Cooperative Research Centre for Viticulture

Australian Government
Grape and Wine Research and Development Corporation

Examination of factors contributing to onset and degree of pre-harvest weight loss in Shiraz berries



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Final Report to Grape and Wine Research & Development Corporation
On:
Examination of factors contributing to onset and degree of pre-harvest weight loss in Shiraz berries

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Cover picture:
Shiraz berries showing pre-harvest weight loss.

The Cooperative Research Centre for Viticulture is a joint-venture between Australia's viticulture industry and leading research and education organisations. It promotes cooperative scientific research to accelerate quality viticultural management from vine to palate. Australian grapegrowers and winemakers are key stakeholders in the CRCV, contributing levies matched by the Commonwealth Government and invested by the Grape and Wine Research and Development Corporation in the Centre.

Table of Contents

1. Abstract 6

2. Executive summary 6

3. Background 10

4. Project aims and performance targets 10

5. Methods 11
- 5.1 Xylem and phloem inflow 11
  - 5.1.1 Element accumulation 11
  - 5.1.2 Dye tracers 11
  - 5.1.3 Xylem and phloem structure 12
  - 5.1.4 Pedicel girdling and excision 12
  - 5.1.5 Bunch stem sap flow 13
- 5.2 Wax morphology and berry transpiration 13
  - 5.2.1 Cuticle characteristics 13
  - 5.2.2 Quantification of epicuticular wax 13
  - 5.2.3 Transpiration of detached berries 13
  - 5.2.4 Transpiration of attached bunches 13
- 5.3 Backflow 14
  - 5.3.1 Diurnal contraction 14
  - 5.3.2 Dye tracers 14
- 5.4 Cultural impacts on berry weight loss 15
  - 5.4.1 Nitrogen and irrigation 15
  - 5.4.2 Rootstock and irrigation 16

6. Results 17
- 6.1 Xylem and phloem inflow 17
  - 6.1.1 Element accumulation 17
  - 6.1.2 Dye tracers 30
  - 6.1.3 Xylem and phloem structure 33
  - 6.1.4 Pedicel girdling and excision 35
  - 6.1.5 Bunch stem sapflow 37
- 6.2 Wax morphology and berry transpiration 39
  - 6.2.1 Cuticle characteristics 39
  - 6.2.2 Quantification of epicuticular wax 42
  - 6.2.3 Transpiration of detached berries 44
  - 6.2.4 Transpiration of attached bunches 44
- 6.3 Backflow 46
  - 6.3.1 Diurnal contraction 46
  - 6.3.2 Dye tracers 50
- 6.4 Cultural impacts on berry weight loss 52
  - 6.4.1 Nitrogen and irrigation 52
  - 6.4.2 Rootstock and irrigation 55

7. Discussion 59
- 7.1 Xylem and phloem inflow 59
- 7.2 Wax morphology and berry transpiration 60

7.3 Backflow 62
7.4 Cultural impacts on berry weight loss 63

8. Summary 66

9. Recommendations 67

Appendix 1: Communication 68

Appendix 2: References 70

Appendix 3: Staff 74

Appendix 4: Budget reconciliation 75

Sources

Excerpts from the following publications were incorporated into this report:

Sections: 5.4.1, 5.4.2, 6.4.1, 6.4.2, 7.4:
Rogiers SY, Hatfield JM and M Keller. 2004. Irrigation, nitrogen, and rootstock effects on volume loss of berries from potted *Vitis vinifera* L. cv. Shiraz vines. Vitis 43: 1-6.

Sections: 5.2.1, 5.2.2, 5.2.3, 6.2.1, 6.2.2, 6.2.3, 7.2:
Rogiers SY, Hatfield JM, Jaudzems VG, White RG and M Keller. 2004. Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and pre-harvest weight loss. American Journal of Enology and Viticulture 55: 121-128.

Sections: 5.1.2, 5.1.4, 6.1.2, 6.1.4, 7.1
Rogiers SY, Smith JP, White R, Keller M, Holzapfel BP and JM Virgona. 2001. Vascular function in berries of *Vitis vinifera* (L) cv. Shiraz. Australian Journal of Grape and Wine Research 7: 47-51.

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1. Abstract

Shiraz berries can undergo shrivelling during late ripening. Shrivelling can begin at around 85 days after flowering in the South-west slopes region of NSW, and more than 85% of the weight loss is in the form of water. The physiological cause for this berry weight loss was examined, and the impacts of irrigation, rootstock and nutrition on onset and severity of weight loss was assessed. A combination of element accumulation patterns, dye tracer infiltration, anatomical observation, gas exchange measurements, berry volume and sap flow sensors were used to deduce rates of water movement into and out of berries. Water flow into the bunch through the xylem stream was diminished after veraison in three out of four seasons. After maximum berry size, water flow through the xylem increased again. The phloem's contribution to the berry's water budget increased after veraison, then diminished after maximum berry size. Water loss from the berry through transpiration diminished after veraison, but was ongoing. Water flow through the xylem from the berry to adjacent berries in the bunch did occur, however flow back to the vine was very restricted, while backflow through the symplasm was not detected. These results indicate that shrivelling in Shiraz was likely due to ongoing slow transpiration combined with decreased water flow into the berry through the phloem. The trigger for the onset of shrivelling was not regulated by day length or temperature. Cultural management of the rate in weight loss from berries was possible through irrigation and rootstocks. Standard irrigation ameliorated the rate of post-weight maximum volume loss as compared to deficit irrigation, and the onset of the weight loss was delayed by one week when Ramsey was used as a rootstock as compared to 101-14 Mgt.

2. Executive Summary

The occurrence of shrivelling during late ripening of grape berries has been reported for several varieties, but cv. Shiraz seems to be particularly prone to this phenomenon. In Shiraz plantings of the South-west slopes, NSW, Australia, shrivelling has occurred in 5 out of the past 6 years prior to commercial harvest, with yield losses of up to 25%. Symptoms include loss in berry turgidity and wrinkling of the skin, without any apparent exterior physical or pathological damage to the surface of the berry, the pedicel or the bunch stem. The absence of such symptoms points to a physiological cause for shrivelling.

In all seasons examined, increase in fresh weight of Shiraz berries followed a typical double sigmoid curve. Weight loss began at 80 or more days after flowering, and in one season berries were commercially harvested at 24˚ Brix before any weight loss set in. Over 85% of the weight loss from Shiraz berries was in the form of water. The weight loss was concurrent with a cessation in the accumulation of sugars and dry weight. ˚Brix continued to increase, however, due to the concentration effect during shrinkage.

Accumulation of mineral elements into Shiraz berries was followed through development in order to understand the changes in contribution of the vascular streams from pre-veraison to post-veraison growth and subsequent berry shrinkage. Phloem mobile elements accumulated most strongly post-veraison, however this lessened during the shrinkage phase. Seeds were the strongest sinks for P, Ca, S, Mg, Mn and Zn, while the pulp and skin were the strongest sinks for K and B. Seeds stopped accumulating the phloem mobile elements prior to veraison. After a temporary cessation, xylem mobile elements continued to accumulate in seeds of post-veraison berries. Pre-veraison accumulation of xylem mobile elements tended to be stronger than post-veraison accumulation. After the berry weight maximum, xylem mobile element accumulation into the berry returned positive. Cumulatively, these data indicate that, late ripening shrinkage was likely due to decreased phloem flow into the berry. The regain of net positive xylem flow into the shrinking berry did not compensate for the loss in phloem flow and thus shrinkage was not averted as transpiration continued.

The xylem pathway into the berry was investigated at several stages of development using three water soluble dyes. Regardless of stage of development or irrigation regime the dyes had moved from the pedicel to the brush region of the berry. The dyes, however, were not always present in the peripheral or central vascular bundles. As berries reached the post weight maximum phase the presence of the dyes in these bundles was diminished. The xylem and phloem of the berry were also examined for anatomical changes which may lead to shrivel. No plugs were observed on the sieve plates of the sieve tube members in the pedicel, brush, central of peripheral vasculature at any stage of development. Stretching of the spiral thickenings of xylem tracheids, however, was apparent after veraison in the central and peripheral bundles.

A deficit irrigation treatment to potted Shiraz vines indicated that post-veraison berries were not as responsive to drought and watering cycles as pre-veraison berries. However, pedicel girdling of berries from the same vines indicated a continuing xylem connection since volume of these berries was 30% greater than excised berries after 30 days. These data indicate that post-veraison there was water movement through the xylem into the berry but only to the region of the brush. At this point water may diffuse into the pericarp through a non-vascular route. Sap flow sensors were used as a non-intrusive method to monitor sap flow changes through the bunch stem in response to environmental changes. Flow rates through the bunch stem correlated well with vapour pressure deficit, even post-veraison and post-weight maximum.

Berry transpiration and cuticle morphology were examined for their roles in water loss from Shiraz berries. We have shown through scanning electron microscopy the presence of very few but functional stomata on young berries, wax filled stomata on older berries, and aside from slight cracks along the stomatal protuberance, no fissures in the surface of the berry that may lead to increased transpiration rates. Pre-veraison berry epicuticular wax platelets were defined and intricate, while post-veraison and shrivelled berry surfaces had large areas of amorphous waxes. Post-veraison, the area of amorphous wax relative to intricate wax was not correlated with berry age or degree of shrivel. Extraction of surface waxes revealed that total wax on a surface area basis decreased during veraison, then remained stable as the berries ripened and entered the weight loss phase. Berry transpiration during this final shrivelled phase was 16% of the pre-veraison rate on a per berry basis. Nevertheless, berry transpiration could account for an average 15 mg loss in fresh weight per berry per day.

One of the objectives was to understand the role of backflow, water flow from the berry back to the vine, in Shiraz berry shrivel. In order to examine if back-flow occurs in Shiraz, and the possible routes of this back-flow, tracer dyes were applied to the berry and the extent of their movements were monitored. The results indicated that backflow to adjacent berries did occur through the apoplasm, however it was limited. Prior to veraison the back-flow can occur from below the seeds, but after veraison the back-flow is restricted from the brush region of the berry. There was no evidence for symplastic back-flow from pre-veraison berries. No tracer was found in the leaves surrounding the berries.

Volume sensors were used to monitor diurnal changes in berry contraction and expansion. Pre-veraison berries of vines grown under standard irrigation did not contract during the day. Berries in the lag phase expanded during the late afternoon and contracted during the morning and middle part of the day. At the onset of the second phase of growth, daily contraction was reduced. Post weight maximum, contraction during the early part of the day resumed, without a concomitant regain in volume during the latter part of the day. Reducing the VPD around the bunch by placing the bunch within a sealed bag eliminated the daily contractions, indicating that shrinkage was due to transpiration rather than backflow.

A summary of the results indicates that weight loss during late ripening of cv. Shiraz berries was due to decreased phloem flow into the berry combined with continued ongoing slow transpiration. The trigger for the onset of weight loss was not regulated by day length since glasshouse vines in the late ripening stage, grown without supplementary light, can undergo shrivelling at any time of year. Likewise, temperature does not appear to be a trigger since berries will shrivel even when vines are grown under a constant temperature regime in the glasshouse over the entire developmental period.

Irrigation regime, nitrogen application rate and rootstock were tested for their effects on the onset of volume loss and extent of volume loss. Maximum berry volume correlated well with volume of berries at 35 days after flowering, the end of the cell division phase of berry growth. Post-maximum berry shrinkage was not as severe for vines grown with split-root irrigation as compared with standard irrigation. However, these berries were smaller at harvest due to less pre-veraison expansion. Berries grown on vines with standard irrigation had greater post-maximum volume loss than those berries grown in a deficit irrigation treatment. Despite this, maximum berry volume correlated well with final volume in all treatments. N application rate had an effect on the onset of post-veraison expansion but not on the amplitude of maximum berry volume or final berry volume. Deficit irrigation delayed the onset of volume loss by 11 days. Rootstock also had an effect on the onset of volume loss with berries from vines grafted on the rootstock 101-14 Mgt losing volume 7 days earlier than berries from vines grafted on Ramsey. These results suggest that onset and degree of volume loss in Shiraz is sensitive to external influences.

This work was partly funded by the ARC-SPIRT Scheme which provided the stipend for Charles Sturt University PhD student, Nicole Dimos. Financial support was also provided by NSW Department of Primary Industries.

Background

Shiraz berry 'shrivel' can result in up to 20% loss in tonnage of fruit in Australia and aside from decreasing yield this condition may also reduce the quality of the crop. There are reports of poor berry colour and decreased acidity in association with the weight loss. The occurrence of shrivelling during late ripening of grape berries has been reported for several varieties, but cv. Shiraz seems to be particularly prone to this phenomenon. McCarthy (1999) reported that the onset of weight loss began at ca. 90 days after flowering in vineyards of Waikerie, SA, regardless of season, weather pattern or irrigation regime. We have found that in Shiraz plantings of the Riverina, NSW, shrivelling has occurred in 5 out of the past 6 years with yield losses of up to 25% prior to harvest. Symptoms include loss in berry turgidity and wrinkling of the skin, without any apparent exterior physical or pathological damage to the surface of the berry, the pedicel or the bunch stem. The absence of such symptoms points to a physiological cause for shrivelling. Since 90% of the weight loss is in the form of water, some insight into a cause may be obtained by an examination of the berry's water relations.

Project Objectives/Outcomes

Objectives:

1. Examine environmental and internal signals in triggering onset of berry weight loss in Shiraz
2. Examine environmental and physiological factors contributing to the degree of weight loss

Outcomes:

1. Better understanding of environmental and physiological factors impacting on Shiraz berry development, ripening, quality and volume.
2. Strategies to eliminate or ameliorate weight loss in Shiraz grapes and increase quality and overall yield

Outputs and Performance Target

Outputs	Performance Target
1. Information on anatomical and physiological causes for onset and degree of weight loss	1. Vascular transport and microscopy studies completed by June 2003
2. Information on environmental and management factors which impinge on the onset and degree of weight loss.	2. Temperature, day-length and evaporative demand studies completed by June 2004
3. Strategy to decrease incidence and severity of weight loss	3. Data collated and summarised by December 2004
4. Informed growers and other members of the industry	4. Article submitted to industry and scientific journal by January 2005 and seminar to at least 100 growers in two of the most heavily affected regions by January 2005

5.0 Methods

5.1 Xylem and Phloem inflow

5.1.1 Element accumulation

Five bunches were harvested at random three times weekly from a plot of 400 own-rooted, 11 year-old, spur pruned cv. Shiraz clone PT23/N/Griffith vines (Block A) at the Charles Sturt University Vineyard, Wagga Wagga, NSW from flowering to harvest in the 1999-2000, 2000-2001 and the 2001-2002 seasons. A second plot of 17 year-old Shiraz vines fitting the above description (Block B) was also sampled weekly in the same manner in the 2001-2002 and 2002-2003 seasons. The fresh weight of a 50 berry sample was taken from each of 5 bunches at each sampling date. Subsequently the sample was juiced and °Brix was measured using a hand-held refractometer (ATAGO Co., Ltd., Tokyo, Japan).

Five harvest dates, spanning the 2000-2001 season were chosen for berry tissue segregation. These were 24/12/2000 (pre-veraison, 23 DAF), 6/01/2001 (pre-veraison, 48 DAF), 18/01/2001 (post-veraison, 60 DAF), 2/02/2001 (mid-ripening, 75 DAF), 16/02/2001 (late ripening, 89 DAF). The 50 berry sample was separated into torus, skin, pulp, brush and seeds. The brush was punched out of proximal half of the frozen berry using a capillary pipette, and subsequently the skin was pulled from the pulp. The seeds were removed from the pulp and rinsed in de-ionized H_2O

Frozen samples were thawed, ground and mineral elements of the whole berries were sent to a commercial laboratory for analysis. The samples were digested with nitric acid and finished with hydrochloric acid. Then they were analysed by inductively coupled plasma atomic emission spectrometry (ICPAES).

5.1.2 Tracing of xylem transport with dyes

At weekly intervals during grape enlargement and ripening, shoots with one or two bunches were cut from Shiraz grapevines (as described above) in the Charles Sturt University Vineyard, Wagga Wagga, New South Wales, during the 2000-2001 growing season. The cut end of the shoot was rapidly immersed into deionized water and recut again. The shoots were taken to the laboratory and transferred to a 0.1% aqueous solution of acid fuchsin. The shoots were left to transpire from 30 minutes to 24 hours. Berries were cut either transversally or longitudinally in paraffin oil. Sections were observed with bright-field optics in an SZ-PT Olympus microscope.

In a separate study, two other tracers were applied to potted 4 year-old own-rooted Shiraz PT23 vines. These were Lucifer Yellow-CH (LyCH) and Sulphorodomine G (SRG), both of which trace xylem flow. The dyes were applied by threading a piece of cotton through the top of the pedicel. One end of the thread was placed in the vial containing the dye while the other end was left to evaporate. The dye was applied for 12 hours. Berries were hand sectioned into four components: upper brush, longitudinal through the berry, peripheral across the berry, and the torus. Sections were viewed by florescence microscopy (Nikon eclipose 1000, Nikon, Tokyo) under bright field, green light (SRG) and blue light (LyCH).



Figure 5.1.2.1 Application of dye tracer to grape pedicel to monitor xylem transport. Photo courtesy of N. Dimos.

5.1.3 Central and peripheral xylem structure

Berry central and peripheral vascular bundles were cleared at the pre-veraison and post-veraison stages of development. Hand sections of two berries were immersed in lactophenol and stored overnight in a sealed container at 4˚C. The berries were viewed under bright-field on a Nikon Microscope.

5.1.4 Berry pedicel girdling and excision

Potted Shiraz vines were utilized in this study. These vines, which were self-grafted as part of a larger study, were in their third season of growth and first season of fruit with roughly 2 bunches per shoot. The vines were trained to three upright shoots in a bird proof enclosure, and the 26 L pots were fertilised monthly with a complete liquid fertiliser. The vines were exposed to two water treatments: (1) daily watering, and (2) deficit irrigation, where from fruit set on, watering to field capacity occurred at the point of leaf wilting. One berry from each of two bunches was chosen at random from 4 vines for successive height and width measurements using hand callipers. Measurements were made at least twice weekly in the early morning from 33 to 95 DAF at which point the bunches were harvested at roughly 22° Brix. A berry pedicel girdling and excision treatment was given at 67 DAF. Using the same bunches as above, two berries were randomly chosen for each of the treatments. Girdling was achieved by manually scraping a 2-3 mm ring around the pedicel with the blunt end of a razor until the tough tissue was reached. Subsequent transverse sections of these girdled pedicels were viewed by microscopy and verified that all phloem was removed and there was minimal damage to the xylem. Excised berries were resuspended on the bunch using cotton thread tied to the pedicel. The pedicel was dipped in mineral oil to prevent water loss from this tissue.

5.1.5 Bunch stem sap flow

Stem-flow gauges (Dynamax SGA2 and SGA3, Dynamax Inc, Houston, Texas) were used to measure sap flow through grape bunch stems during the 2003-2004 season. The gauges monitor power transfers from a heater strip to the ambient, to the stem, and to the flowing sap. The advantage of the stem heat balance method is that it requires no calibration or stem intrusion by temperature probes. Sap flow was computed by a formula using the heat applied to the stem, the radial energy from the stem, and temperature differences of sap above and below the strip heater.

The sap flow gauges were placed around the bunch stem of Shiraz vines grown in a glasshouse. Sap flow was logged every 10 minutes throughout the day. HOBO® (Onset Computer Corp, MA) relative humidity and temperature data loggers were placed within the canopy of the vines. Correlations between vapour pressure deficit (VPD) and sap flow were carried out using Sigmaplot (version 8.0).

5.2 Wax morphology and transpiration

5.2.1 Scanning electron microscopy of berry waxes

Shiraz berries were sampled during the 1999-2000 season at random from the field vines described above, taking care not to make contact with the surface of the berry. cvs. Cabernet Sauvignon and Chardonnay berries were also sampled from the same vineyard. Some berries were plunged into liquid nitrogen, and 1 cm^2 skin sections were freeze dried, attached to aluminum stubs, and sputter coated with gold at 0.05 mbar and 25 mA for 3 minutes prior to scanning electron microscopy (SEM). In a second method, a 1 cm^2 slice was cut from each berry, attached to a flat brass stub, quick-frozen at approx. -170˚C on the cold stage of a Jeol 6400 SEM, gold-coated then observed.

5.2.2 Quantification of epicuticular wax

Fifty berries from 3 bunches of each sampling date of the 1999-2000 season were weighed and briefly rinsed in water to remove dirt and dust. They were dried on filter paper and then dipped in chloroform three times in separate vials (30 s, 10 s, then 1 s). Chloroform extracts were combined in tarred vials and allowed to evaporate at 22˚C under nitrogen flux overnight, they were then dried at 40˚C for 5-7 days until constant weight. Water-soluble exudates were partitioned from the waxes by dissolving in water (5 mL), then chloroform (5 mL). The water layer was removed with a pipette and the chloroform layer was dried as described above.

5.2.3 Transpiration of detached berries

Berries of 5 developmental stages (6 replicates) covering pre-veraison to shrivelled were harvested at random from the field vines described above during the 2001-2002 season. Height and width of each berry was measured with callipers and surface area was estimated according to the modified equation for a sphere: surface area = π(width)(height). Pedicels were dipped in paraffin wax to prevent water loss from this exposed surface. Berries were suspended in an incubator at 25˚C with silica gel to maintain a constant 23% RH without air turbulence. Berries were weighed twice daily over 3 days and transpiration rates were calculated from the slope of the linear regression of weight loss over time (the r^2 of each slope was 0.994 or higher), and taking into account an average daily respiration rate of 1.9 mg CO_2 per berry.

5.2.4 Transpiration of attached bunches

A bunch chamber was constructed from a 3 L poly-propylene 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 in and out of the bunch chamber was monitored by an infra-red gas analyser in an open configuration. The LCA4 monitored chamber air temperature and the incoming and outgoing chamber water vapour pressure.

Six vines grown in pots in a glasshouse were used to measure bunch transpiration over the season. Measurements were taken twice weekly on one bunch per vine for all six vines at a VPD of 1.0 KPa. VPD was controlled by a water bubbler placed in a refrigerated circulator bath. All measurements were carried out from 0600-1100 hours. Bunch transpiration rates were averaged over a ten minute period.



Figure 5.2.4.1 Open gas exchange system with bunch chamber to monitor transpiration of grape bunches during development. Photo courtesy of N. Dimos.

5.3 Backflow

5.3.1 Diurnal contraction

Fruit growth sensors (FI-XSM, PhyTech, Israel) was used to monitor growth of berries during the season. The sensor includes a linear displacement transducer (LVDT) and is supplied with an electronic signal conditioner. The sensor was attached to a berry within the bunch and logged every minute throughout the day.



