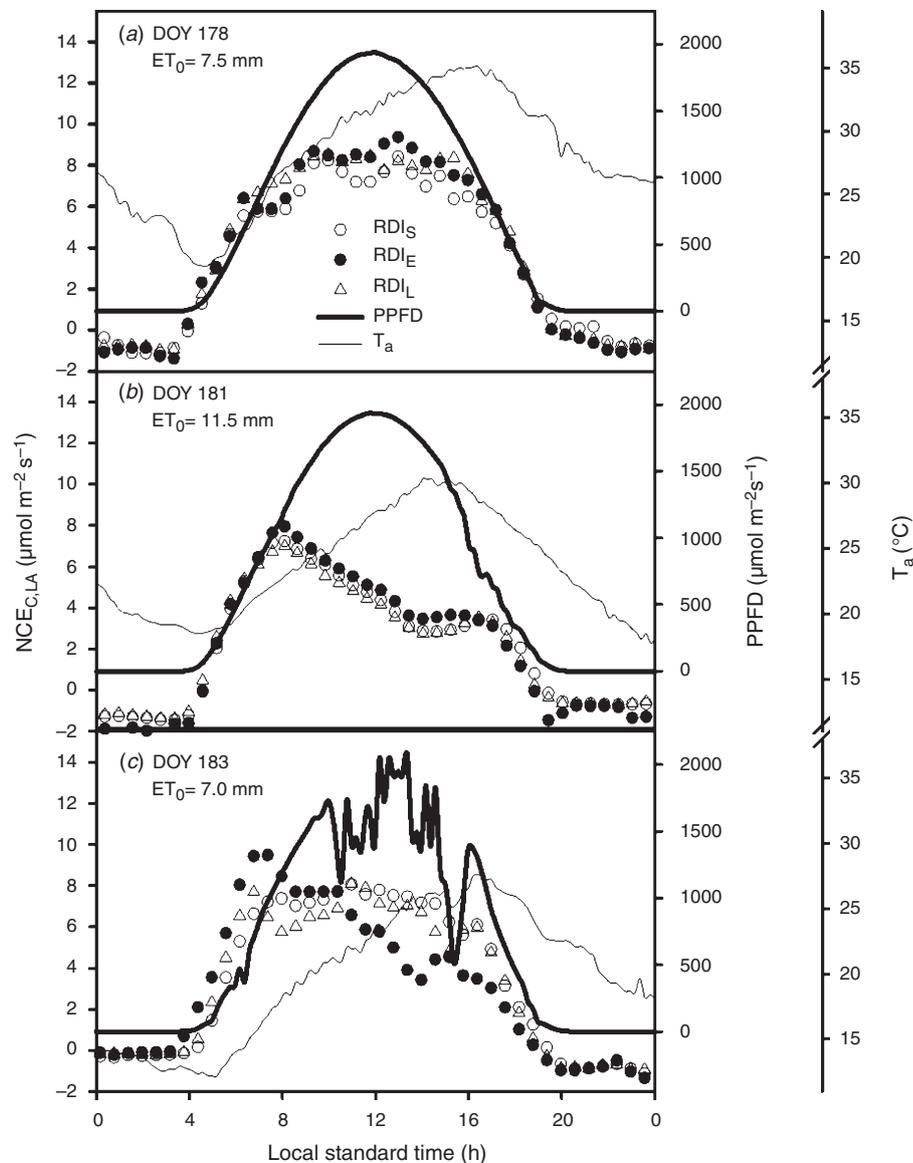

Fig. 1. Whole-canopy *Vitis vinifera* net CO_2 exchange rate per unit leaf area ($NCE_{C,LA}$), photosynthetic photon flux density (PPFD), and air temperature (T_a) during the 'fruit set' measurement run, 2003. Symbols represent the mean of two vines. Abbreviations: RDI, regulated deficit irrigation; RDI_S, industry standard RDI; RDI_E, early additional deficit; RDI_L, late additional deficit; ET_o, reference evapotranspiration.

average daily ET_c of ~8 mm in mid-summer, a water deficit could have been generated within 3 days with more than 50% of available water transpired in 2 days.

Shortly before veraison, 5 weeks of additional water deficit in RDI_E vines resulted in lower midday rates of $NCE_{C,LA}$ than in those vines without the additional water restriction (RDI_S, RDI_L) regardless of irradiance, temperature, or ET_o (Fig. 2). For example, from DOY 210 to 212, all plots were irrigated but RDI_E vines received 54% (7.8 mm) of the water applied to RDI_S and RDI_L vines (14.4 mm); lower mid-day rates of $NCE_{C,LA}$ in RDI_E vines are apparent on d_m DOY 213 and DOY 215 (Fig. 2a, b), the latter of which was characterised by lower evaporative demand and variable cloudiness. The effect of the early additional deficit is most evident at the dry

end of the weekly cycle (DOY 218): in vines under RDI_E, mid-day rates of $NCE_{C,LA}$ were about half those of RDI_S and RDI_L vines. No irrigation had been applied to RDI_S and RDI_L during the previous 3 days or to RDI_E during the previous 5 days.

