

Deficit Irrigation Alters Grapevine Growth, Physiology, and Fruit Microclimate

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Abstract: A deficit irrigation trial was conducted with field-grown Cabernet Sauvignon grapevines in the Columbia Valley of southeastern Washington. Four irrigation regimes were applied in four replicated blocks to replace various fractions of crop evapotranspiration (ET_c) between fruit set and harvest. These treatments were designated ET_{100} (100% ET_c), ET_{70} (70% ET_c), ET_{25} (25% ET_c), and $ET_{25/100}$ (25% ET_c before veraison and 100% ET_c thereafter). Leaf water status and gas exchange, canopy growth and microclimate, and yield formation were evaluated over three years. Despite yearly variation in growing season temperatures, irrigation treatment effects were consistent among years. Overall, deficit irrigation did not enhance water-use efficiency. The ET_{100} and ET_{70} regimes rarely differed in vine physiology and performance. The ET_{25} regime, however, strongly limited gas exchange and led to a decline in vine capacity and productivity, suggesting that this degree of water deficit was economically unsustainable. In addition, this treatment was associated with small berries on small clusters, very high fruit-zone sunlight exposure, and elevated cluster temperature. The $ET_{25/100}$ regime was generally intermediate in vine physiology, growth, and yield components. This treatment resulted in open canopies and small berries without the penalty in vine capacity and yield that was incurred with ET_{25} . Potential effects of water deficit on fruit composition may be related to altered canopy size and microclimate, in addition to decreased berry size.

Key words: canopy microclimate, gas exchange, regulated deficit irrigation, *Vitis vinifera*, water potential, yield components

The majority of the world's grape production regions are located in seasonally dry climates with varying degrees of summer drought. In regions where summer rainfall does not compensate for water loss through evapotranspiration, vineyards experience increasingly severe water deficit as the growing season progresses. Grape production in such regions requires irrigation. This puts growers at risk from water shortages but also permits them to adjust water supply to control shoot growth, manipulate fruit composition, and conserve irrigation water (Chaves et al. 2010, Keller 2010). Regulated

deficit irrigation (RDI) is a common dry climate irrigation management strategy with the production goal of fine-tuning canopy development and improving fruit quality attributes depending on wine style (Matthews and Anderson 1988, Dry et al. 2001, Keller 2005, Acevedo-Opazo et al. 2010, Romero et al. 2013). Under RDI, less water is applied than a vineyard loses to evapotranspiration during a portion of the growing season. Deficit irrigation may result in red wine with more fruit and less vegetal aromas, more anthocyanin pigments, and sometimes lower astringency (Matthews et al. 1990, Chapman et al. 2005, Castellarin et al. 2007a). Much of the impact of water deficit on fruit composition may be mediated by reduced vigor, which can increase light interception in the fruit zone (Castellarin et al. 2007b, Chaves et al. 2007).

In response to reduced amounts of available water, grapevines adjust their growth to promote water uptake and minimize water loss. The nature and degree of adjustments depend on the timing, duration, and severity of the water deficit. Long-term responses to water shortage include reduced canopy size, increased root-to-shoot ratio, improved water-use efficiency, and altered fruit composition (Chaves et al. 2010). Prolonged and severe water deficit may reduce vigor, yield, and wine quality, and may have cumulative effects on growth and yield formation in subsequent years (Matthews and Anderson 1989, Romero et al. 2010, Dayer et al. 2013). Nevertheless, earlier work suggests that relatively severe deficit irrigation not only saves considerable amounts of water, but also may have limited additional effects on vine performance compared with moderate water deficit (Keller et al. 2008, Edwards and Clingeleffer 2013). This raises two important questions. First, how severe is too severe? And

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second, can we save even more water and still improve grape quality without sacrificing long-term vine productivity?

Another issue related to irrigation management is the fear that increased water supply during grape ripening might increase berry size and compromise wine quality. This fear may be due in part to the observation that in non-irrigated regions, dry years tend to be associated with good vintage quality (e.g., Van Leeuwen et al. 2009). As a consequence, in irrigated regions the practice of reducing or stopping water supply at veraison remains relatively commonplace. The Guidelines for Integrated Production of Grapes from the International Organization for Biological and Integrated Control declares that “irrigation of vines for wine production must not be applied after veraison (BBCH-Scale 81-85) or highly restricted as specified by the regional guidelines in order to guarantee the good quality of the wine” (Malavolta and Boller 2009). Such recommendations contrast with warnings to avoid inappropriate water stress during ripening beyond what is needed to control shoot growth (Dry et al. 2001), and with physiological studies suggesting that late-season irrigation may merely alleviate drought-induced berry shrinkage rather than increasing berry size (Keller et al. 2006, 2015, Castellarin et al. 2007a). However, little research on increased water supply during ripening has been conducted in the field (Coombe and Monk 1979, Matthews and Anderson 1989, Mendez-Costabel et al. 2014). The evidence in favor of a “berry dilution” effect of late-season water supply seems to come from production regions where high water supply is associated with rainfall rather than irrigation. During rainfall or overhead sprinkler irrigation, ripening grape berries may absorb water through their skin (Becker and Knoche 2011). It is unknown, however, whether the berries also import excess water that has been taken up by the roots following drip or flood irrigation. Further, it remains unclear whether excess water close to harvest may lead to an increase in berry size and whether this may alter wine composition.

The objective of the present study was to answer some of these questions. A field trial was conducted in southeastern Washington, a continental climate characterized by warm, very dry summers associated with average annual precipitation of ~200 mm due to the rain-shadow effect of the Cascade Range and sporadic cold winters due to the occasional influx of Arctic air masses (<http://weather.wsu.edu>). The trial was designed to vary the timing and extent of water deficit between fruit set and harvest from none to severe. The trial was conducted in a vineyard that had already experienced different RDI regimes during the three years leading up to this study (Casassa et al. 2015). The overall goal of the present trial was to determine the effects on fruit and wine composition of more widely contrasting irrigation regimes than were previously applied (Casassa et al. 2013). Here, we report on the effects of these regimes on vine performance over three years. Measurements of growth and yield formation were supplemented with physiological measurements of plant water status and gas exchange and a characterization of canopy microclimate. Effects on fruit and wine composition will be reported separately (Harbertson et al. in preparation).