Figure 5.3.1.1 FI-XSM PhyTech LVDT sensor attached to post-veraison berry. Diurnal trends in volume were monitored throughout development.

5.3.2 Dye tracers

The apoplastic dye, acid fuchsin, and the symplastic dye, 5(6)-carboxyflourescein diacetate (CFDA) were applied by threading cotton through the brush of the berry, or below the seeds in a similar manner as described in 5.1.2. Treatments were applied pre-veraison, at veraison, post-veraison and post-weight maximum. The dye was applied for 16 hours, berries were then hand sectioned and viewed by fluorescence microscopy.

5.4 Cultural impacts on berry weight loss

5.4.1 Nitrogen and Irrigation

Potted Shiraz (clone PT23) vines were utilised in this study. These vines were on their own roots, their second season of growth, first season of fruit and grown in a medium of river sand: loam: peat moss at 2:2:1. The vines had two shoots that were trained upright with one bunch per plant and were placed in a bird proof enclosure. The pots were 11 L in size and those used for the SRI treatment had a partition down the middle. The vines were exposed to three N application treatments and three irrigation treatments in a factorial design. After an initial base application of 0.5 g prior to bloom (14 November), a total of 0 g, 1.5 g or 3.0 g of N was applied as NH_4NO_3 at 8 intervals from bloom to veraison. Vines were either daily irrigated (STD), deficit irrigated (DI), or exposed to daily irrigation of half the root zone. In this split-root irrigation (SRI) treatment, irrigation was alternated weekly between the two halves of the split roots from bloom to harvest. The DI treatment was started at bloom (18 November), where at the first sign of leaf wilting water was applied to field capacity. This treatment was terminated at the beginning of veraison (9 January) and followed with STD irrigation until harvest. The SRI treatment was initiated at bloom and terminated at harvest. One berry from one bunch was chosen at random from 5 vines per treatment for height and

width measurements twice weekly using hand callipers, to calculate berry volume. Shoot length was measured at harvest, and yield components were determined by weighing the bunches and counting the number of berries per bunch. In this study, 50% bloom occurred at 18 November, 2000 and 50% veraison occurred 61 days subsequent to this (18 January, 2001). Volume was last measured at 102 days after flowering (DAF, 28 February) and berries were harvested at 118 DAF (16 March).

5.4.2 Rootstock and Irrigation

Three year-old potted Shiraz vines (clone PT23) grafted to own roots, Ramsey or 101-14 Mgt were used for this component of the study. The vines were trained to three upright shoots in a bird proof enclosure, and where possible selected for two bunches per shoot. The 26 L PVC pots were fertilised monthly with a complete liquid fertiliser (MEGAMIX PLUS®, Rutec, Tamworth, Australia), providing approximately 4g nitrogen during the season plus other nutrients. The vines were exposed to two irrigation treatments: daily irrigation (STD) and deficit irrigation (DI), where from fruit set on, watering to field capacity occurred at the point of leaf wilting. In this study, 50% bloom occurred at 12 November, 2000 and 50% veraison occurred 57 days subsequent to this (8 January, 2001). The first drying cycle of the DI treatment commenced on 12 December, and continued on an average of seven day cycles for the remainder of the season. However, if leaf wilting occurred prior to the 7 day interval the vines were watered. Leaf photosynthesis and stomatal conductance were measured with a portable LCA-4 system (Analytical Development Company, Hoddesdon, Herts, England) as a non-destructive indicator of plant water status. Measurements were taken midway through the drying cycle of the last fully expanded leaf at mid-morning. Across the three rootstocks, photosynthesis averaged at 9.83 in the STD treatment and 4.12 μmol CO_2 $m^{-2}s^{-1}$ in the DI treatment. Stomatal conductance (g_s) averaged at 231 in the STD treatment and 56 mmol H_2O $m^{-2}s^{-1}$ in the DI treatment. To assess rootstock and irrigation effect on berry growth, one berry from each of two bunches was chosen at random from 4 vines for successive height and width measurements. Measurements were made at least twice weekly in the early morning from 33 to 95 DAF at which point the bunches were harvested (15 February).

6. Results

6.1 Xylem and phloem inflow

6.1.1 Element accumulation 1999-2003

Budburst was early, at 24 August, in the 1999-2000 season. There were no extreme hot periods during this season and monthly rainfall was average (Figure 6.1.1.1). The flowering period extended over 24 calendar days (1 November to 25 November), and veraison extended over 12 days (13 January to 25 January). Sixty eight days passed between 50% flowering and 50% veraison (6.5 ˚Brix), and 115 days passed between 50% flowering and harvest (March 6).

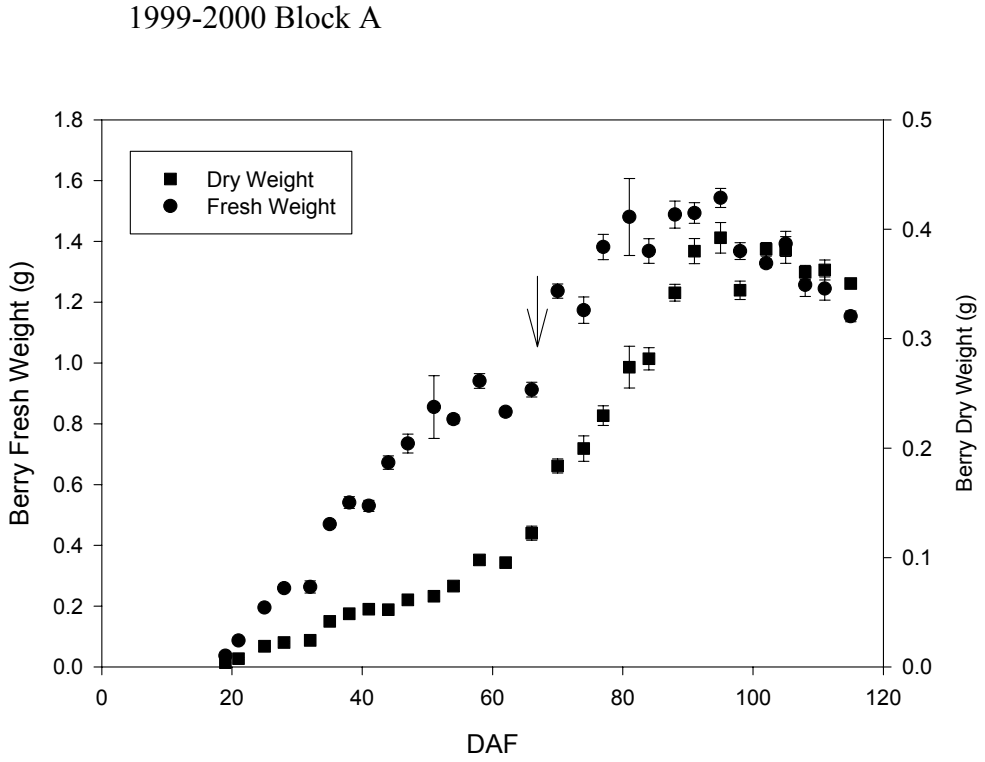


Figure 6.1.1.1. Berry fresh and dry weight during development and ripening of Shiraz berries. Arrow points to 50% veraison date. Rogiers et al., 2000.

Berry fresh weight increased with a lag phase before veraison and was at a maximum between 81 and 95 days after flowering at 1.6 g (Figure 6.1.1.1). Through the fresh weight plateau berry ˚Brix increased from 15.5 to 21.7. Fresh weight then declined to 75% of the maximum by harvest and ˚Brix levels climbed further to 25.7. The lag in fresh weight accumulation before veraison was not accompanied by a lag in dry weight accumulation. Post-veraison the rate of dry weight accumulation was faster than pre-veraison and a maximum occurred at 95 days after flowering at 0.39 g/berry. The dry weight maximum occurred 14 days after the onset of the fresh weight plateau. Dry weight then declined to 90% of maximum values by 115 days after flowering. Based on an average daily respiration rate of 1.9 mg CO_2 evolution per berry (Niimi and Torikata, 1979) this decline in dry weight can be attributed to respiratory loss.

In the 1999-2000 season, K accumulation into the berries was slow pre-veraison at 0.02 mg/day and increased 3.5-fold this rate post-veraison (Figure 6.1.1.2). Accumulation of this mineral was well described by a logistic equation (r^2=0.96). On a per berry basis, K increased from 0.18 mg at 19 days after flowering to 5.6 mg at 115 days after flowering. Ca accumulation was linear throughout the entire developmental and ripening period (r^2=0.83) and increased from 0.07 mg/berry at 19 days after flowering to 0.66 mg/berry at 115 days after flowering (Figure 6.1.1.2). Ca accumulation during the season was much more variable than K accumulation. This may be explained by variations in xylem flow which is dependent on the water-potential gradient from the soil through the vine to the atmosphere. There was a 3.4 fold increase in the K/Ca ratio

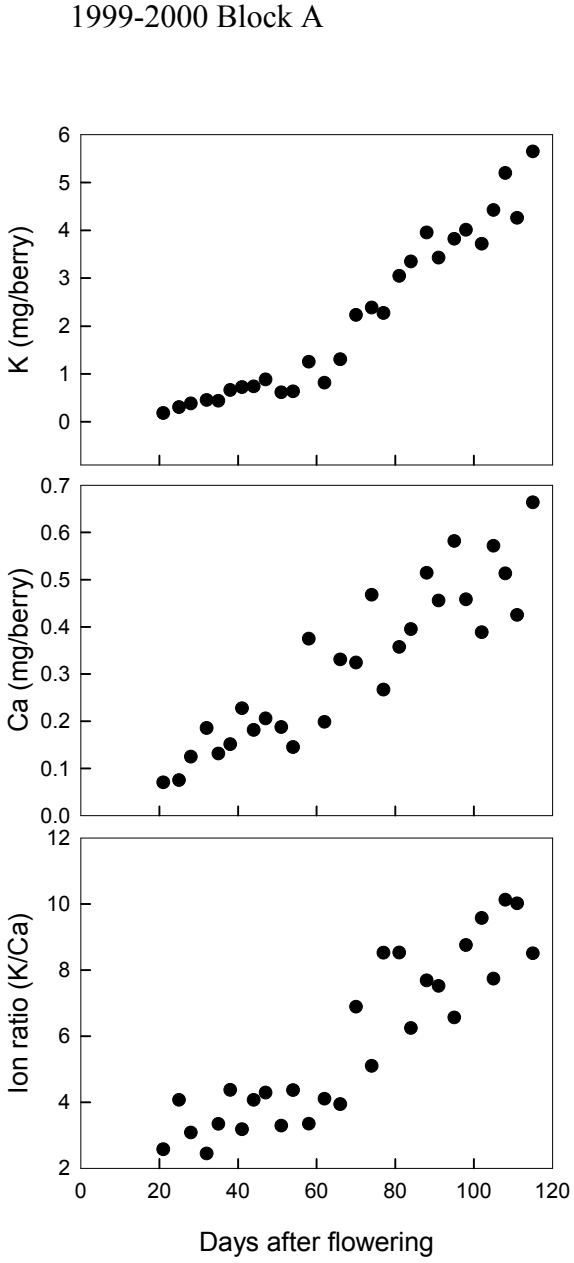


Figure 6.1.1.2: K content, Ca content and K/Ca ratio of Shiraz berries during development and ripening in the 1999-2000 season. Rogiers et al., 2000.

In 2000-2001, increase in fresh weight of Shiraz berries from Block A followed a typical double sigmoid growth curve with veraison occurring at 55 DAF (Jan 13, 2001) (Figure 6.1.1.3). Subsequently weight loss set in at 87 DAF. These berries were harvested 94 DAF (21 February) when the fresh weight was 1.5 g and the soluble solids was 24.1 °Brix. The trend in TSS accumulation was very similar to the fresh weight accumulation curve, except near harvest when fresh weight accumulation ceased (Figure 6.1.1.3). The 2000-2001 season was the hottest on record in Wagga and this may be why sugar accumulation rates were very high and berries reached an adequate Brix level (24.1) within 94 days of flowering.

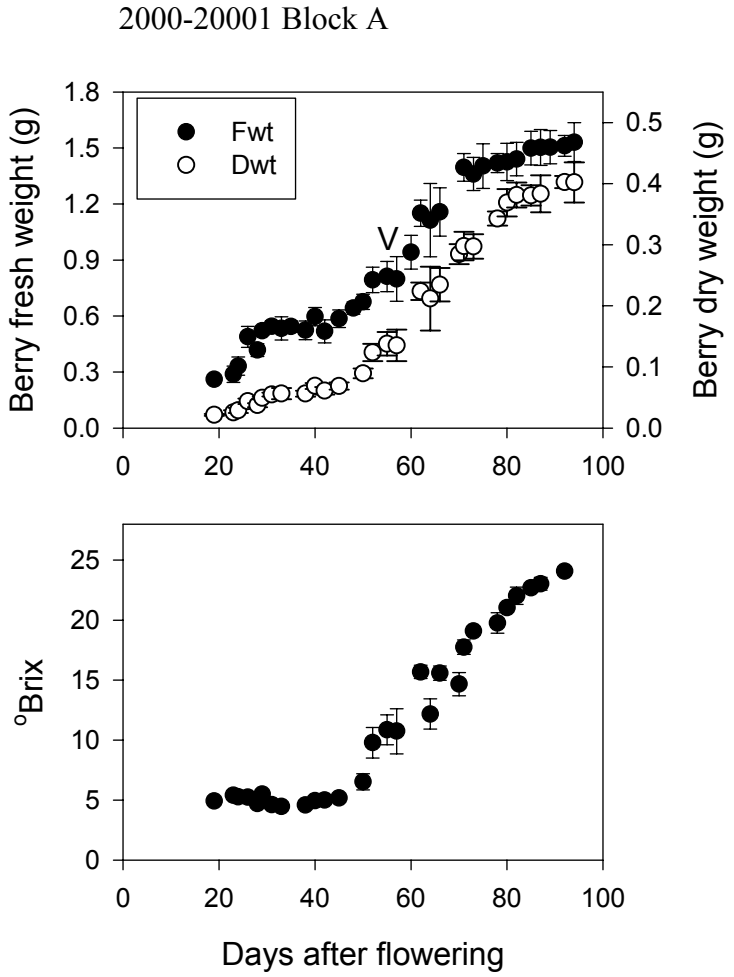


Figure 6.1.1.3: Fresh weight (g) and TSS (°Brix) during development and ripening of cv. Shiraz berries from field vines of Block A in 2000-2001. V=veraison. Bars indicate standard error of the means.

Phloem mobile elements accumulation

The phloem mobile elements K, P, S and Mg accumulated in a linear fashion from 20 to 70 days after flowering during the 2000-2001 season (Figure 6.1.1.4). Between 70 and 95 DAF accumulation tapered off, except for S where accumulation continued. Over the entire sampling period (75 days), total amount of these elements per berry increased from 6 to 10.5 fold depending on the element. Because Shiraz berry volume increases and then decreases during ripening the concentration of an element (mg of element per kg of fresh berry tissue) is often different from trends in the accumulation of that element. For instance, while post-veraison amounts of S and Mg per berry were higher than pre-veraison amounts, the concentrations of these elements within the berry were lower post-veraison than pre-veraison (Figure 6.1.1.5).

The elements accumulated strongly in the pulp and skin until 90 DAF (the last sampling date for the partitioning analyses), however accumulation had tapered off in the seeds between 80 and 90 DAF for P, K, and Mg and between 50 and 60 DAF for S (Figure 6.1.1.6). K and Mg levels did not increase in the torus during ripening, while P and S levels declined. Amounts in the brush either increased (Mg), decreased (K), or did not alter (S, P).

Xylem mobile elements accumulation

The xylem mobile elements Ca and Mn, accumulated strongly until 50 DAF (Ca) or 69 DAF (Mn) during 2000-2001, and beyond this tapered off (Figure 6.1.1.4). Ca levels per berry decreased after veraison until 94 DAF and this was followed with an upsurge. Total accumulation into the berry increased 3.7 fold for Ca, and 2.3 fold for Mn. The concentrations of these elements within the berry, however, decreased until 70 DAF and then remained stable (Figure 6.1.1.5). The seeds accumulated most of the Ca and Mn (Figure 6.1.1.6). There was a downward and then an upward trend in the pulp, skin and brush. The amount in the torus also increased at 92 DAF for both elements. Amounts of Ca in the brush remained at 1%, and in the torus at 10%, of the total Ca in the berry.

Variable phloem mobile elements accumulation

B, a variable phloem mobile element, accumulated nearly linearly except for a lag phase prior to veraison and then tapered off at 82 DAF (Figure 6.1.1.4). Total accumulation increased by 15.7 fold over the 75 days. Like the phloem mobile elements, amounts were highest in the pulp and the skin. Accumulation into the seed continued until 92 DAF and there was a downward trend in the amounts of this element in the torus and the brush (Figure 6.1.1.6).

B is considered to have conditional or low mobility in the phloem (Shelp et al., 1995). It is primarily transported in the xylem but is re-translocated in phloem in the sink organs that do not readily transpire (Shelp et al., 1995). Grape berries generally do not transpire at very high rates and that is likely why it behaved as a phloem mobile element in this study.

Cu and Fe are other variable phloem mobile elements. Cu accumulated in a similar manner to P. In 2000-2001, Fe accumulated in a similar fashion as Mn, but in this case accumulation occurred in the pulp, not the seed (Figure 6.1.1.6). Fe and Mn did not accumulate in the subsequent seasons (data not shown). Na did not accumulate over time and amounts were quite variable in the 2000-2001 season.

2000-2001 Block A

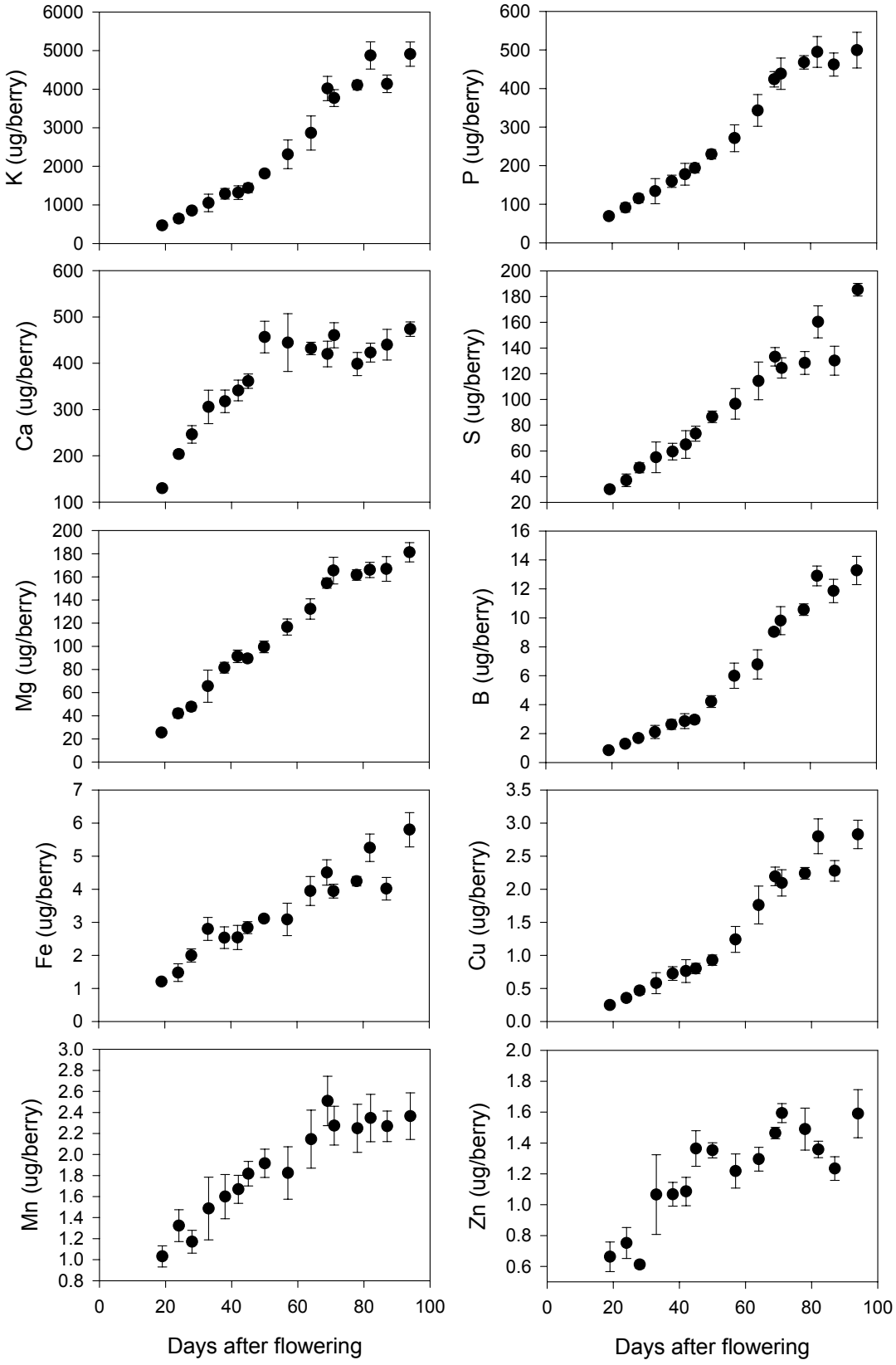


Figure 6.1.1.4. Accumulation of elements (µg berry^{-1}) in berries from field vines of Block A in 2000-2001. Bars indicate standard error of the means.

