Water Flux of *Vitis vinifera* L. cv. Shiraz Bunches throughout Development and in Relation to Late-Season Weight Loss

Dennis H. Greer[1]* and Suzy Y. Rogiers[1,2]

Abstract: *Vitis vinifera* (L.) cv. Shiraz commonly loses a significant amount of bunch water during late ripening, with attendant yield losses. The objective of the study was to quantitatively assess the hypothesis that this sustained weight loss was due to decreased vascular flow and increased transpiration. Transpiration, fresh and dry weights, and water content of whole attached grape bunches were measured from flowering to maturity on vines grown in controlled environments and in the vineyard. Seasonal changes in net water fluxes into and out of bunches were determined. A simple technique of weighing bunches in situ also provided independent measurement of daily rates of water gain and loss. Bunch transpiration rates were high just after flowering but declined to 0.2 g g (dry wt)$^{-1}$ d^{-1} at about harvest date. Bunch net water import rates also showed a 90% decrease with development from 1.0 to 0.1 g g (dry wt)$^{-1}$ d^{-1}. Comparisons of these rates revealed net water import exceeded transpiration throughout early and midbunch development. However, at 60 to 80 days after flowering, import rates had declined to an extent that transpiration now exceeded import and an overall loss of water occurred. Quantitatively comparable rates of water gain and loss determined on Shiraz bunches over four growing seasons on vineyard-grown vines conformed closely with those rates determined as above. Changes in diurnal bunch water fluxes supported the conclusion that net water import exceeded transpiration losses throughout bunch development until the late stage of ripening. The hypothesis that sustained weight loss in late-ripening Shiraz grape bunches occurred because bunch water fluxes shifted from a net import to a net loss by transpiration was confirmed.

Key words: grape berry, phenology, transpiration, water import, shrinkage

The ripening process of *Vitis vinifera* cv. Shiraz is characterized by a well-known persistent and sustained loss of weight occurring over several weeks before harvest (McCarthy 1999, Rogiers et al. 2000, 2004a, 2004b). Weight loss can, in some seasons, equal ~20% of maximum fresh weight (McCarthy 1999, Rogiers et al. 2000) and, therefore, has a major impact on final crop yield. The extent to which this weight loss can be prevented remains unknown because the physiological causes of the sustained weight loss remain uncertain.

Water rather than dry matter loss has been considered the primary cause of the decrease in fresh weight of Shiraz grapes (McCarthy and Coombe 1999, Rogiers et al. 2006a) and transpiration has been hypothesized to account for this water loss (McCarthy and Coombe 1999). The more well-known short-term diurnal fruit shrinkage that occurs in many other fruit such as apple (Jones and Higgs 1982, Lang 1990), peach (Morandi et al. 2007), tomato (Bussières 1994), and kiwifruit (Dichio et al. 2003) is also caused by atmospherically driven transpiration. However, none of these different fruit show a sustained loss of weight in the late-ripening stage of development, so it remains uncertain if short-term fruit shrinkage is physiologically similar to the more sustained shrinkage that occurs in Shiraz grapes.

Vascular flow into the fruit is also an important contribution to fruit growth, both through the xylem and the phloem. In some fruit such as peach, xylem is the predominant supply of water to fruit growth (Morandi et al. 2007), while in tomato phloem is the predominant supply route (Ho et al. 1987, Liu et al. 2007). Grapes, by contrast, vary the water supply routes with development; from predominantly xylem supply before veraison (onset of ripening) to predominantly phloem supply postveraison (McCarthy and Coombe 1999, Rogiers et al. 2000, Tyerman et al. 2004, Bondada et al. 2005). Several studies with the use of vascular tracer dyes and other approaches have implied that xylem may become nonfunctional at the time of veraison, thus the phloem becomes the predominant flux of solutes and water (Düring et al. 1987, Greenspan et al. 1994, 1996, McCarthy and Coombe 1999, Rogiers et al. 2006a, 2006b). Consistent with this, stretching of xylem tracheids in the peripheral and axial bundles has been shown to occur in grapes of maturing bunches (Findlay et al. 1987). However, it has been more

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recently suggested that decreases in hydrostatic pressure-driven xylem flow cause the decline in the xylem contribution to grape growth (Bondada et al. 2005, Keller et al. 2006). Anatomical studies of late-ripening Shiraz berries confirm that some xylem vasculature continues to function beyond veraison (Rogiers et al. 2001). Therefore, there is considerable consensus that phloem flow provides the water and solute fluxes for growth of grapes in the rapid post-veraison growth stage. The hypothesis then is that impediments in phloem fluxes in the late-ripening stage contribute to the weight loss in Shiraz grapes (McCarthy and Coombe 1999). However, the possibility that backflow of water from the bunch to the vine has also been raised (Tyerman et al. 2004, Keller et al. 2006), given that hydraulic conductance between bunches and vine remains high after veraison (Tyerman et al. 2004, Bondada et al. 2005).

Transpiration rates of fruit are generally much lower than leaf rates (Lang 1990) because of much lower skin conductances (Jones and Higgs 1982, Morandi et al. 2007). Consistent with this, stomatal and lenticel densities on grape berries are low and become occluded with wax after fruit set (Mullins et al. 1992). For example, in Sangiovese grapes at midripening, bunch transpiration rates were generally less than 5% of leaf transpiration rates (Poni et al. 2001). More specifically, for Shiraz grapes, across the growing season berry weight loss rates (determined with detached berries) declined six-fold, from 62 nmol s^{-1} before veraison to 10 nmol s^{-1} during late ripening (Rogiers et al. 2004b). This latter rate is equivalent to a berry water loss of 15 mg d^{-1} and compares well with other estimates of 5 to 8 mg d^{-1} (McCarthy and Coombe 1999) and up to 20 mg d^{-1} (McCarthy 1999). There are contrasting views about whether or not these rates are adequate to explain sustained water loss in the late-ripening stage of Shiraz berries (McCarthy and Coombe 1999, Rogiers et al. 2004b). However, most rates of transpiration have been determined with individual detached berries, and it remains unclear if these adequately account for transpiration rates of attached berries within whole bunches.

