DYNAMICS OF GRAPE BERRY VOLUME CHANGE DURING RIPENING

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of MARCO BIONDI find it satisfactory and recommend that it be accepted.

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DYNAMICS OF GRAPE BERRY VOLUME CHANGE DURING RIPENING

Abstract

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Vitis vinifera L. (c.v. Merlot) and *Vitis labruscana* Bailey (c.v. Concord) were used in this study to better understand the dynamics of fruit volume change during grape ripening. Berry diameter was monitored during dry-down and re-watering cycles at veraison. The diameter of green berries declined during dry-down and increased when the skin changed color even though water was not available in the soil. The increase in berry size which was accentuated by re-watering may be due the increase of phloem influx. Leaf photosynthesis also increased within two hours of rewatering. Pressurizing the root system of Concord vines during veraison showed that berries respond to pressure by increasing their volume up to a soluble solids concentration of 9 Brix and sometimes cracked at >11 Brix. When the xylem-mobile dye, basic fuchsin, was infused through the shoot base, the dye penetrated through the berry's vascular system. Dye penetration decreased as the berry skin changed color from green to blue. This trend could be reversed by pressurizing the shoot base. When dye was infused through the stylar end of post-veraison berries, it moved back to the plant only when pressure applied to the root was released. This proves that the xylem vessels are perfectly functional and the berry is

not hydraulically isolated from the rest of the plant during ripening. Berries immersed in distilled and tap water absorbed water through the skin and pedicel regardless of the variety, water source and maturity stage. Post-veraison berries were likely to crack and when they did, they lost up to 300 mg of sugar. Cracked berries gained more weight in distilled water than in tap water which might be explained by the difference of mineral concentration in the water source. Even if the berries did not crack, sugar leached out of the berry from the pedicel. When clusters were girdled or detached shortly before veraison, berries changed color similar to intact clusters. However, this was only true when the berries shriveled. Berry color change may be trigged by the sugar concentration which increases due to water loss or phloem influx.

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Dedication

This thesis is dedicated to my family, especially to my Mom and Dad, who throughout my whole life have provided encouragement to always achieve my dreams.

Grazie!

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 General Aspects of the Washington Wine and Grape Industry

Production of wine in the State of Washington has more than doubled over the past decade, making wine grapes the state's fourth largest fruit crop. In Washington State there are 350 wine grape growers, with more than 12,000 ha of *Vitis vinifera* L. vineyards (Ball *et al.,* 2003). Major red-wine cultivars planted are Cabernet Sauvignon (2448 ha), Merlot (2420 ha), Syrah (849 ha), and Cabernet Franc (750 ha), while the white-wine cultivars are Chardonnay (2687 ha), Riesling (890 ha), Sauvignon Blanc (287 ha), and Gewürztraminer (271 ha) (Washington Agricultural Statistics Service, 2006). The nine American Viticulture Areas (AVAs) currently located in the state are Yakima Valley, Columbia Valley, Walla Walla Valley, Puget Sound, Red Mountain, Columbia Gorge, Horse Heaven Hills, Wahluke Slope, and Rattlesnake Hills. Today, the Washington State wine industry affects the U.S economy by $ 3 billion annually (http://www.wawgg.org), and the acreage planted to wine grapes continues to grow each year. Washington State is also the leading producer of Concord grapes (*Vitis labruscana* Bailey) with ~ 10,000 hectares supplying more than half of the U.S total (Ball *et al.,* 2004). Concord is used mostly for juice and jelly and is highly appreciated in United States.

1.2 Climate of Washington State

Climatic conditions in Washington State are unique when compared with other regions of the world. West of the Cascades, temperatures are milder without the danger of extreme cold in the winter, but in eastern Washington, vineyards are susceptible to killing frosts and cold temperatures. On the east side of the Cascade Mountains a rainshadow effect prevails, so that much of the region is semi-arid with less than 250 mm of rain per

year. In Prosser, where this study has been conducted, average annual rainfall is 198 mm with 75% of the rain events occurring from October through April. There is about 10 ˚C range between day and night temperatures especially during the growing season. The growing season is short (158 frost free days) and the average cumulative "Class A" unscreened pan evaporation is 1266 mm for April through October (Naor and Wample, 1994) with maximum daily reference evapotranspiration (grass) during the growing season of about 9 mm d^{-1} (Evans *et al.*, 1993). The combination of a short growing season, low rainfall, hot summers and cold winters challenges Washington grape growers in several ways. One challenge is the necessity of irrigation to grow grapes due to the large deficit between evapotranspiration and rainfall.

1.3 Vegetative Development of Grapevines

Several distinct developmental stages such as dormancy, budbreak (budburst), bloom (anthesis, flowering), fruit set (berry set, setting), veraison (berry softening, color change, onset of ripening), harvest (ripeness, maturity), and leaf fall (abscission) occur in the annual vine growth cycle (Appendix 1). The vegetative cycle starts with bleeding in March, which refers to the exudation of xylem sap from pruning surfaces and is a transition from dormancy to active growth. Bleeding is caused by root pressure, which is generated by remobilization of nutrient reserves (especially carbohydrates) and pumping of sugar into the xylem (Sperry *et al.*, 1987). Root pressure serves to dissolve and push out air bubbles that have formed in the xylem vessels during the winter. In temperate climates budbreak and shoot growth are induced by mean daily temperatures above 10˚C (Galet, 2000). Budbreak is affected also by the grape variety, the time of pruning, the

vigor of the vine, water stress and cold hardiness (Mills *et al.,* 2006). Budbreak is followed by a period of exponential shoot growth (Williams and Matthews, 1990). This phase is characterized by strong apical dominance, whereby release of auxin by the growing main-shoot tip inhibits lateral shoot growth. However, lateral shoots can supply up to 40% the total leaf area (Candolfi-Vasconcelos and Koblet, 1990) and lateral leaves are important contributors to sugar accumulation in the fruit and starch accumulation in the wood (Candolfi-Vasconcelos and Koblet, 1990; Mabrouk *et al.,* 1997; Schultz *et al.,* 1996).

Unlike many fruit trees which produce specialized fruiting buds and vegetative buds, grapevines produce both fruit and foliage from the same buds. Buds are particularly numerous and complex. Latent buds can remain dormant for several years, before some event activates them. In the axil of each leaf on the main shoot there are lateral buds which can development and enrich leaf area (Pratt, 1971). The petiole is the leaf stem that connects the leaf to the shoot and contains multiple vascular bundles from a separate shoot vascular bundle (Pratt *et al.*, 1974). As perennial plants, grapevines annually store and remobilize carbohydrates and mineral nutrients in response to changing conditions (Galet, 2000). After budbreak when the cells of the young shoot are dividing, growth is slow. Then, as mean temperatures rise, growth and shoot elongation accelerates from day to day (Williams *et al.,* 1987). Unless growth conditions become unfavorable (e.g. with water stress), the shoots will continue to grow (Winkler *et al.*, 1974). Shoots and leaves position themselves on the outside of the vine's canopy to capture sunlight. The plant leaf area depends on the training system, plant vigor, pruning intensity and genotype

(Williams, 1987; Mabrouk *et al.,* 1997; Palliotti *et al.*, 2000). The basic function of the leaf is to capture sunlight for energy (ATP) production and carbon dioxide (CO_2) for carbohydrate production to support the vine's metabolism.