2000-2001 Block A

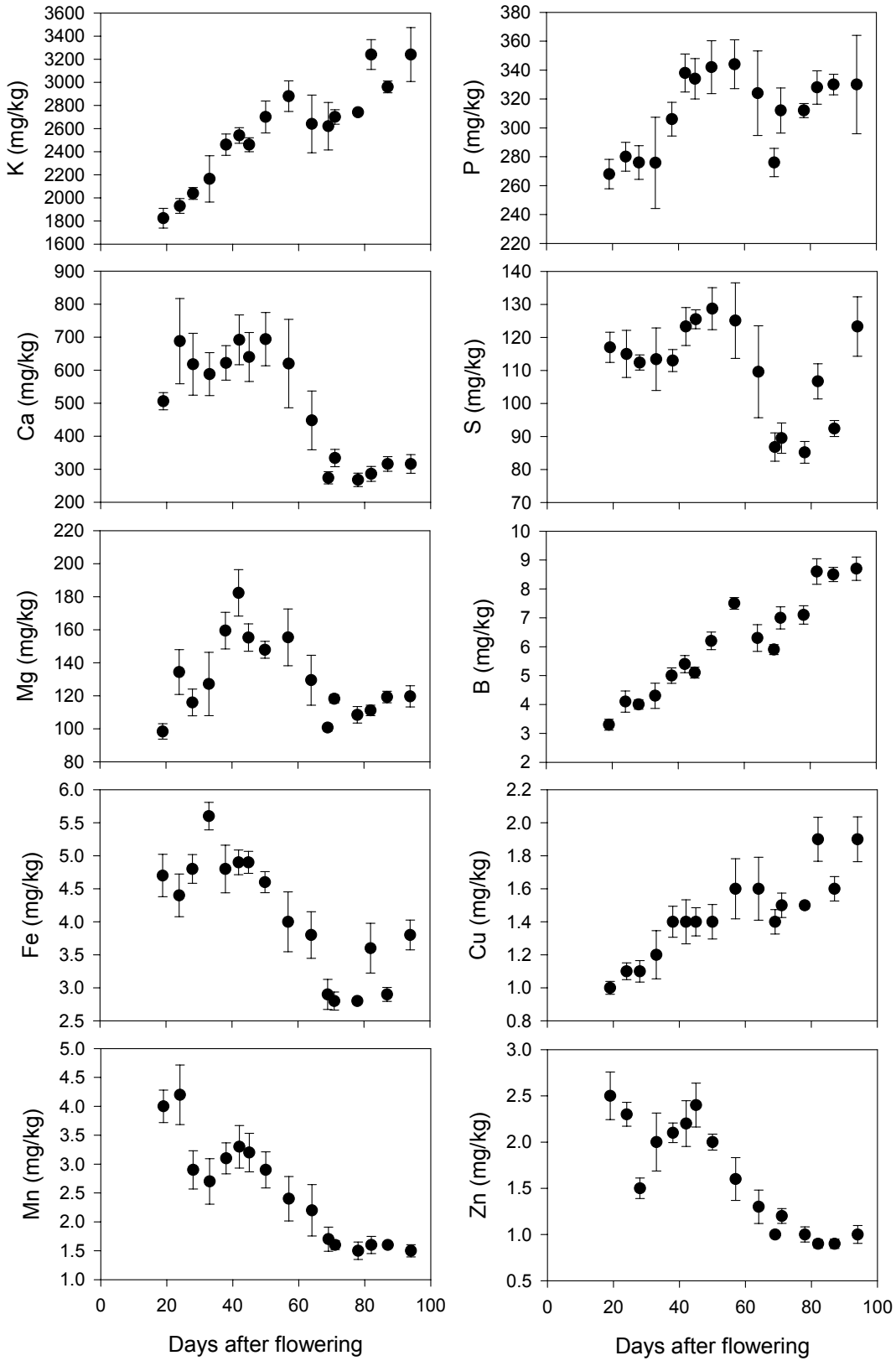


Figure 6.1.1.5. Changes in concentration of elements (mg kg^{-1}) in berries from field vines of Block A in 2000-2001. Bars indicate standard error of the means.

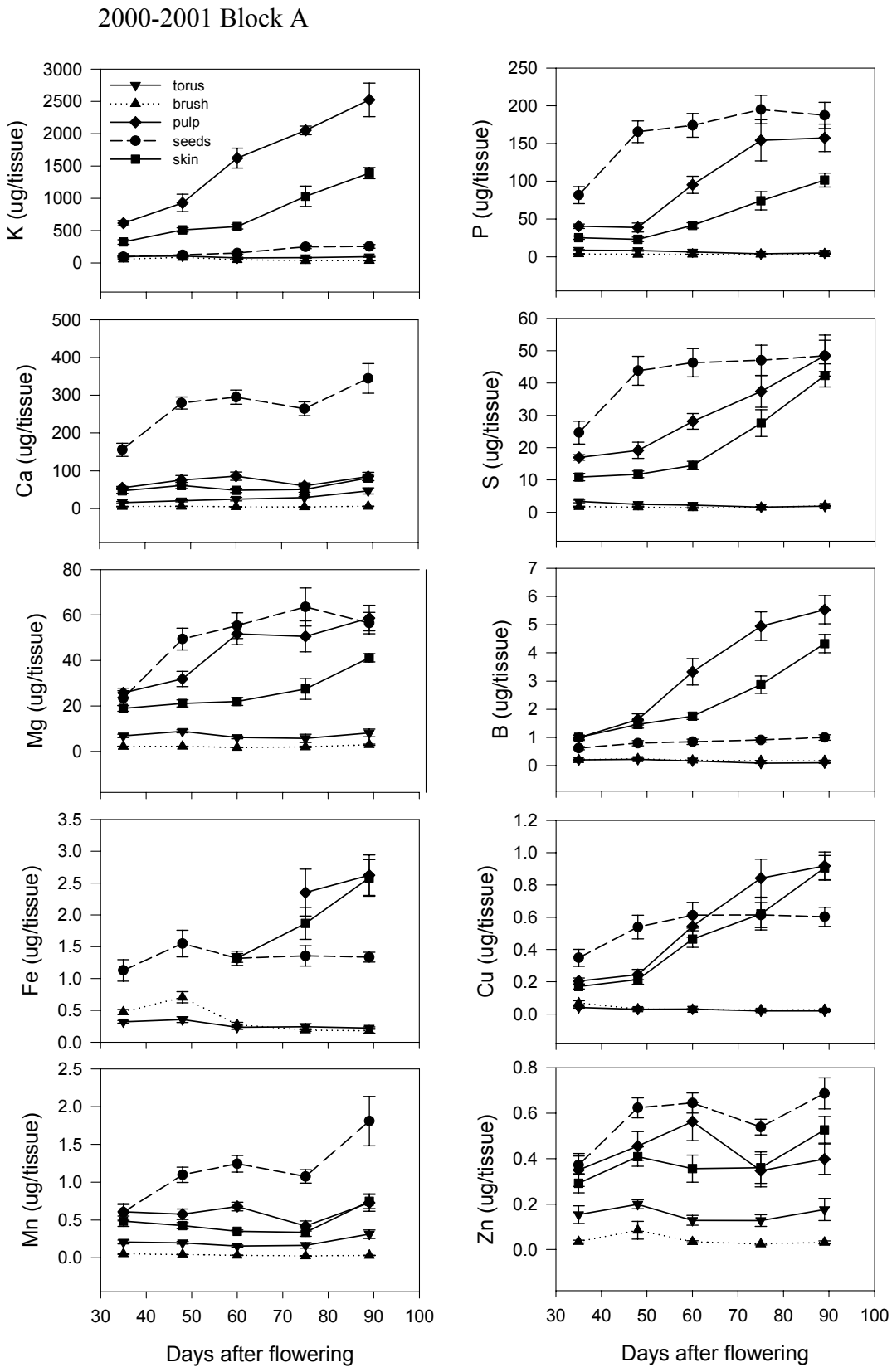


Figure 6.1.1.6. Accumulation of elements (µg tissue^{-1}) in torus, brush, pulp, seeds and skin during development of berries from field vines of Block A in 2000-2001. Bars indicate standard error of the means.

Relative order of elements at harvest

By the 2001 harvest, K was the most abundant element at 4.91 mg/berry (Figure 6.1.1.4). This is equivalent to 0.3 % of the berry's fresh weight. P and Ca were the next two most abundant elements at harvest (499 and 473 µg/berry respectively), approximately one tenth of the K amounts. S and Mg levels were similar at 185 and 181 µg/berry, respectively. This was followed with B (13.2), Na (12.4), Fe (5.8), Cu (2.8), Mn (2.3) and Zn (1.6 µg/berry). Six other elements were detected in minute amounts in the Shiraz berries. They include: zinc (0.7 to 3.2 ppm), aluminium (<1 ppm), nickel (<0.3 ppm), molybdenum (<0.2 ppm), cobalt (<0.2 ppm), cadmium (<0.09 ppm).

The amounts and concentrations of the elements were compared for berries at the same ripening stage (22 °Brix) over 2 seasons from Block A and 2 seasons from Block B. Even though trends in accumulation and concentration were fairly constant, actual amounts and concentrations varied between seasons and blocks (Table 6.1.1.1).

Table 6.1.1.1: Amount (µg/berry) and concentration (mg/kg) of elements in Shiraz berries at 22 Brix over 3 seasons.

	2000-2001 (Block A)		2001-2002 (Block A)		2001-2002 (Block B)		2002-2003 (Block B)	
	µg/berry	mg/kg	µg/berry	mg/kg	µg/berry	mg/kg	µg/berry	mg/kg
K	4873 ±352	3240 ±128	2217 ±323	2338 ±121	3574 ±247	2195 ±53	3140 ±165	2288 ±79
P	495 ±40	328 ±12	302 ±54	313 ±19	498 ±35	306 ±10	327 ±31	236±16
Ca	423 ±20	286 ±22	448 ±44	484 ±39	570 ±64	348 ±24	367 ±23	267±11
Mg	166 ±7	111 ±3	165 ±16	177 ±8	264 ±15	163 ±5	129 ±7	94±3
S	160 ±12	107 ±5	211 ±31	229 ±31	370 ±76	229 ±47	99 ±8	72±6
Na	11 ±2	7 ±1	24 ±5	24 ±2	174 ±25	110 ±20	54 ±3	39.1±0.4
B	12.9 ±0.7	8.6 ±0.4	21 ±5	21 ±5	32 ±7	20 ±4	35 ±5	25 ±2
Fe	5.3 ±0.4	3.6 ±0.4	2.8 ±0.4	3.2 ±0.6	4.6 ±0.6	2.8 ±0.2	6.0 ±0.5	4.4 ±0.3
Cu	2.8 ±0.3	1.9 ±0.1	1.8 ±0.5	1.8 ±0.3	2.2 ±0.2	1.4 ±0.01	0.35 ±0.04	0.25±0.02
Mn	2.3 ±0.2	1.6 ±0.1	2.1 ±0.4	2.2 ±0.3	1.4 ±0.1	0.83 ±0.06	1.1 ±0.1	0.8±0.1

In the 2001-2002 season, berries from Block A were relatively small compared with Block B (Figures 6.1.1.7 and 6.1.1.8). In Block A, 50% veraison occurred at 60 DAF and the berry weight maximum was 1.06 g at 87 DAF. Berry fresh weight decreased to 0.92 g (14%). Berries from Block B were harvested at 119 DAF (1.2 g) and a weight maximum point (1.5 g) was defined at 103 DAF, 36 days after 50% veraison (Figure 6.1.1.8). Weight loss was 12% over the 16 days prior to harvest. Trends in accumulation of berry TSS were not different between Block A or B. At the weight maximum, berries were 21.1 °Brix and this increased to 24.0 °Brix by harvest.

Berries from Block B were smaller during the 2002-2003 season as compared to the previous season (6.1.1.9). Veraison occurred at 68 DAF (January 9) and the berry weight maximum occurred at 87 DAF (January 27, 03). Berry weight then declined from 1.2 g to 1.0 g (17%) over the subsequent 16 days before harvest (February 12, 03). At the weight maximum berries were 21.9 °Brix and by harvest berries were 26.6 °Brix. Sugar per berry (°Brix multiplied by berry weight) ceased to accumulate after the weight maximum.

In the four sets of vines examined from 2001-02 and 2002-2003, Ca accumulation was strong until veraison. Following veraison, the Ca levels per berry decreased by about 100 µg every season until the weight maximum (Figures 6.1.1.7 to 6.1.1.9). At the weight maximum, Ca accumulation was positive again until harvest. This was not as clear in Block A of the 2001-2002 season because of the early harvest (Figure 6.1.1.8). Prior to the upsurge there was a short, but pronounced spike in Ca amounts per berry (Figures 6.1.1.7 and 6.1.1.9), but these deviations from the curve were not significant at the 5% level.

In the four sets of vines examined from 2001-02 and 2002-2003, there was a stronger emphasis in post-veraison accumulation of K as compared with pre-veraison accumulation (Figures 6.1.1.7 to 6.1.1.9). Cessation in K accumulation occurred at the weight maximum in all four sets of vines (Figures 6.1.1.7 to 6.1.1.9).

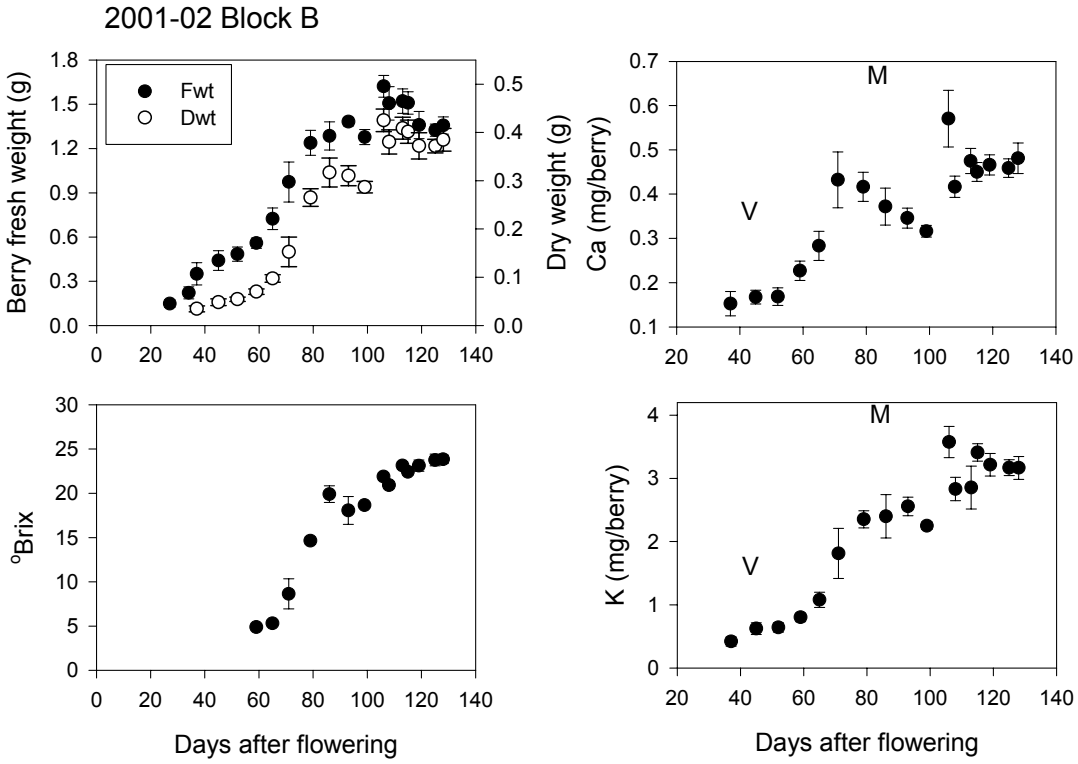


Figure 6.1.1.7. Changes in berry fresh weight (g), TSS (°Brix), Ca (µg berry^{-1}) and K (µg berry^{-1}) during development and ripening of cv. Shiraz berries from field vines of Block B in 2001-2002. V=veraison.

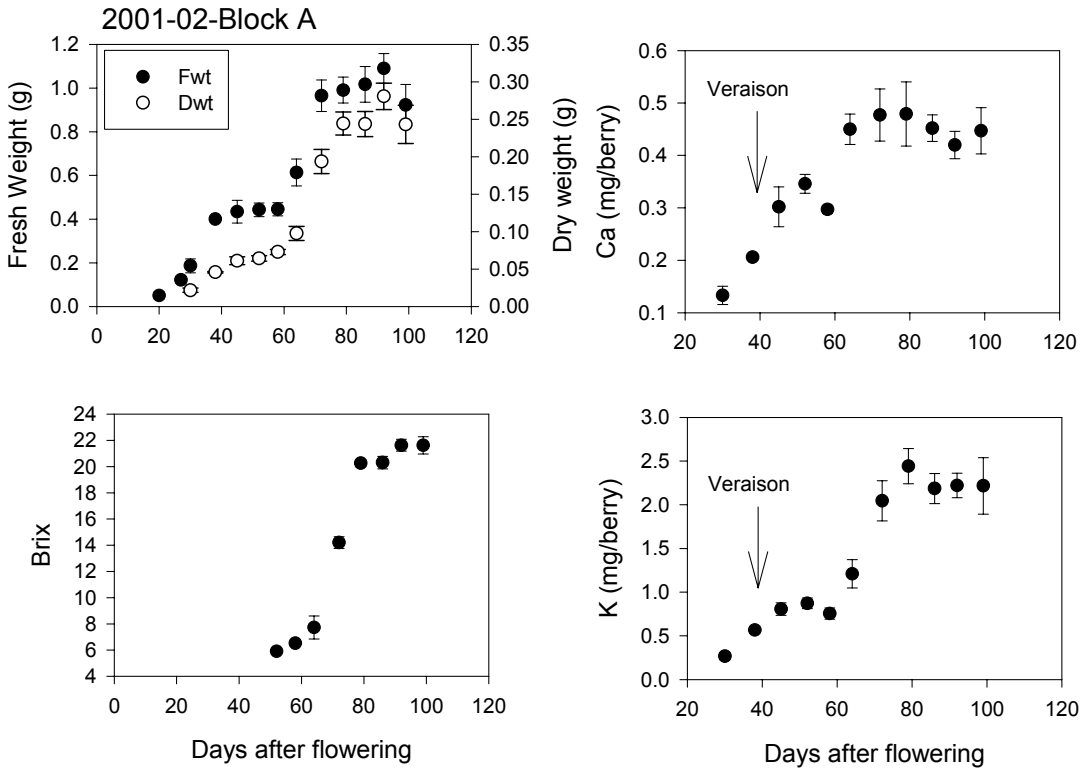


Figure 6.1.1.8. Changes in berry fresh weight (g), TSS (°Brix), Ca (µg berry^{-1}) and K (µg berry^{-1}) during development and ripening of cv. Shiraz berries from field vines of Block A in 2001-2002. V=veraison.

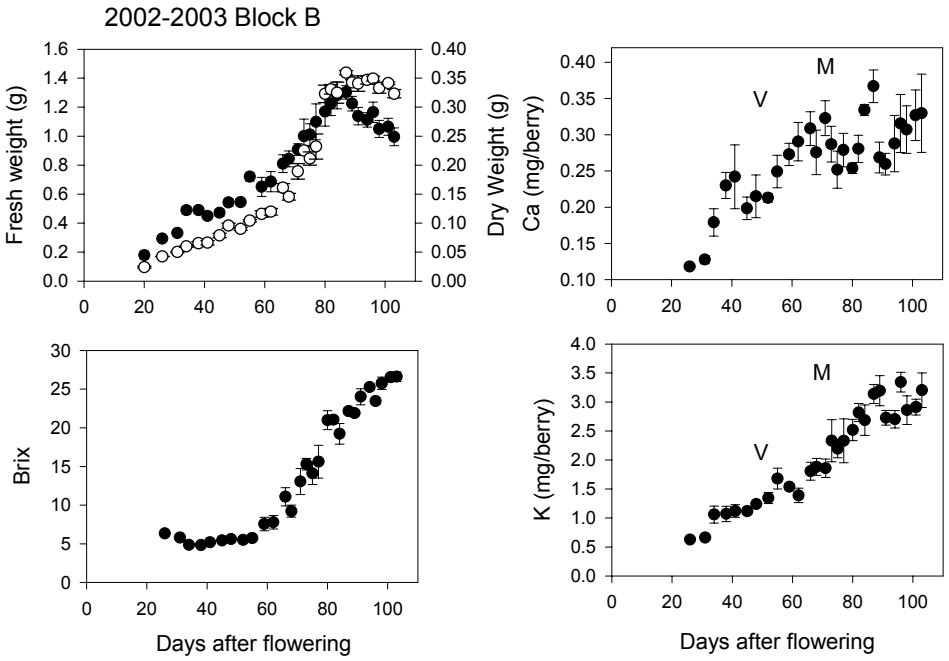


Figure 6.1.1.9. Changes in berry fresh weight (g), TSS (°Brix), Ca (µg berry^{-1}) and K (µg berry^{-1}) during development and ripening of cv. Shiraz berries from field vines of Block B in 2002-2003. V=veraison.

6.1.2 Dye tracers

The movement of the xylem tracer, acid fuchsin, through the shoot was easily visible by the naked eye. The tracer appeared in the leaf veins within half hour of application. In pre-veraison berries the dye accumulated in the axial and peripheral vascular bundles (Figure 6.1.2.1A and B). The accumulation had an uneven and patchy distribution and so it was difficult to trace the path of movement through the berry. Post-veraison the dye accumulated only in the axial bundles between the pedicel and the seeds (Fig 6.1.2.1C). It did not move down the axial bundles past the seeds, nor into the peripheral bundles. At and after maximum berry weight dye accumulation was only in the brush area as in berries directly at and after veraison. We compared the behaviour of Shiraz berries to two other varieties: Merlot and Muscat Gordo Blanco. The movement of the xylem tracer in berries of both these varieties was identical to that of Shiraz berries. Pedicels also accumulated dye and this was seen in both pre and post-veraison berries in all three varieties. In very immature berries the dye in the receptacle radiated outward to the epidermis to form a red band visible from the exterior. This may be the result of the staining of the remnants of the bundles that radiated out to the flower parts.

The xylem tracers, SRG and LyCH were applied to berry pedicels at three stages of development and in two irrigation regimes (Table 6.1.2.1). Regardless of stage of development or irrigation regime the dye had moved from the pedicel to the brush region of the berry. The dyes, however, were not always present in the peripheral or central vascular bundles. SRG did not cross in to the central or peripheral strands post-weight maximum, and was also absent from the peripheral strands at veraison in the deficit irrigation treatment. LyCH was absent from the central bundles at veraison and post-weight maximum in the deficit irrigation treatment, but was present in these strands in the standard irrigation treatments. LyCH was absent from the peripheral strands post weight maximum in the standard irrigation treatment.