Materials and Methods

Vineyard site and experimental design. The study was conducted in 2011, 2012, and 2013 on own-rooted *Vitis vinifera* L. cv. Cabernet Sauvignon clone FPS 08 in the Cold Creek vineyard (lat. 46.579° N; long. 119.805° W; 310 m asl) of Ste. Michelle Wine Estates. The vineyard is located in the Columbia Valley American Viticultural Area of southeastern Washington and was planted in 1981 at a vine spacing of 2.1 m within rows and 3.0 m between rows, oriented north to south, on a <5% south-facing slope. Vines were trained to bilateral cordons at 1.1 m, spur-pruned in winter to 67 nodes. Shoots were loosely positioned between two foliage wires located 0.3 m above the cordon. All fertilizers, herbicides, and other soil and pest management practices were applied using commercial standards and as uniformly across the vineyard as possible. The soil is a deep (≥90 cm) Warden silt loam with a field capacity of ~23% (v/v) and permanent wilting point of ~8%, as estimated by company staff using the neutron scattering method. The vineyard was drip-irrigated using pressure-compensated emitters (flow rate 4 L/hr) spaced 1.1 m apart. Precipitation during winter was usually insufficient (long-term average 63 mm from November through April; Table 1) to replenish soil water content; thus, the root zone was irrigated to near field capacity prior to budbreak. Irrigation was interrupted before bloom to dry the soil sufficiently to control shoot growth (Keller et al. 2008).

When shoot growth stopped, which typically occurred soon after fruit set, four irrigation regimes were implemented that were intended to replace different portions of full-vine or crop evapotranspiration ($ET_c = ET_0 \times K_c$). The reference crop (grass) evapotranspiration (ET_0) was calculated using the Penman-Monteith equation (Allen et al. 1998) using data collected by an on-site weather station (Campbell Scientific, Logan, UT) located <400 m from the trial block. A variable crop coefficient (K_c , varying from 0.3 at the onset of treatments to 0.85 prior to veraison to 0.4 by harvest) developed for fully irrigated Cabernet Sauvignon in southeastern Washington (Evans et al. 1993) was used to calculate ET_c . The current industry standard was used as a control to replace 70%

Table 1 Summary of weather conditions in the Cold Creek vineyard in southeastern Washington. Data were collected by an on-site weather station installed in late 1994 and located <400 m from the experimental vineyard block.

Year	GDD (°C) ^b	Seasonal temperatures (d) ^a				Precipitation (mm)	
		>30°C PV/RP	>35°C PV/RP	<15°C S/F	<10°C S/F	Annual	Seasonal ^c
2011	1644	32/16	2/2	20/9	1/1	91	54
2012	1836	50/4	15/0	7/9	1/4	102	45
2013	1960	55/19	16/1	10/6	0/0	47	37
1995 to 2013	1803	53	13	10/9	0/2	120	57

^aNumber of days from 1 April to 31 Oct with maximum temperatures above or below four threshold temperatures (PV: preveraison; RP: ripening; S: spring; F: fall).

^bCumulative growing degree days (>10°C) from 1 April to 31 Oct.

^cCumulative rainfall from 1 April to 31 Oct.

ET_c from fruit set to harvest (ET_{70}). The three other irrigation regimes were calculated to replace 100% ET_c (ET_{100}) or 25% ET_c (ET_{25}) from fruit set through harvest, or 25% ET_c from fruit set to veraison followed by 100% ET_c through harvest ($ET_{25/100}$). The experiment was designed as a randomized complete block with four replicated blocks, each comprising 30 to 40 rows whose vine number decreased from 65 to 40 vines from east to west. Each irrigation regime was randomly assigned to six to 10 rows within each block; the number of rows increased as the row length shortened to provide similar amounts of fruit for winemaking (Casassa et al. 2013). A water management zone 0.9 m deep by 1.1 m wide down the vine row was used to calculate the required volume of irrigation water to be applied each week. Water was applied in 4-hr to 8-hr sets over one to four days, depending on the total amount of water required per treatment. The root zone was refilled with irrigation water after harvest to minimize freeze-induced root injury during winter (Keller et al. 2008).

Weather data and plant physiology. Daily weather data were obtained from the on-site weather station. Growing degree days (GDD) for 1 April through 31 Oct were estimated from daily maximum and minimum temperatures, applying a base temperature of 10°C. The number of days between 1 April and 31 Oct with maximum temperatures above three threshold temperatures (hot: $T_{max} > 30^\circ\text{C}$; very hot: $T_{max} > 35^\circ\text{C}$; and extremely hot: $T_{max} > 40^\circ\text{C}$) were counted separately for preveraison and ripening and days with T_{max} below two threshold temperatures (cool: $T_{max} < 15^\circ\text{C}$ and cold: $T_{max} < 10^\circ\text{C}$) were counted for spring (before fruit set) and fall (after veraison). Gas exchange and stomatal conductance (g_s) were measured on a fully expanded, sun-exposed leaf on two vines per treatment replicate using a CIRAS-2 portable system (PP Systems) with a PLC6 universal leaf cuvette, air flow rate of 200 mL/min, and reference CO_2 concentration set at 380 $\mu\text{mol/mol}$. Measurements were typically taken the day before the weekly irrigation began, two to three times before and two to three times after veraison between 1000 and 1200 hr local standard time under clear skies and photosynthetic photon flux (PPF) $>1000 \mu\text{mol/m}^2\text{s}$. Preliminary diurnal measurements indicated that gas exchange values reached a plateau that lasted for about the two hour duration of our measurements. Immediately after each measurement, the leaf was enclosed in an aluminum-coated plastic bag and ≥ 1 hr later, the midday stem water potential (Ψ_{md}) was determined using a pressure chamber (PMS Instrument Company). All physiological measurements were taken on the leaf above the upper cluster on a two-cluster shoot of each vine.