Grapevine leaves reach light saturation at photosynthetic photon fluxes between 700 and 1200 μmol m^{-2} s^{-1}, which is well below the photon flux of full sunlight (up to ≥2000 μmol m^{-2} s^{-1}) (Mullins *et al.,* 2003). However, the conditions under which vines are grown may cause some change in the value for light saturation (Kriedemann, 1968). Temperature is another important environmental factor that can influence the rate of photosynthesis in grape leaves. The optimum leaf temperature for photosynthesis of field grown vines is generally between 25 and 35˚C (Kriedemann, 1968). Vine leaves typically have between 100 and 400 stomata per mm^2 (Mullins *et al.,* 2003). Stomata are responsible for regulating the gas exchange (mainly CO_2 for photosynthesis, oxygen (O_2) for respiration, and water (H_2O) vapor from transpiration) between the leaf and the atmosphere.

Vines, like other plants, are stationary so they cannot move from place to place to find better food sources. Because uptake of water and nutrients by the roots from the surrounding soil quickly depletes available resources, the roots must keep growing throughout the vine's life and the season in order to maintain the supply of these raw materials (Williams. *et al.,* 1991; Araujo *et al.,* 1988).

1.4 Reproductive Development of Grapevines

Grapevines require two consecutive growing seasons for flower and fruit production. Inflorescences are initiated the year before bloom. Environmental conditions required for the formation of the maximum number of inflorescence include high light intensity, optimal temperature (25-30˚C), and adequate water and nutrient availability (Mullins *et al.,* 2003). Shoots and inflorescence develop between inflorescence initiation and anthesis. Depending on the species and cultivar, flowers might be female, male, or hermaphroditic (also known as "perfect", which means that each flower contains both male and female structures). By far, the majority of cultivated varieties are hermaphroditic (Pratt, 1971). Anthesis is the period during which a flower is fully open and functional. After bloom, fertilized flowers set fruit, although about 2/3 of the individual florets in the cluster drop off. Grape fruit set and growth is triggered by pollination and correlates with elevated endogenous auxin indole-3-acetic acid (IAA) levels (Cawthon and Morris, 1982). As berries develop, the fruit starts to be a greater 'sink' for photosynthetic products (Coombe, 1989; Ho 1988; Conradie, 1980), and the growth rate of shoots declines. Unfavorable environmental conditions (e.g. cloudy, cool, wet bloom period, water or nutrient stress), insufficient or inefficient leaf area (e.g. due to hail, insect or disease attack) or excessively vigorous shoot growth (competing with inflorescences for assimilates) often result in poor fruit set and loose clusters.

Growth of some fleshy fruit such as grape exhibits a double sigmoid pattern in which there are two periods of growth separated by a lag phase (Coombe, 2001; Harris *et al.,* 1968; Ollat *et al.,* 2002) (Figure 1). The first stage is the cell division phase (phase I)

throughout mid July. Cell division and cell enlargement both contribute to pericarp growth in the early post-anthesis stage. Cell division in the grape pericarp begins 5-10 days before anthesis and continues for approximately 25 days (Harris *et al.,* 1968). Following the cell division phase, is a pause in berry growth called the "lag phase" or phase II, during which seed formation progresses. Berry turgor pressure also declines about tenfold during this period (Matthews and Shackel, 2005). The length of the lag phase (one to six weeks) depends largely on the cultivar and is important in determining the time of fruit maturity (early vs. late ripening varieties). After the lag phase, the number of cells in each berry is set. Further berry growth is due to cell expansion only (Hardie *et al.,* 1996; Ollat *et al.,* 2002; Coombe 1976; Pratt, 1971).

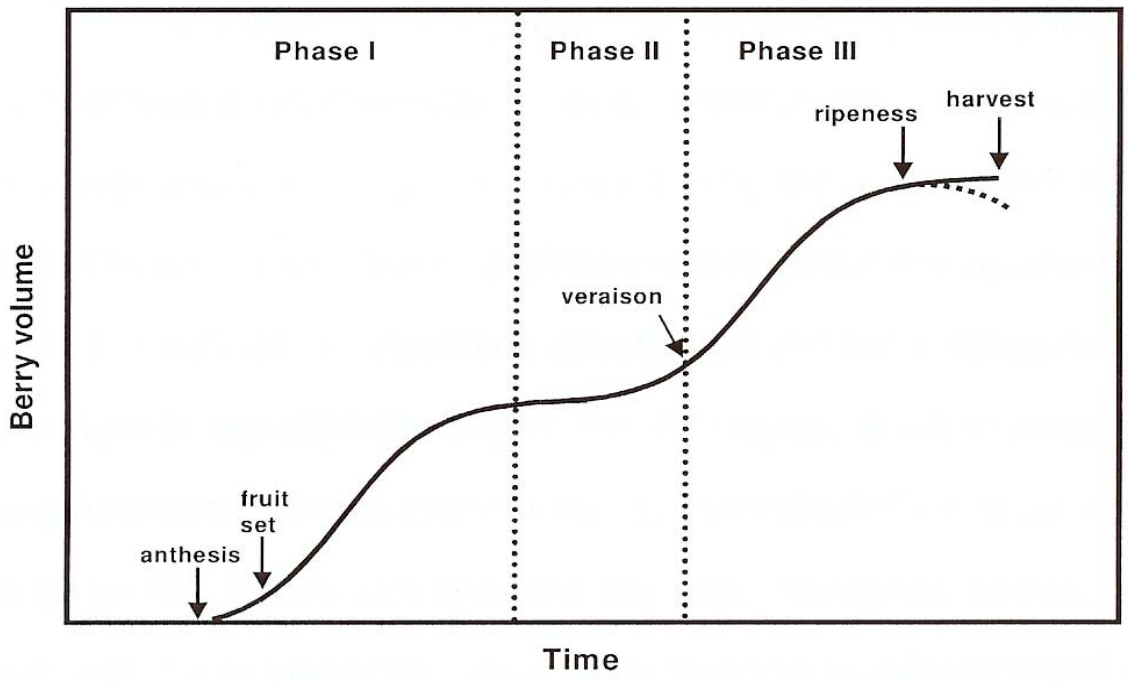

Figure 1: Diagram of a typical double-sigmoid pattern of growth by a grape berry, from anthesis to harvest. As noted in the figure above, in some cultivars, berries may shrivel between ripeness and harvest (redrawn from Coombe, 2001).