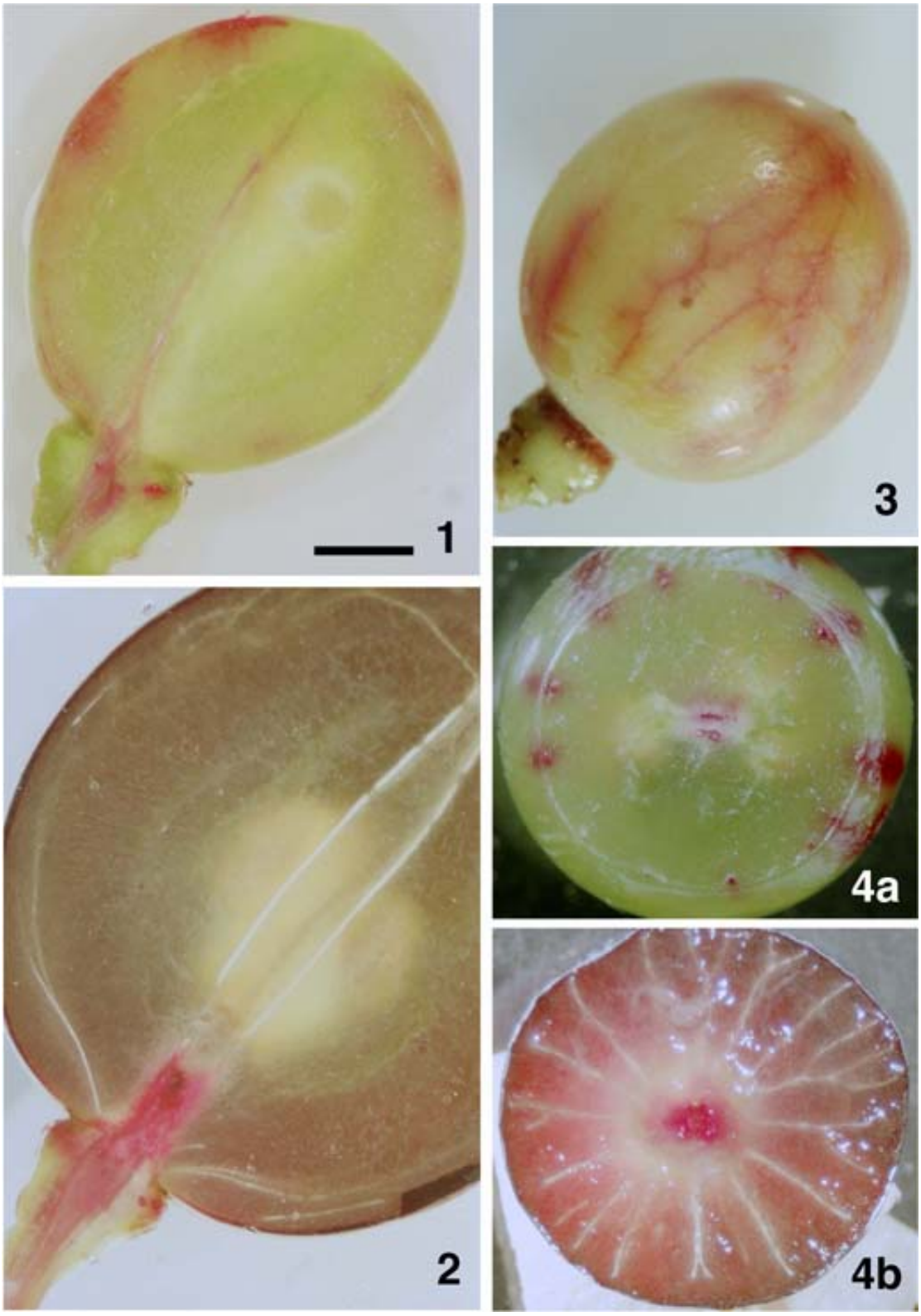


Figure 6.1.2.1: (1)Longitudinal section of a pre-veraison berry showing patterns of acid fuchsin accumulation. (2) Surface coloration of pre-veraison berry showing a patchy distribution of acid fuchsin in peripheral vascular bundles. (3) Longitudinal section of a post-veraison berry showing a restricted pattern of acid fuchsin accumulation within brush tissues. (4a) Transverse section of the distal end of pre-veraison berry. (4b) Transverse sectio of the proximal end of a post-veraison berry. Scale bar = 2mm.

					Tracer applied to berry pedicel								
				SRG					LyCH				
Tissue		**Control**		**Deficit irrigation**				**Control**			**Deficit irrigation**		
	Pre-ver	Veraison	Post-max	Pre-ver	Veraison	Post-max	Pre-ver	Veraison	Post-max	Pre-ver	Veraison	Post-max	
Pedicel	P	P	P	P	P	P	P	P	P	P	P	P	
Brush	P	P	P	P	P	P	P	P	P	P	P	P	
Central	P	½ P	A	P	P	B	P	P	P	P	A	A	
Peripheral	P	B	A	P	A	A	P	P	A	P	P	P	

Table 6.1.2.1: Tracer detection in Shiraz berries. Three tracers were used: SRG and LyCH which traces xylem water flow and CFDA which traces phloem water flow (next page). The dye was loaded into berry pedicels for 12 hours at either pre-veraison, veraison and post-maximum diameter under well-watered and deficit water conditions. The berry and corresponding branch was harvested and hand sections were employed to the four berry parts as described in figure 3.1b. The tissue was then viewed under fluorescent microscopy for tracer detection. P= present, A= absent, B= bleeding. Bleeding is defined as dye leakage, where the dye has spread from the xylem. Source: PhD Thesis, N. Dimos.

6.1.3 Xylem and phloem structure

Xylem and phloem of Shiraz berries were investigated for anatomical features which may lead to blockages in transport through either of these routes. The phloem sieve tube members of the pedicel, brush, central and peripheral bundles of pre-veraison, post-veraison and post-weight maximum appeared to be capable of transport since neither p-protein or callose plugs were evident (Figure 6.1.3.1). The xylem conduits of within the peripheral and central bundles of the pre-veraison berries were intact, however at veraison stretching of the spiral thickenings was evident in both the peripheral and central bundles (Figure 6.1.3.2). In the brush region of the pre-veraison berries blockages were visible within the xylem tracheids. These blockages were frequent in the post-veraison berry and post-weight maximum berry. Cell walls of the xylem also appeared thicker in the later developmental stage (data not shown).

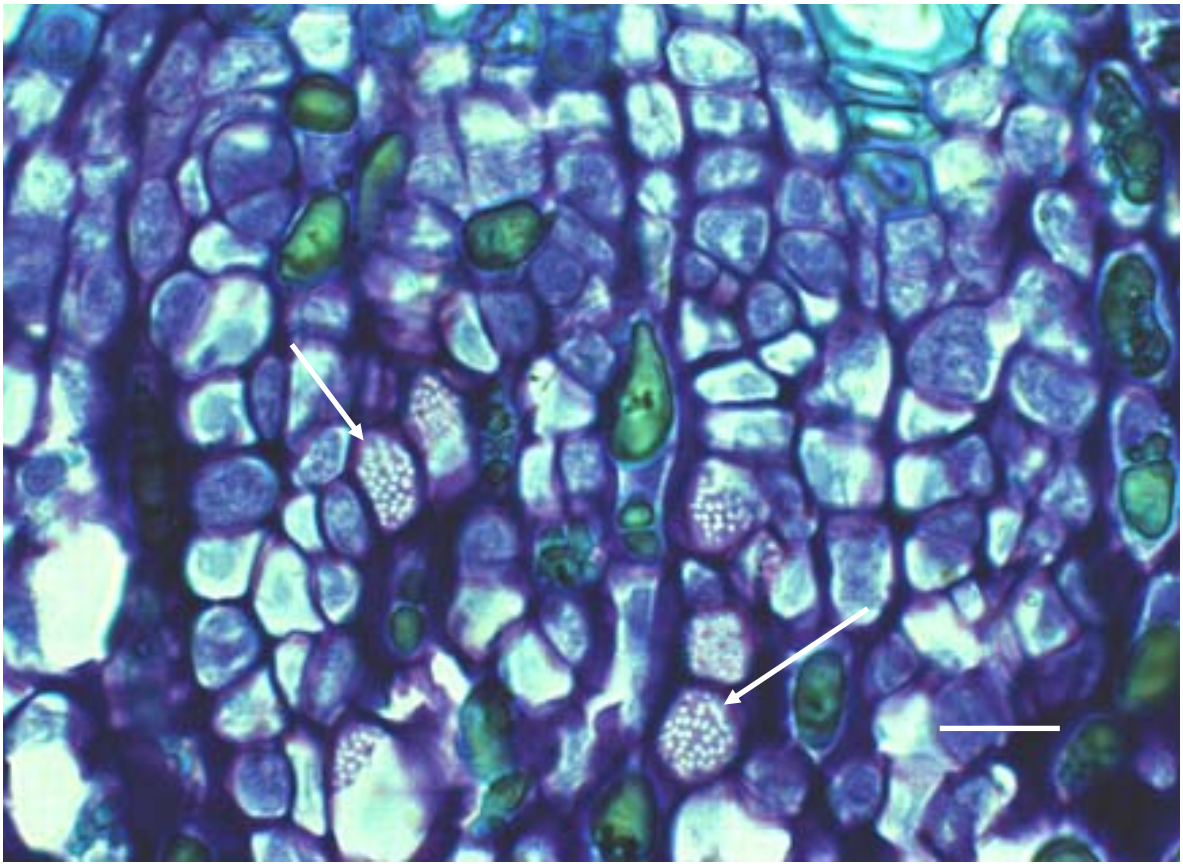


Figure 6.1.3.1. Vascular bundle in brush region of Shiraz berry at the weight maximum. Scale bar represents 550 µm. Note open pores of sieve plate. Source: PhD Thesis, N. Dimos.

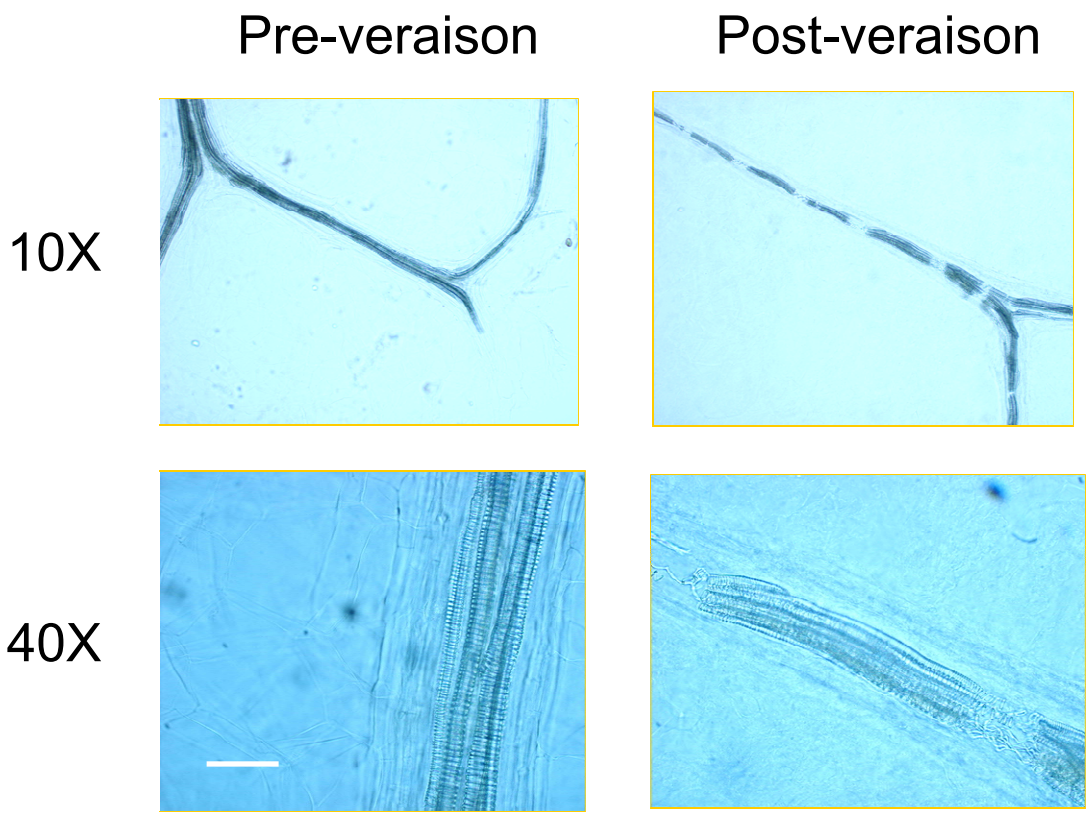


Figure 6.1.3.2. Peripheral vascular bundles of pre-veraison and post-veraison Shiraz berry. Scale bar represents 60 µm. Note stretching of spiral thickenings of xylem tracheids in post-veraison berry. Source: PhD Thesis, N. Dimos.

6.1.4 Pedicel girdling and excision

The objective of the water regime study was to determine if berry volume is responsive to water availability after veraison. We were particularly interested in determining if berries that had shrivelled during periods of water stress are capable of plumping up after veraison upon watering. Maximum and final berry volume was affected by watering frequency. Vines that were watered daily (control) had berries which reached 1.8 cm^3 while vines grown in deficit irrigation had berries which only reached 1.5 cm^3 (Figure 6.1.4.1). The control berries reached their size maximum at 70 to 80 DAF while deficit irrigation berries reached their peak at 80 to 90 DAF. Berry volume then declined in both treatments. Despite a greater loss in total volume for berries in the daily irrigation treatment, deficit irrigation berries were 10% smaller at harvest. Prior to veraison, berries exposed to deficit irrigation responded to the watering and drought cycles with expansion and shrinkage. A drop in berry volume occurred after 5 days without water (40 DAF) and this was followed with a jump in volume to match the size of the control berries when vines were watered daily (42 to 50 DAF). Veraison (50%) occurred at 57 DAF. At veraison the vines exposed to the deficit irrigation treatment had not been watered for 8 days and these berries were substantially smaller than the control berries. The lack of water did not affect the average date of veraison however. After veraison berry volume increased in both water treatments. In the deficit irrigation treatment there was a slight jump in volume upon watering (67 DAF) after a 5 day drought cycle. This occurred for every singly berry that was measured. Subsequent to this berry volume continued to increase despite 3 (60 and 64 DAF), 4 (70 DAF) or 5 (78 DAF) days without water. At the same time watering did not cause a jump in the rate of volume increase (81 DAF). At 88 DAF the downward trend in berry volume set in. At this point the berries had not been watered for 7 days but we cannot ascertain if volume would have dropped in spite of this.

The lack of response to the drought and watering cycles during later ripening is further evidence for hindered or discontinuous xylem flow into the berry. The continued increase in berry volume despite lack of irrigation may be an indication of replacement of water flow in the xylem to one in the phloem. Phloem flow is likely to be far less responsive to drought than is xylem flow. Phloem flow (sugar import) to the berry needs to be maintained despite water stress.

Berry pedicel girdling and berry excision resulted in an immediate drop in volume (Figure 6.1.4.2). The rate of volume decrease was more rapid for the excision treatment than the girdling treatment. Final berry volume was only 25% of the control for excised berries in both the daily watering treatment and the deficit irrigation treatment. Girdled berries were 90% of the control in the daily watering treatment and 57% of the control in the deficit irrigation treatment. Therefore girdled berries did not have nearly the same rate of water loss as the excised berries. This is indicative of a xylem connection into the berry and is further supported by the observation that girdled berries lost volume at a greater rate in the deficit irrigation treatment as compared to the daily watering treatment. The phloem is likely making a contribution to the volume of the berry because phloem removal (girdling) resulted in smaller berries.

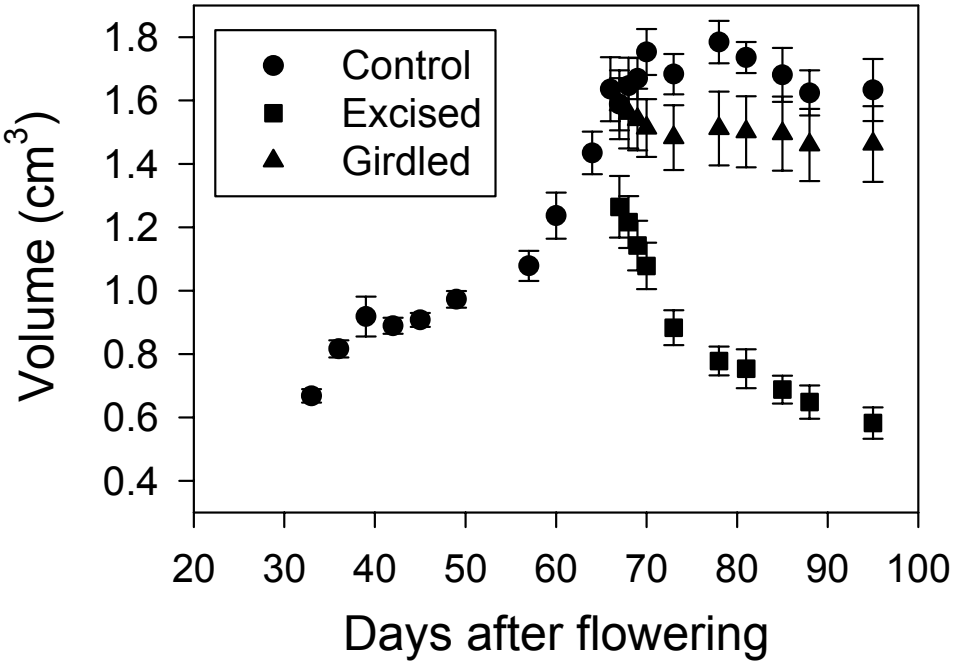


Figure 6.1.4.1. Volume of control (•), girdled (▲) and excised (■) berries. Veraison occurred around 57 DAF. Each symbol is the mean of 8 berries; vertical bars represent standard errors x2. Source: Rogiers et al., 2001.

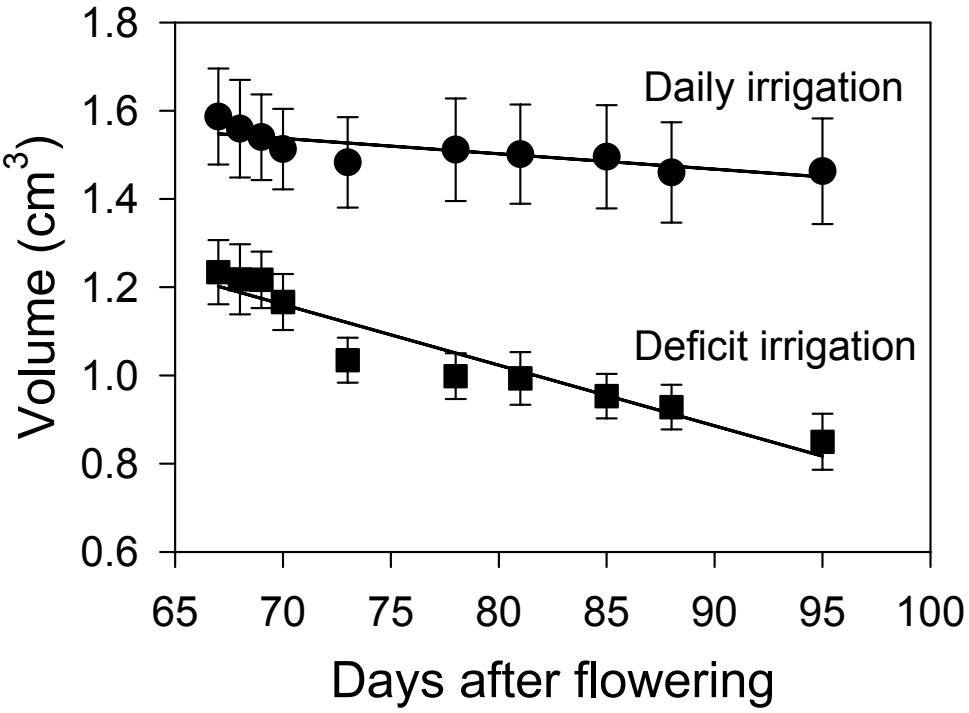


Figure 6.1.4.1. Volume of girdled berries subsequent to berry volume maximum. All measured berries were held on girdled pecidels and were sustained by vines that were subjected to either daily irrigation (•) or deficit irrigation (■). Each symbol is the mean of 8 observations. Source: Rogiers et al., 2001.

6.1.5 Sap flow through bunch stem

Sap flow through post-veraison Shiraz grape bunch stems cycled between 0 to 2.5 g/h with a peak during the middle of the day (Figure 6.1.5.1). Sap flow cycling mimicked daily cycling of VPD and a linear regression between VPD and sap flow was significant ($P<0.01$, $r^2=0.91$) from 0 to 4 KPa (Figure 6.1.5.2). Similar diurnal patterns were observed for pre-veraison and post-weight maximum berries. When VPD was reduced by encasing the bunch in a plastic bag the peak in sap flow was halved (Figure 6.1.5.3).

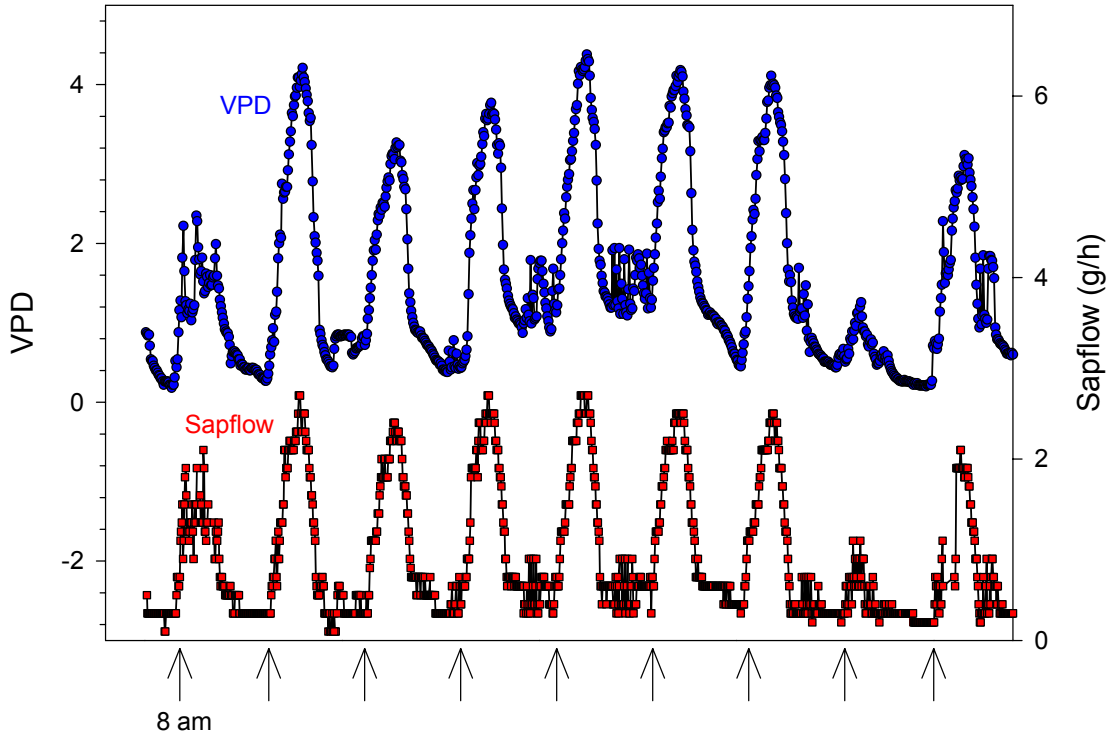


Figure 6.1.5.1. VPD (kPa) and Sap flow (g/h) through post-veraison Shiraz bunch stems of vines grown in a glasshouse under standard irrigation. Each arrow (8:00 am) indicates the start of a daily cycle.