At veraison, RDI_E vines were returned to standard RDI and the additional deficit was initiated in RDI_L plots. The post-veraison measurement run (Fig. 3) was conducted about 3 weeks after this reversal. Instantaneous rates of $NCE_{C,LA}$ were initially low across treatments under overcast skies and rain (2.3 mm; Fig. 3a); consequently, differences among RDI regimens were not apparent. Later under mostly clear skies, maximum instantaneous rates of $NCE_{C,LA}$ were at the highest values of the season (~12–14 $\mu mol\ m^{-2}\ s^{-1}$; Fig. 3b, c). At the
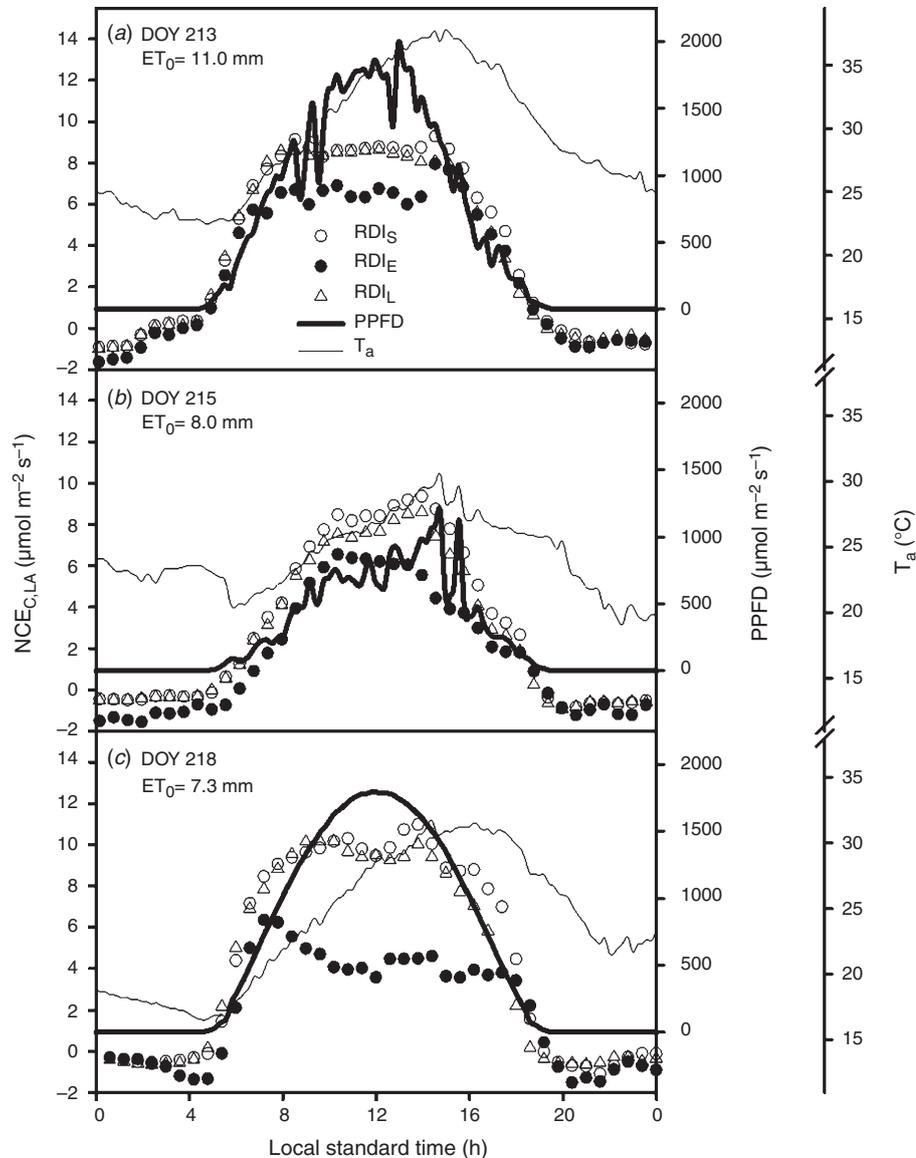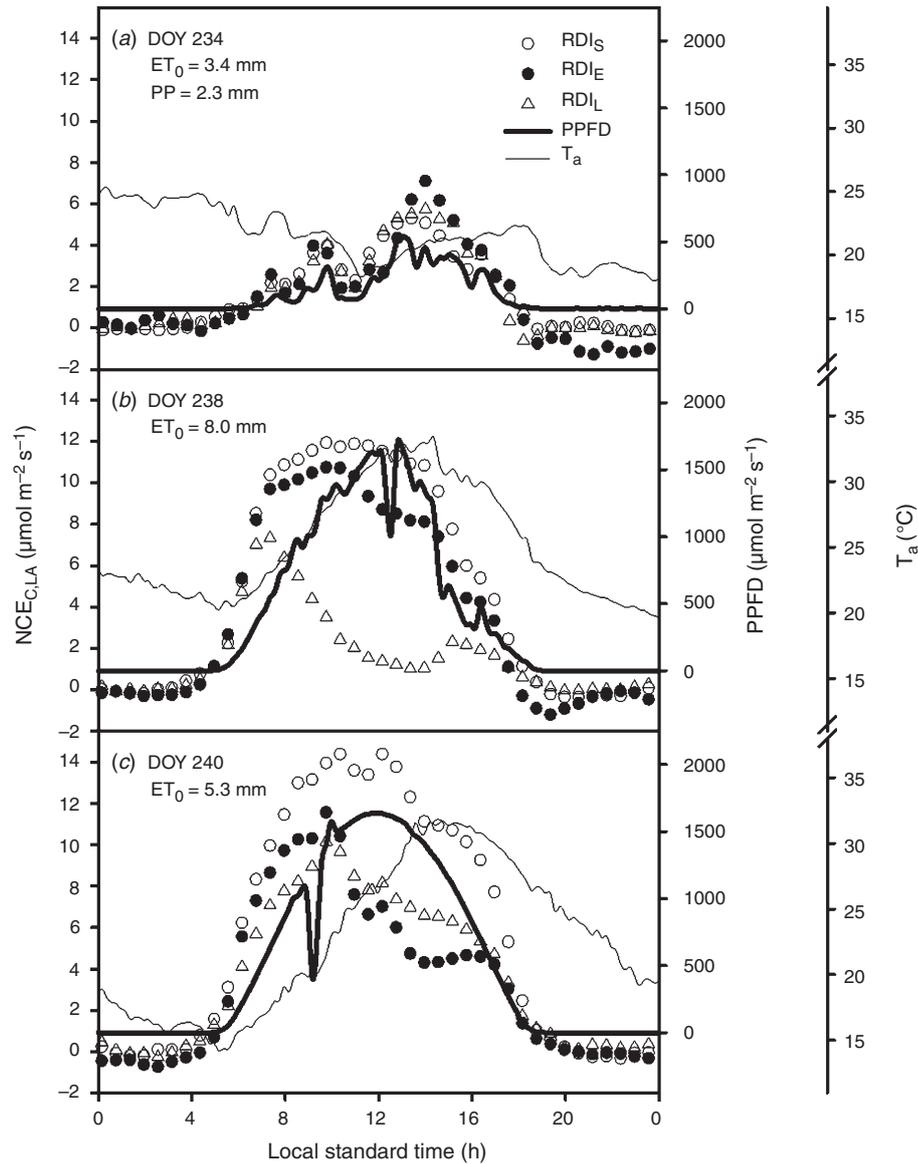