At the end of the quiescent lag phase, veraison (a French word meaning the commencement of berry color change) marks the beginning of ripening. During veraison the berries soften and skin changes in red-fruited cultivars from green to purple, while in white-fruited cultivars the berries acquire a more translucent appearance. The veraison process can continue for two weeks until all berries in the cluster complete the color change (Harris *et al*., 1968). Water import through the xylem declines gradually while the berry changes color. Although the xylem appears to remain functional (Bondada *et. al*., 2005; Keller *et al.,* 2006), most of the water for the berry is now provided through the phloem. Berry turgor pressure is low (<0.5 bar), but remains positive and relatively constant throughout ripening (Matthews and Shackel, 2005). The last phase or phase III is characterized by a further increase in berry volume, which is initially very rapid but slows progressively towards fruit maturity. Red pigments (anthocyanins) accumulate in the exocarp, and sugars (glucose and fructose) accumulate in the pericarp, while organic acids (malate) and chlorophyll are degraded (Coombe, 2000; Ollat *et al*., 2002). Unlike in many other (so-called climacteric) fruits, the "fruit-ripening" hormone ethylene does not appear to play a prominent role in grape ripening (Coombe, 1976).

1.5 Anatomy of the Grape Berry

The grape berry has three major types of tissue (Figure 2): seed, skin and flesh. Grape berries can have a maximum of four seeds, although in practice the seed number is usually one or two (Cawthon *et al.,* 1982, Coombe, 1987). Seeds are made up of the outer and inner integument, which together form the testa (seed coat), the nucellus surrounding

the developing endosperm, and the embryo (with two cotyledons, epicotyl, hypocotyl, and radicle). After fertilization the pistil develops into the fruit, with the ovary wall (pericarp) becoming the skin and flesh of the grape berry. The pericarp consists of three anatomically distinct tissues: the exocarp, mesocarp, and endocarp (Coombe, 1987). The exocarp forms the grape's dermal system, or 'skin', which makes up between 5% and 18% of the fresh weight of mature berries. It is made up of a cuticle-covered (single-layer) epidermis and the underlying outer hypodermis (Considine and Kriedemann, 1972). Epicuticular wax covers the surface of the cuticle forming a strongly hydrophobic layer that protects the berry from water loss (Rogiers *et al.,* 2004). The structure of the epicuticular wax changes with age of the berry. Although the wax is normally crystalline, the crystals appear to degrade slowly over time (Rogiers *et al.,* 2004). The wax material is rather soft and can be altered or removed by the impact of rain, by abrasion from wind-blown particles, or by contact with other berries and leaves.

The mesocarp, which is commonly called the 'flesh' or 'pulp' of the grape berry, consists of 25 to 30 layers of thin-walled and highly vacuolated parenchyma cells. Vacuoles can make up as much as 99% of the cell volume in ripe grape berries and contain sugars and organic acids. While the mesocarp cell walls remain intact, their polysaccharide components (such as cellulose) are modified at veraison to enable the berry to soften (Coombe,1987; Ollat *et al.,* 2002). Moreover, the incorporation of soluble proteins (especially glycoproteins, i.e. proteins attached to sugar molecules) reinforces the cell walls, so that cellular integrity can be maintained during softening (Davies *et al.,* 1999). The outer mesocarp contains the tissues outside the network of peripheral vascular

bundles of the pericarp; the inner mesocarp is inside the network and, at maturity, makes up almost two thirds of the berry volume (Coombe, 1987). The innermost tissue in the pericarp is the septal tissue, or the endocarp, which surrounds the seeds. Throughout the flesh and under the skin are vascular bundles. The ovular vascular bundles that previously served the ovary give rise to a complex network of vascular traces (axial and peripheral) that supply the seed and the pericarp (Pratt, 1971). The central vascular bundles (axial) and their associated parenchyma cells are termed the 'brush' and remain attached to the pedicel when a ripe grape is plucked from the cluster. Vascular bundles are composed of xylem with the phloem next to it and are responsible for water and nutrients transport through the plant (Matthews and Shackel, 2005).

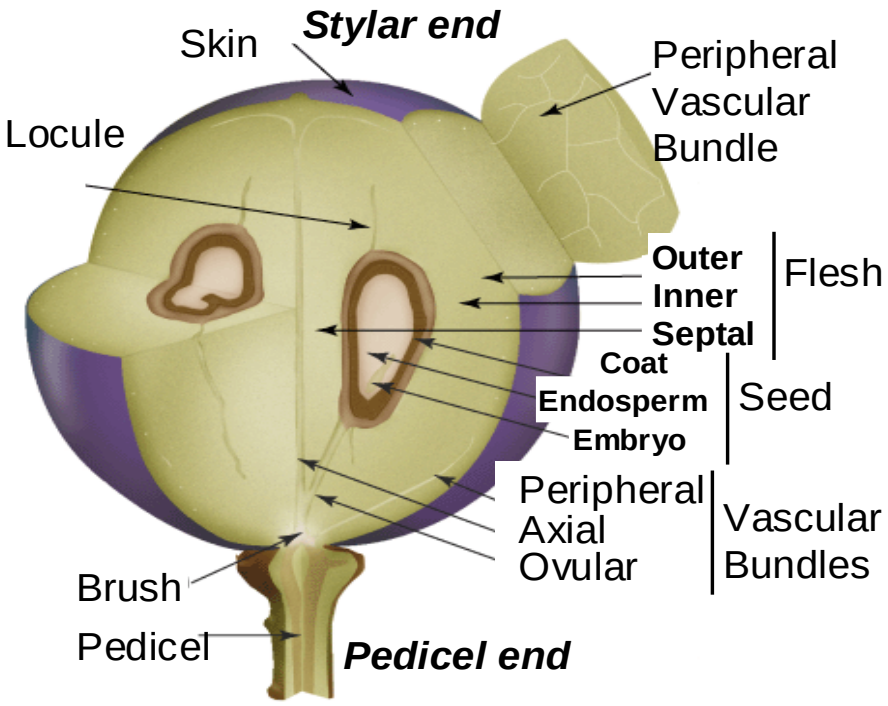

Figure 2: Structure of a ripe grape berry partially sectioned on the long and central axis to show internal part (A) (illustration redrawn from Coombe, 2001).

1.6 Water Flow and Vascular Transport

Much of the following information was gathered from the text book "Plant Physiological Ecology" (Lambers *et al.,* 1998). In plants, exchange of water between the fruit and the plant occurs primarily through the phloem and xylem tissues (Matthews and Shackel, 2005) which represent the vascular transport. The xylem and phloem are interconnected along their entire length, so can readily exchange water and solutes (Evert, 2006; Zwieniecki *et al.,* 2004). Water relations of developing fruit play an important role in determining fruit growth and composition. Grapevine's fresh mass consists between 70% and 95% of water. Most of this water serves as a solvent for ions and organic molecules in vine's cells. Water can diffuse freely (but relatively slowly) across the phospholipid bilayer of cell membranes. Membranes are selectively permeable or semipermeable so, special gates (protein channels and transporters or pumps) are required for molecules other than H_2O to pass through membranes. Because the concentration of solute in the cell such as sucrose, malic acid, and potassium (K^+) and chloride (Cl^-) are always higher than that of the exterior, there is relatively less water inside the cell than outside. This water concentration gradient causes water to diffuse through aquaporins (Steudle *et al.,* 2000) into the cell and this diffusion is termed osmosis.