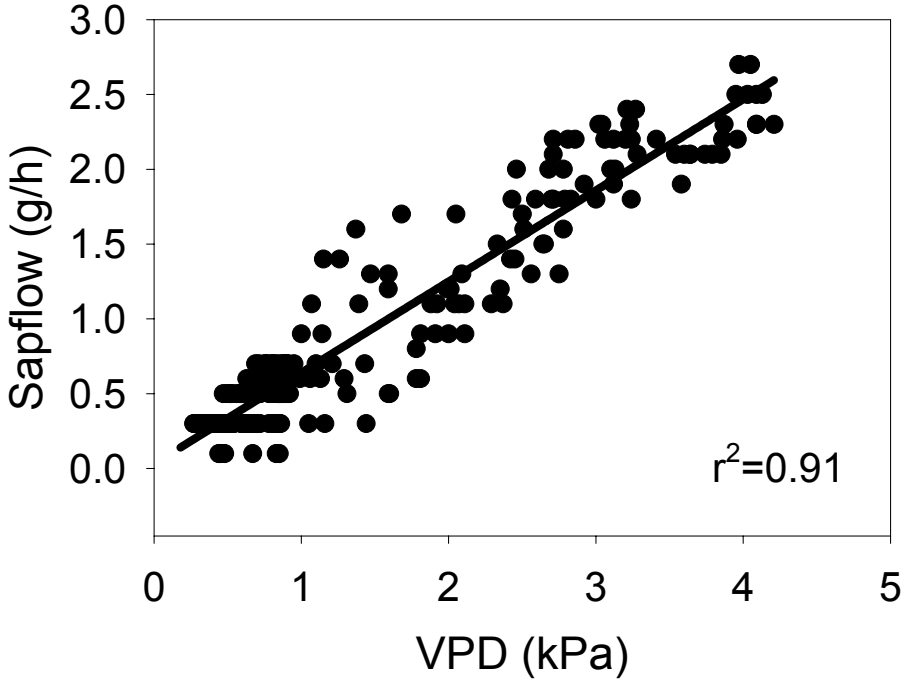


Figure 6.1.5.2. Linear regression of VPD (kPa) and Sap flow (g/h) through post-veraison Shiraz bunch stems of vines grown in a glasshouse under standard irrigation. The regression was significant ($P<0.01$, $r^2=0.91$).

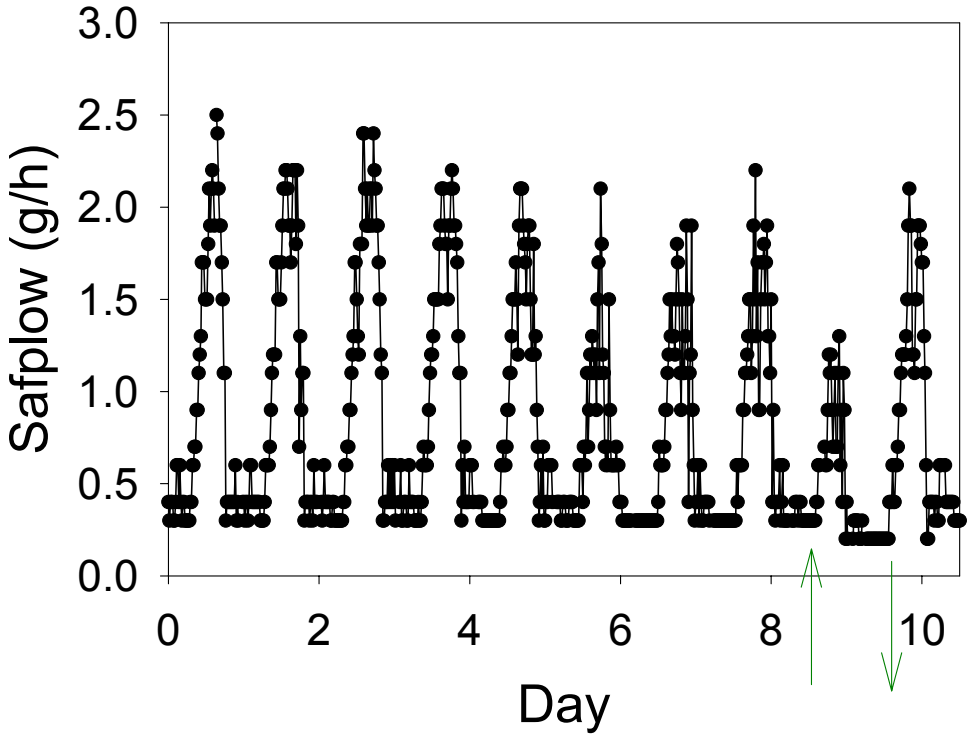


Figure 6.1.5.3. Sap flow (g/h) through post-veraison Shiraz bunch stems of vines grown in a glasshouse under standard irrigation. In order to reduce VPD levels around the bunch a plastic bag, lined with aluminium foil, was sealed over the bunch. The arrows indicate when plastic bag was placed on the bunch and removed from the bunch.

6.2 Wax morphology and berry transpiration

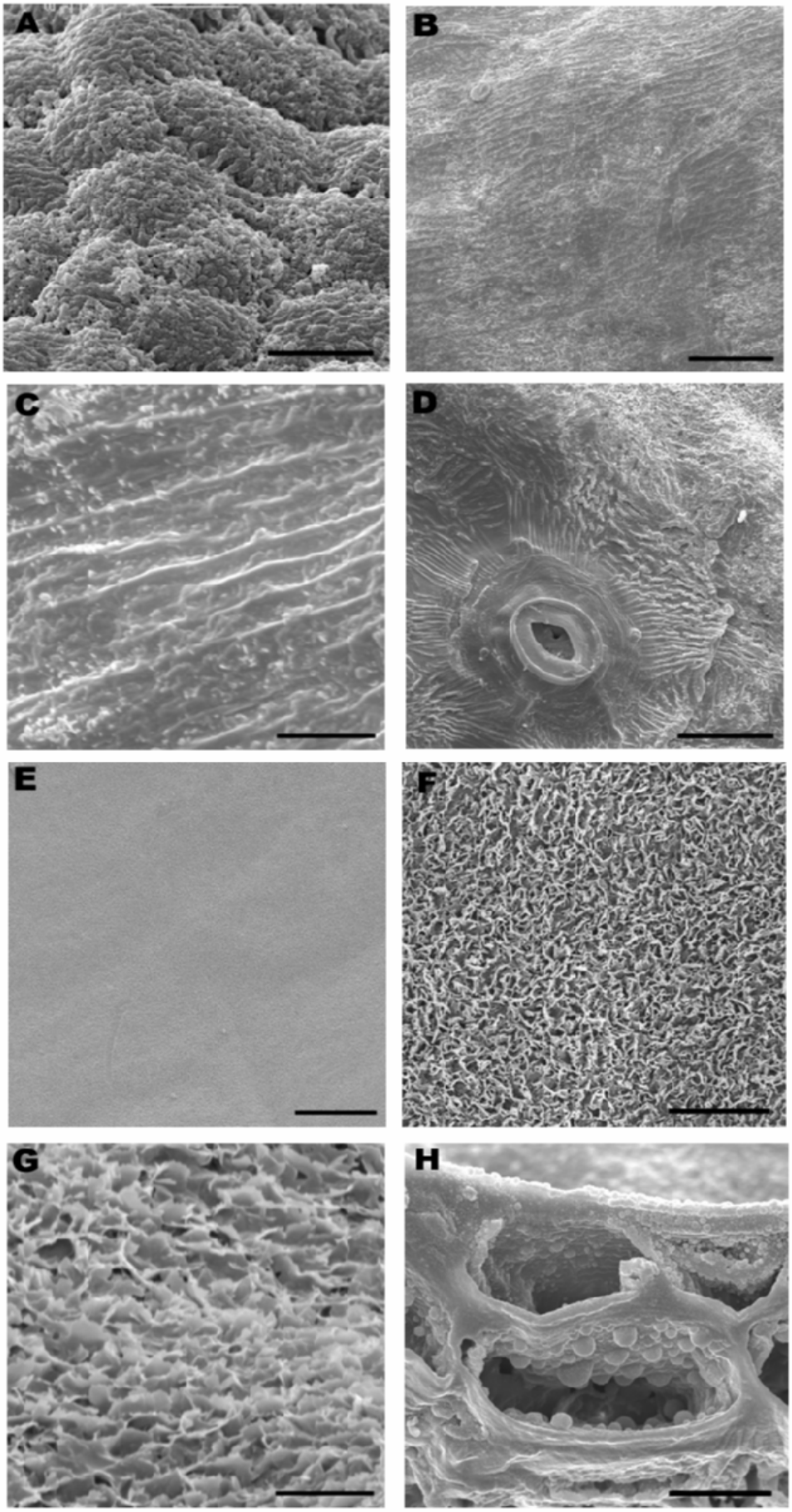
6.2.1 Cuticle Characteristics

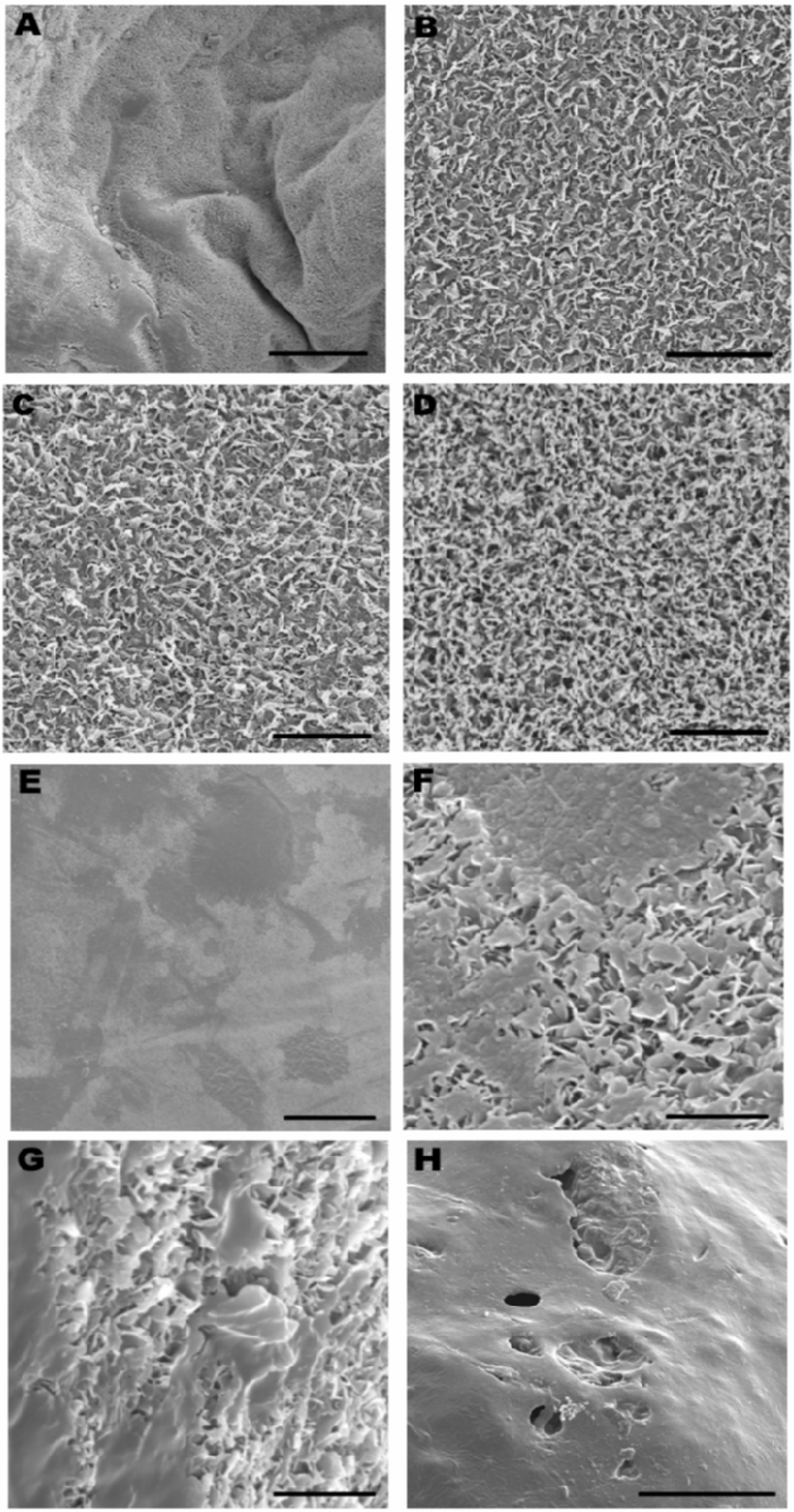
The morphology of the grape berry changed during fruit ontogeny and ripening. At anthesis the ovary was covered in cuticular ridges (Figure 6.2.1.1 A), which had spread out by 20 DAF (Figure 6.2.1.1 B) and wax was present as grains (Figure 6.2.1.1 C). Occasional protuberant stomates (on average less than 5 per berry) were also visible (Figure 6.2.1.1 D). Stomates were visible on the flower cap prior to bloom, on the ovary and on the pre-veraison berry. Once the epicuticular waxes covered the berry these stomates were hidden. One or two stomates could be seen on post-veraison berries but they were mostly filled by the wax (data not shown). At 40 DAF the waxes were in the form of very well defined plates with fringed edges and were so dense that the cuticle membrane underneath was no longer visible (Figures 6.2.1.1 E-G). In the post-veraison berry, the cuticle and epicuticular wax was approx. 2µm thick, and can be seen in cross section in Figure 6.2.1.1 H. The defined vertical platelets also featured in the post-veraison and post-weight maximum berries (Figures 6.2.1.2 A-C) and were similar to those seen on cv. Cabernet Sauvignon (Figure 6.2.1.2 D) and cv. Chardonnay (data not shown). From veraison onwards, large areas of amorphous wax were also observed in all three varieties (Figure 6.2.1.2 E). The top ends of the vertical platelets appeared to spread out into umbrella-like horizontal plates which condensed together to form a smooth crust (Figure 6.2.1.2 F and G). These smooth areas covered from 10 to 80% of the berry and the area was not dependent on the age of the post-veraison berry or the degree of shrivel. However, sun-exposed berries had larger areas of the amorphous wax than shaded berries. The wax was also amorphous in those areas where berries were in contact with other berries.

Cavities or fissures were not visible in plump, slightly- or moderately - shrivelled Shiraz berries. However, there were tiny cracks along some of the stomatal protuberances in several of the post-veraison berries. Moreover, very tightly shrivelled berries also had cavities in the surface (Figure 6.2.1.2 H).

Figure 6.2.1.1. Scanning electron micrographs of grape berry surfaces at various developmental stages. (A) Cuticular ridges on the surface of cv. Shiraz ovary at anthesis. (B) Surface of the cv. Shiraz pre-veraison berry at 20 DAF. (C) Close-up of (B) showing cuticular ridges and rudimentary wax granules. (D) Stomate on berry surface at 20 DAF. Note the lack of wax granules near the opening. (E-H) Typical berry surface at 40-120 DAF featuring upright intricate scale-like wax platelets. (H) Transverse section of the dermal and cuticle layers of a post-veraison berry. Note that the cuticle, including the epicuticular wax layer, was approximately 2 µm thick. (A, F and H) Bar indicates 10 µm. (B and D) Bar indicates 20 µm. (C and G) Bar indicates 5 µm. (E) Bar indicates 100 µm. Source: Rogiers et al., 2004a.

Figure 6.2.1.2. Scanning electron micrographs of cv. Shiraz grape berry surfaces. (A) Moderately shrivelled berry at 100 DAF (B) Close up of (A) showing intricate wax platelets (C) Intricate wax platelets of severely shrivelled berries at 120 DAF. Note fungal hyphae. (D) Wax platelets of the cv. Cabernet Sauvignon post-veraison berry are very similar to that of cv. Shiraz (E) Amorphous wax formation seen on portions of cv. Shiraz, cv. Chardonnay and cv. Cabernet Sauvignon berries 60-120 DAF. These amorphous waxes can cover up to 80% of the berry. (F and G) Close up of (E) showing formation of the amorphous waxes on top of the platelets. (H) Cavities in the surface of tightly shrivelled and decaying berries. These were not present in mildly or moderately shrivelled berries. (A,H and E) Bar indicates 100 µm. (B, C and D) Bar indicates 10 µm. (F and G) Bar indicates 5 µm. Source: Rogiers et al., 2004a.


A
B
C
D
E
F
G
H


A
B
C
D
E
F
G
H

6.2.2 Quantification of epicuticular wax

In the 1999-2000 season, gain in berry fresh weight was bi-phasal with a central transition point (Staudt et al. 1986, and Blanke 1992) one week before veraison (67 Days after Flowering) (Figure 6.2.1.1 A). Mean berry fresh weight reached a maximum at 95 DAF (1.5 g), and then declined by 20% to 1.2 g at 115 DAF giving an average weight loss of 15 mg per berry per day. In the 2001-2002 season, veraison again occurred at 67 DAF and the berry weight maximum occurred at 106 DAF (1.6 g). Berries were harvested at 133 DAF when they were 1.2 g. Weight loss was 25% with an average weight loss of 15 mg per berry per day as in the 1999-2000 season.

Mass of the primary chloroform extract per unit berry surface area prior to water extraction was 1.4 ± 0.1 μg mm^{-2} and did not change through development (Figure 6.2.1.1B insert). After removal of water-soluble berry surface exudates from the chloroform extract, mass on a surface area basis ranged between 1.1 and 1.2 μg mm^{-2} from 35 to 50 DAF, then declined by 90 DAF (berry weight maximum) to 17% of peak values (0.2 to 0.4 μg wax mm^{-2}) (Figure 6.2.1.1B) and remained low through the weight loss phase until berries were harvested at 110 DAF. In contrast, the water-soluble exudates increased from approx. 18% of the total surface exudates at 35-50 DAF to 83% of exudates by 90 DAF.

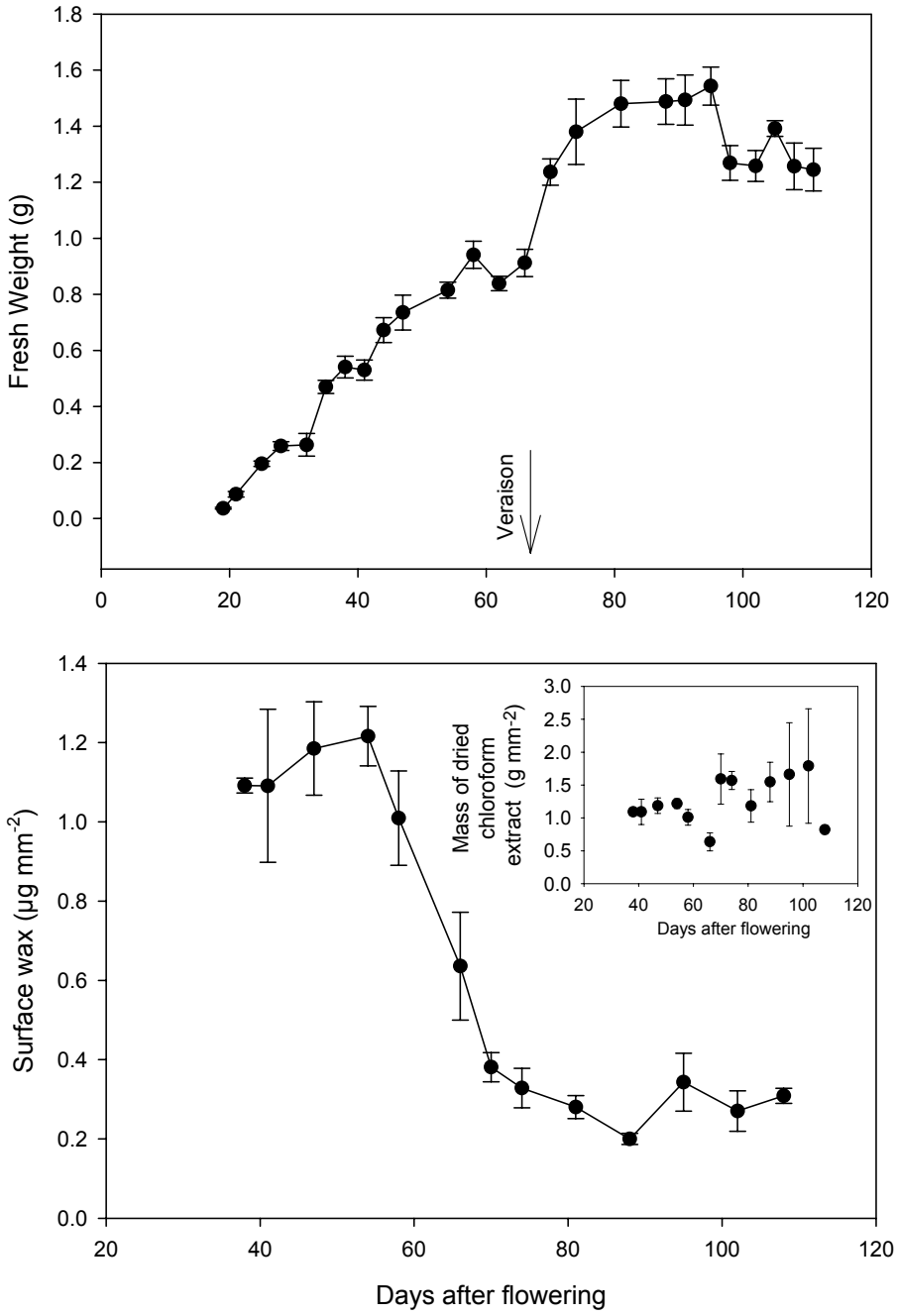


Figure 6.2.1.1. (A) Berry fresh weight (n=5, 50 berries per sample) and (B) berry epicuticular wax amounts (n=3, 50 berries per sample) during development and ripening. Inset in (B) refers to mass of chloroform extract prior to partitioning with water to remove berry exudates (n=3, 50 berries per sample). Bars indicate standard error of the mean. Source: Rogiers et al., 2004a.

6.2.3 Transpiration of detached berries

From the pre-veraison to the post-veraison phases of berry development, transpiration rates per unit surface area decreased to 19% of original rates (Table 1). This then decreased by another 3% during the shrivelled phase. On a per berry basis, transpiration of shrivelled berries was 16% of pre-veraison rates and was equivalent to 15.2 mg berry^{-1} day^{-1}.