Fig. 2. Whole-canopy *Vitis vinifera* net CO_2 exchange rate per unit leaf area ($NCE_{C,LA}$), photosynthetic photon flux density (PPFD), and air temperature (T_a) during the 'pre-veraison' measurement run, 2003. Symbols represent the mean of two vines. Abbreviations: RDI, regulated deficit irrigation; RDI_S, industry standard RDI; RDI_E, early additional deficit; RDI_L, late additional deficit; ET_o, reference evapotranspiration.

Fig. 3. Whole-canopy *Vitis vinifera* net CO_2 exchange rate per unit leaf area ($NCE_{C,LA}$), photosynthetic photon flux density (PPFD), and air temperature (T_a) during the 'post-veraison' measurement run, 2003. Symbols represent the mean of two vines. Abbreviations: RDI, regulated deficit irrigation; RDI_S, industry standard RDI; RDI_E, early additional deficit; RDI_L, late additional deficit; ET_o, reference evapotranspiration. On DOY 234, 2.3 mm of rain (PP) fell.

end of a weekly irrigation cycle in which 20.4 mm had been applied to both RDI_S and RDI_E plots, rates of $NCE_{C,LA}$ in the RDI_E vines approached those of RDI_S vines (Fig. 3*b*; DOY 238). By contrast, 8.4 mm of water had been applied to RDI_L plots, causing $NCE_{C,LA}$ to decline over the course of the day from an early morning maximum. An irrigation scheduling error (DOY 239, 2003) that omitted irrigation (3.8 mm) in RDI_E plots induced enough cumulative water stress (4 days without irrigation) that both instantaneous rates of $NCE_{C,LA}$ and the daily cumulative $NCE_{C,LA}$ on the following d_m (DOY 240) resembled those of RDI_L vines that had been intentionally subjected to additional deficit (Fig. 3*c*).

Immediately before harvest, instantaneous rates of $NCE_{C,LA}$ in RDI_S and RDI_E vines were similar, and higher than those in RDI_L vines, consistent with more severe water deficit in RDI_L (Fig. 4*a*). Only 4 mm had been applied to RDI_S and RDI_E in the 7 days before DOY 256, resulting in a diurnal pattern of $NCE_{C,LA}$ consistent with limiting soil water. After approaching a daily maximum instantaneous rate of ~10 μmol m^{-2} s^{-1} (RDI_S, RDI_E) and ~6 μmol m^{-2} s^{-1} (RDI_L) at around 0800 hours, rates of $NCE_{C,LA}$ declined until late afternoon (~1500 hours), when vines accessed water that was being applied on that day (3.5–4.6 mm total on DOY 256). Under variable cloud cover and lower T_a, instantaneous rates of $NCE_{C,LA}$ were lower in all RDI regimens
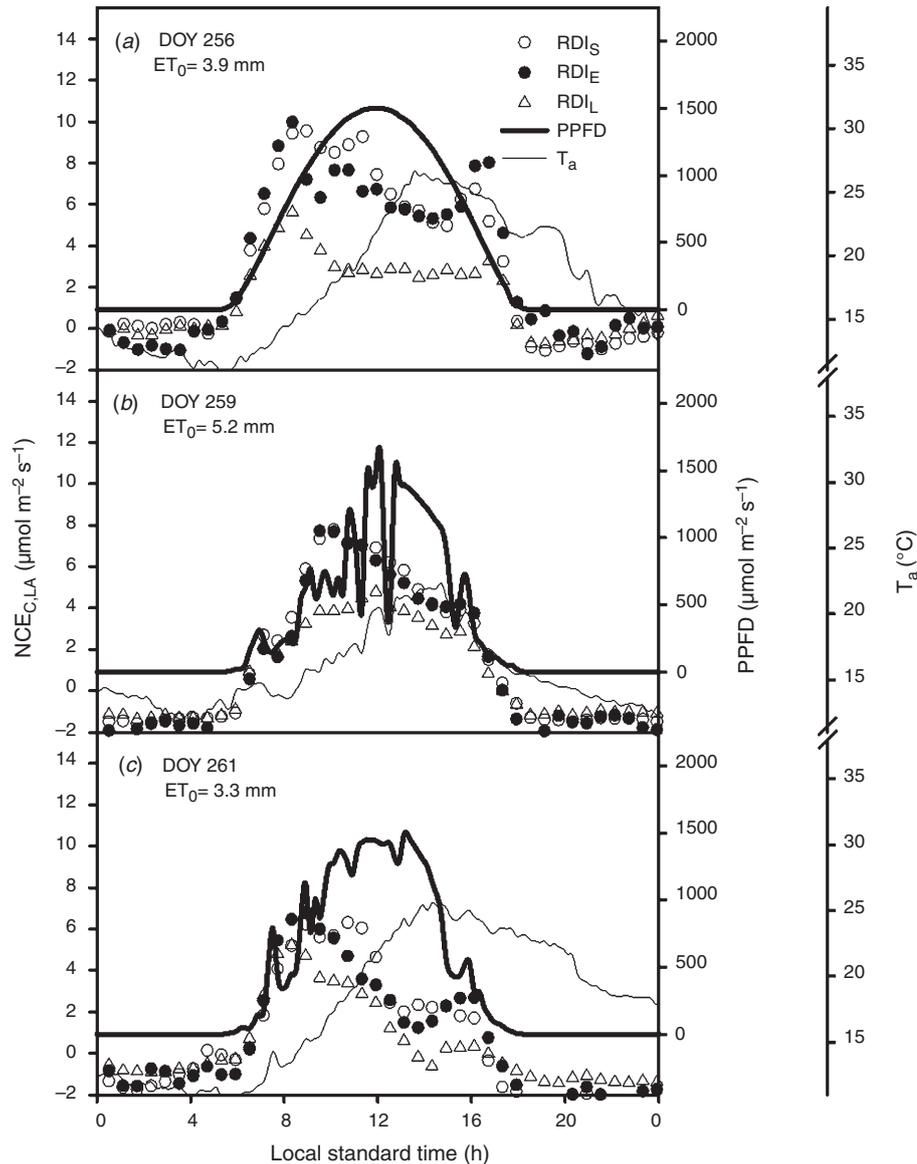
Fig. 4. Whole-canopy *Vitis vinifera* net CO_2 exchange rate per unit leaf area ($NCE_{C,LA}$), photosynthetic photon flux density (PPFD), and air temperature (T_a) during the 'pre-harvest' measurement run, 2003. Symbols represent the mean of two vines. Abbreviations: RDI, regulated deficit irrigation; RDI_S, industry standard RDI; RDI_E, early additional deficit; RDI_L, late additional deficit; ET_o, reference evapotranspiration.