Osmotic pressure (π) is defined as the hydrostatic pressure required to stop the net flow of water across a membrane separating solutions of different compositions. The movement of water during osmosis will always be from a region of lower solute concentration (i.e. greater water potential) to one of higher solute concentration (i.e. lesser water potential). When water moves into a cell, the wall pressure balances the

osmotic force. The cell wall raises the energy of the water inside the cell until it becomes equal to the water outside the cell. At this point the cell's internal hydrostatic pressure, termed turgor pressure, is equal to π inside – π outside and water flow stops. The solute and pressure forces in plants and soils are described as free energy per unit volume (i.e. equivalent to force per unit area, or pressure) which are termed water potential (Ψ expressed in MPa).

Water potential is the sum of the component potentials arising from the effects of turgor pressure (i.e. turgor potential, $\Psi_P = P$) and solutes (i.e. solute potential, Ψ_s or osmotic potential, $\Psi_\pi = -\pi$) in addition to interactions with matrices of solidss (cell walls) and macromolecules (i.e. matrix potential, Ψ_M), as described by the equation: $\Psi = \Psi_P + \Psi_\pi + \Psi_M$. The matrix potential is very close to zero in well-watered plant tissues and is therefore insignificant unless the tissue is dehydrated (e.g. loss of 50% of the tissue water). Pure water has the highest water potential which equals zero ($\Psi = 0$). Therefore, the water potential of aqueous solutions is always negative ($\Psi < 0$).

Water flow from the soil, roots, leaves to pre-veraison berries, is caused by a water potential gradient ($\Delta\Psi$) between the soil and the vine (Keller 2005). In addition, during the transition from dormancy to active growth of a grapevine in early spring, water uptake by the roots and transport to the shoots is driven by positive root pressure (Sperry *et al.*, 1987). Root pressure is induced by remobilization of stored nutrients and starch and unloading of osmotically active solutes into the xylem.

Using Ohm's law, the flow (F) of water from the soil to the leaves can be described by the equation $F = \Delta\Psi\ r_h^{-1}$ where $\Delta\Psi = \Psi_{soil} - \Psi_{leaf}$. The parameter r_h describes the hydraulic resistance to water flow due to friction between water and conduit walls and between the H_2O molecules themselves. Most of the r_h is imposed by the vine's hydraulic architecture which is determined by the shape, size, and arrangement of xylem vessels as well as the total length of the flow pathway and the number and shape of bends. Also the opening and closing of stomata generates a resistance (stomatal resistance). During the course of the day, stomatal opening (i.e. stomatal conductance, g_s) normally follows the daily change in light intensity, which peaks around midday. Conversely, stomata close when the leaf runs out of water. Therefore, low Ψ_{leaf} (indicating water stress) causes stomata to close partially to protect the xylem conduits (vessels and tracheids) from cavitation (Jones, 1998). In fact, cavitation occurs when the water column inside the xylem is put under too much tension so the water column breaks and fills up with H_2O vapor or air bubbles (Figure 3-A). Gas blockages (embolisms) greatly increase r_h which renders the vessels nonfunctional and can lead to canopy desiccation (Schultz and Matthews, 1988). For the gases to dissolve and reestablish xylem functionality, the xylem pressure must rise to near atmospheric pressure (0.1 MPa) or above (Sperry *et al*., 2002).

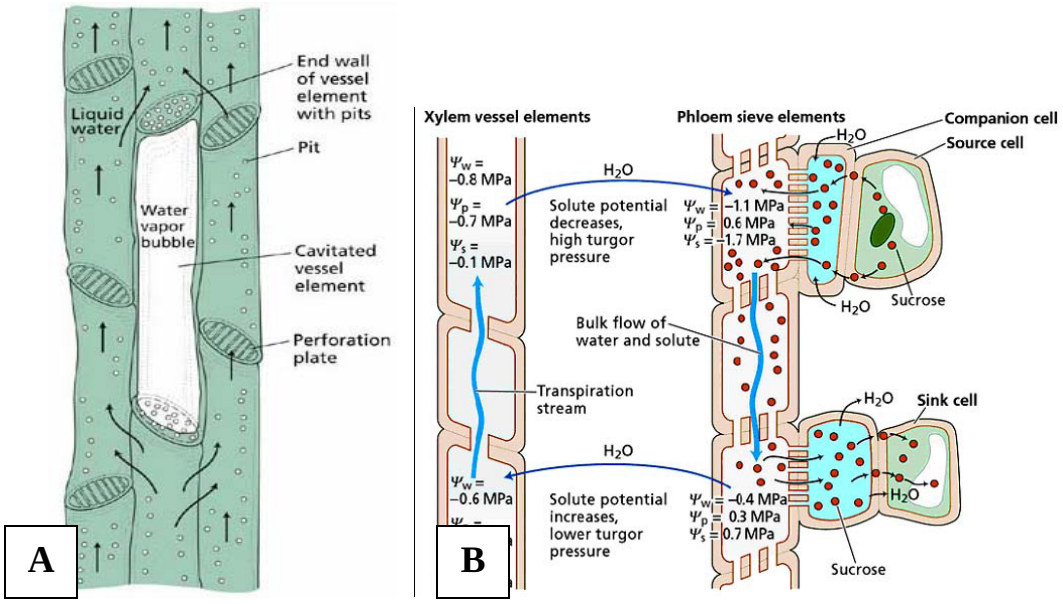

Figure 3: Cavitation of xylem vessel by water vapor bubble (A) (Taiz and Zeiger, 1998); Vascular flow (B) (Nobel, 1999).

Pre-veraison berries transpire due to the presence of stomata in the skin. Transpiration has been estimated at ~ 550 μL d^{-1} in pre-veraison berries and <100 μL d^{-1} in post-veraison berries (Greespan *et. al.,* 1996, Rogiers *et al.,* 2004). Fruit transpiration declines during development, implying that evaporative water loss during ripening ceases to function as the driving force for water influx (Rogiers *et al.,* 2004). Also a $\Delta\Psi$ in the xylem is the driving force for water movement to pre-veraison berries, whereas phloem flow is caused by the differences in positive pressure resulting in the loading and unloading of sugars in source and sink regions (Figure 3-B) (Lang and Düring 1991). Phloem unloading in grape berries (similar to tomato fruit), switches at veraison from the standard symplastic (through the cell cytoplasm) route to an apoplastic (extra-cellular including cell walls) path, in which sucrose is released to the apoplast (Zhang *et al.,* 2006).

Scientists believe that during veraison the grape berry becomes hydraulically isolated, so there is no fluid exchange between the vine and the berry through the xylem vessel (Düring *et al.,* 1987; Findlay *et al.,* 1987; Creasy *et al*., 1993). Evidence for a xylem breakdown comes mainly from studies of dye tracer perfusion through the pedicel, originally by Findlay *et al.* (1987) and Düring *et al.* (1987) and later confirmed for different grape cultivars by Creasy *et al.* (1993) and Rogiers *et al.* (2001). In fact, hydraulic isolation of the fruit sink is now often regarded as a necessity for apoplastic phloem unloading (Patrick 1997), as seen in grape (Sarry *et al.,* 2004), apple (Zhang *et al*., 2004), kiwifruit (Dichio *et al.,* 2003), or tomato (Davies *et al.,* 2000) and such isolation is considered a prerequisite to prevent loss of solutes via the xylem.