Table 6.2.3.1: Transpiration rates of five developmental stages of Shiraz berries sampled on the same day. Loss in fresh weight of detached berries was monitored over three days at 25˚C and 23% RH (VPD=2.45 x 10^{-2} MPa). Transpiration rate was calculated by subtracting a daily respiratory loss of 1.9 mg CO_2 per berry (Niimi and Torikata, 1979) as carbon from fresh weight loss. Values are means ± standard errors. Source: Rogiers et al., 2004a.

Berry maturity level	Soluble solids[1] (°Brix)	Transpiration rate (µmol m^{-2} s^{-1})	Transpiration rate (µmol berry^{-1} s^{-1})
Green (pre-veraison)	-	129 ± 3	0.062 ± 0.002
50% pink	22.6 ± 0.7	62 ± 2	0.030 ± 0.001
100% pink	22.6 ± 0.7	47 ± 2	0.023 ± 0.001
Blue (post-veraison)	31.2 ± 0.9	24 ± 1	0.0117 ± 0.0006
Shrivelled	44.0 ± 0.8	21 ± 1	0.0098 ± 0.0006

[1]Soluble solids readings were taken after 3 days of transpiration measurements. Pre-veraison berries did not contain adequate amounts of juice for a soluble solids assessment.

6.2.4 Transpiration of attached bunches

Transpiration rates of bunches still attached to the vine had similar trends to the transpiration of detached berries. Transpiration was higher at the start of the growing season, reaching a maximum of 4.4 g bunch^{-1} d^{-1} and then declined to 1.7 g bunch^{-1} d^{-1} at 80 DAF (Figure 6.2.4.1). From this time onwards transpiration rates were no longer significantly different.

Diurnal trends in transpiration of post-veraison Shiraz bunches were monitored (Figure 6.2.4.2). Rates of transpiration were relatively low during the early morning (0.13 ug g dry wt^{-1} s^{-1}). After 6 am transpiration rates increased, and a maximum of 0.68 ug (g dry wt)$^{-1}$ s^{-1} was transpired at noon. Thereafter, rates declined until 6 am.

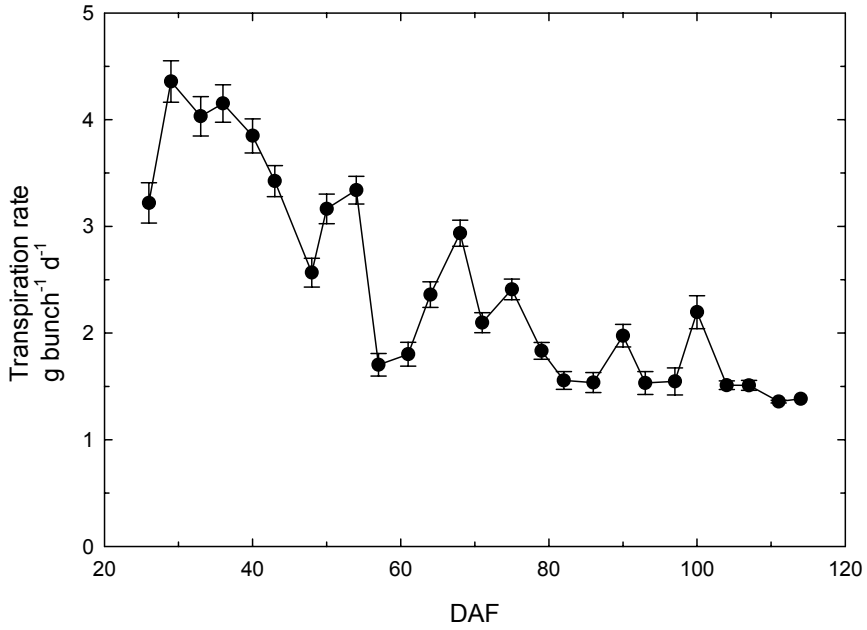


Figure 6.2.4.1: Bunch transpiration (mean ± SE) over the growing season. Transpiration rates of six grape bunches on potted glasshouse vines were measured twice weekly from 26 DAF until harvest (118 DAF). Source: PhD Thesis, N. Dimos.

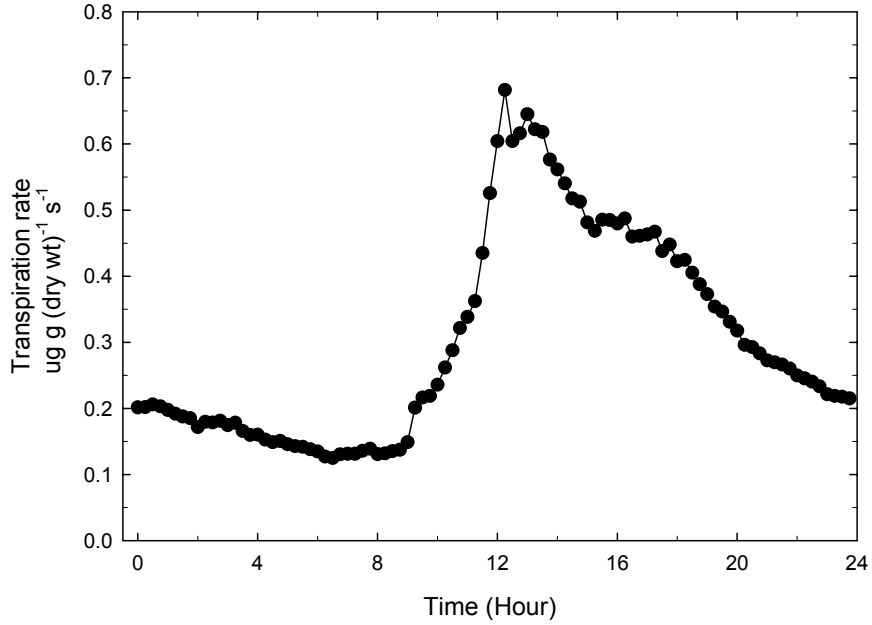


Figure 6.2.4.2: Diurnal transpiration of attached post-veraison bunches from potted outdoor Shiraz vines. Source: PhD Thesis, N. Dimos.

6.3 Backflow

6.3.1 Diurnal contraction

Berry volume was monitored continuously from pre-veraison through veraison, weight maximum and post-weight maximum. There was no gain in diameter of the pre-veraison berry from 1:00 pm until 5:30 pm, but subsequent to this berry diameter increased substantially (Figure 6.3.1.0). There was no diurnal contraction despite temperatures reaching to 43˚C in the glasshouse. Diurnal contraction and expansion of berries occurred during the lag phase, post-veraison and post weight- maximum stages (Figures 6.3.1.1-6.3.1.3). Contractions (up to 0.9 mm) occurred from 11 am to 5 pm and this was followed with a regain in volume until 11 pm (Figure 6.3.1.1). A plastic bag enclosed over the bunch prevented diurnal fluctuations in VPD in the atmosphere around the bunch, and this eliminated the diurnal contractions at both the veraison (Figure 6.3.1.1) and post weight-maximum (Figure 6.3.1.3) stages of development. These data indicate that contractions are due to VPD driven transpiration as opposed to flow from the berry back to the vine. Placing the vine under water stress did not induce diurnal contractions during this time. When the vine was watered there was an immediate increase in diameter (0.3 mm) of the post-veraison berry. In the post weight maximum berry, the overall declining trend in diameter was due to ongoing diurnal contractions without any diurnal expansion (Figure 6.3.1.3). Berries shrank at a rate of 1 mm per week under the glasshouse conditions.

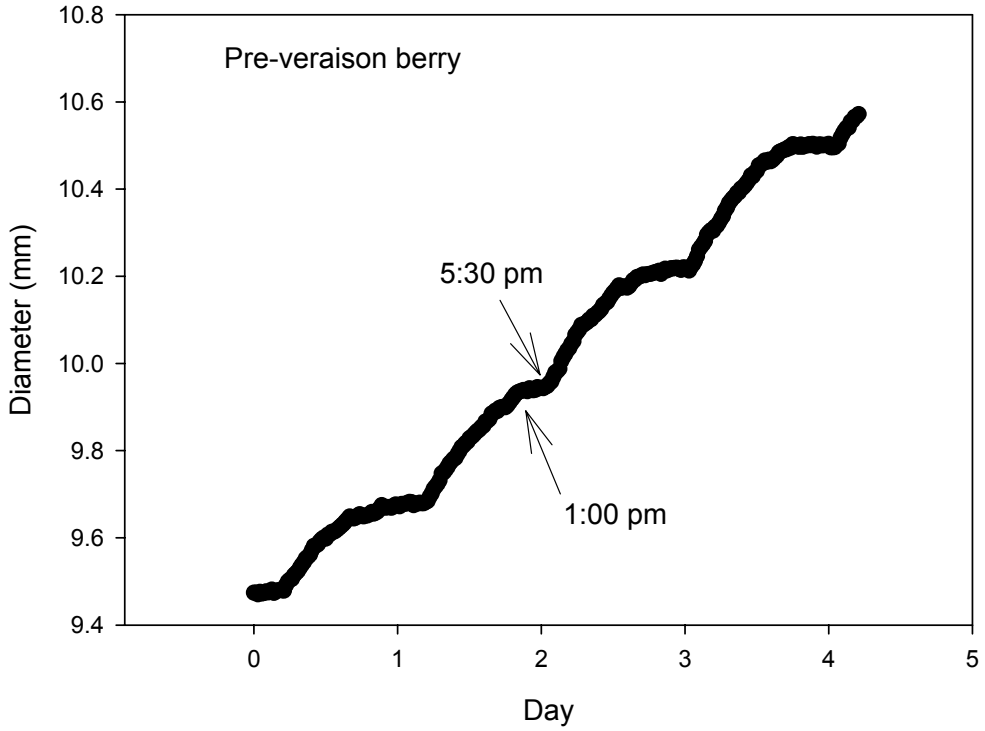


Figure 6.3.3.0 Daily trends in growth (diameter) of pre-veraison Shiraz berry.

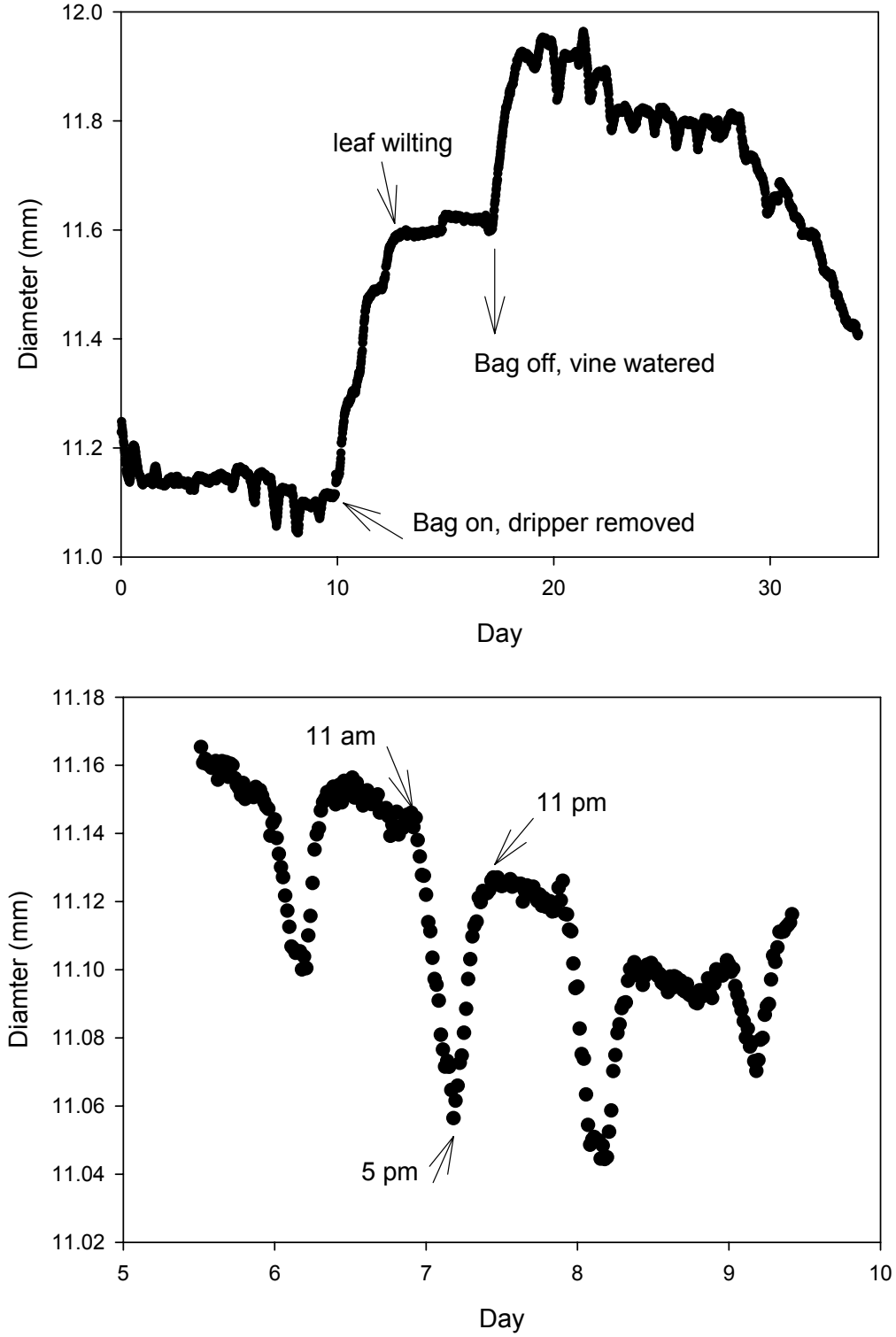


Figure 6.3.1.1. (A) Changes in berry diameter, pre-veraison. At day 10, VPD driven fluctuations in bunch transpiration were eliminated by enclosing the pre-veraison bunch in a plastic bag. The irrigation dripper was also removed. At day 12 the leaves showed visible signs of wilting. At day 17 the bag was removed from the bunch and the vine was watered. Veraison had occurred between day 12 and day 17. (B) Expansion of the above data (days 6 to 9) showing diurnal shrinkage and expansion of pre-veraison berry.

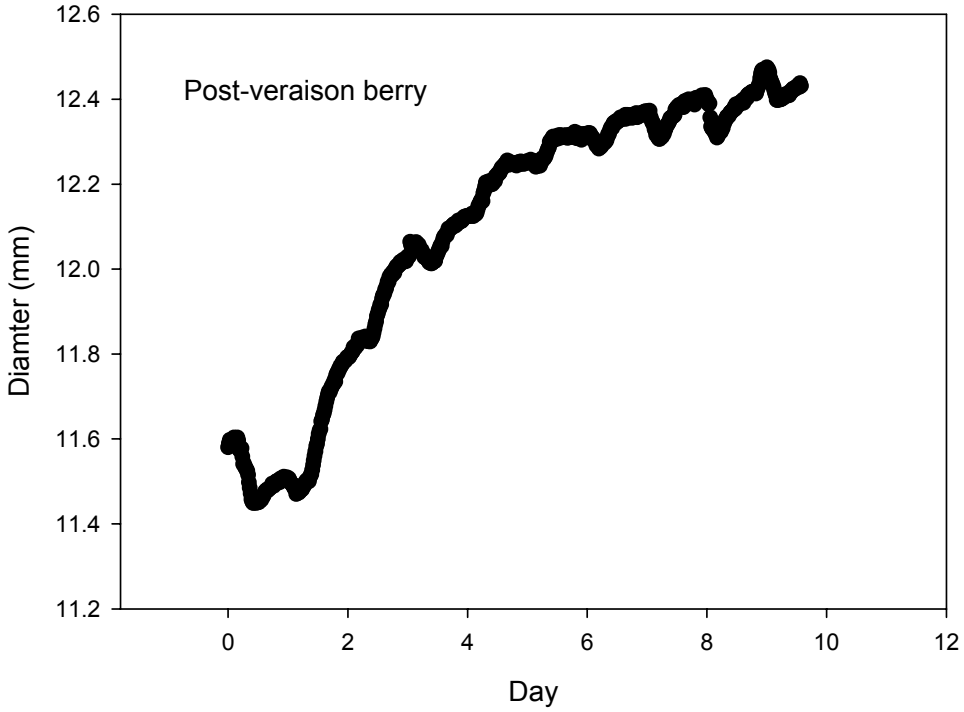
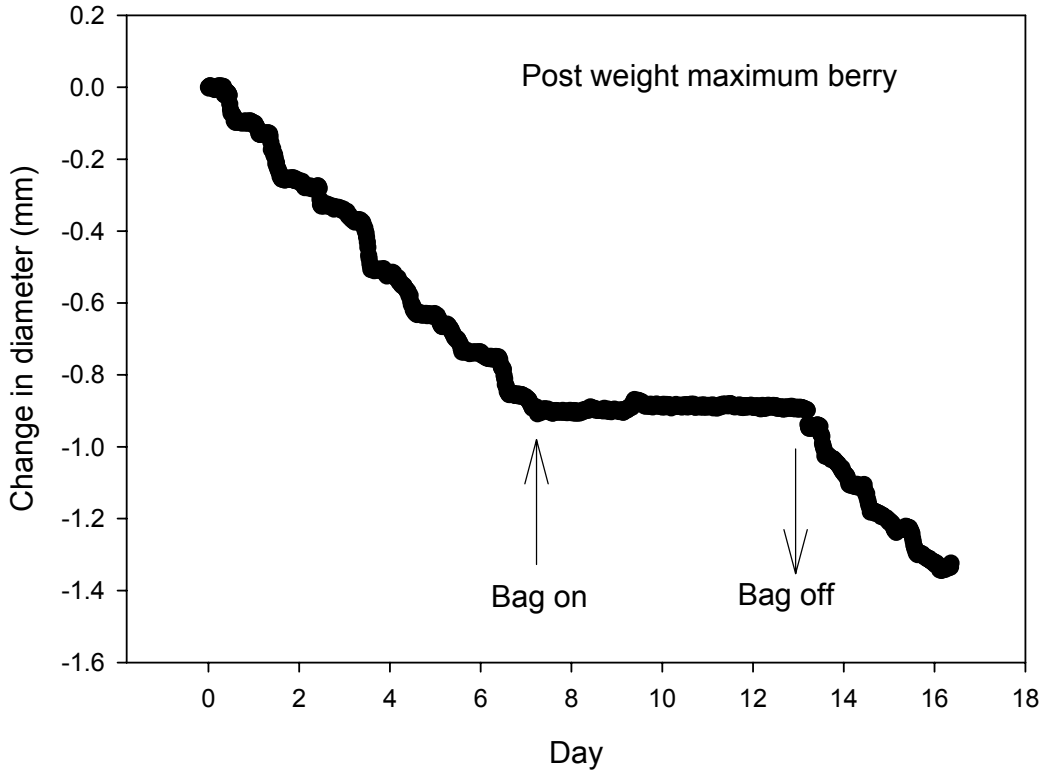


Figure 6.3.1.2. Daily changes in diameter of post-veraison Shiraz berry.



6.3.1.3. Daily changes in diameter of post weight maximum berry. VPD driven fluctuations in bunch transpiration were eliminated by enclosing the bunch in a plastic bag on day 7. It was removed on day 13.

6.3.2 Dye tracers

An apopoplastic tracer, acid fuchsin, and a symplastic tracer, CFDA, were used to assess the extent of backflow from berries to the vine. Acid fuchsin had travelled from the berry to adjacent berries at the pre-veraison and veraison stages of development, regardless of whether it was applied through the brush or below the brush (Table 6.3.2.1). In post-veraison and post-weight maximum berries, however, acid fuchsin had travelled from the berry to adjacent berry pedicels only if the dye was applied to the brush. CFDA did not move from the berry to other parts of the bunch at any stage of development (Table 6.3.2.1). CFDA only travelled from the berry to its own pedicel in pre-veraison and veraison fruit. In shrivelled fruit the CFDA did not move from the berry at all.

		Acid Fuchsin				CFDA			
Developmental stage	**Application site**	**Pedicel**	**Adjacent Pedicels**	**Adjacent Berries**	**Rachis/ Peduncle**	**Pedicel**	**Adjacent Pedicels**	**Adjacent Berries**	**Rachis/ Peduncle**
Pre-veraison	Through brush	P	P	P	P	P	A	A	P
	Below seeds	P	P	P	A	P	A	A	P
Veraison	Through brush	P	P	P	A	P	A	A	A
	Below seeds	P	P	P	A	P	A	A	A
Post-veraison	Through brush	P	P	? (Bleeding)	P	P	A	A	A
	Below seeds	A	A	A	A	A	A	A	A
Shrivelled	Through brush	P	P	?	A	A	A	A	A
	Below seeds	A	A	A	A	A	A	A	A

Table 5.2: Acid fuchsin (AF) traces xylem water flow. Carboxy-fluorescein diacetate (CFDA) traces phloem water flow. The tracer was loaded into the berry, either through the brush or through the pericarp (below seeds) for 12 hours at four developmental stages. Hand sections of the berry, pedicel, adjacent pedicel and berry and bunch stalk were viewed under fluorescence. Identification of the dye is noted as P (present) or A (absent). (n=3). Source: PhD thesis, N. Dimos.

6.4 Cultural Impacts on berry weight loss

6.4.1 Nitrogen and Irrigation

Berry volume increased in two growth phases, reached a maximum and subsequently declined. Irrigation strategy had an effect on pre-veraison and maximum berry size on own-rooted vines (Figure 6.4.1.1A). At the onset of berry volume measurements (34 DAF), berries from the SRI and the DI treatments were 0.1 cm^3 (17%) smaller than berries from the STD treatment. Maximum volume occurred 79 to 88 DAF in all three irrigation treatments, and berries from the SRI treatment were again smaller (by 0.17 cm^3 equivalent to 12%) than the berries from the STD treatment. Maximum volume was positively correlated with volume at 34 DAF (Figure 6.4.1.2A). The extent of post-veraison expansion was not however affected by irrigation treatment (Table 6.4.1.1). Post maximum volume loss was 1.6 fold greater for berries of vines grown in the STD as compared to the SRI or DI treatments (Table 6.4.1.1). The counter effects of less expansion between fruit set and 30 DAF and less post maximum volume shrinkage resulted in a non-significant difference in final berry volume, at 102 DAF (Figure 6.4.1.1A). Final berry volume was also positively correlated with maximum volume (Figure 6.4.1.2B), although on a less than 1:1 relationship, which shows that larger berries lost more volume than smaller berries.