on DOY 259 and 261 (Fig. 4*b, c*); nonetheless, diurnal patterns were indicative of water deficit in all treatments, as no irrigation had been applied since DOY 256. In the post-harvest measurement run, on d_m with mostly clear skies, daily maximum instantaneous values of $NCE_{C,LA}$ ranged from 6.0 to ~9.5 $\mu mol\ m^{-2}\ s^{-1}$ across RDI regimens, with no differences apparent between treatments (data not shown). Frost defoliated the vines ~40 days after harvest.

NCE_C: cumulative values

Significant differences in cumulative NCE_C per day ($g\ CO_2\ day^{-1}$) were detected among RDI regimens whether NCE_C was expressed per plant or per unit LA (Table 4),

despite some differences in LA_V (Table 2). Thus, canopy size was not the main determinant of the observed responses to additional water deficit. As one would expect from the treatment structure, daily cumulative NCE_C was affected by RDI regimen when those vines were subjected to the additional water deficit ($P = 0.001$). When all vines were irrigated identically (i.e. fruit-set and post-harvest runs) there were no differences among regimens. The seasonal pattern was consistent between years (data not shown), where daily cumulative NCE_C was highest around veraison when the canopy was most fully developed (Table 4). Mean daylength during those measurement runs was 14.6 h (pre-veraison) and 13.7 h (post-veraison). After being subjected to the additional

Table 4.   Daily cumulative net carbon exchange (NCE) by grapevine canopies under three regimens of regulated deficit irrigation (RDI) near key developmental stages, 2003

Data are expressed per vine (NCE_C; top; $g\,CO_2\,day^{-1}$) and per unit leaf area ($NCE_{C,LA}$; bottom; $g\,CO_2\,day^{-1}\,m^{-2}$). The NCE values are means of six vines averaged across all measurement days (d_m) in each developmental stage. Abbreviations: RDI_S, industry standard RDI; RDI_E, early additional deficit; RDI_L, late additional deficit; DOY, day of year; Trt, treatment. Values followed by different letters within rows are significantly different at $P \le 0.05$ by the Tukey–Kramer test

Developmental stage	Sampling period (DOY)	RDI regimen			P-value	
		RDI_S	RDI_E	RDI_L	d_m	Trt $\times d_m$
			NCE_C			
Fruit set	177–184	101	94	113	0.001	0.532
Pre-veraison	212–219	125a	67b	131a	0.001	0.048
Post-veraison	233–241	127a	90b	85b	0.001	0.001
Pre-harvest	255–262	55	37	33	0.001	0.146
Post-harvest	274–281	51	52	45	0.082	0.484
P-value			0.001[A]		0.001[B]	
			$NCE_{C,LA}$			
Fruit set	177–184	11.5	11.5	11.7	0.001	0.730
Pre-veraison	212–219	14.9a	8.4b	13.4a	0.003	0.151
Post-veraison	233–241	15.3a	11.0b	8.8b	0.001	0.007
Pre-harvest	255–262	7.8	5.6	3.8	0.001	0.259
Post-harvest	274–281	8.7	7.1	6.9	0.174	0.935
P-value			0.001[A]		0.001[B]	

[A]For pooled data, effect of developmental stage.
[B]For pooled data, interaction between RDI regimen and developmental stage.

deficit for ~5 weeks, over a week-long irrigation cycle RDI_E vines fixed an average of 43–46% less CO_2 per vine per day than did RDI_S vines. When RDI_L vines had been under the additional water deficit for ~3 weeks, those vines fixed on average ~33% less CO_2 per day than did RDI_S vines. Immediately before harvest and across all RDI regimens, the average daily cumulative NCE_C was ~40% of its value 3 weeks earlier.