Canopy density and microclimate. Canopy density was assessed on two vines per treatment replicate at veraison and before harvest by the point-quadrat method to estimate the leaf layer number (LLN) and the proportion of sun-exposed clusters (Smart and Robinson 1991). A copper rod (1 m \times 3 mm) was inserted horizontally across the canopy in the fruit zone ~ 10 cm above the cordon and ~ 50 cm on either side of the trunk. Contacts with leaves and clusters and canopy gaps were recorded. Due to the sprawling canopy architecture, the amount of light in the fruit zone, expressed as PPF relative to

ambient PPF, was estimated using an AccuPAR LP-80 ceptometer (Decagon Devices) inserted like the point quadrat within 30 min of solar noon at ambient PPF $>1000 \mu\text{mol/m}^2\text{s}$. Both canopy density and light were estimated as the average of two positions per vine. Thermochron DS1922L-F5 iButton temperature loggers (diam. 16 mm, width 6 mm; Maxim Integrated) were used to simulate cluster temperature between fruit set and harvest in ET_{25} and ET_{100} . Two iButtons were wrapped in a layer of Parafilm and embedded in two clusters per vine on one vine per replicate; one logger per cluster facing the exterior of the canopy, the other facing the interior. To standardize comparisons, the cumulative number of hours during which the loggers recorded temperatures above or below four threshold temperatures ($>40^\circ\text{C}$, $>35^\circ\text{C}$, $<20^\circ\text{C}$, $<10^\circ\text{C}$) were calculated for the first three weeks of fruit ripening.

Plant growth and yield components. Growth and yield measurements were taken on two representative shoots each on two vines per treatment replicate. Measurements taken at fruit set, veraison, and harvest included primary shoot length, leaf area, number of nodes, number of internodes with brown periderm, number of lateral shoots, and number of lateral leaves. The area of all primary leaves for each shoot was estimated from a regression of midvein length against leaf area ($r = 0.96$, $p < 0.001$, $n = 158$), which was determined on leaves from adjacent vines using a LI-3100C area meter (Li-Cor Biosciences). Lateral leaf area was estimated from a regression of leaf number against leaf area ($r = 0.94$, $p < 0.001$, $n = 80$). Vines were hand-harvested near a target soluble solids level of 25 to 26 Brix, as determined by routine maturity sampling by company staff, and the clusters were counted and weighed. Berry weight was determined from a 30-berry sample that was also used for analysis of fruit composition (Casassa et al. 2013). Pruning weight, number of canes (separated into canes with ≤ 5 nodes and canes with >5 nodes), average cane weight, and number of retained nodes were determined during winter pruning.

Data analysis. Data were analyzed using Statistica 64 (version 12; StatSoft). Measurements taken on a single date in each year were analyzed using analysis of variance (ANOVA). Two-way ANOVA, applying a repeated-measures design, was also used to test irrigation treatment effects over time within each year. Because significant time \times treatment interactions were common, those data were also analyzed using one-way ANOVA within dates. Duncan's new multiple range test was used for post-hoc means comparisons when irrigation treatment or year effects were significant. Relationships between key response variables were tested using Pearson product moment correlation analysis.

Results

The field trial spanned three disparate growing seasons that ranged from cool (2011) to average (2012) to warm (2013; Table 1). Cool days ($T_{max} < 15^\circ\text{C}$) during the growing season were more common in spring than in fall, and were especially frequent in 2011. However, with the single exception of 22 May 2013, the cool days were confined to April and the postharvest period in October. Cold days ($T_{max} < 10^\circ\text{C}$) were

very uncommon during any of the three growing seasons. Hot days ($T_{\max} > 30^{\circ}\text{C}$) and, especially, very hot days ($T_{\max} > 35^{\circ}\text{C}$) were much more frequent before than after veraison (Table 1). Nevertheless, in 2013, vines experienced almost three weeks of hot days during fruit ripening. This was also the only year with an extremely hot day: $T_{\max} = 40.3^{\circ}\text{C}$ on 2 July 2013, 48 days before veraison. There was virtually no rainfall during the growing season. Despite the differences in temperature and thus GDD and ET_0 , the total seasonal irrigation water supply varied relatively little from year to year (Figure 1). Irrigation water supply was highest in ET_{100} , varying from 402 (2013) to 413 mm (2012). Under the ET_{25} regime, the vines received between 145 (2011) and 201 mm

(2013) irrigation water per year. Seasonal water supply varied from 265 (2012) to 313 mm (2013) in $ET_{25/100}$, and from 315 (2011) to 326 mm (2013) in ET_{70} . Thus, on average, deficit irrigation reduced the total water supply by 22% (ET_{70}), 31% ($ET_{25/100}$), or 56% (ET_{25}) ($p < 0.001$).