The objective of the study, therefore, was to quantitatively test the hypothesis that decreased vascular flow and continued transpiration leads to weight loss in Shiraz berries (Rogiers et al. 2004b). Our approach was to measure transpiration in situ by gas exchange and to measure water content of whole attached Shiraz bunches to quantify rates of water flux into and out of the bunch throughout development, but particularly during the ripening process. Direct and frequent measurements of diurnal changes in bunch fresh weight were also made so that water fluxes could be ascertained. We also compared rates of bunch water fluxes on vines grown in controlled environment conditions with those on vineyard-grown vines over several growing seasons.

Materials and Methods

Plant material. This study was carried using potted vines (*Vitis vinifera* L. cv. Shiraz clone PT23/N/Griffith) grown in a controlled environment chamber (TPG-6000-TH; Thermoline Scientific Equipment, Smithfield, Aust.) and field vines of the same clone growing on their own roots and drip-irrigated at the Charles Sturt University (CSU) Vineyard, Wagga Wagga, NSW, Australia (Rogiers et al. 2006a).

Controlled environment study. Four-year-old, potted Shiraz vines were initially grown outdoors in a birdproof enclosure. Vines were grown in a potting medium of river sand:loam:peat moss at 2:2:1 by volume and had four shoots trained upright with an average of 6 bunches per vine. The vines were fertilized with a complete liquid fertilizer (Megamix Plus; Rutec, Tamworth, Aust.) and watered twice daily with automatic drip irrigation. Just after budbreak, five vines were transferred to a growth chamber under an 11-hr photoperiod and an irradiance of 650 µmol m^{-2} s^{-1} at midcanopy height. Temperature was held constant at 25/15°C day/night and vapor pressure deficits at 1.0/0.6 kPa. The temperature and vapor pressure change was set to occur one hour before lights turned on and one hour after lights turned off. A screened data logger (HOBO, Onset Computer, Bourne, MA) was placed within the canopy of the vines to measure air temperatures and humidities at regular intervals throughout. No symptoms of water stress were evident throughout the growth period as indicated by the health of the tendrils. Flowering on each shoot of the vines was tagged as it occurred and the color of berries recorded visually throughout to determine the color change associated with veraison. Each bunch contained ~60 berries on average.

Bunch gas exchange. A bunch chamber was constructed from a 3-L polypropylene beaker (145 x 160 mm) and a Perspex lid with a slot (8 x 92 mm) through which the bunch peduncle could be passed. The lid was attached to the chamber with butterfly clips. The chamber included inlet and outlet gas exchange ports, two rotary fans, and a thermistor to measure air temperature. Gas exchange (transpiration and respiration), chamber air temperature, and vapor pressures were monitored by an infrared gas analyzer (LCA4; ADC BioScientific, Hoddesdon, UK) in an open configuration. The bunch was sealed into the chamber with blue tack, and diurnal gas exchange rates were logged at 5-min intervals continuously throughout a complete 24-hr period. Gas exchange was measured on bunches from as early as six days after flowering (DAF) and continued at regular intervals until bunches reached about 120 DAF. A new bunch was used for each measurement and repeated on at least three bunches. At the conclusion of each daily measurement, the bunch was weighed and dried using a drying oven set at 60°C for up to 10 days and then dry matter measured and water content determined. Bunch transpiration, which included berries, pedicles, and the peduncle, was determined on a dry weight basis because surface area of the berries in situ on the bunch was not readily assessable and enabled comparison with other measurements of water flow. Transpiration was not measured on a relative bunch basis because of inherent differences in size between bunches.

Diurnal changes in bunch fresh weight. An attached, vertically hanging bunch on a vine growing in the controlled environment chamber was lifted and supported horizontally on a digital balance (PG503-S; Mettler-Toledo, Port Melbourne, Aust.) for 6 to 8 days. The forces related to suspending the bunch on the balance were assumed to remain constant throughout this time. Furthermore, plant turgidity (relaxing and tensioning of the shoot) was assumed to have no consistent affect on the bunch weight. Bunch weights were logged (COMDebug; Windmill Software, Manchester, UK) at 5- to 10-min intervals continuously throughout each day. At the end of six days, each bunch was detached, weighed, oven-dried as above, and the water content (g g[dry wt]$^{-1}$) determined. The measurements were repeated at least twice more on a new bunch and measured at three stages of bunch development (early, mid, and late). Further measurements were undertaken at the late stage of development when three attached bunches were excised from the vine by cutting the peduncle and subsequent changes in weight loss followed over several days.

This technique enabled the daily increments and decrements in bunch weight to be measured over several days. For each day, the gain in weight during the night and the loss in weight during the day were determined and each accumulated over the course of the experiment. Rates of water gain or loss were then calculated from the difference in day and night weight changes over the time interval and corrected to a dry weight basis in accordance with transpiration rates.

Vineyard study. Five vines at the CSU Vineyard were randomly selected for study over four growing seasons: 1999/2000, 2002/2003, 2003/2004, and 2004/2005. The same Shiraz clone as in the controlled environment experiments was used and a different set of vines was used in each of the growing seasons. Five to 10 whole bunches were randomly sampled from these vines at intervals of three times per week from the date of flowering until the date of harvest within each season (up to 130 days). These bunches were placed in plastic bags and transferred to the laboratory on ice, where the fresh weight was determined. A sample of 50 berries was cut from each bunch, fresh weight determined, and the sample dried as outlined above to determine dry weight. The sample dry matter content was then used to determine dry matter and estimate water content of each bunch. Linear regressions were used to determine rates of change in water content in each case.