1.7 Water Excess and Deficiency

Water and nutrients are basic requirements for plant growth and leaf water supply constitutes the limitation on plant productivity. Increasing soil moisture stimulates vigor, which can lead to a denser canopy and shaded fruit (Kriedemann and Goodwin 2003). Abundant water supply ordinarily delays veraison and slows the rate of fruit ripening. High availability of water and nitrogen to the vine has been associated with excessive vegetative growth (vigor). Excessive vigor may have undesirable consequences, such as vegetative growth competing for assimilate (Wade *et al.,* 2000). This can influence many of the processes which affect yield and quality, including budbreak, inflorescence initiation, fruit set, berry growth and the balance between sugars, acids, aroma and flavor compounds which collectively describe fruit quality (Loveys *et at.,* 1998).

Under natural conditions, water is supplied by snow and rainfall and temporarily stored in the soil for extraction by plant roots. In addition, soil water holding capacity and hence the amount of plant-available water varies with soil depth, texture, and organic matter content. Variation in soil moisture due to differences in water holding capacity and effective rootzone has a pronounced impact on vine performance (Hall *et al.,* 2002). Water deficit typically reduces yield (Williams and Matthews, 1990) and fruitset (Hardie and Considine, 1976) and can increase or decrease berry sugar content, acidity, pH, and color (Matthews and Anderson, 1988) depending on the extent and timing of the deficit. Pre-veraison water stress tends to have a greater effect on berry size reduction as compared to post–veraison water stress (Matthews and Anderson, 1988). Mild water deficit (-1.2 MPa) is ordinarily applied before veraison to limit shoot growth which may increase sugar accumulation in the berry by reducing canopy density (Kriedemann and Goodwin, 2003). However, water stress can delay berry development because of a reduction in photosynthesis or, in extreme cases, leaf drop (Kriedemann and Goodwin, 2003). In addition, water stress induces the formation of air bubbles in the xylem vessel called cavitation which stops xylem flow. Therefore root pressure pushes out air bubbles and reestablishes xylem functionality.

To produce premium wines, growers frequently receive bonus payments for fruit with above-average sugar and/or color concentration. Grape growers and winemakers are often concerned about the “dilution of grape quality or even cracking of berries” by late season irrigation or rain. This popular belief, written into the law in Europe, prohibit or strictly regulate irrigation after veraison (Galet, 2000; Keller *et al.,* 2006).On the other

hand, grape berries tend to shrivel at the end of maturity losing up to 25 % of the predicted yield (Rogiers *et al.,* 2006; McCarthy and Coombe, 1999; McCarthy, 1999).

1.8 The Hypothesis

Our hypothesis is that xylem is functional in the post-veraison stage and is responsible for recycling excess water from the berry (Bondada *et al.,* 2005; Keller *et al.,* 2006) while phloem flow is responsible for transporting sugar diluted in water into the berry (Greespan *et. al.,* 1994, Rogiers *et al.,* 2004). Consequently, irrigation later in the season could actually help berry ripening due to high photosynthetic activity and could maintain berry weight without an increase or decrease in berry-size due to water flow. Moreover, xylem functionality during ripening combined with natural root pressure may increase berry size and dilute the quality of the grape. Root pressure, which is the driving force for sap flow in early spring (Fisher *et al.,* 1997), is generated by a degradation of starch in the root in glucose which then actively moves from the parenchyma cells to the xylem vessel resulting in hydrostatic pressure. Our hypothesis is that the same starch degradation process may happen during ripening when the plant is under water stress. If the stressed plant with high concentration of sugar in the xylem vessel could find water available due to late season rainfall or irrigation, the root pressure generated by osmosis may be higher than the xylem backflow from the berry resulting in an influx of water. An increase of water inside the berry before harvest may result in increased berry volume, which could lead to dilution of berry sugar concentration or even cracking. Also, during rainfall, berries may take up water directly through the skin (Lang and Thorpe, 1989),

which suggests overhead sprinkler irrigation or rainfall might effectively dilute berry solutes.

Knowing how water flows in and out of the berry during ripening is important for both wine and juice grape industries. In wine grapes, winemakers desire small berries that have a high sugar concentration (~25 Brix soluble solids), a high proportion of skin and seed derived compounds, and high skin-pulp ratio for good color extraction which are requisites for high quality wine. In juice grapes, growers want to maintain a sugar content above 16 Brix, and keep the weight of the berries as high as possible. Since rainfall is likely to occur late in the season (September-October), knowing how water moves into and out of the berry and through the skin and how much it affects berry size would be helpful to viticulturists and winemakers for scheduling harvest time. In regard to health of the vines, water stress would decrease photosynthetic rate which would cause a lower accumulation of sugar inside the berry and a depletion of starch reserves in roots resulting in poor cold hardiness and budbreak (Mullins *et al.,* 2003).

The experiments described in this thesis were set up to examine if: berry size responds to dry-down and re-watering cycles during veraison; berry size responds to rapid changes in plant water status during veraison; xylem connections between the berries and the rest of the plant are intact; irrigation or rainfall affects berry weight at different maturation stages; water can enter in the berry through the skin and pedicel; xylem backflow is an overflow mechanism to balance the amount of water inside the berry; sugar can leach out from the pedicel; and sugar can be exported between berries.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Location of the experiments

The study was conducted from 2005 to 2007 on own-rooted grapevines *V. vinifera* (Merlot, Chardonnay, Muscat Blanc), and *V. labruscana* (Concord). Grapevines were grown in white 20.0 liter PVC pots and black 3.0, 5.0, 10.0 liter PVC pots containing a mixture of 50% sandy loam, 25% peat moss, 25% pumice, and 30 g/L dolomite. The vines were grown outside in the full sun light (photosynthetic photon flux ~1820 μmol m-2 s-1 under clear sky at midday) (Figure 4) then moved inside in the air-conditioned glass houses (Figure 5) at the Irrigated Agriculture Research and Extension Center in Prosser, Washington, USA (46˚ 17' N; 119˚ 44' W; elevation 270 m). Prosser is located in Yakima Valley which has a semi-arid climate. The growing season in this region is characterized by warm days and cool nights with a long-term (1954-2004) average accumulation of 1344 growing degree days (base 10˚C) and low rainfall (199 mm/year) (Figure 6) (www.weather.wsu.edu). Meteorological conditions in the glasshouse were recorded with a HMP45 (Vaisala, Oyj, Finland) temperature and relative humidity sensor (Figure 7). In addition to the potted plants, clusters were also collected from Merlot, Muscat Blanc, Chardonnay and Concord grapevines from two vineyards at the Irrigated Agriculture Research and Extension Center in Prosser, Washington. One vineyard was planted in 1984 in north-south oriented rows on a ~5% south facing slope at an elevation of 270 m. The other vineyard was planted in 1999 in north-south oriented rows at an elevation of 363 m. Both were spaced at 1.8 m (within rows) by 2.7 m (between rows) and were trained to a bilateral cordon with loose vertical shoot positioning.