Treatment	Post-veraison expansion (cm^3)	Post-maximum volume loss (cm^3)	Shoot length at harvest (cm)
Irrigation	ns	$P < 0.05$	ns
SRI	0.52	0.18 b	276
DI	0.55	0.18 b	288
STD	0.63	0.28 a	313
N rate	ns	ns	*P<0.001*
0 g	0.62	0.21	195 b
1.5 g	0.49	0.25	360 a
3.0 g	0.60	0.17	322 a

Table 6.4.1.1. Effects of irrigation and N application rate on amplitude of post-veraison expansion, amplitude of post-maximum volume loss and total shoot length at harvest of pot-grown Shiraz grapes. Main effects are shown (ns = not significant); values followed by the same letter do not differ significantly. There were no significant interactions. Source: Rogiers et al., 2004b.

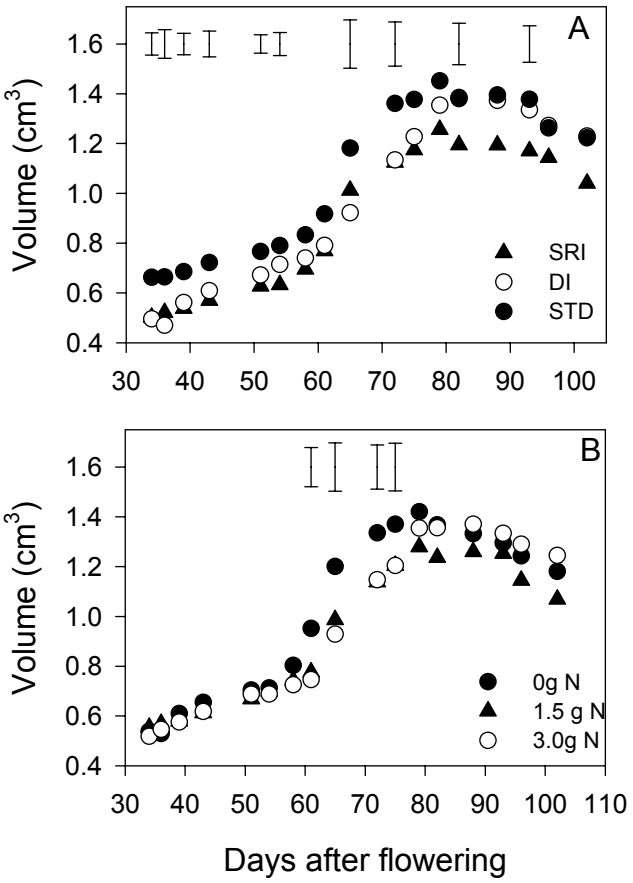


Figure 6.4.1.1. Changes in berry volume of Shiraz on their own roots during the post fruit set to harvest period. Bars represent least significant differences ($p < 0.05$) between means of irrigation treatment (A) or N fertiliser application rate (B); n=15; absence of bars indicates no significant differences. Source: Rogiers et al., 2004b.

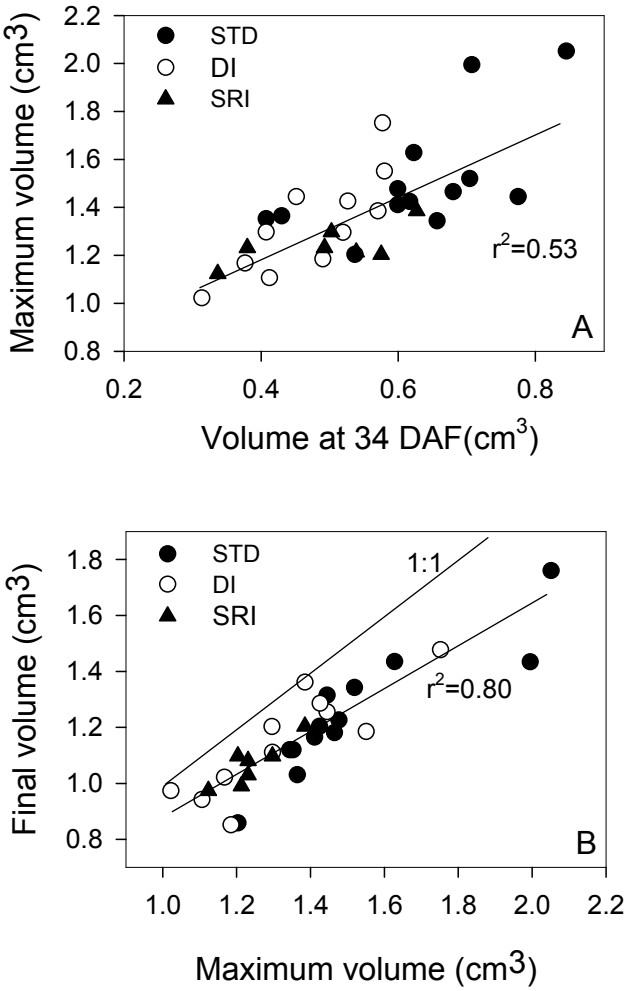


Figure 6.4.1.2. Linear correlation of maximum volume with volume at 34 DAF ($p<0.0001$) (A), and final volume with maximum volume ($p<0.001$) (B) of berries from Shiraz vines on their own roots treated with SRI, DI, or STD irrigation regimes. The 1:1 line was added to show the relationship that would occur if all berries lost volume equally, regardless of their size. Final volume refers to volume at harvest. At harvest, Brix was 23.0 for STD vines and 19.0 for DI treated vines. Source: Rogiers et al., 2004b.

Shoot length at harvest was not affected by irrigation (Table 6.4.1.1), however yield (g/vine) was less for vines grown in the SRI treatment as compared to the STD treatment (Table 6.4.1.2). This can be explained through berry number because there were 35% less berries in the SRI treated vines than the STD treated vines. Yield and berry number of DI treated vines was in between these two extremes. Soluble solids was highest for berries grown in the STD treatment (27.8 ˚Brix) and lowest in the SRI treatment (25.8˚Brix).

Treatment	Yield (g/vine)	Number of berries per bunch	Soluble solids °Brix
Irrigation	$P < 0.05$	$P < 0.01$	$P < 0.05$
SRI	41 b	55 c	25.8 b
DI	48 ab	73 b	26.9 ab
STD	58 a	86 a	27.8 a

Table 6.4.1.2. Effect of irrigation on yield and soluble solids of ungrafted Shiraz grapes (ns = not significant); values followed by the same letter do not differ significantly. There was no significant effect of N on these parameters. Source: Rogiers et al., 2004b.

N application rate did not affect pre-veraison volume (Figure 6.4.1.1B), amplitude of post-veraison expansion (Table 6.4.1.1) or post-maximum berry volume loss (Figure 6.4.1.1B and Table 6.4.1.1), however there was an effect on the onset and *rate* of post-veraison expansion (Figure 6.4.1. 1B). Berry expansion began earlier (at 58 DAF as compared to 65 DAF) and was 1.77 fold faster in the early post-veraison period of the treatment receiving no additional N as compared to the other N treatments. Neither N nor irrigation, however had a significant effect on the timing of the volume maximum (Figure 6.4.1.1). Shoot length at harvest (including lateral growth) was affected by N, with vines given 0g of N having 40 to 50% less shoot growth than vines receiving the other two N treatments (Table 6.4.1.1). N did not have an effect on yield, berry number or soluble solids.

6.4.2 Rootstock and Irrigation

Irrigation also affected berry volume on grafted vines. Vines watered daily had a larger maximum volume as compared to vines under a deficit irrigation regime (1.74 vs 1.56 cm^3, Figure. 6.4.2.1). This difference in volume was no longer apparent beyond 85 DAF. Those berries which were larger at the onset of measurements (33 DAF) tended to be larger at the volume maximum (Figure 6.4.2.2A). There was also a significant irrigation effect on the timing of the volume maximum (Table 6.4.2.1). The maximum occurred 11 days later in the DI than the STD treatment. In this study water stress was not applied until after fruit-set and there was no effect of irrigation on yield per vine or number of berries per bunch.

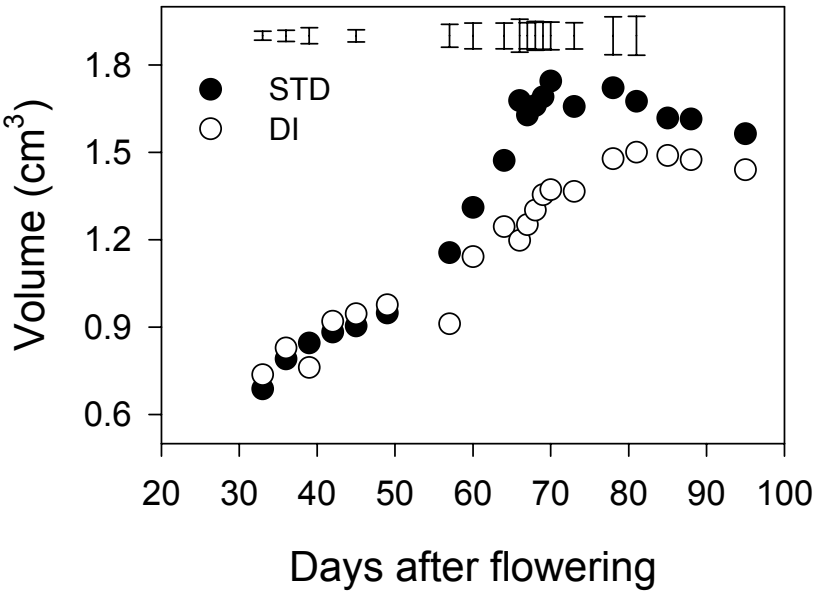


Figure 6.4.2.1. Changes in berry volume of Shiraz during the post fruit set to harvest period. Bars represent least significant differences ($p < 0.05$) between means of irrigation treatment; data of the three rootstocks were pooled (n=24); absence of bars indicates no significant differences. Source: Rogiers et al., 2004b.

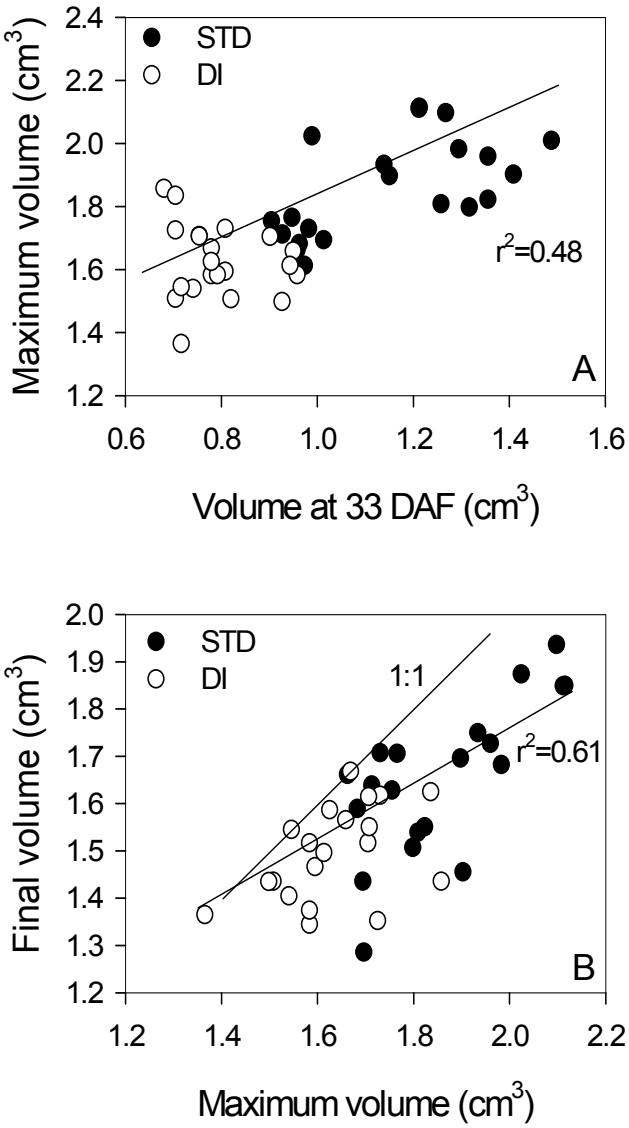
Treatment	Days to volume maximum	Post-veraison expansion (cm^3)	Post-maximum volume loss (cm^3)
Irrigation	$P < 0.01$	ns	$P < 0.05$
DI	85	0.68	0.16
STD	74	0.70	0.31
Rootstock	$P < 0.01$	$P < 0.05$	ns
Ramsey	83 a	0.63 c	0.19
101-14 Mgt	80 b	0.70 b	0.32
Shiraz	76 c	0.76 a	0.19

Table 6.4.2.1. Effect of irrigation and rootstock on post-veraison expansion, post-maximum volume loss of Shiraz grapes. Main effects are shown (ns = not significant); values followed by the same letter do not differ significantly. There were no significant interactions. Source: Rogiers et al., 2004b.

There was no significant effect of rootstock on maximum berry volume or final volume, however there was a significant effect on the timing of the volume maximum (Table 6.4.2.1). 101-14 Mgt advanced and Ramsey delayed the volume maximum compared with self-grafted Shiraz.

There was a significant effect of rootstock on the degree of post-veraison expansion (Table 6.4.2.1). Berries on Shiraz grafted to Ramsey had the least expansion (0.63 cm^3), while those on self-grafted Shiraz had the greatest (0.76 cm^3). There was no effect of rootstock on post-maximum volume loss but, as in ungrafted vines, irrigation treatment did have a significant effect (Table 6.4.2.1). Post-maximum volume loss in the STD treatment was double that of the DI treatment (0.31 vs 0.16 cm^3). There was no relationship between post-veraison expansion and the degree of volume loss, or maximum volume and the degree of volume loss. However, as in the ungrafted vines, final volume was closely correlated to maximum volume (Fig. 6.4.2.2B).

Rootstock impacted on yield per vine and berry number per bunch. Vines grafted onto Ramsey had 25% more berries compared with either 101-14 Mgt or Shiraz, yet there were no correlations between yield and post-veraison expansion, maximum volume, post-maximum volume loss, or final volume. Shoot length at harvest did not differ between treatments and averaged at 1.94 m. There were no significant correlations between maximum volume, final volume, or days to volume maximum with shoot growth from flowering to veraison, shoot growth from veraison to harvest or average shoot length at harvest (data not shown). As in the ungrafted vines, Brix was higher in the STD treated vines than the DI treated vines (23.0 vs 19.0). Self-grafted vines had 22.4˚Brix at harvest and this was significantly higher than the other two rootstocks.



6.4.2.2. Linear correlation of maximum volume with volume at 33 DAF ($p<0.0001$) (A), and final volume with maximum volume ($p<0.001$) (B) of berries from grafted Shiraz vines grown in STD or DI. The 1:1 line was added to show the relationship that would occur if all berries lost volume equally, regardless of their size. Source: Rogiers et al., 2004b.

7. Discussion

7.1 Xylem and phloem inflow

Element accumulation

Shiraz grapes are susceptible to weight loss during later stages of ripening. The onset of this weight loss begins at 70 to 90 days after flowering (DAF) depending on the season and may lead to berry shrinkage or 'shrivel'. Weight loss may be the result of a breakdown in the vine to berry vascular continuity. A loss of xylem flow into the berry at veraison has been hypothesised to be followed by a discontinuity in the phloem shortly after the weight maximum (McCarthy and Coombe 1999). Continued berry transpiration would then lead to shrinkage.

The objective of this study was to determine which vascular systems contribute to post-veraison berry growth and to ascertain if the flows through these systems change during post weight-maximum shrinkage. By monitoring the movement of the phloem mobile elements into the berry it was clear that phloem did flow into the berry pre-veraison but that it was stronger in the early post-veraison period. This has been reported several times (Hrazdina et al. 1984; Lang and Thorpe, 1989; Creasy et al. 1993; Ollat and Gaudillère 1996; Rogiers et al. 2000) and is due to the high sink strength of the berry. The rate of accumulation of the phloem mobile elements slowed or ceased during the weight plateau and during the shrinkage phases of berry growth. This may indicate a disruption in phloem flow, or that other sinks may have become more predominant and phloem flow is simply slowed as phloem tracers are produced towards other areas of the vine.

The strongest sinks for the phloem mobile elements *within* the berry varied for each element. For instance K accumulation into the pulp and skin was much higher than into the seeds, while most of the P accumulated in the seeds. Seeds, did however, stop accumulating all the phloem mobile elements prior to veraison. The seeds may become isolated from the phloem connection prior to veraison. Grape seeds are thought to acquire the ability to germinate by veraison, although storage reserves such as starch and lipids are still accumulated in the endosperm after veraison (Currle et al. 1983). That the elements stopped being deposited into the seeds at different developmental stages, (ie S was earlier) indicates that unloading must be preferential, rather than a total discontinuity into all unloading. That none of these elements accumulated in either the brush or torus indicates that these two tissues are not a depositing site for these elements.

Ca accumulation into the pre-veraison berry was rapid in all seasons examined. High rates of Ca accumulation into the berry is likely related to the high transpiration rates. A flattening of the Ca curves at veraison was evident in the 2000-2003 seasons, and this was followed with a decline in Ca levels in the berry. Because of the less frequent sampling and higher variability between replicates in the 1999 season it was hard to resolve any flattening of the curve at verasion. The xylem stream is influenced by daily changes in temperature, water vapour pressure deficits, and irrigation. The great level of variation in Ca accumulation between seasons and between replicates within a year may be explained by the fact that xylem mobile element accumulation is more responsive to environmental factors than phloem mobile element accumulation. The decline in berry Ca levels after veraison was unexpected. However, upon re-examination of Ca accumulation data sets in the literature, loss in calcium was present (but not discussed) in de Chaunac grape (Hrazdina et al. 1984), Chardonnay (Possner and Kliewer 1985), and Pinot noir (Creasy et al. 1993). Loss of Ca from berries after veraison was highlighted in a study of 5 varieties (Schaller et al. 1992). Backflow to the vine could be a cause for this loss in Ca. Backflow can occur because the hydrostatic pressure of the berry is slightly positive while that in the vine is negative (Lang and Thorpe 1989) especially under conditions of high evaporative demand. As inferred from diurnal berry

contraction patterns, backflow was more enhanced in pre-veraison berries (Greenspan et al. 1994 and 1996). We did not observe net loss of Ca from pre-veraison berries probably because xylem inflow was greater than backflow at this stage of development. In post-veraison berries, however, it is likely that xylem inflow during the night was negligible compared to xylem backflow during the day.

The resumption of Ca accumulation into the berries after this backflow period points to a second phase of xylem inflow late in ripening. This trend was also observed in the above data sets and highlighted by Schaller et al., 1992. The resumption of this xylem inflow was coincident with the loss in phloem flow. It is likely that the highly negative water potential of the berry, brought on by cell senescence and decompartmentation, will draw water from the vine. We suggest that when the phloem was no longer available during this very late stage of ripening, the xylem was drawn on instead. The sharp spike in Ca amounts in the berry may represent the sudden surge of xylem flow into the berry after a period of sequestration. It's disappearance suggests ongoing backflow during the day even with the resumption of xylem inflow during the night.

That net xylem flow became positive during this shrivel phase indicates that there was likely no blockage or permanent embolism formed after veraison. The blockage or embolism would also hinder backflow after veraison. An accumulation of xylem mobile elements in the brush or torus would provide evidence for a xylem discontinuity in this site. The data in this study indicate no such blockage in this region after veraison since most of these xylem mobile elements were transported to the seeds until late ripening.

Dye tracers

These results of the dye studies are in accordance with water flow studies in Riesling berries where dye also accumulated only in the axial bundles after veraison (Düring et al. 1987). Multiple breakages were observed in the peripheral xylem and these opened up progressively as berry growth continued. In Merlot and Pinot Noir, dye in the peripheral strands of post-softening berries was less regular than that of pre-softening berries and axial strands also stained less predictably after softening (Creasy et al. 1993). Perfusion of eosin in Muscat Gordo Blanco grapes was blocked at the outer edge of the brush in ripe berries and this appeared to be associated with stretched tracheids (Findlay et al. 1987). It appears that Shiraz is not different from these varieties in vine to berry and within-berry xylem continuity. With a discontinuity or blockage to the bundles servicing the pericarp at veraison it can be assumed that overall xylem flow into the berry is diminished. However, this does not necessarily indicate a complete cessation of xylem exchange between the vine and the berry since water could potentially flow into the berry through the xylem up until the brush and then continue along symplastic routes from the brush into the pulp and skin regions.

7.2 Wax morphology and berry transpiration

Cuticle characteristics

The epicuticular wax development on Shiraz berries in this study was similar to that described earlier for other varieties (e.g. cv. Thompson Seedless, Rosenquist and Morrison 1988; and cv. Palomino fino, Casado and Heredia 2001). Cuticular ridges were present at flowering and these spread out and fused as the berry expanded. Rosenquist and Morrison (1988) suggested that the cuticular ridges function as a storage form of cuticular material, which spreads out as the berry expands. The first epicuticular wax appeared as small grains and these then formed into intricate crystalline platelets 20-40 days after flowering. This was followed by the development of an amorphous wax layer on top of the existing platelets post-veraison. The berry surface that was covered with amorphous wax was larger on sun-exposed berries than on shaded berries. This could be related to the substantially higher temperature experienced

by the exposed berries. Such berries often heat up to as much as 15°C above ambient temperature, whereas shaded berries are generally close to ambient temperature (Smart and Sinclair 1976, Spayd et al. 2002). The surface wax of 'Valencia' orange fruit changed from upright plates to amorphous during development, and this change occurred earlier for exposed fruit (El Otmani et al. 1989). In our study there were extensive areas of amorphous wax on post-veraison berries. However, shrivelled berries did not have more extensive amorphous wax than plump post-veraison berries, suggesting that shrivelling in Shiraz berries is not the result of amorphous cuticular wax development.

Fissures on skins of berries may lead to increased transpiration rates. Fissures were observed in cvs. Optima (Percival et al. 1993) and Pinot noir (Comménil et al. 1997) skins at maturity. Our investigation revealed no fissures running along the surfaces of post-veraison, mildly shrivelled or moderately shrivelled Shiraz berries. Cabernet Franc berries are relatively small in size, and like Shiraz berries in this study, these did not show any fissures traversing the cuticle (Percival et al. 1993). Cuticles of varieties with larger berries may be more prone to fissures because there is lack of adequate mechanical support (Percival et al. 1993). In some varieties small cracks can develop at the base of stomatal protuberances during veraison (Blanke et al. 1999). We also observed these cracks on some post-veraison berry stomata but since this phenomenon occurs in many varieties it is unlikely that it would lead to the shrivelling that is unique to Shiraz. The cavities in the severely shrivelled berries were likely a by-product of shrivel as opposed to a cause of shrivel because they were not apparent in earlier stages of shrivelling.