Statistical analysis and calculations. All weight data were analyzed statistically using a general linear model and least squares means and standard errors determined using SAS software (version 9.1; SAS Institute, Cary, NC). Fixed terms in the model for a variable included an overall mean and a linear trend across days after flowering. Significance of all fixed terms was assessed at 5%. Rates of fresh and dry weight change and water gain and loss were also calculated using SAS software from linear regressions fitted from the first sampling date to the lag phase, from the lag phase to maximum weight, and from maximum weight to harvest. Additional analyses integrating daily transpiration rates were performed using Origin software (version 6; Microcal, Northampton, MA).

The water content of bunches was determined as the fresh weight-dry weight. Rates of gain or loss in bunch water were determined from the slopes of the regression equations fitted to the water contents over time and then corrected by the dry weight of the bunch at the same time in the different stages of bunch development. Rates of water import into the bunch were then determined as the difference between the rate of gain or loss in water-rate of transpiration at the different stages of bunch development, based on the assumption that the net rate of change in weight was the sum of the rate of water import and the rate of loss by transpiration.

Results

Bunch development in controlled environment. Budbreak was initiated immediately before vines were placed in the controlled environment conditions and flowering occurred shortly after. Bunches followed the normal developmental process, except that it was faster than occurred in the vineyard. For instance, berry color changes occurred at ~30 DAF, consistent with veraison occurring at this time. Bunch fresh weight accumulation commenced about five days after flowering (Figure 1). At ~30 DAF, bunch fresh weight increased more rapidly in a linear pattern until ~60 DAF when fresh weight then commenced to decline, again following a linear pattern such that at 120 DAF, the fresh weight of bunches was equivalent to that at 40 DAF. By contrast, dry matter of bunches increased very slowly until ~30 DAF when it began to increase more rapidly, although

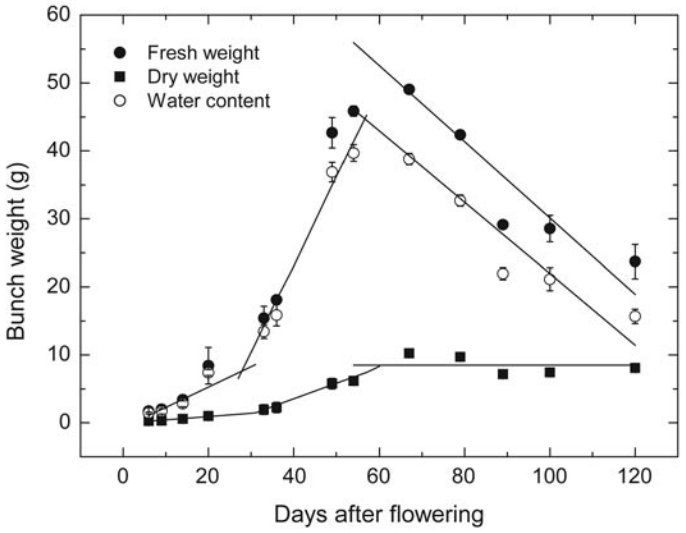

Figure 1 Changes in bunch fresh and dry weights (mean ± SE, N = 3) and water content throughout development for Shiraz vines grown in controlled environment for 120 DAF. Veraison occurred at ~30 DAF and no lag phase in growth occurred. Lines are linear regressions ($p < 0.01$; $r^2 = 0.8$ - 0.9) fitted to these data, except for dry weights between 6 and 30 DAF and 60 and 120 DAF, where the regressions were not significant ($p > 0.05$) and the line is the mean dry weight for each period. Some regression lines have been excluded for fresh weight change to improve figure clarity.

this dry matter increase was also completed by 60 DAF when the bunch dry matter became more or less constant. The net water content of the bunch changed over the period of bunch development in an almost identical pattern to the fresh weight changes.

Bunch transpiration throughout development. Bunch transpiration rates varied diurnally in response to the diurnal changes in the controlled environment chamber conditions (Figure 2), notably irradiance and vapor pressure deficit. Shortly after flowering, maximum daily rates of transpiration exceeded 300 mg g (dry wt)$^{-1}$ h^{-1} during the day and were between 50 and 100 mg g (dry wt)$^{-1}$ h^{-1} during the night. Similar patterns occurred as the bunch developed, although by about veraison the maximum daily rates were reduced to ~30 mg g (dry wt)$^{-1}$ h^{-1} and late in development were further reduced another ~50%. At all stages of bunch development, however, transpiration remained detectable during the night. These results showed bunch transpiration rates dropped markedly across the growing season.

Diurnal patterns in transpiration were mathematically integrated to give total daily transpiration for each bunch. Across the growing season, this declined in an exponential pattern (Figure 3), with very high transpiration losses of ~8 ± 0.9 g g (dry wt)$^{-1}$ d^{-1} around 6 to 8 DAF. Even before veraison had occurred, bunch transpiration had declined markedly to less than 2 ± 0.1 g g (dry wt)$^{-1}$ d^{-1} and to ~1 ± 0.1 g g (dry wt)$^{-1}$ h^{-1} by veraison (30 DAF). Thereafter, the transpiration losses continued to decline slowly to reach 0.2 ± 0.01 g g (dry wt)$^{-1}$ d^{-1} at harvest.

Bunch respiration throughout bunch development. Respiration for young (7 DAF) and middevelopment (36 DAF) bunches was significantly lower during the day at 25°C than at night and at 15°C (Figure 4), indicating that green bunches were capable of photosynthesis, although not enough to fully counteract respiration. Thus, a net loss of CO_2 occurred throughout the day in each case. Rates of respiration at 15°C averaged 3 ± 0.4 mg g (dry wt)$^{-1}$ h^{-1} on young bunches and declined by middevelopment to ~0.8 ± 0.1 mg g (dry wt)$^{-1}$ h^{-1}. It was notable for both bunches that for the brief period when the lights were turned off and the temperature remained transiently at 25°C (20:00 hr), rates of respiration peaked at 9 ± 0.5 and 1.5 ± 0.2 mg g (dry wt)$^{-1}$ h^{-1} for bunches at 7 and 36 DAF, respectively. Once veraison had occurred, photosynthesis was no longer detectable and respiration rates in bunches late in development (115 DAF) were very low, ranging from 0.05 to 0.17 ± 0.01 mg g (dry wt)$^{-1}$ h^{-1} at 15 and 25°C, respectively.