Variable weather and irrigation scheduling imposed some confounding effects on measurements of NCE_C because up to 5 days were required to apply the week's irrigation to a soil of low water-holding capacity, the number of days depending on time of year (i.e. ET_c and K_c) and RDI regimen (i.e. proportion of K_c). To better interpret the data in light of unavoidable field conditions, NCE values from the optimal d_m in each run were segregated. Here, daily cumulative NCE_C also responded to the additional deficit and only during the time that the additional deficit was applied to its respective regimen (Table 5). For example, in the post-veraison measurement run when all d_m were pooled, RDI_E vines apparently fixed significantly less CO_2 per day than did RDI_S vines (Table 4) despite having been returned to the standard irrigation schedule. This outcome was not the case (Table 5) when DOY 240 (Fig. 3c) was excluded, where the anomalously low values in RDI_E were known to have been caused by a missed irrigation set. There was a higher potential cumulative reduction in carbon fixed (v. RDI_S) under RDI_E (35%) than under RDI_L (12%) because RDI_E included the highest K_c of the season.

NCE_L

There was a significant ($P < 0.001$) linear association ($r^2 = 0.61$; $P < 0.001$) between NCE_L and $NCE_{C,LA}$ (Fig. 5), where NCE_L

generally overestimated $NCE_{C,LA}$ regardless of RDI regimen. In both years, leaf-level measurements confirmed our observations from the whole-canopy system: differences in NCE_L among RDI regimens reflected those in $NCE_{C,LA}$ (Fig. 6a–d). Both NCE_L and g_s were lower during the time that the additional deficit was applied to RDI_E or RDI_L, respectively. Maximum instantaneous values of NCE_L occurred at veraison (pre- and post-veraison measurement runs), with daily maxima (~15–17 $\mu mol\,m^{-2}\,s^{-1}$) generally around mid-morning. During the post-veraison measurement run, NCE_L was lower in RDI_L vines than in either RDI_E or RDI_S vines in 2003 (Fig. 6), but RDI_E and RDI_L were not significantly different from one another in 2002 (data not shown). The apparent inconsistency between years is due to a single irrigation application error in 2002 that delivered the RDI_S allotment. Instantaneous rates of NCE_L were strongly associated with g_s ($r = 0.85$ to 0.97; Fig. 6e–h). At pre-veraison, mid-day values of g_s in vines under additional deficit ranged from ~75 to 125 $mmol\,H_2O\,m^{-2}\,s^{-1}$, whereas in vines under standard RDI, g_s was between ~150 and 250 $mmol\,H_2O\,m^{-2}\,s^{-1}$. During the post-veraison measurement run, g_s ranged from ~50 to 100 $mmol\,H_2O\,m^{-2}\,s^{-1}$ in vines under the additional deficit.

Nonstructural carbohydrates, yield, fruit quality

Concentrations of SS and starch in leaves did not respond consistently to RDI regimen or developmental stage in either year (data not shown). We observed no diurnal changes in leaf SS in 2002. In 2003, SS concentrations in all regimens were higher ($P < 0.05$) at the first sampling time of a given day (t_1; ~0800 hours) than at the last (t_4–t_6; up to ~1700 hours). However, there were no differences among treatments. Leaf starch

Table 5. Daily cumulative (top) and estimated potential maximum (bottom) net carbon exchange per vine (NCE_C) by grapevine canopies under three regimens of regulated deficit irrigation (RDI) near key developmental stages, 2003

Potential maxima estimated by linear interpolation between developmental stages using data from measurement days (d_m) with clear skies. Abbreviations: RDI_S, standard RDI; RDI_E, early additional deficit; RDI_L, late additional deficit; PPFD, photosynthetic photon flux density; DOY, day of year; Trt, treatment. Values in parentheses (bottom) are percent of total over the 97-day period. Values followed by different letters within rows are significantly different at $P < 0.05$ by the Tukey–Kramer test

| Developmental stage or interpolation period	d_m (DOY)	Daily cumulative NCE_C			Daily cumulative PPFD ($mol\,m^{-2}\,d^{-1}$)
		RDI_S	RDI_E ($g\,CO_2\,d^{-1}\,vine^{-1}$)	RDI_L	
Stage					
Fruit set	178	111	120	141	62.5
Pre-veraison	218	140a	60b	149a	54.3
Post-veraison	238	145a	124a	60b	38.7
Pre-harvest	256	81	66	49	40.6
Post-harvest	278	54	59	57	28.7
		Potential cumulative NCE_C			
Period	Duration (d)	RDI_S	RDI_E ($kg\,CO_2\,period^{-1}\,vine^{-1}$)	RDI_L	
Fruit set to veraison	46	5.79 (54)	2.77 (40)	6.68 (71)	
Veraison to harvest	32	3.64 (34)	3.04 (43)	1.75 (19)	
Harvest to post-harvest	19	1.29 (12)	1.19 (17)	1.01 (11)	
Total	97	10.73 (100)	7.01 (100)	9.45 (100)	
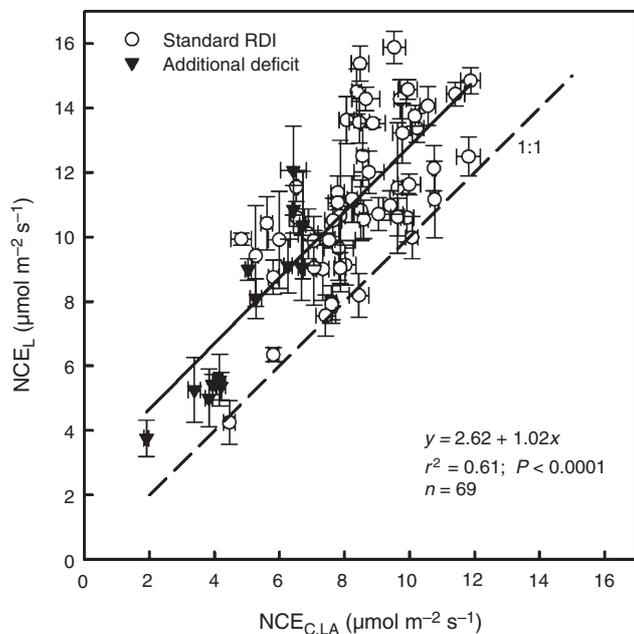
Fig. 5. Linear association between rate of *Vitis vinifera* net CO_2 exchange at the single-leaf level (NCE_L) and at the canopy level expressed per unit leaf area ($NCE_{C,LA}$), for vines under an industry standard practice of regulated deficit irrigation or under an additional deficit that reduced the standard irrigation application by half. Symbols represent means over 1 h of simultaneous measurement ($n = 6$ for NCE_L, $n = 10$ for NCE_C), Error bars are ±s.e. Data are from all developmental stages in 2003 except harvest, when there were no coincident measurements.