Figure 4: Vines grown in white 20.0 liter PVC pots at the Irrigated Agriculture Research and Extension Center in Prosser, Washington, USA.

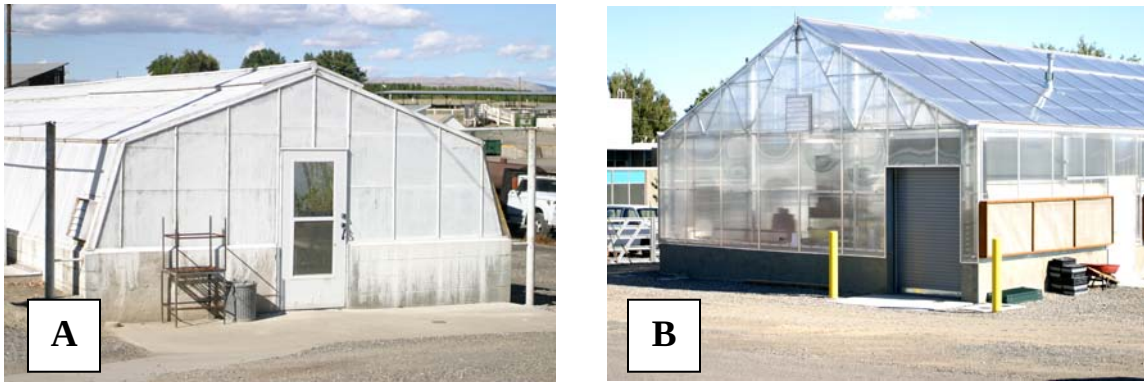

Figure 5: Glasshouses used for experiments at the Irrigated Agriculture Research and Extension Center in Prosser, Washington, USA. (Light intensity in glass-house A= photosynthetic photon flux ~ 860 µmoL m^{-2} s^{-1} under clear sky at midday; glass house B= photosynthetic photon flux ~1180 µmoL m^{-2} s^{-1} under clear sky at midday).

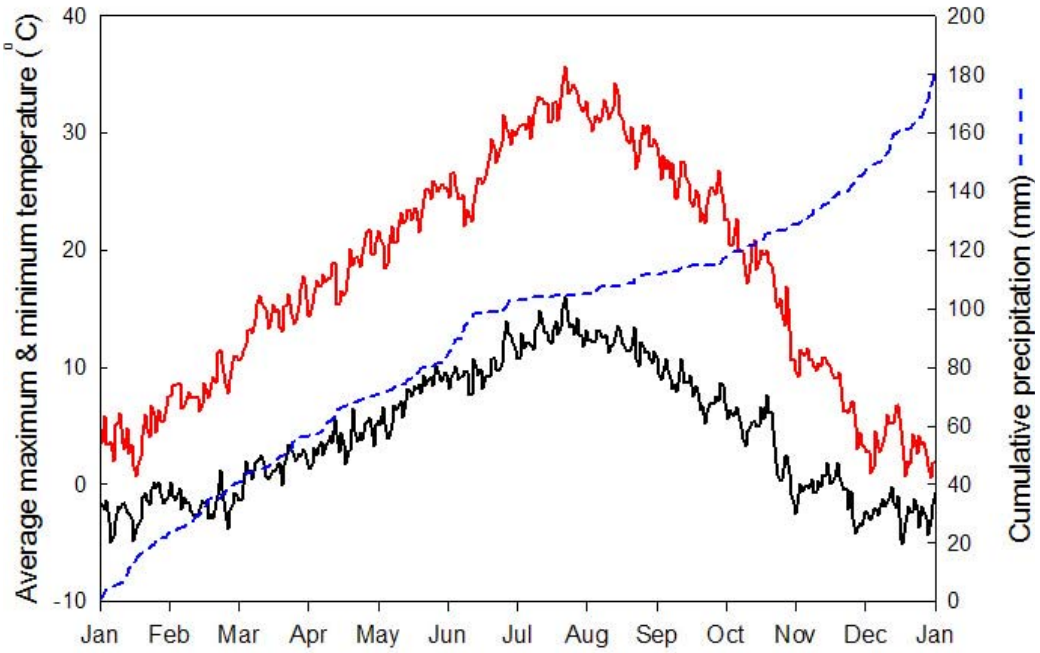

Figure 6: Climate of 2006 at IAREC Prosser, WA. Data from AgWeatherNet, Prosser, WA, USA.

2.2 Dry- down and re-watering experiment

A dry down and re-watering experiment was conducted to determine the response of berry size to dry-down and re-watering cycles during veraison to understand whether the vine is sensitive to plant water status at that stage. Concord and Merlot were used in this study to determine and understand how the genus *Vitis* behaves. Berry size was monitored daily with a digital caliper (General Tools, New York, USA; 0.01 mm resolution) to identify the lag phase. During the "lag phase" berries stop growing for several days just prior to veraison. When the berries reached the lag phase, vines were no longer irrigated resulting in berry shriveling while the berries were at soluble solids level from 5 to 7 Brix for Concord and 6 to 8 Brix for Merlot. Three plants of each variety were used for the experiment and the experiment was repeated three times over two seasons. During each dry-down experiment changes in berry diameter were recorded on

three berries per vine, using FI-XSM linear variable displacement transducers (Phytech, Rehovet, Israel, figure 7-B) inside the glasshouse. Transducers were connected via an AM416 relay multiplexer (Campbell Scientific, Logan, UT, USA) to a CR10X data logger (Campbell Scientific, Logan, UT, USA), to the computer (Figure 8). The calibration for the sensors was preformed by attaching the sensors to different diameter surfaces (0, 0.96 mm, 3.18 mm, 6.45 mm, 9.99 mm) and reading the sensor measurement in mV. The plants were also placed on digital balances (Rice Lake Weighing System, Rice Lake, WI, USA) (Figure 7-A) connected via an AM 416 relay multiplexer to a CR10X data logger to record transpiration. The digital balances were calibrated by measuring known weight in mV. The pot surfaces were sealed with a plastic bag to prevent water loss by evaporation from the soil (Figure 7-A). Transpiration was calculated by subtracting the daily weight of the pot from the initial weight. During veraison berries with similar color to the berries analyzed by the sensors were collected and soluble solids content (Brix) was measured with the refractometer (Pocket PAL-1, Atago, Tokyo, Japan) to determine their stage of ripening. When the berries changed color to red-purple, each vine was irrigated with 2 L of water. The experiment was terminated two days after irrigation. At the end of each experiment, the selected berries were removed, and the soluble solids content was measured.