Across the whole period of bunch development, respiration decreased in an exponential pattern (Figure 5), declining rapidly initially from 5 to 7 ± 0.8 mg (g dry wt)$^{-1}$ h^{-1} at 7 DAF to 1 to 2 ± 0.3 mg g (dry wt)$^{-1}$ h^{-1} by 20 DAF. Thereafter, bunch respiration continued to decline to less than 0.5 mg ± 0.1 (g dry wt)$^{-1}$ h^{-1} through the remainder of development. These results indicate bunch metabolic activity was low throughout ripening and conform with grapes being a nonclimacteric fruit.

Relationships between bunch weight changes and transpiration. Linear regressions of the changes in bunch water content over time at the early, mid, and late stages of development were all statistically significant ($p < 0.01$, r^2 = 0.8 to 0.9) (Figure 1). The regression slopes (the net rates of

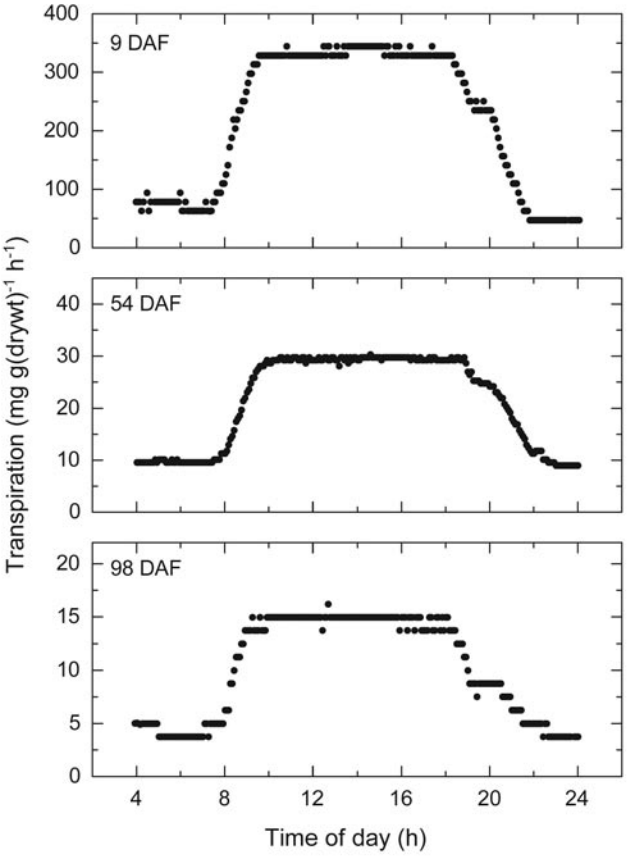

Figure 2 Diurnal changes in Shiraz bunch transpiration on a selected day at the early, mid, and late stages of development for vines grown in controlled environment with a day/night temperature of 25/15°C and at an irradiance of 650 µmol m^{-2} s^{-1}. Photoperiod occurred from 0700 to 1800 hr.

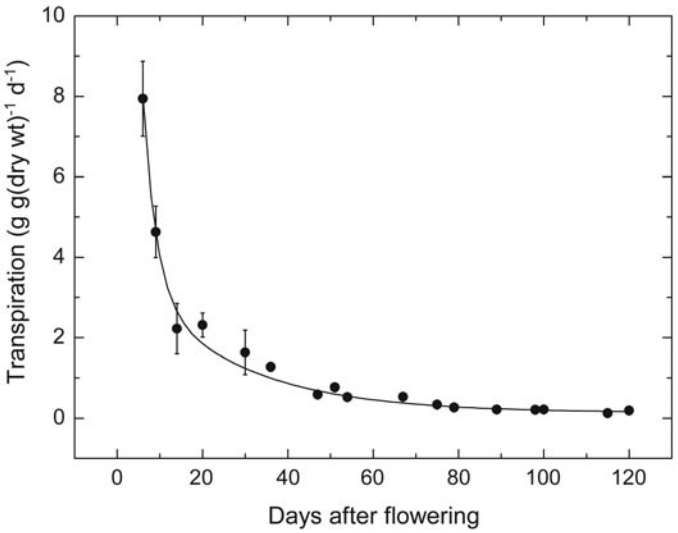

Figure 3 Changes in daily transpiration of Shiraz bunches throughout development from 6 to 120 DAF. Each value is the mean ± SE of two to three measurements. The fitted line is a 3rd order exponential decay ($p <$ 0.0001, r^2 = 0.997). Veraison occurred at ~30 DAF.

gain in water corrected to a dry weight basis) were high in the early stage of bunch development (18 to 30 DAF) then declined ($p < 0.01$) at the mid stage (30 to 60 days) (Table 1). This decline in the *rate* of water gain per gram of bunch dry matter appeared at odds with the increase in bunch weight; however, the decline in the rate was a mathematical function of the longer duration and the higher dry matter of the postveraison stage compared with the preveraison stage. In the late-ripening stage (60 to 120 DAF) the rate declined further to become a net loss of water from the bunch. Calculated rates of water import declined significantly ($p < 0.01$) throughout bunch development but remained higher than the rates of transpiration until the ripening process had begun, when the difference was not significant (Table 1). Thereafter, transpiration rates exceeded the rates of water import. Notably, however, these data suggest import of water continued to occur, albeit at slow rates, even when bunches were losing weight.