concentrations were higher in the afternoon than in the morning in both years and across all RDI regimens. In 2002, leaf starch concentrations differed among RDI regimens only at t_1 ($P < 0.05$)

and only at pre-veraison: under RDI_E, concentrations were 63–64% of those that were under standard RDI. In 2003, only at t_6 were leaf starch concentrations lower in vines that were under the additional deficit: 36% of RDI_S at pre-veraison (i.e. RDI_E) and 83% of RDI_S at post-veraison (i.e. RDI_L). Neither SS nor starch concentrations in dormant cane tissue differed among RDI regimens in either year (Table 3). However, the mass of canes pruned was 27% lower in RDI_E vines than in either RDI_S or RDI_L vines, so total non-structural carbohydrate content was lower in the wood of RDI_E vines.

In both years, crop loads (fruit : pruning) were low. Fruit : pruning differed among irrigation regimens in the order $RDI_E > RDI_L > RDI_S$ ($P < 0.001$; Table 3), although most values fell within a range generally thought to indicate sufficient leaf area to ripen the crop (i.e. 5–10; Kliewer and Dokoozlian 2005). Considered alternatively as LA_v : fruit mass, the order of relative differences was conserved in the inverse: $RDI_E \approx RDI_L < RDI_S$ ($P < 0.001$). Given the mean yield that we observed (4.7 kg vine^{-1}) and assuming that berries comprise ~25% dry matter with 50% C content (RP Schreiner, unpublished data), the fruit we harvested represented on average ~2.15 kg CO_2 fixed. Under this assumption and scaling from our NCE_C measurements to an estimated net maximum of CO_2 fixed between fruit set and harvest, the C sequestered in fruit (RDI_S) would have accounted for ~23% of total NCE_C. Under the same average yield scenario, but scaling from NCE_C on RDI_L vines, fruit would have accounted for ~26% of total NCE_C. By contrast, in RDI_E vines, fruit C would have accounted for ~37% of NCE_C.

At harvest (DOY 262, both years) there were more shoots and thus more clusters per vine in 2003, but on average, clusters were lighter in 2003 (Table 3). Pooled across years, yield variation was explained mainly by average cluster mass ($r^2 = 0.56$; $P \leq 0.001$) followed by the number of clusters per vine ($r^2 = 0.21$; $P \leq 0.001$). Average berry mass did not differ
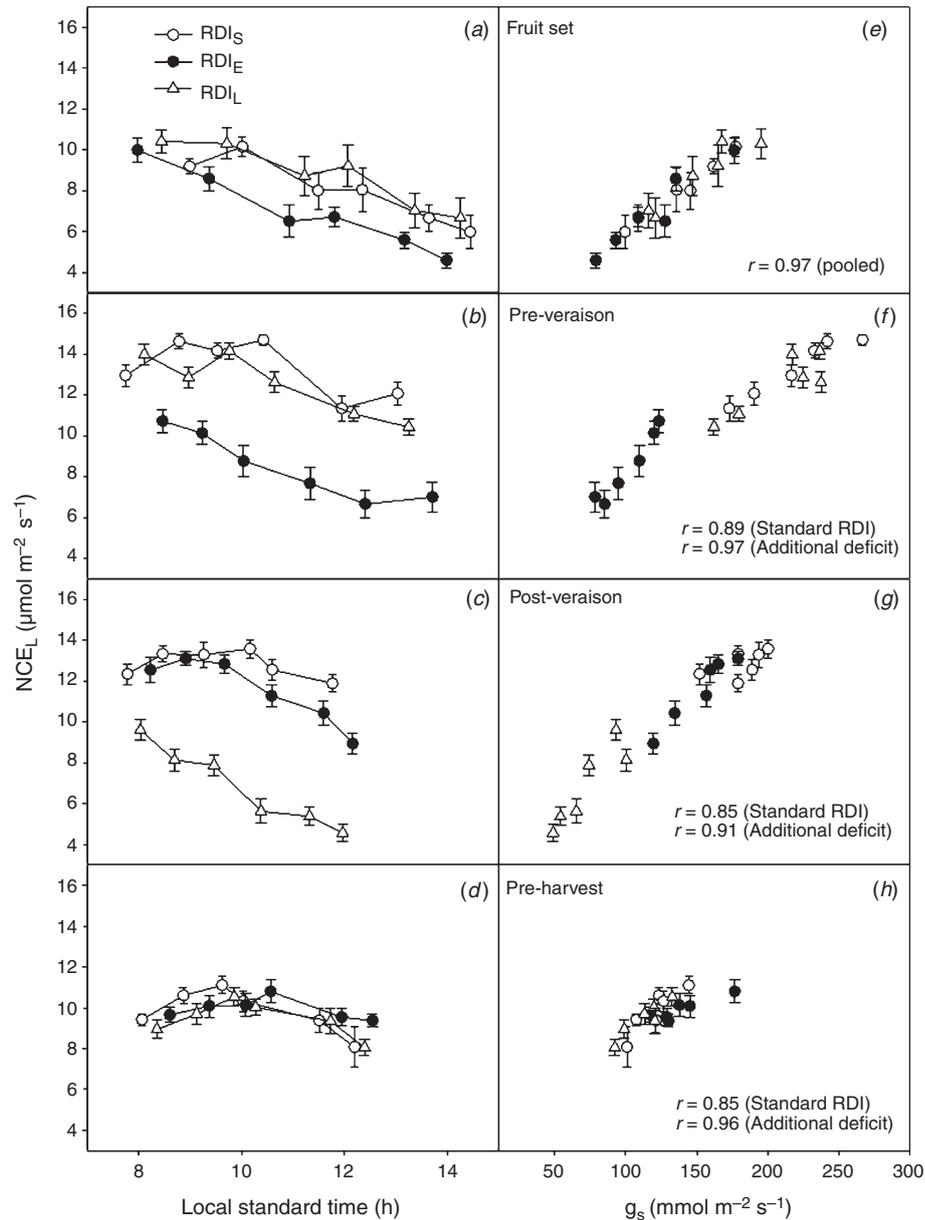
Fig. 6. Instantaneous rates of *Vitis vinifera* net CO_2 exchange at the single-leaf level (NCE_L; *a–d*) as a function of time of day, and stomatal conductance (g_s; *e–h*) in 2003, for vines under three regimens of regulated deficit irrigation (RDI). At each developmental stage, correlation coefficients between NCEL and g_s were computed separately for vines under standard RDI (RDI_S) and those under the additional water deficit (RDI_E (early additional deficit) at pre-veraison; RDI_L (late additional deficit) at post-veraison and pre harvest). Symbols represent mean of measurements on three vines; error bars are ±s.e.