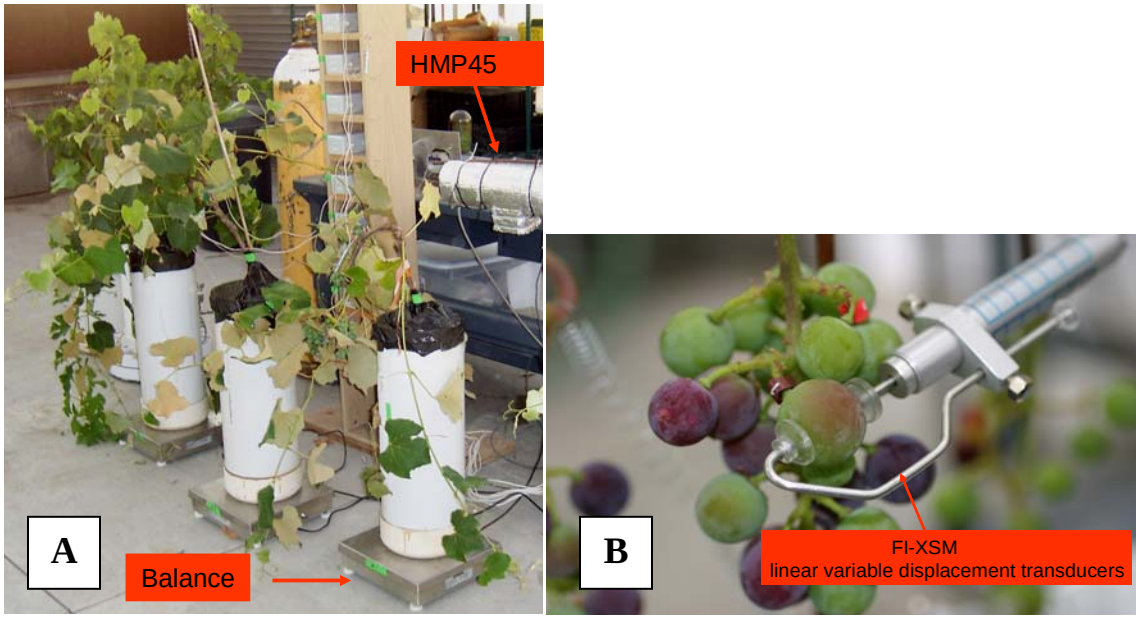

Figure 7: Plants placed on digital balances and the pot surfaces sealed with a plastic bag to prevent water loss by evaporation from the soil (A). Meteorological conditions in the glasshouse recorded using an HMP45 temperature and relative humidity sensor (A). Also, berry diameter recorded with FI-XSM linear variable displacement transducers (B).

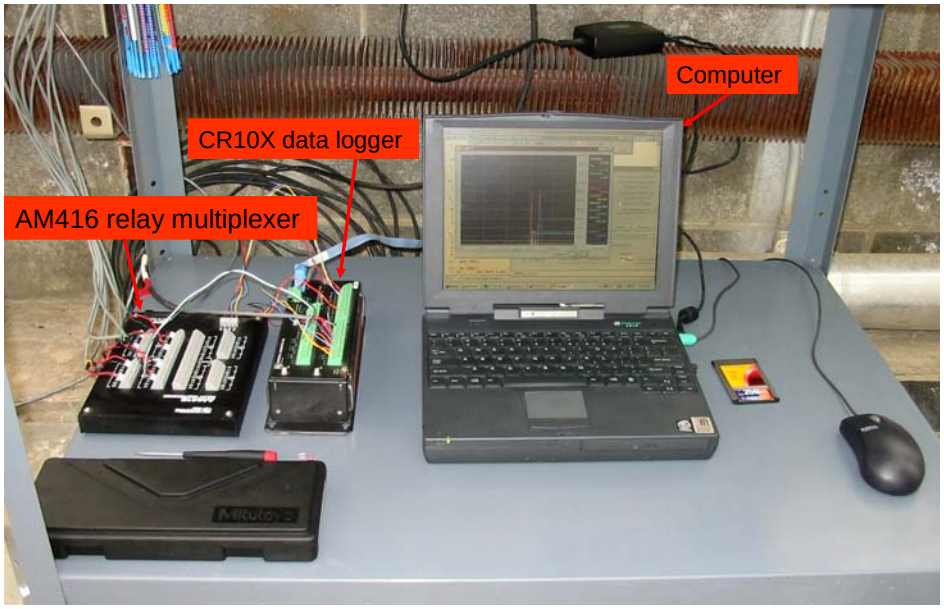

Figure 8: FI-XSM linear variable displacement transducers connected via an AM416 relay multiplexer to a CR10X data logger which was connected to the computer.

2.3 Root pressurization experiments

2.3.1 Berry size response

This experiment was conducted to determine the effect of rapid changes in plant water status on berry size during veraison. Pressure was applied to three Concord vines with a population of berries colored from green to blue (6.2 to 13.2 Brix) after having been dried down for five days and re-watered. When pressure is applied to a plant's root system, the entire xylem becomes pressurized (Wei *et al.,* 2000). A custom-built 26-L metal root pressure chamber, based on the design by Yong (2000) and modified by Smith (2004) (Figure 9) was used in this study. The chamber was designed with a split lid such that the entire white 20.0 L PVC pot could be placed inside the chamber and the lid sealed around either side of the vine trunk. The join line of the lid was covered with a fast setting two-part silicon epoxy and the two halves of the lid were pulled together forming an air-tight seal. A split silicon bung (Dow Corning Silastic 3481, Wiesbaden, Germany) with a hole to match the trunk diameter was then pushed into the well in the center of the lid and a compression collar bolted down to seal around the stem (Figure 9-B). Pneumatic pressure was applied to the chamber under manual control with compressed air and was monitored with an analog pressure gauge. Gas flow to the chamber was regulated via metering and 3-way valves. The procedure was a rapid one-step increase to 10 bar in root pressure to bring the plant to full hydration ($\Psi_{leaf} \approx 0$ MPa) within a few minutes. This pressure was held for approximately 3 hours and 30 minutes with adjustments made manually to maintain the vine at full hydration. Seven FI-XSM sensors were used to monitor berry diameter. At the end of each experiment, the selected berries were removed, and the soluble solidss content was measured with a refractometer.