Diurnal changes in bunch weights. Across all stages of development, diurnal changes in bunch fresh weight (Figure 6) occurred in direct response to the daily fluctuation in the growth conditions. The pattern of weight change each day, in both early and mid-developing bunches, included a relatively sharp increase in weight, coinciding with the switch from day to night conditions, followed by a more gradual increase during the night. This was followed by an equally sharp decrease in weight, coinciding with the switch from night to day conditions and then a period of little to no weight gain occurred throughout the rest of the day. Bunches thus gained weight over time. These apparently abrupt changes (occurring over 2-hr periods) were highly correlated with the changes in vapor pressure deficit between day and night but also were highly coincidental with the diurnal changes in transpiration rate (Figure 2).

Similar diurnal weight changes occurred in the late-ripened bunches (Figure 6), except that a significant loss

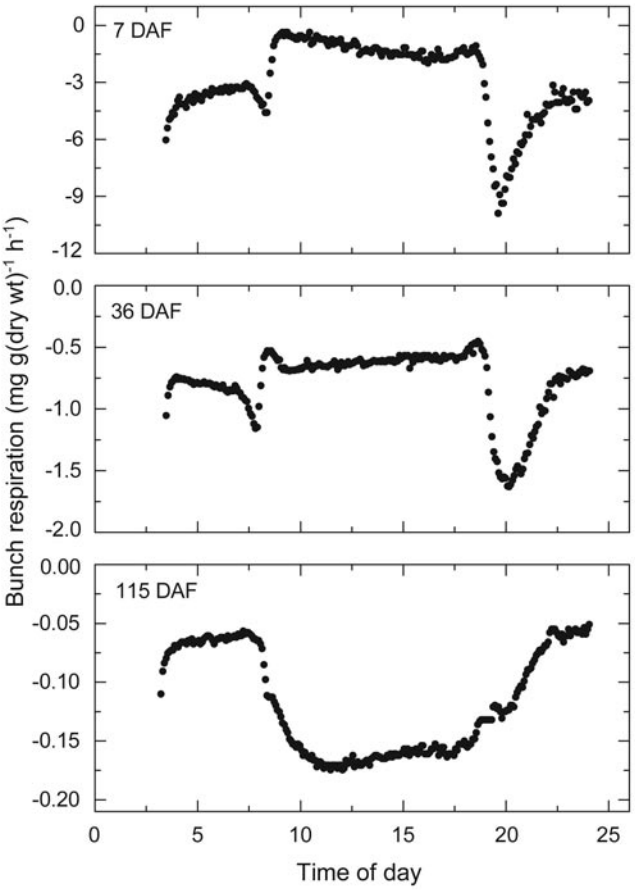

Figure 4 Diurnal changes in Shiraz bunch respiration on a selected day at the early, mid, and late stages of development.

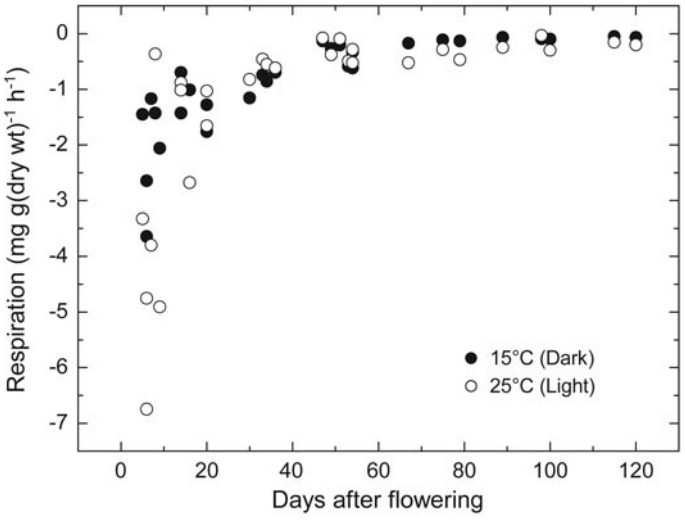

Figure 5 Changes in Shiraz bunch maximum respiration rates throughout the growing season. At 25°C, rates were those occurring during the light period, and at 15°C, rates were those occurring during the dark conditions. Each point represents one day of measurement.

Table 1 Estimated rates of water import and the measured rates of transpiration and gains (+ve) or losses (-ve) in bunch water content (means ± SE; N = 5) for Shiraz grapes at three different stages of bunch development on vines grown in controlled environment conditions.

Growth period (DAF)	Water import[a] (g g [dry wt]$^{-1}$ d^{-1})	Transpiration[b] (g g [dry wt]$^{-1}$ d^{-1})	Net gain or loss[c] (g g [dry wt]$^{-1}$ d^{-1})
18 to 30	2.53 ± 0.03	-1.98 ± 0.24	+0.55 ± 0.06
30 to 60	0.54 ± 0.10	-0.32 ± 0.05	+0.29 ± 0.05
60 to 120	0.15 ± 0.03	-0.19 ± 0.02	-0.033 ± 0.008

[a]Slopes of the linear regressions fitted to the water content data over the different stages of bunch development (Figure 1) and corrected to a dry weight basis.
[b]Mean rates determined over the same period from data in Figure 3.
[c]Rates determined by subtraction of the transpiration losses from the rates of water gain or loss at each stage.

of weight occurred during the day such that, over time, these bunches declined markedly in weight. Nevertheless, changes showed an increment in weight continued to occur each night despite the more general weight loss, indicative of some flow occurring into the bunch.

It was notable that the pattern of diurnal weight change in each case remained relatively constant across several days. These weight changes between the different stages of bunch development were all different ($p < 0.01$). Thus the diurnal weight gain decreased more than 10-fold from early to late ripening, while the comparable diurnal weight loss decreased about three-fold. Of more significance perhaps was the relative balance between these weight changes, which shifted progressively from a net daily gain at the early stage of bunch development to a net daily loss in weight at the late stage of ripening. These data conform well to the weight loss occurring at the late stage of development (Figure 1). Furthermore, the rates of water gain or loss determined from the diurnal weight changes (Table 2) generally conformed to the measured rates of transpiration and the estimated rates of import and weight gains/losses (Table 1).