among RDI regimens in either year. The RDI_L vines produced the highest yield in both years due to more shoots, thus more fruit clusters per vine. Berry soluble solids was the only fruit quality attribute that differed between years, reflecting higher T_a during ripening in 2003 than in 2002 (Table 3). Among RDI regimens, there were no differences in berry soluble solids, colour density, or colour hue. The magnitudes of the observed differences in T_a and pH among RDI regimens were unlikely to have influenced fermentation practices (J Lee, pers. comm.).

Discussion

Canopies of grapevines that were managed under more restrictive irrigation than the industry's current RDI standard approach fixed less CO_2 during the period in which the respective additional deficit was imposed. Weather and the timing of individual irrigation applications (up to five per week) influenced the day-to-day dynamics of NCE_C in all RDI regimens. The influence of the additional water deficit was most evident at the end of a weekly irrigation cycle, when

instantaneous rates of NCE_C declined markedly from a mid-morning daily maximum, and without recovery later in the day. Similar patterns in NCE have been observed elsewhere in drying soils (Medrano *et al.* 2003; Poni *et al.* 2009). The water deficits in our study were characterised by rapid drying and re-wetting of the soil, facilitated by its high infiltration rate and low water holding capacity. There is evidence that all vines, whether irrigated under RDI_S or the additional deficit, responded to water application within 1 day indicated by higher daily cumulative NCE_C and a diurnal course of NCE_C that more closely reflected the diurnal course of irradiance.

The sensitivity of NCE_C to frequent water application and soil water depletion suggests that this vineyard was managed under water stress of 'moderate' or 'transitional' severity, described elsewhereas 'Stage 2' water deficit and characterised by daily maximum g_s between 50 and 150 mmol H_2O m^{-2} s^{-1} (Flexas *et al.* 2002*a*; Lovisolo *et al.* 2010). A *Vitis* hybrid that was subjected to moderate water deficit recovered overnight after re-watering, but vines subjected to severe water deficit ('Stage 3'; daily maximum g_s <50 mmol H_2O m^{-2} s^{-1}) recovered slowly during the week after re-watering and did not attain the same maximum instantaneous rates of NCE as before the deficit had been imposed (Flexas *et al.* 2009). Vines in our study recovered quickly after rewatering; thus, there did not appear to have been a detrimental impact on the photosynthetic apparatus. Our measurements of NCE_L support the interpretation that the vineyard was managed under moderate water stress: mid-day values of g_s in sunlit leaves approached 50 mmol H_2O m^{-2} s^{-1} but did not fall below this ostensible threshold. Some discussion in the literature about the responses of water-stressed vines to re-watering is driven by inconsistencies among publications; the variation in results often can be attributed to variation in experimental conditions, cultivar, environment, and their interactions with the severity of water deficit (see review by Lovisolo *et al.* 2010).

After 5 weeks of a 50% reduction in irrigation from existing levels of deficit, the rapid recovery in RDI_E vines to values of NCE_C similar to those in RDI_S vines also indicates that the additional deficit caused only moderate water stress and that there was not persistent damage to the photosynthetic apparatus. Only under severe water deficits are non-stomatal limitations on NCE thought to be consequential, particularly when the deficit coincides with intense solar radiation and high temperature (Flexas *et al.* 2002*b*). More marked declines in g_s and NCE than in electron transport rates (Medrano *et al.* 2003) support this hypothesis. We observed close associations between NCE_L and g_s in all RDI regimens. Overall, our data from mature field-grown vines support the body of evidence in the literature that points to stomatal limitation as the dominant driver of lower rates of NCE in grapevines under moderate water deficit.