The repeated measurements of Ψ_{md} and leaf gas exchange were averaged over pre- and postveraison (Table 2). Before veraison, Ψ_{md} generally decreased as the fraction of replaced ET_c decreased. However, except in the first year, the Ψ_{md} in the $ET_{25/100}$ regime was similar to that of the ET_{70} regime. After veraison, ET_{100} continued to be associated with the highest Ψ_{md} and ET_{25} with the lowest. The increased water supply in $ET_{25/100}$, however, led to full recovery in Ψ_{md} only in 2012. Stomatal conductance (g_s) usually decreased with decreasing ET_c replacement and Ψ_{md} . While g_s sometimes became extremely low in ET_{25} , g_s was consistently higher in ET_{70} and ET_{100} but rarely differed between the two latter treatments. In agreement with the Ψ_{md} data, postveraison recovery of g_s in the $ET_{25/100}$ regime was often incomplete. There was a strong positive correlation across treatments and years between the net photosynthesis rate (P_n) and g_s (Supplemental Figure 1). The relationship between g_s and P_n was essentially linear up to $g_s = 300 \text{ mmol H}_2\text{O/m}^2\text{s}$, with the values for ET_{25} concentrated near the lower end. Consequently, P_n was usually lowest in ET_{25} but rarely differed between ET_{70} and ET_{100} (Table 2). In general, the decreased transpiration rate (E) due to lower irrigation water supply was similar to the decrease in P_n . Since the changes in P_n were approximately proportional to the changes in E ($r = 0.70$, $p < 0.001$, $n = 192$), there was no gain in instantaneous water-use efficiency ($\text{WUE}_{\text{inst}} = P_n/E$) at lower irrigation rates (data not shown). Nonetheless, the intrinsic water use efficiency ($\text{WUE}_i = P_n/g_s$) was usually higher in ET_{25} than in the other irrigation regimes (Table 2). Overall, WUE_{inst} and WUE_i were poorly correlated ($r = 0.24$, $p < 0.001$). Because lower seasonal irrigation water supply was associated with lower yield ($r = 0.62$, $p = 0.03$, $n = 12$), there were no differences among irrigation regimes in terms of irrigation WUE (yield per unit irrigation water applied; mean \pm SE: $2.7 \pm 0.2 \text{ t/ML}$), irrigation water footprint (irrigation water applied per unit yield; $411 \pm 43 \text{ m}^3/\text{t}$), or total water footprint (rainfall plus irrigation water per unit yield; $530 \pm 59 \text{ m}^3/\text{t}$).

Most shoot growth occurred prior to fruit set. The primary shoots grew on average only 4 cm from fruit set to veraison and there was no primary shoot growth after veraison, irrespective of RDI treatment. Thus, shoot length at harvest was a tight linear function of shoot length at fruit set (Figure 2). Moreover, greater shoot vigor was associated with longer internodes, more main leaves, and more lateral leaves, which led to greater leaf area per shoot (Supplemental Figure 2). Shoots grew more vigorously during the cool 2011 growing season than in 2012 or 2013 (Table 3). Irrigation effects on vine size, vigor, and canopy density were consistent from year to year: there were no significant treatment \times year interactions (Tables 3 and 4). Vine vigor was similar in ET_{100} and ET_{70} and greater than in ET_{25} and $ET_{25/100}$. The ET_{25} regime, in particular, resulted in very weak vines as indicated by low

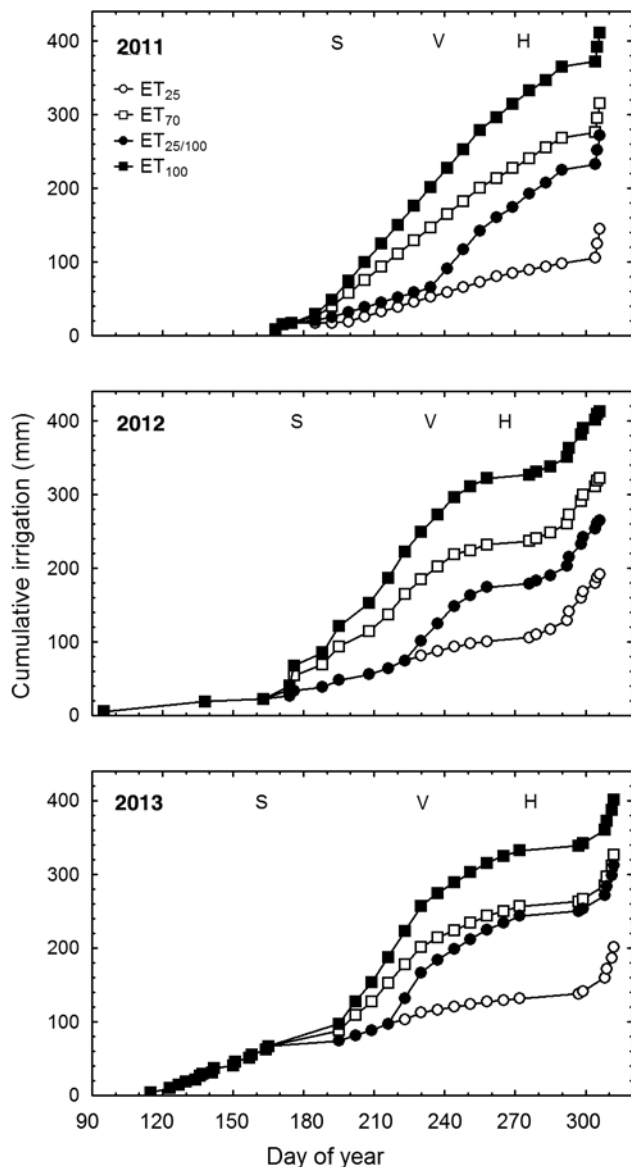


Figure 1 Cumulative amounts of irrigation water applied over three years to field-grown Cabernet Sauvignon irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set (S) to veraison (V) and harvest (H), and at identical rates before fruit set and after harvest. $ET_{25/100}$ denotes 25% ET_c before veraison and 100% ET_c thereafter.

pruning and cane weights, a high proportion of canes with ≤ 5 nodes, and short shoots with few laterals and low leaf area (Table 3). Although the primary shoots stopped growing before veraison, lateral shoots continued to grow through harvest, but only in ET₁₀₀ and ET₇₀ ($p < 0.001$). In addition, there were fewer leaf layers across the canopy and more light in the fruit zone in ET₂₅ than in the other RDI treatments (Table 4). Cluster sun-exposure was very high across RDI regimes (average fruit-zone PPF = $282 \pm 18 \mu\text{mol}/\text{m}^2\text{s}$ or $28 \pm 2\%$ of ambient light), and was only slightly reduced by ET₁₀₀. Each year, there were negative correlations between pruning and cane weight and both the absolute amount and proportion of light intercepted by the fruit zone ($r \leq -0.40$, $p < 0.05$, $n = 32$). Confirming this trend, the greater pruning weight in 2013 (Table 3) was also associated with lower fruit-zone PPF (Table 4). Although all RDI regimes received the same

amount of water before fruit set and abundant water after harvest (Figure 1), treatment carryover effects on shoot vigor in the following year were apparent in the shorter internodes and fewer main and lateral leaf numbers at fruit set in ET₂₅ (Figures 2 and 3; see also Supplemental Figure 2). Greater shoot vigor due to increased water supply did not compromise shoot periderm formation (Table 3): across years and treatments, there was a positive correlation between the number of nodes per shoot and the number of brown internodes at harvest ($r = 0.72$, $p < 0.001$, $n = 96$).