Effect of bunch peduncle excision on weight loss. A further indication that water flow into the bunch occurred while bunches were otherwise losing weight was apparent when the peduncle was cut and an immediate increase in the weight loss occurred (Figure 7). Before the cut, the diurnal fluctuations in bunch weight were similar to those reported in Figure 6, with the rates of water gain (0.095 ± 0.019 g g [dry wt]$^{-1}$ d^{-1}), loss (-0.13 ± 0.02 g g [dry wt]$^{-1}$ d^{-1}) and the net loss (-0.033 ± 0.001 g g [dry wt]$^{-1}$ d^{-1} similar to the rates reported in Table 2. However, once the peduncle was cut, bunch weight declined rapidly at an average rate of -0.182 ± 0.006 g g (dry wt)$^{-1}$ d^{-1}, that is, nearly six times faster than when the peduncle was attached. If it were assumed that only solute flow was stopped by this treatment, then the net rate of loss would increase to ~0.22 g g (dry wt)$^{-1}$ d^{-1}, which is consistent with that actually measured.

Bunch weight changes in the vineyard. Across all four growing seasons, bunches lost an average of 26% of the fresh weight during late ripening. In all seasons, comparable changes in bunch development occurred; bunch fresh and

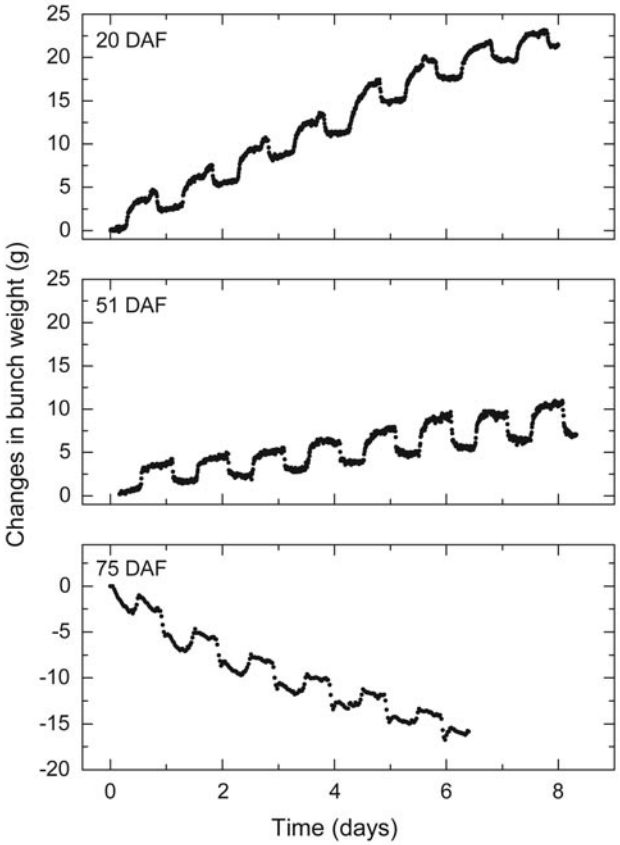

Figure 6 Diurnal changes in fresh weight (mean ± SE, N = 3) of Shiraz bunches across several days at early, mid, and late stages of development; for vines grown in controlled environment.

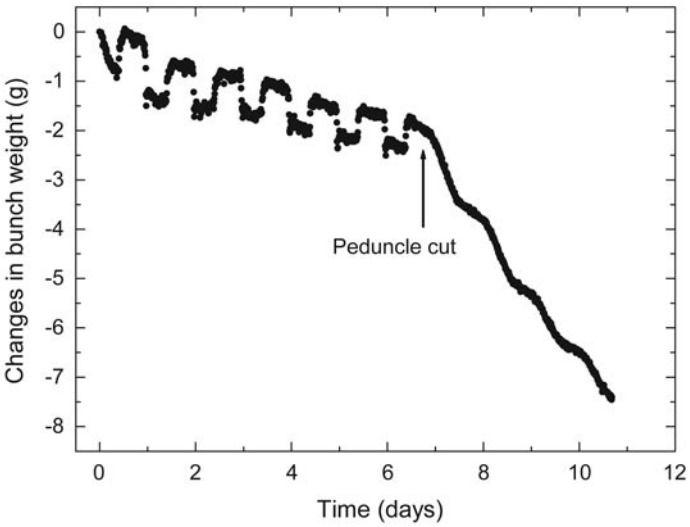

Figure 7 Diurnal changes in fresh weight loss (mean ± SE, N = 3) of a Shiraz bunch measured from ~70 DAF with the peduncle attaching the bunch to the vine completely cut 7 days later as indicated.

Table 2 Mean daily rates of bunch fresh weight gain (import) and loss (transpiration) in water and the difference between these rates (mean ± SE, N = 3) at selected stages of bunch development for Shiraz grapes on vines grown in controlled environment conditions. Rates calculated from bunch weights measured throughout each 24-hr period, with weight increases occurring during the night and weight decreases during the day and weight gain or loss calculated as the difference between these. All data corrected to a dry weight basis. Over the short time intervals used, the fresh weight changes were assumed to be primarily water.