Quantification of epicuticular wax

In order to determine whether decreasing wax quantities contributed to Shiraz shrivel, chloroform was used to remove the surface wax from the grape berries. The main component of berry wax is a 'hard wax', oleanic acid, whereas 'soft wax' is a mixture of long chain fatty acids, alcohols, aldehydes, esters and hydrocarbons (Radler and Horn 1965, Yamamura and Naito 1983, Comménil et al. 1997). For example, Yamamura and Naito (1983) found that 'Delaware' berry surface wax was predominantly hard wax during all growth stages (87% at maturity). Since chloroform not only solubilized the waxes but also other exudates on the berry's surface, these exudates were removed from the chloroform extract by washing in water and the remaining chloroform layer was then dried down to constant weight. Berry exudates consist of phenolic compounds and malic acid in immature fruit, and as the fruits mature they contain sugars, malic acid, potassium and sodium as well (Padgett and Morrison 1990).

The total mass of primary chloroform extract remained unchanged, on a surface area basis, throughout development. This result contrasts with the finding of Palliotti and Cartechini (2001) who reported a substantial increase in chloroform-extracted wax (from 1 up to 5 μg mm^{-2}) from post-flowering to post-veraison cv. Cabernet Sauvignon berries. This contrast is starkly enhanced by removal of the water soluble extracts which revealed a substantial decrease in wax, on a surface area basis, from veraison until the berry weight maximum (1.2 to 0.3 μg wax mm^{-2}). This may at first seem surprising considering that the SEM images indicated the post-veraison development of an amorphous layer on top of the existing wax platelets. However, it was observed in cv. Pinot noir grapes that, despite the formation of this layer, cuticle thickness decreased as the berry expanded (Comménil et al. 1997). The density of the wax platelets may also decrease as the berry expands and this may result in an overall decrease in wax quantities per surface area.

As indicated above, in other varieties there appears to be no consistent correlation between berry development and wax thickness or quantity per surface area. For example, the wax of 'Delaware' grapes increased during the early pre-veraison stage (until 30 DAF) and then remained constant thereafter (Yamamura and Naito 1983). In cv. Thompson Seedless,

however, no change in the total wax (1 μg mm^{-2}) during berry growth was reported (Radler 1965) and an increase in the weight of wax per unit surface area occurred during development of cv. Pinot Noir grapes (Comménil et al. 1997). Our results for cv. Shiraz suggest that the formation and deposition of epicuticular wax occurs early during berry development and ceases before the central transition point of berry growth. These conflicting reports on amount of berry surface wax may be due to differences in methods of wax extraction. Furthermore, the length of the developmental period investigated and environmental factors such as temperature, light exposure, relative humidity and irrigation (Rosenquist and Morisson 1989) will have an effect on trends.

Transpiration

Intuitively, one might assume that the amount of epicuticular wax will have an effect on cuticular transpiration. In this study, however, there was no negative correlation between amount of wax and berry transpiration. Berry transpiration per surface area decreased from pre-veraison to post- veraison and then decreased even further during the shrivelling phase of ripening. Similarly, transpiration of post-veraison cv. Müller-Thurgau (Leyhe and Blanke, 1989) and cv. Cabernet Sauvignon (Greenspan et al. 1994 and 1996) berries was less than that of pre-veraison berries. The composition of the cuticle and the epicuticular wax may be more important than its thickness or density for determining transpiration rates. The physical structure of the wax, such as crystallinity may also have an effect on transpiration rates (Reynhardt and Riederer 1991), but since there was no correlation between area of amorphous wax and incidence of shrivel, it seems that other factors are at play.

Loss of stomatal functioning may also contribute to the decreased transpiration during ripening of grape berries (Palliotti and Cartechini 2001). Shortly after fruit set, the stomata were raised on a conical protuberance and appeared to be functional. An "aureole peristomatique" was first described for the grape berry by Bessis (1972) and its protuberance discovered and named "stomatal protuberance" by Blanke and Leyhe (1988). The very few stomata we observed in post-veraison berries in this study had been transformed into non-functional lenticels and were mostly filled with wax. The continued decrease in berry transpiration after veraison is further evidence that shrivel is not caused by fissures or cavities in the skin. Such physical damage would be expected to increase transpirational water loss rather than to decrease it.

Transpiration rates of shrivelled berries were equal to the rates of weight loss observed in field berries (on average 15 mg water vapour $berry^{-1}$ day^{-1}). However, since transpiration rate decreased on both a surface area and whole berry basis during ripening, it is unlikely that this alone would be the cause for shrivel. In a growing post-veraison berry (prior to the weight maximum), net water flow into the berry must exceed net water loss. In a shrivelling berry, the rate of water loss through transpiration was slightly less than that of the growing post-veraison berry, and this indicates that net water flow into this shrivelling berry must have been less than that of the growing post-veraison berry. If transpiration rates had suddenly increased during shrivelling, transpiration alone could have accounted for the transformation of a plump berry into a shrivelled one.

7.3 Backflow

Diurnal berry contraction

One of the objectives was to understand the role of backflow, water flow from the berry back to the vine, in Shiraz berry shrivel. Soft fruit of many species, including grapes, may show diurnal shrinkage. This shrinkage may be due to vascular back-flow at times of water stress. Water is then regained during the night when the water potential of the shoot is no longer below that of the berry. The diurnal shrinkage in Shiraz may be accentuated- the volume of

the berry is not fully regained during the night and an overall trend of weight loss occurs. Back-flow may occur through the xylem elements and the interconnecting cell walls of cells (apoplastically) or through the cytoplasm of cells (symplastically).

Although not thought to be important after veraison (Greenspan et al. 1994), backflow has been reported in grapes (Lang and Thorpe 1989). Ongoing berry contraction under conditions where transpiration is reduced would provide evidence for backflow. However, in our study diurnal contractions were eliminated when the post weight- maximum bunch was placed in a highly humid environment where transpiration would be negligible. These data indicate that, unlike transpiration, backflow is not likely a predominant avenue for water loss from the berry.

Dye tracers

The results of the tracer study indicate that phloem backflow does not likely occur. Xylem backflow did occur, but it was limited to the pre-veraison and veraison stages of development. The xylem backflow was not extensive since the dye was only present in berries adjacent to the one treated with the dye. It was not detected in the shoot to which the treated bunch was attached to. These results indicate that backflow is not a likely cause of Shiraz berry shrivel.

7.4 Cultural impacts on berry weight loss

Water stress can impact on grape berry development. Deficit irrigation can reduce final berry size in cv. Shiraz (McCarthy, 1997) but it is uncertain if it affects the degree of weight loss during the later phase of ripening. Weight loss likely results in grape berries when water inflow into the berry cannot keep pace with evaporative water loss. It is possible that this could be ameliorated by reducing the extent of post-veraison xylem disruption inside the berry. While water deficit clearly interferes with both cell division and cell enlargement in grapes (Williams et al., 1994), resulting in smaller maximum berry volumes, continued xylem connection to the post-veraison berry could enhance water uptake by the berry and therefore reduce volume loss. Indeed, mild soil water deficit applied to tomato during the early stages of fruit development resulted in increased xylem connection into the ripening fruits, whereas partial rootzone drying (PRD) imposed during the same period had the opposite effect (Davies et al. 2000). Therefore, one objective of the present study was to vary water levels during flowering and early berry development to see if it changed the extent of volume loss in ripening Shiraz berries.

In addition to soil water status, nitrogen availability can also have an effect on berry size (Keller et al. 2001), as can rootstock (Keller et al. 2001), along with an influence on yield and composition (Keller et al. 2001). Ramsey, for instance, is a vigorous rootstock producing high yields (Hedberg et al., 1986; Cirami et al., 1984) and conferring drought tolerance (McCarthy et al., 1997). 101-14 Mgt is a low-moderate vigour rootstock, while Shiraz on its own roots results in a medium to high vigour canopy (Dry and Gregory, 1991). Both nitrogen and rootstocks may affect berry volume either through their influence on vegetative vigour or through an effect on fruit set and thus berry numbers.

The objective of this work was to gain information on factors that impact on the onset of volume loss and/or the degree of volume loss in Shiraz berries. We examined the impact of deficit irrigation, N application and rootstock on berry volume during development. A pot system was used to test the effects of these treatments because non-destructive, precise and accurate measurements of berry volume changes in the field are extremely difficult. It is also easier to carry out experiments on berry volume in a controlled situation where effects of rain and soil differences can be minimised. However, before any results from such studies can be integrated into a practical vineyard situation, rigorous testing of specific treatment effects in specific field situations will be required.

Deficit and split-root irrigation reduced berry volume throughout development of both own-rooted and grafted Shiraz vines. That these differences were apparent very early in development (by 35 DAF) indicates that cell division was inhibited. The increase in volume of grape berries after bloom can be attributed to an increase in cell number (2-fold) and to an increase in cell volume (300-fold; Coombe, 1976). Cell division in the mesocarp is thought to cease 20-30 DAF (Harris et al., 1968) and in the skin 35-40 DAF (Nakagawa and Nanjo, 1966). Thus the first volume measurements in this study were taken at approximately the end of the cell division phase. The effect of water stress on cell division after flowering has also been proposed by Hardie and Considine (1976). In the present study, there was a significant correlation between volume of berries at 30-35 DAF and the maximum volume (Fig's 2A and 4A). Therefore the cell division phase of berry growth has a significant impact on the potential maximum size of berries. Berries from the SRI and the DI treatments may have had a smaller maximum volume than berries from the STD treatment because there were fewer pericarp cells to expand initially and there was less expansion of those cells that were present. The effects of irrigation on maximum berry volume seen here are comparable to those of a large irrigation study of field vines where differences in maximum berry weight were attributed to a combination of irrigation treatments and seasonal conditions (McCarthy, 1997 and 1999).

Deficit irrigation did not delay the volume maximum of berries on own-rooted Shiraz, but resulted in an 11-day delay in grafted vines. The absence of an effect of water deficit on the onset of weight loss in own-rooted vines is consistent with results of the field trial cited above (McCarthy, 1997), where the timing of the volume loss in Shiraz berries was at 90 DAF, regardless of irrigation regime. This potential difference between grafted (even self-grafted) and ungrafted vines deserves further investigation. The final volume of berries from deficit-irrigated vines in this study was not different from the STD treatment and this may be attributed to a greater degree of post-maximum volume loss for berries from the STD treatment. The pericarp cells of the STD berries may have had more water available for loss. Moreover, larger berries also have a larger surface area and could thus be more prone to evaporative water loss than smaller berries. A study on the rates of cell division and expansion during DI would provide useful insights into the dynamics of berry growth. It is also possible that berries that grow more slowly are able to maintain an increased hydraulic connection to the vine due to reduced xylem disruption. This would allow them to alleviate evaporative water loss in the very latest stages of ripening through continued xylem import.

DI consistently reduced maximum berry volume and the extent of volume loss. This supports the finding by Davies et al. (2000) that soil drying during flowering and early fruit development enhanced the hydraulic (xylem) connection between tomato fruit and the rest of the plant. In addition, irrigation can also have an indirect effect on berry volume through its effect on berry number. Water stress during flowering and fruit set can result in lower berry numbers through fruit abscission and this may lead to larger berries since competition for photosynthate supply and water is reduced. In this study there were 15 to 35% fewer berries on vines grown in the SRI or DI treatments and they were smaller, not larger than berries of vines grown in STD irrigation. The larger berry size in the STD treatment was thus not due to a smaller berry number per vine.

Shoot growth can also have an impact on berry volume. Extensive shoot growth can divert the water and photosynthate supply away from berry growth and ripening. In this study, there were no negative correlations between maximum berry volume and shoot growth between fruit set and veraison or shoot growth between veraison and harvest. This is likely because overall yield compared with leaf area (i.e. the crop load) was very low. The differences in maximum berry size between the irrigation treatments were therefore not due to competition with shoot growth.

Vines grown in the 11L pots (nitrogen x irrigation study) had berries which were on average smaller than those of the 26L pots (rootstock x irrigation study). Even though the soil of the vines grown in the 11 L pots was wetted to field capacity daily, the overall volume of water may not have been sufficient to allow for optimal berry growth. If these vines did indeed experience a water stress, however, it was not severe since there were no signs of leaf wilting in the STD or SRI treatments at any time.

The effect of SRI on minimising berry size is comparable to studies on tomato fruit. A SRI treatment enhanced the extent of hydraulic isolation of the fruit and there was a reduction in the size and the fresh weight of the fruit (Davies et al., 2000). However, our data is currently insufficient to support or otherwise contradict this conclusion. The authors suggested that reduced fruit size may be the result of growth-retarding, root-borne signals emanating from those roots in contact with the drying soils. The effect on fruit size relative to the vegetative parts of the plant was reduced, however, and this may be because xylem-borne signals may not be able to penetrate the fruit as effectively late in development as the phloem-derived water supply dominates. Recent studies on phloem and xylem continuity into Shiraz berries during and after the volume maximum also indicated that phloem inflow relative to xylem inflow increased. Since the variability in post-veraison expansion only contributed 34% ($r=0.58$, $P< 0.001$) to the variability in maximum berry size, root signals prior to veraison could have accounted for most of the difference in berry size.

In this study, N had an effect on rate of post-veraison berry expansion but not on maximum berry volume. The accelerated rate of post-veraison expansion with 0 g of N could be the result of less vigorous vegetative growth in this treatment leaving more water and photosynthates available for berry growth. A decrease in vegetative growth may also increase relative sink strength of the fruit (Davies et al., 2000; Dry et al., 1996). Low N at bloom usually reduces fruit set, particularly in combination with water stress (Keller et al. 1998). In this study, however, there was no effect of N on number of berries per vine (data not shown), likely because these vines had a very light crop load. Therefore, the N effect on the rate of post-veraison expansion was not a consequence of berry number.

Not only irrigation, but rootstock also had an influence on the timing of the volume maximum, with the earliest maximum occurring for berries grown on 101-14 Mgt. 50% flowering varied over 5 days within this study, with no influence of rootstock or irrigation on its timing. Therefore, time of anthesis did not contribute to the variation in the timing of the volume maximum. A smaller berry number may lead to more photosynthates and water available for the remaining berries, however there was not a consistently lower berry number for 101-14 Mgt compared to the other rootstocks. 101-14 Mgt is considered a low-vigour rootstock compared to Shiraz and Ramsey. In this study, however, not shoot length at veraison nor final shoot length were smaller for vines on this rootstock as compared to Shiraz on own roots or Ramsey. If smaller canopies had been produced by 101-14 Mgt this could have led to less competition for water between berries and shoots and thus advancing the volume maximum. However, the lack of any significant correlations between maximum berry volume, final volume, or days to volume maximum and shoot growth from flowering to veraison, shoot growth from veraison to harvest or average shoot length at harvest indicate that there was little effect of vegetative growth on berry volume.

It should be noted that comparisons of berry volumes were made at a particular berry age, as opposed to a particular soluble solids concentration, because berry volume itself has an effect on Brix levels (i.e. higher Brix was probably a consequence of volume loss rather than a cause). The present data do indicate that while there was an effect of irrigation on *maximum* berry volume there was no effect on *final* berry volume. Therefore, larger berries lost more volume than smaller berries.

8.0 Summary

Shiraz berry volume increases in a double sigmoidal fashion and this may be followed by shrinkage during the later stages of ripening. A loss of xylem flow into the berry at veraison has been hypothesised to be followed by a discontinuity in the phloem shortly after the weight maximum (McCarthy and Coombe 1999). The data in this study indicate a cessation in phloem flow but a resurgence of some xylem flow into the berry. This xylem flow, however, was not enough to keep up with the transpirational loss from the berries and hence shrinkage ensued. The trigger for the loss in phloem flow did not likely involve a day length or temperature component since berries of vines forced to grow during the winter also followed the shrivelling trend. The loss in phloem flow did not involve any obvious blockages within the berry. Further anatomical and physiological investigations of phloem connections from the shoot to the bunchstem are required to understand how phloem flow into the berry is regulated.

Xylem	Phloem
• Xylem breakages within berry after veraison • Xylem dye tracer moves into berry but progressively less around berry after veraison • Ca accumulation is variable but stopped at veraison in 3 out of 4 seasons • Ca accumulation restarts at weight-maximum	• K accumulation stops at weight-maximum • Sugar accumulation stops at weight-maximum • Dry weight stops accumulation at weight-maximum • No sieve tube plate plugs present before or after weight-maximum

Table 8.1 Summary of results for xylem and phloem flow into the berry.

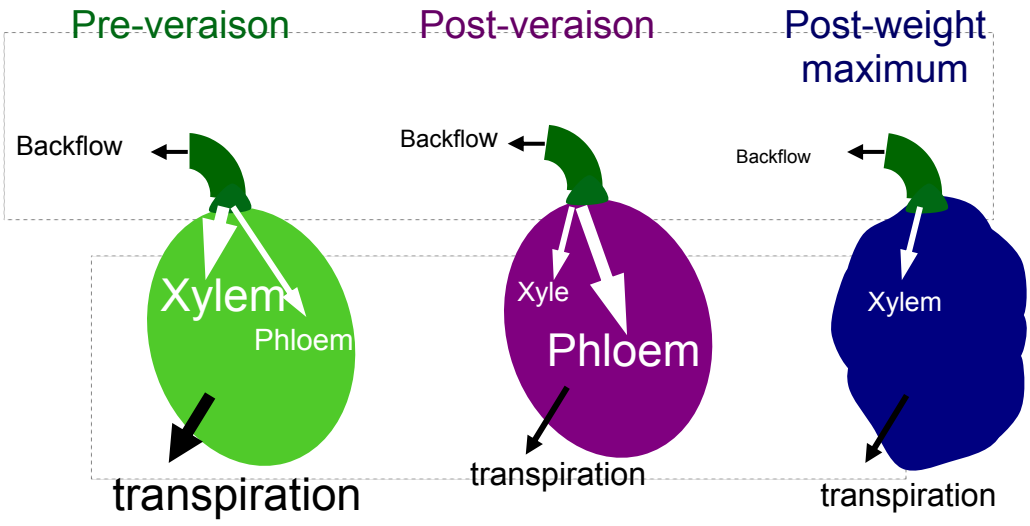


Figure 8.1 Summary of changes in water relations of Shiraz berries during development and weight loss. The pre-veraison berry has high rates of water import through the xylem and some import through the phloem. Transpiration rates are very high and some backflow may occur. The berry expands because net amounts of water entering the berry through the xylem and phloem in a day exceeds that lost by transpiration and backflow. The post-veraison berry has high rates of phloem import and a small amount of import through the xylem.

Transpiration rates of the post-veraison berry are less than that of the pre-veraison berry and backflow is also reduced. Again, because net amounts of water entering the post-veraison berry through the phloem and xylem in a day exceeds that lost by transpiration and backflow, the berry expands. In the post-weight maximum berry, phloem flow into the berry has ceased, there is some xylem inflow and ongoing low transpiration. The berry shrinks because the net amount of water entering through the xylem is not enough to balance that lost by transpiration.

9.0 Recommendations

This physiological study on causes for shrivelling in Shiraz has relevance to the understanding of grape ripening in general. Knowledge on the contribution of the vascular streams to the growth and development of the grape berry would enable development of more specific management practises, especially in regards to irrigation and nutrition. But method development for better quantification of xylem and phloem transport is essential for progress in this field.

Further field studies are required to monitor the influence of irrigation on rates of weight loss during late ripening. The potential for rootstock utilisation in the control of shrivel also warrants further study. An examination of wine quality at different points along the berry weight loss trend would also provide useful information to winemakers and viticulturists.

Appendix 1: Communication

Refereed Publications

Rogiers SY, Hatfield JM and M Keller. 2004. Irrigation, nitrogen, and rootstock effects on volume loss of berries from potted Vitis vinifera L. cv. Shiraz vines. Vitis 43: 1-6.

Rogiers SY, Hatfield JM, Jaudzems VG, White RG and M Keller. 2004. Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and pre-harvest weight loss. American Journal of Enology 55: 121-128.

Rogiers SY, Smith JP, White R, Keller M, Holzapfel BP and JM Virgona. 2001. Vascular function in berries of *Vitis vinifera* (L) cv. Shiraz. Australian Journal of Grape and Wine Research 7: 47-51.

Rogiers SY, Keller M, Holzapfel BP and JM Virgona. 2000. Accumulation of potassium and calcium by ripening berries on field vines of *Vitis vinifera* (L) cv. Shiraz. Australian Journal of Grape and Wine Research 6: 240-243.

Industry Publications

Dimos N, Rogiers SY, Greer D, Gray J and R White. 2003. Shiraz shrivel update: the results. Australian and New Zealand GrapeGrower and Winemaker 474: 17-19.

Dimos N, Gray J, Greer D, Rogiers SY and R White. 2003. Composition of shrivelled Shiraz grapes. Australian Viticulture. November-December p57-58.

NF Dimos, SY Rogiers, D. Greer, J. Gray and R. White. 2002. Shiraz shrivel update. The Australian Grapegrower and Winemaker magazine. 466: 31-32.

SY Rogiers. 2002. Physiology of Shiraz berry shrivel. Wagga Wagga Agricultural Institute Field Day Publication.

Symposium Presentations

SY Rogiers, N Dimos, J Gray, D Greer. 2004. Shiraz berry shrivel: all the latest. NWGIC Industry day, June 24, 2004, Wagga Wagga, NSW

SY Rogiers, JM Hatfield. 2003. Water flows into and out of shrinking Shiraz berries. 2nd Australian Grapevine Physiology Workshop, October 3, 2003, Sunbury, Vic.

SY Rogiers, N Dimos, J Gray and D Greer. 2002 Update CRCV project 2.4.2.- Examination of factors to onset and degree of pre-harvest weight loss in Shiraz berries. CRCV Shiraz berry shrivel meeting, October 11, 2002, Wagga Wagga, NSW.

SY Rogiers, J Hatfield and N Dimos. 2002. Is shrivel the way to go? Interactive display. NWGIC Annual Meeting, 11th-13th June 2002, Wagga Wagga, NSW.

SY Rogiers. 2002. Summary of year 2 CRCV project 2.4.2.- Examination of factors to onset and degree of pre-harvest weight loss in Shiraz berries. CRCV Program 2 annual meeting, Merbein, June 18-20, 2002

N. Dimos, SY Rogiers. R White. J Gray, and D Greer. 2002. Water transport out of developing grape berries. Australian Grapevine Plant Physiology Workshop, Research School of Biological Sciences, Canberra, October 4, 2002

Rogiers, SY. 2000. Pre-harvest weight loss in Shiraz: an investigation of the berry's water budget. National Wine and Grape Industry Centre Seminar Series, Wagga Wagga, NSW

Posters

Dimos N, R White, Rogiers SY, , Gray J, Greer D and. 2004. Vascular continuity in Shiraz berries. 12th Australian Wine Industry Technical Conference , July 24-29, Melbourne, Vic

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