During the daytime, simulated cluster temperatures, especially on the sun-exposed side, were often 10 to 15°C warmer than the ambient temperature, but at night, cluster temperatures were often 1 to 3°C below ambient (Figure 4). Nevertheless, cluster temperatures below 10°C were extremely rare (<2 hr) in any year. During the first three weeks of ripening

Table 2 Midday stem water potential (Ψ_{md}) and leaf gas exchange averages pre- and postveraison of field-grown Cabernet Sauvignon grapevines irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set to harvest over three years; ET_{25/100} refers to 25% ET_c before veraison and 100% ET_c thereafter.

	Preveraison			Postveraison		
	2011	2012	2013	2011	2012	2013
Ψ_{md} (MPa)						
ET ₂₅	-1.13 c ^a	-1.22 c	-1.35 b	-1.20 c	-1.48 c	-1.41 d
ET _{25/100}	-1.09 c	-1.08 b	-1.27 ab	-0.87 b	-0.97 a	-0.79 b
ET ₇₀	-0.88 b	-1.05 b	-1.17 a	-0.87 b	-1.28 b	-0.89 c
ET ₁₀₀	-0.73 a	-0.87 a	-1.15 a	-0.70 a	-1.07 a	-0.67 a
SE ^b	0.02	0.04	0.05	0.04	0.08	0.04
<i>p</i>	<0.001	<0.001	0.013	<0.001	<0.001	<0.001
Stomatal conductance (mmol H₂O/m²s)						
ET ₂₅	80 c	57 c	72 b	91 c	91 c	38 c
ET _{25/100}	103 b	96 b	80 ab	138 b	177 b	88 b
ET ₇₀	168 a	143 a	88 a	143 ab	229 ab	127 a
ET ₁₀₀	160 a	171 a	91 a	167 a	290 a	127 a
SE	8	17	6	10	31	12
<i>p</i>	<0.001	<0.001	0.04	<0.001	<0.001	<0.001
Photosynthesis rate (μmol CO₂/m²s)						
ET ₂₅	6.2 b	6.1 c	7.2 c	9.5 b	12.2 b	4.0 c
ET _{25/100}	7.3 b	10.0 b	8.2 b	12.7 a	15.6 ab	7.4 b
ET ₇₀	10.9 a	11.7 ab	8.7 b	12.7 a	18.6 a	10.2 a
ET ₁₀₀	11.0 a	12.9 a	10.0 a	13.3 a	19.1 a	10.8 a
SE	0.5	0.8	0.4	0.6	1.6	0.7
<i>p</i>	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Transpiration rate (mmol H₂O/m²s)						
ET ₂₅	2.4 c	2.0 c	3.2 c	2.4 c	2.3 b	1.3 c
ET _{25/100}	2.9 b	3.1 b	3.5 bc	3.3 b	3.8 a	2.9 b
ET ₇₀	3.9 a	3.4 ab	3.9 ab	3.3 b	4.8 a	3.5 ab
ET ₁₀₀	4.2 a	4.1 a	4.1 a	3.9 a	4.8 a	3.8 a
SE	0.2	0.4	0.2	0.2	0.5	0.3
<i>p</i>	<0.001	<0.001	0.003	<0.001	<0.001	<0.001
Intrinsic water-use efficiency (μmol CO₂/mol H₂O)						
ET ₂₅	79 a	122 a	132 a	96 a	138 a	119 a
ET _{25/100}	74 b	122 a	127 a	77 b	96 b	86 b
ET ₇₀	67 c	104 ab	122 a	80 b	84 b	85 b
ET ₁₀₀	69 bc	92 b	120 a	72 b	70 b	87 b
SE	2	11	18	3	12	8
<i>p</i>	<0.001	0.04	0.74	<0.001	<0.001	<0.001

^aMeans followed by different letters within columns differ significantly at $p < 0.05$ by Duncan's new multiple range test.

^bLargest standard error (SE) of any irrigation treatment mean.

in 2011, outward-facing iButtons recorded 87 hr above 35°C and 42 hr above 40°C, while inward-facing iButtons recorded 63 hr above 35°C and only nine hours above 40°C ($p < 0.001$). Restricting water supply was consistently associated with warmer clusters during the day but not at night. The sun-exposed side of clusters in ET₂₅ was up to 5°C warmer during midday than in ET₁₀₀, while the temperatures of the shaded side did not differ among treatments (Figure 4). During the first three weeks of ripening in 2011, clusters on ET₂₅ vines experienced 84 hr above 35°C (32 hr above 40°C) and 107 hr below 20°C, while ET₁₀₀ clusters experienced 66 hr above 35°C (19 hr above 40°C) and 116 hr below 20°C ($p < 0.001$). Inconsistent logger performance in 2012 and 2013 did

not permit a detailed comparison with 2011. Nevertheless, the data were adequate to show that ripening ET₂₅ clusters were warmer than ET₁₀₀ clusters in both 2012 ($p = 0.045$) and 2013 ($p = 0.001$). In both years, the ET₂₅ clusters experienced about double the hours above 35°C and fewer hours below 20°C than did ET₁₀₀ clusters. Across years, there was a positive correlation between the PPF in the fruit zone and the number of hours that the simulated cluster temperature was above 35°C ($r = 0.54$, $p = 0.01$, $n = 22$), and a negative correlation between PPF and the number of hours below 20°C ($r = -0.44$, $p = 0.04$).