Growth period (DAF)	Weight gain (g g [dry wt]$^{-1}$ d^{-1})	Weight loss (g g [dry wt]$^{-1}$ d^{-1})	Net gain or loss (g g [dry wt]$^{-1}$ d^{-1})
18 to 30	1.09 ± 0.08	-0.57 ± 0.05	+0.51 ± 0.08
30 to 60	0.51 ± 0.03	-0.40 ± 0.01	+0.11 ± 0.01
60 to 120	0.10 ± 0.01	-0.18 ± 0.02	-0.07 ± 0.02

dry weights for the 2004-2005 growing season are shown (Figure 8). Furthermore, the bunches followed a developmental pattern comparable to that of the vines grown in a controlled environment, except for an extended lag period between ~20 and 50 DAF when there was little to no change in weight. The water content of these bunches also increased rapidly in a linear pattern both before and after the lag phase and declined in a linear pattern from ~80 DAF. Similar patterns occurred during the 1999/2000, 2001/2002, and 2003/2004 growing seasons (data not shown), but the slopes from the regression lines fitted to the various stages were all highly significant ($p < 0.01$) (Table 3). Across the four years, bunches at the early stage of development gained water at an average net rate of 0.76 ± 0.11 g g (dry wt)$^{-1}$ d^{-1} and at the mid stage declined to 0.11 ± 0.025 g g (dry wt)$^{-1}$ d^{-1}. During late ripening, bunches lost water across all growing seasons at an average net rate of -0.035 ± 0.006 g g (dry wt)$^{-1}$ d^{-1}. These rates compare extremely favorably with the net rates of change in bunch water content at the different developmental stages of the controlled environment vines (compare Tables 1 and 2 with Table 3).

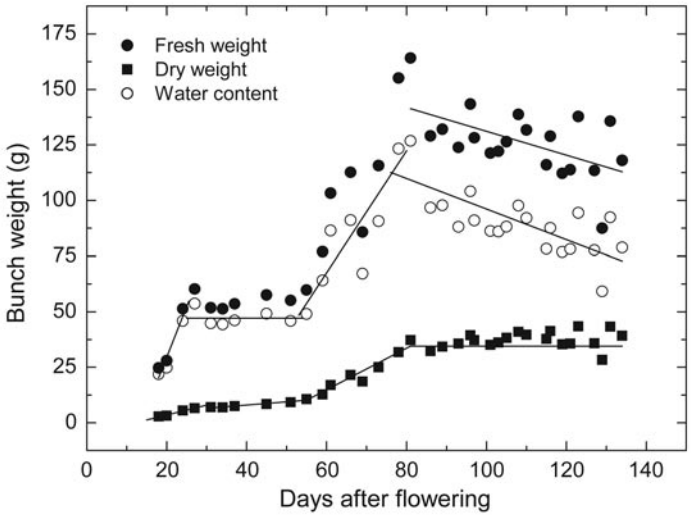

Figure 8 Seasonal changes in Shiraz bunch fresh weight, dry weight, and water content, calculated by the difference between the fresh and dry weights for vineyard-grown vines over the 2004–2005 season (each value is mean ± SE, N = 50, with SE smaller than the symbols). An extended lag phase occurred from ~25 to 55 DAF and veraison occurred shortly after. Each fitted line is linear regression ($p < 0.01$, $r^2 = 0.85 – 0.9$), except for changes in dry matter from ~30 to 50 DAF and from 80 to 130 DAF and also for the fresh weight and water content from ~25 to 50 DAF, where the fitted line is the mean value through each period. For figure clarity, some lines are omitted for fresh weight changes.

Discussion

Shiraz grape bunches grown on vines in controlled environment conditions developed in a generally similar pattern to bunches grown in the vineyard. However, an exception was that bunch development progressed faster in controlled conditions because no obvious lag phase occurred. For example, veraison was reached at least 20 DAF earlier than that occurring in the vineyard and the time of maximum weight was 20 to 25 DAF earlier than typically occurred in the vineyard (Rogiers et al. 2000, 2004b). Both air and soil temperatures influence grape bunch phenology, particularly time of veraison (Tesic et al. 2002). It was likely, therefore, that over the controlled growth conditions, pot soil temperatures were consistently higher than for vines growing in vineyards and high root-to-shoot ratios of potted vines preferentially support bunch growth, consistent with the more rapid development of the bunches shown here.

Whole bunch transpiration rates declined markedly with development of the Shiraz bunch, consistent with the decreased rates of water loss observed previously (Rogiers et al. 2004b). The decline was such that by the time the grapes reached maturity, these rates appeared negligible, at least compared with rates during the 10 to 15 days immediately after flowering. This pattern of change in bunch transpiration is consistent with other fruit, including grapefruit (Huang et al. 1992), strawberry (Blanke 1992), and pear (Zhang and Deng 2006) and other grape varieties (Blanke and Leyhe 1987). Given that the vines were grown in constant conditions with little change in evaporative demand, the long-term decline in transpiration across the growing season could not be related to vapor pressure deficit as is commonly asserted for diurnal shrinkage in other fruit (Lang 1990, Morandi et al. 2007) but rather to increased resistance to transpiration.

Some stomata are present in Shiraz berries but accumulation of epicuticular waxes early in development generally occludes these (Mullins et al. 1992, Blanke and Leyhe 1987, Rogiers et al. 2004b). Furthermore, bunches actively transpired at night, daily changes in transpiration were in direct accord with the diurnal fluctuation in VPD, and the low

Table 3 Rates of net bunch water gain (+ve) and loss (-ve) at three growth periods of Shiraz bunch development over each of four growing seasons. Rates and standard errors were determined from linear regressions ($p < 0.01$, $r^2 = 0.95$) fitted to the water content of grapes, determined as the difference between fresh and dry weights, over the duration of each stage of bunch development.