Rates of carbon fixation may be source limited under water deficit. It seems reasonable to infer that the vines under early additional deficit in this study directed less carbohydrate to roots and permanent structures, and may have ended the season with less root biomass and carbohydrate reserves than occurred in those vines managed under RDI_S or RDI_L (Schreiner *et al.* 2007). Our observations of lower pruning mass in RDI_E vines in the absence of lower starch or SS concentrations indicates a lower total carbohydrate content in permanent structures. There have been mixed observations on the movement of labelled carbon from leaves to trunks, which could be reduced under severe water stress, although perhaps not under moderate water stress (Bota *et al.* 2004). Roots reportedly are a low priority sink for carbon during fruit ripening (Candolfi-Vasconcelos *et al.* 1994), but measurements of fine root length density in our companion study (Schreiner *et al.* 2007) appear to contradict that notion because regardless of RDI regimen, fine root length density increased over time with a maximum value at harvest. Nonetheless, there was less total production of fine roots in RDI_E vines because the timing of the additional deficit resulted in the greatest cumulative reduction in NCE_C and because the additional deficit was imposed when roots were growing most rapidly.

We found that NCE_L generally overestimated $NCE_{C,LA}$, supporting others' conclusions that leaf-level measurements can be misleading if extrapolated to the whole canopy (Edson *et al.* 1993, 1995; Intrieri *et al.* 1997; Poni *et al.* 2003, 2009). For example, an increase in intrinsic water use efficiency (i.e. NCE/g_s) computed from leaf-level data was not conserved in canopy-level data (Poni *et al.* 2009), leading those authors to suggest caution in scaling-up, especially for estimating potential water conservation or carbon sequestration under various RDI approaches. At high values of NCE_L, one might expect to record lower concurrent values $NCE_{C,LA}$ because a portion of the canopy comprises shaded leaves (see discussion in Petrie *et al.* 2009), very young leaves or senescing leaves, and because of contributions to canopy respiration from non-photosynthetic organs. To estimate NCE_C accurately from single-leaf measurements, they must be temporally and spatially extensive, which imposes logistical limitations on time and equipment. Alternative approaches to scaling-up NCE_L may involve modelling radiation interception by the canopy (Petrie *et al.* 2009) and estimating the varying responses to drought of light-saturated and non-saturated photosynthesis (Escalona *et al.* 2003). However, the relative differences among treatments that we detected in NCE_C were reflected in NCE_L data. Leaf-level techniques are valuable in this context, as whole-canopy systems are more complex and expensive to maintain and operate, and are not suited to investigation of the physiology of the photosynthetic apparatus itself.

The vineyard in our study produced yields (7.8–11 T ha^{-1}) that were lower than average for Cabernet Sauvignon in the district (~12–14 T ha^{-1}). Rather than source limitation *per se*, the vines may have responded also to sink limitation in all RDI regimens, as there was consistently high LA_v : fruit mass (>15 cm^2 g^{-1}) or conversely, low fruit : pruning (i.e. crop load; ~3.3–6.5). Source–sink dynamics admittedly are complex (Minchin and Thorpe 1996; Génard *et al.* 2008) and their description is beyond the scope of our present measurements. Soluble solids accumulation in grape berries may be impeded only when LA_v : fruit mass falls below ~6 cm^2 g^{-1} (Intrieri *et al.* 1997). Under low crop load, the source limitation associated with mild water stress may not adversely affect yield (Poni *et al.* 1993, 2009) and fortuitously may improve fruit composition in red wine cultivars that are reputedly sensitive to water stress (e.g. 'Tempranillo'; Intrigliolo and Castel 2008).

We observed no consistent effect of the additional water deficit on berry mass or composition. Other evidence suggests that water deficit affects both berry growth and metabolism (Roby *et al.* 2004; Castellarin *et al.* 2007), depending in part upon the timing of the deficit (i.e. pre- or post-veraison). Our understanding is far from complete. In other Cabernet Sauvignon berries, both pre- and post-veraison water deficits resulted in higher concentrations of anthocyanins at maturity, particularly tri-hydroxylated moieties (Castellarin *et al.* 2007). Those authors reported little effect of deficit timing on concentrations of proanthocyanidins and flavonols, or on related gene expression products. Our observations indicate that the additional water deficit represented by RDI_E and RDI_L induced measureable and significant reductions in NCE_C, but that the water stress imposed probably did not exceed what would be categorised in grapevines as moderate. Thus there is substantial opportunity to conserve water by further reducing irrigation in commercial vineyards that are carrying lower crop loads and that do not appear to be affected detrimentally (i.e. crop development and ripening) by the limited water supply.

Conclusion

When mature Cabernet Sauvignon grapevines were given only ~50% of the water that was supplied to vines irrigated under the industry standard RDI approach, instantaneous and daily cumulative rates of NCE_C were inhibited. Compared with vines under RDI_S, the early additional deficit resulted in 35% lower cumulative NCE_C and the late additional deficit 12% lower cumulative NCE_C between fruit set and harvest. Canopy size did not account for these treatment effects. Both the daily maximum instantaneous rates of NCE_C and daily cumulative NCE_C responded rapidly (~1 day) to high frequency dry-down/re-wetting cycles. Relative differences among RDI regimens were detected in both whole-canopy and single-leaf measurements. We did not observe any consistent effect of the additional water deficits on yield, berry mass, or berry composition, the latter two having been the original intended outcomes of the vineyard owner. Low crop load contributed to the apparent lack of effect on yield. A secondary outcome of the additional deficits was water savings: from fruit set to harvest, nearly 40% less water was applied to vines managed under RDI_E and ~20% less to vines managed under RDI_L than to those under the industry standard RDI practice. By managing vineyards with more restrictive RDI approaches than current practices, further water conservation may be possible without negative effects on yield and fruit composition, a key consideration in arid grape-growing regions.

Acknowledgement

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