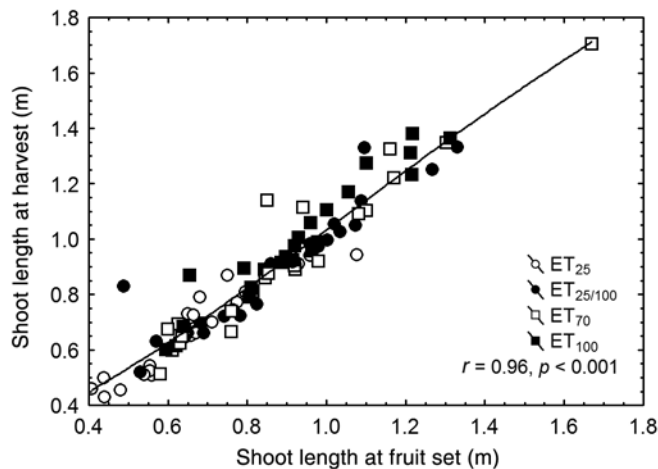


Figure 2 Correlation between the length of main shoots at fruit set and at harvest in field-grown Cabernet Sauvignon irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set to harvest. ET_{25/100} refers to 25% ET_c before veraison and 100% ET_c thereafter. Data were pooled for 2011, 2012, and 2013 ($n = 96$).

Table 4 Preharvest canopy density of field-grown Cabernet Sauvignon grapevines irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set to harvest over three years. ET_{25/100} refers to 25% ET_c before veraison and 100% ET_c thereafter.

	LLN ^a	Exposed clusters (%)	PPF ^b (% ambient)
ET ₂₅	2.0 b ^c	100 a	38 a
ET _{25/100}	2.9 a	100 a	26 b
ET ₇₀	3.0 a	92 ab	25 b
ET ₁₀₀	3.1 a	87 b	22 b
<i>p</i>	0.02	0.03	<0.001
2011	3.0	94	32 b
2012	2.8	93	39 a
2013	2.5	96	13 c
<i>p</i>	0.31	0.77	<0.001
SE ^d	0.3	6	4
I × Y ^e	0.33	0.87	0.39

^aLeaf layer number across canopy.

^bPhotosynthetic photon flux in the fruit zone.

^cMeans followed by different letters differ significantly at $p < 0.05$ by Duncan's new multiple range test.

^dLargest standard error (SE) of any irrigation treatment or year mean.

^eSignificance (p value) of irrigation treatment (I) × year (Y) interaction.

Table 3 Shoot vigor and vine size of field-grown Cabernet Sauvignon grapevines irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set to harvest over three years. ET_{25/100} refers to 25% ET_c before veraison and 100% ET_c thereafter.

	Shoot length ^a (m)	Lateral leaves (/shoot)	Shoot leaf area ^b (m ²)	Periderm ^c (/shoot)	Pruning wt (g/m)	Cane number (/m)	Cane wt ^d (g)	Weak canes ^e (%)
ET ₂₅	0.69 b ^f	10 b	0.17 c	8 c	207 c	27	10.3 c	33 a
ET _{25/100}	0.90 a	16 b	0.26 b	9 bc	365 b	28	16.8 b	25 b
ET ₇₀	0.91 a	23 a	0.35 a	11 b	429 ab	27	19.6 ab	27 ab
ET ₁₀₀	0.98 a	22 a	0.34 a	13 a	522 a	29	21.2 a	22 b
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.56	<0.001	0.004
2011	1.03 a	23 a	0.203 a	nd	335 b	25 b	13.8 b	nd
2012	0.78 b	15 b	0.114 b	10	356 b	31 a	15.9 b	30 a
2013	0.80 b	15 b	0.097 c	11	455 a	27 b	21.3 a	23 b
<i>p</i>	<0.001	0.003	<0.001	0.31	0.02	<0.001	<0.001	<0.001
SE ^g	0.06	3	0.01	1	48	2	2.0	3
I × Y ^h	0.77	0.64	0.50	0.29	0.50	0.12	0.32	0.13

^aLength of primary shoots at harvest.

^bTotal shoot leaf area (main and lateral leaves) at harvest.

^cNumber of brown internodes per shoot at harvest (not determined in 2011).

^dMean weight of canes with >5 nodes.

^eProportion of canes with ≤5 nodes (not determined in 2011).

^fMeans followed by different letters differ significantly at $p < 0.05$ by Duncan's new multiple range test.

^gLargest standard error (SE) of any irrigation treatment or year mean.

^hSignificance (p value) of irrigation treatment (I) × year (Y) interaction.

Crop yield and its components varied considerably from year to year (Table 5). The light crop in 2011 was associated with relatively small clusters (i.e., few berries per cluster) and large berries. Although cluster numbers were similar among RDI treatments, the ET₂₅ regime reduced yield by decreasing cluster weight due to smaller and fewer berries per cluster. While the differences in berry weight were significant each year, differences in berry numbers only became apparent after the first year. Berry size also decreased, albeit to a lesser extent, in ET_{25/100} compared with ET₇₀ and ET₁₀₀. Because the pruning weight was similarly impacted by the irrigation regimes, the yield:pruning-weight ratio remained constant across treatments (Table 5).

Discussion

This study demonstrated that reducing irrigation water supply from 100 to 70% ET_c between fruit set and harvest,

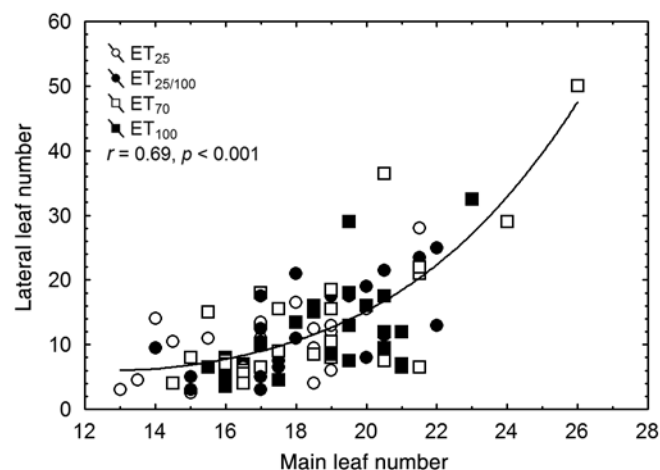


Figure 3 Correlation between the number of main leaves and the number of lateral leaves at fruit set in field-grown Cabernet Sauvignon irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set to harvest. ET_{25/100} refers to 25% ET_c before veraison and 100% ET_c thereafter. Data were pooled for 2011, 2012, and 2013 (n = 96).