	Bunch net water gain or loss (g g [dry wt]$^{-1}$ d^{-1})		
Season	**18 to 30 DAF[a]**	**55 to 80 DAF**	**80 to 130 DAF**
1999–2000	1.06 ± 0.13	0.10 ± 0.03	-0.058 ± 0.011
2002–2003	0.55 ± 0.12	0.15 ± 0.03	-0.048 ± 0.009
2003–2004	0.58 ± 0.09	0.07 ± 0.01	-0.013 ± 0.003
2004–2005	0.85 ± 0.10	0.12 ± 0.03	-0.020 ± 0.002

[a]Between ~30 and 55 DAF, the regressions between bunch water content and days after flowering for each growing season were not significant ($p > 0.05$), indicating no net gain or loss in water content occurred during the lag phase of bunch development.

density of stomata conform with the transpiration pathway from grapes being cuticular in nature (Possingham et al. 1967, McCarthy and Coombe 1999). Cuticular transpiration has been shown to occur in other fruit, including apple (Jones and Higgs 1982), currants (Blanke 1995), chilli pepper (Blanke and Holthe 1997), and tomato (Vogg et al. 2004, Liu et al. 2007). Some of the seasonal decrease in transpiration with Shiraz bunches might be explained by wax accumulation increasing the resistance to water loss from the individual berries (Radler 1965, Possingham et al. 1967). However, Shiraz berry waxes on a surface area basis declined by ~70% after veraison (Rogiers et al. 2004b), so wax changes do not appear to account for the decline in Shiraz bunch transpiration (8 to 0.2 g g^{-1} d^{-1}). Decreasing surface conductance or changes in surface area to volume of these bunches might explain the decline in transpiration rates. However, in situ bunch surface area is difficult to assess and dry weight was used in the present study to base the transpiration rates and other measurements to ensure conformity and comparability of results.

During development of the Shiraz grapes, the rate of water import was determined as the net difference between the rate of bunch water gain or loss and the rate of water loss by transpiration. This analysis suggests that rates of water import into the bunch prior to veraison were ~30% higher than the rates of transpiration. Although the import rates appeared to decline subsequently, rates were still nearly 70% higher than transpiration because of the greater decrease in the latter. The balance in favor of import was consistent with the very rapid gain in bunch weight that occurred between 30 and 60 DAF (Figure 1) and between 50 and 80 DAF (Figure 8). In contrast, after this growth phase the transpiration rates, although very low, exceeded the net import rates into the bunch by nearly 30%, with the outcome that the bunch water content declined at a rapid rate and the symptomatic weight loss occurred. Eliminating inflow (Figure 7) caused the weight loss to increase markedly, giving strong support to the conclusion that, during late ripening when Shiraz berries were otherwise shrinking, some water continued to flow into the bunch. When the berries were shrinking, bunch weight continued to fluctuate diurnally, which also supports this conclusion.

This study could not resolve whether the residual flow was through the xylem or the phloem vasculature, but the evidence may be more consistent with xylem-supplied water flow. On hydraulic grounds, for example, Tyerman et al. (2004) concluded that translocation had ceased by the time Shiraz berries had begun to shrink, as has also been proposed elsewhere (McCarthy and Coombe 1999, Rogiers et al. 2006b). Consistent with these conclusions, across all treatments in the present study, the time when the weight loss commenced was highly coincidental with the time that bunch dry matter accumulation ceased, indicative of phloem shutting down. Respiration rates also were very slow (Figure 5), indicating little metabolic activity was occurring and consistent with dry matter losses not contributing to the weight loss. Furthermore, sugar accumulation also slows markedly or stops completely at the same time (Coombe 1960, Coombe and McCarthy 2000). There is a widely held view that in grape berries, xylem flow slows or even ceases at veraison and phloem flow predominates during berry ripening (Düring et al. 1087, Greenspan et al. 1994, Coombe and McCarthy 2000, Rogiers et al. 2006a). Clearly, a sharp reduction in flow to the berries must have occurred to so dramatically switch Shiraz bunches from increasing to decreasing in weight (Figure 1, Figure 8). The weight of evidence is thus consistent with phloem flow ceasing about the time that Shiraz berries begin to lose weight and perhaps xylem flows continuing to trickle. Recent studies showing postveraison hydraulic connections between berries and vine are consistent with this view (Bondada et al. 2005, Keller et al. 2006).

Sustained weight loss in Shiraz berries was, therefore, not a consequence of increased transpiration (Mccarthy 1999), but rather a marked shift in the water fluxes in favor of transpiration loss over water import into the bunch, thus confirming quantitatively the hypothesis in previous work (Rogiers et al. 2004b). Other research invoked backflow and transpiration to account for the increased weight loss (Tyerman et al. 2004). Because only net fluxes were determined in the present study, the contribution of backflow cannot be determined. However, it was clear that water flow into the bunch must have exceeded any backflow for the net gain of water that was observed to occur when the bunches were otherwise shrinking. That Shiraz shrinkage was caused by an imbalance between water import and transpiration was also strongly supported by the results of the direct bunch-weight technique. Both methods gave quantitatively similar yet independently obtained estimates of the rates of bunch water import and loss (Table 1, Table 2) and would suggest a high degree of reliability in the estimated rates of water flow in and out of the bunch.

This conclusion is further strengthened from the determination of the gains and losses of bunch water in the vineyard throughout four separate growing seasons. For example, the net rate of bunch water gain prior to veraison in the vineyard-grown vines ranged from 0.55 to 1.06 g g (dry wt)$^{-1}$ d^{-1} compared with 0.51 to 0.55 g g (dry wt)$^{-1}$ d^{-1} for controlled-environment vines. After veraison, the vineyard rates ranged from 0.07 to 0.15 g g (dry wt)$^{-1}$ d^{-1} and comparable rates for the controlled vines ranged from 0.11 to 0.29 g g (dry wt)$^{-1}$ d^{-1}. This remarkable consistency across diverse seasons and treatments in determination of rates of water flow in and out of the Shiraz bunches provides strong quantitative support for the conclusion of the study.

Conclusions

Sustained weight loss occurred in late-ripening Shiraz grape bunches because of changes in the net flux of water, that is, import declined relatively abruptly while transpiration losses continued unabated. Both conditions were required for weight loss to occur. Our results also clearly demonstrated that import into the bunches did not cease completely, and indirect evidence suggested this import may

have been xylem-supplied water flow, while impediments to phloem flow appeared to account for the substantial reduction in bunch weight. Further investigation is needed concerning the triggers that cause such a dramatic change in Shiraz bunches from accumulating water to losing water.

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