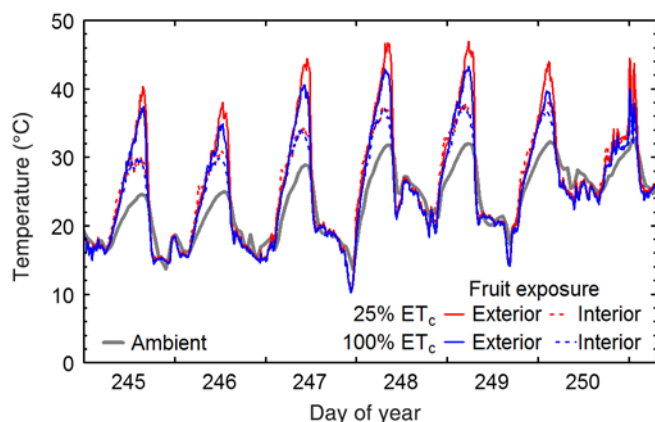


Figure 4 Ambient temperature and simulated cluster temperatures during one week after veraison of field-grown Cabernet Sauvignon irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) in 2011. Each line represents the average of eight iButtons placed on both the exterior and interior faces of different clusters and logging temperature at 10-min intervals. The ambient temperature was logged hourly by an on-site weather station.

while slightly decreasing Ψ_{md} , had virtually no impact on leaf gas exchange, canopy development, and yield formation of Cabernet Sauvignon in the dry climate of southeastern Washington. Restricting water supply to 25% ET_c, however, decreased plant water status and strongly limited gas exchange, shoot growth, and fruit development. This regime also led to leaf yellowing and some leaf abscission. It should be noted that this field trial extended an RDI trial that was conducted in the same vineyard over the preceding three years with the same experimental design, but less extreme treatment differences. In that trial, growth and yield were also limited at an irrigation level of 30 to 35% ET_c (ET₃₅) from fruit set through harvest, although not as severely (Casassa et al. 2015). Since the present ET₂₅ regime replaced the earlier ET₃₅ regime, cumulative effects of low water supply may have increasingly compromised vegetative growth and yield formation over multiple years. No early-season measurements were taken in the previous trial; consequently, we can only assume that ET₃₅ reduced early-season growth in the following year because shoot vigor in ET₂₅ was already low at fruit set in the first year of the present study. However, the decreased berry number per cluster under ET₂₅ in the second and third years of this study suggests that ET₂₅ limited inflorescence differentiation in the buds. This explanation seems more plausible than decreased fruit set under ET₂₅, because water supply was similar across irrigation regimes before fruit set and because berry numbers were high under ET_{25/100}. Carryover effects of water deficit on growth and yield formation in subsequent years were also observed in an irrigation trial with Tempranillo in semiarid southwestern Spain (Uriarte et al. 2015). Similar to a long-term field trial with Malbec conducted in the arid Mendoza region of Argentina (Dayer et al. 2013), the ET₂₅ regime in our study reduced vine capacity and yield over time. Furthermore, Cabernet Sauvignon was reported as more

Table 5 Yield, yield components, and crop load of field-grown Cabernet Sauvignon grapevines irrigated at various fractions of crop evapotranspiration (denoted by subscripts of ET_c) from fruit set to harvest over three years. ET_{25/100} refers to 25% ET_c before veraison and 100% ET_c thereafter.

	Yield (kg/m) ^a	Clusters /vine	Berries/ cluster	Berry wt (g)	Yield: pruning wt
ET ₂₅	1.42 b ^b	57	69 c	0.84 c	7.3
ET _{25/100}	2.63 a	60	89 a	0.95 b	8.2
ET ₇₀	2.69 a	58	76 bc	1.08 a	7.1
ET ₁₀₀	2.98 a	61	80 ab	1.06 a	6.9
<i>p</i>	<0.001	0.56	0.001	<0.001	0.64
2011	1.73 b	53 b	70 c	1.18 a	6.6
2012	2.63 a	66 a	79 b	0.84 c	8.6
2013	2.94 a	58 b	88 a	0.93 b	7.0
<i>p</i>	<0.001	<0.001	<0.001	<0.001	0.11
SE ^c	0.6	4	5	0.06	1.0
I × Y ^d	0.001	0.12	0.48	0.09	0.04

^aCrop yields in t/ha may be obtained by multiplying the values by 3.28.

^bMeans followed by different letters differ significantly at *p* < 0.05 by Duncan's new multiple range test.

^cLargest standard error (SE) of any irrigation treatment or year mean.

^dSignificance (*p* value) of irrigation treatment (I) × year (Y) interaction.

sensitive than Malbec under similar RDI conditions to those employed in the current study (Shellie and Bowen 2014). Although one study concluded that replacing 25% ET_c was a viable strategy for semiarid climates (Uriarte et al. 2015), all measures of vine size and vigor in our study indicated that ET_{25} lead to weak vines by established standards of canopy ideotype (Smart et al. 1990). Thus, ET_{25} is not economically sustainable in the arid climate in which the present study was conducted.

The detrimental impact on vine performance (i.e., photosynthesis, growth, and yield formation) of the ET_{25} regime contrasts with the effects that were noted when water was supplied at 25% ET_c after fruit set and then increased to 100% ET_c at veraison ($ET_{25/100}$). In this treatment, most measures of leaf physiology, canopy development, and yield formation were intermediate between the more extreme irrigation regimes. Importantly, $ET_{25/100}$ decreased berry weights compared with ET_{70} and ET_{100} , but not the other yield components. Thus, small berries were obtained in $ET_{25/100}$ without the severe penalty in yield and canopy size that was incurred with ET_{25} . One possible explanation for the observation that berries in $ET_{25/100}$ were not quite as small as in ET_{25} is that the increased water supply at veraison might have reduced preharvest berry dehydration (Keller et al. 2006). Recent research, moreover, suggests that because the switch from 25 to 100% ET_c occurred when the berries on most clusters ranged from green to blue, photosynthetic recovery following the increased water supply may have led to a rise in phloem flow to the berries, which in turn was associated with a temporary increase in berry expansion and sugar accumulation (Keller et al. 2015). Indeed, the average amount of sugar per berry at harvest, estimated from berry weight and total soluble solids, was also intermediate in $ET_{25/100}$ (242 mg) compared with ET_{25} (213 mg) and ET_{70} or ET_{100} (282 mg; $p < 0.001$). Clearly, increasing the amount of irrigation water supply at veraison did not result in “dilution” of berry composition (see also Harbertson et al., in preparation).

Although Ψ_{md} was usually not very low in our study, gas exchange measurements suggested that vines irrigated at less than 100% ET were often under moderate water stress ($g_s < 150 \text{ mmol H}_2\text{O/m}^2\text{s}$; Flexas et al. 2002, Lovisolo et al. 2010). Replacing only 25% ET sometimes led to severe water stress ($g_s < 50 \text{ mmol H}_2\text{O/m}^2\text{s}$). Despite these marked effects on leaf gas exchange, water deficit in this experiment did not increase WUE_{inst} because P_n decreased in concert with E . There also was no gain in irrigation WUE and irrigation or total water footprint, since lower water supply was associated with lower yield. Irrigating at only 25% ET_c did, however, increase the intrinsic water use efficiency (WUE_i), consistent with other studies investigating water deficit in grapevines (reviewed by Schultz and Stoll 2010). The poor correlation between WUE_i and WUE_{inst} may be due to the high dependence of the latter on evaporative demand driving E (Schultz and Stoll 2010).

The incomplete postveraison recovery of Ψ_{md} and g_s under the $ET_{25/100}$ regime may at first seem surprising. However, although soil moisture was not measured in this experiment, replacing 100% ET_c beginning at veraison should not be ex-

pected to fully replenish water in the root zone following the relatively severe water deficit established by the preveraison 25% ET_c irrigation regime. A full recovery would likely require an increase in water supply above 100% ET_c . The incomplete recovery in $ET_{25/100}$ is also the likely reason that, unlike in ET_{70} and ET_{100} , there was no lateral shoot growth after veraison. Even in the latter two RDI regimes, postveraison lateral shoot growth was minor: on average, only three new lateral leaves per shoot had emerged by harvest. However, although weed growth was not quantified in this experiment, visual inspection showed that weeds grew more abundantly in the ET_{100} regime than in any other treatment. Diversion of some of the extra irrigation water for weed growth increased the need for weed control and may also partly explain why vine growth did not differ between ET_{100} and ET_{70} .

Moderate water deficit in vineyards is generally associated with desirable changes in fruit composition compared with fruit produced under abundant water availability (Chaves et al. 2007, Keller 2005). The smaller berry size due to water deficit is often cited as the main reason for such improvements, but water deficit may also alter the biosynthesis of quality-determining compounds independently of berry size (Castellarin et al. 2007a, 2007b, Roby et al. 2004). Increased light interception by the clusters due to lower shoot vigor under water deficit may be responsible for some of these changes (Castellarin et al. 2007b, Chaves et al. 2007, Romero et al. 2013). Our results support this idea and suggest that changes induced by water deficit may be related to altered canopy size and microclimate in addition to decreased berry size. Midday peak temperatures of sun-exposed ET_{25} clusters were often 2 to 4°C higher than exposed clusters in ET_{100} . The difference in temperature was related to differences in berry size and light intensity in the fruit zone, although the generally open canopy led to only small differences in the proportion of fully sun-exposed clusters. Thus, the higher cluster temperature in ET_{25} may have been due to a combination of a smaller, somewhat more open canopy and smaller berries. The smaller clusters (i.e., fewer and smaller berries) in ET_{25} may have counteracted these effects to some degree: although small berries are heated more by sun exposure than large berries, berries that do not touch one another are heated less because they conduct less heat and lose more heat due to convection than do berries in tight clusters (Smart and Sinclair 1976).

Conclusions

This study evaluated the performance of Cabernet Sauvignon under various RDI regimes in arid southeastern Washington over three years. Despite considerable variation in growing season temperatures from year to year, the irrigation treatment effects were consistent among years. There were very few and only minor differences in vine physiology, growth, and yield formation between regimes that replaced either 100 or 70% ET_c between fruit set and harvest. Supplying only 25% ET_c during the same period, however, was economically unsustainable, as it led to a decline in vine capacity and yield. While yields were rather similar across RDI regimes in

the cool, low-crop year 2011 (5.7 t/ha), at 25% ET_c they were only 43% (4.6 t/ha) of the other regimes (10.6 t/ha) in 2012 and 2013. By contrast, limiting water supply to 25% ET_c early during the berry development period, and then increasing it to 100% ET_c at veraison, proved to be an interesting irrigation management option for Cabernet Sauvignon. This RDI regime limited vigor and berry size and conserved irrigation water, while avoiding detrimental long-term effects on vine growth and yield. Cluster temperature was monitored in the contrasting 25 and 100% ET_c regimes. The former treatment was associated with higher cluster temperatures than the latter. This difference resulted from a combination of smaller berries and greater light intensity in the fruit zone due to a smaller, somewhat more open canopy under 25% ET_c. These results suggest that potential changes in fruit composition due to water deficit may be related to altered canopy size and microclimate, in addition to decreased berry size.

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