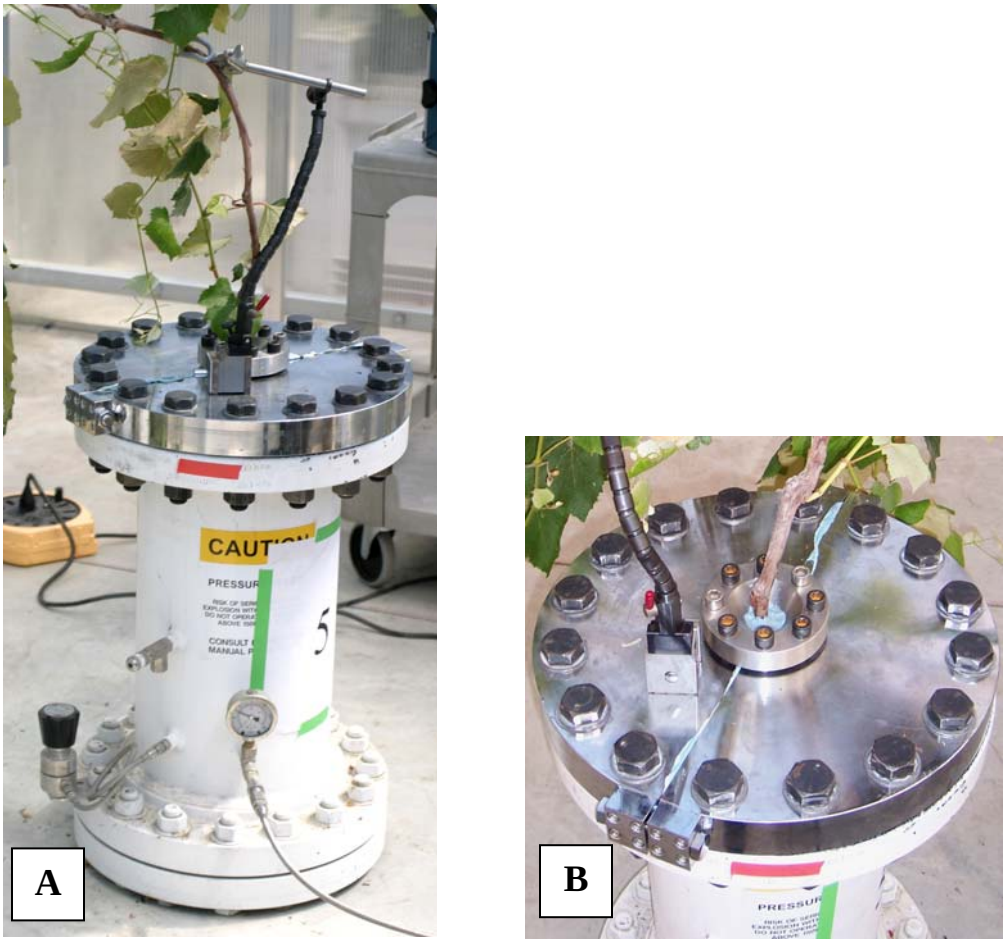

Figure 9: Different perspectives of the custom-built 26-L metal root pressure chamber (A and B), based on the design by Yong (2000) and modified by Smith (2004). The compression collar bolted down to seal around the stem (B).

2.3.2 Xylem sap collection

The root pressure chamber was used to collect xylem sap to determine whether the vine mobilizes and pumps sugar into the xylem vessels to generate root pressure when water stressed. This normally happens in early spring before budbreak when the vine does not have any leaves (Sperry *et al.,* 1987). After veraison, three vines of Concord grown in 20.0 L PVC pots were not irrigated for a week. The Model 615 Pressure Bomb (PMS Instrument Company, Corvallis, Oregon, USA) (Figure 10-A). was used to determine leaf water potential (Ψ_{leaf}). The leaf was cut from the vine using a sharp razor blade (Feather S, 0.1 mm, Tokyo, Japan) and quickly inserted and sealed in the chamber. When the Ψ_{leaf} reached -2 MPa, one vine at the time was irrigated with 2 L of water and inserted into the root pressure chamber (Figure 9). Pressure was applied to keep the vines at full hydration. A cluster was cut off to expose fresh tissue and a length of parafilm TM (American National Can, Chicago, Illinois, USA) was then wrapped around the peduncle for collecting the sap into an HPLC vial (Figure 10-B). Approximately 0.5 mL of xylem sap was collected from each vine after pressurizing at 10 bar for 2 hours. The sample was centrifuged for 1 min at 10,000 rpm in a centrifuge tube. The collected sap was analyzed for sugar concentration by HPLC Agilent-1100 series (Agilent Technologies, Munich, Germany). The sugar analysis was carried out using a solvent system (acetonitrile: water = 80:20) and an injection volume of 8µL. The column temperature was 30˚C and refractive index detector temperature was 30˚C. The flow was 1.1 mL min^{-1} and run time was 11 minutes. The column used was an Agilent Zorbax Carbohydrate analysis column (4.6mm X 150 mm, 5 micron, Agilent Technologies, Chicago, Illinois, USA).

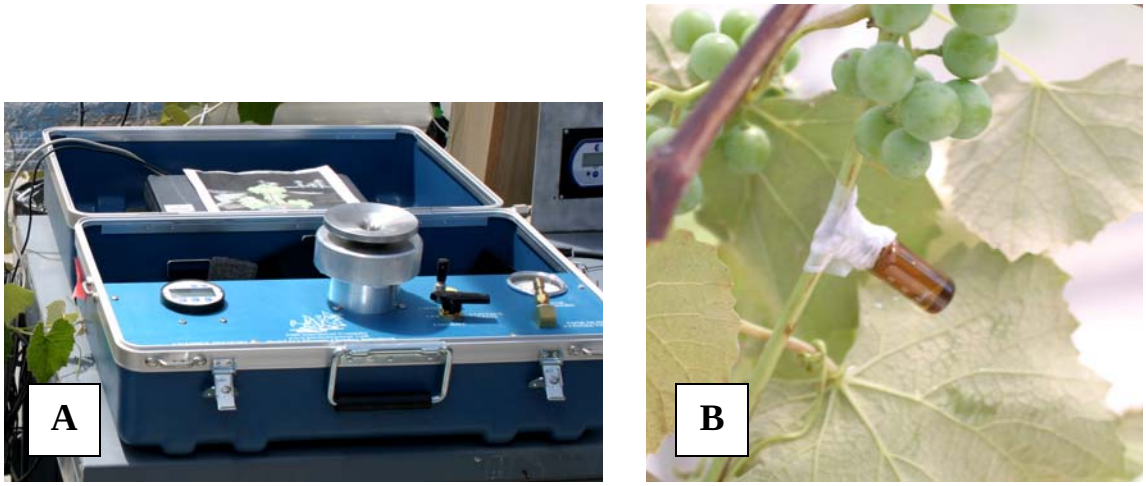

Figure 10: The Model 615 Pressure Bomb used to determine the leaf water potential (A). A length of parafilm TM wrapped around the peduncle of the cut cluster to attach an HPLC vial for collecting the xylem sap (B).

2.3.3 Leaf water potential / photosynthesis rate / xylem sap collection

This experiment was conducted to understand how the water stress and re-watering cycle applied to Merlot and Concord vines undergoing veraison affects the physiological status of the vine. Moreover it was conducted to determine how rapidly the photosynthetic rate recovers after irrigation of water-stressed vines. The experiment was carried out in the glasshouse on a total of four vines at their lag phase of berry growth. The pots were watered at field capacity and then allowed to dry down. The Portable Photosynthesis System CIRAS-2 (PP Systems, Amesbury, Massachusetts, USA) was used to measure the photosynthesis rate (A) (Figure 11). The Model 615 Pressure Bomb was used to determine Ψ_{leaf} as described in 2.3.2. The root pressure chamber described in 2.3.1 was used for the collecting of xylem sap. All data were collected at the beginning of the experiment and after 5, 10 and 15 days. The photosynthetic rate was measured on the same three leaves before and after watering the vines with 500 mL of water. The leaves were selected from the fifth leaf from the tip of the main shoot. Photosynthesis was

recorded from 10 to 11 o'clock before irrigation and from 12 to 13 o'clock after irrigation when the photon flux was ~1180 µmol m^{-2} s^{-1} inside the glasshouse. Then after watering and photosynthesis measurements, root pressure was applied to each vine to bring the plant to full hydration. A length of parafilm TM was wrapped around the petiole of a cut leaf to collect the sap into an HPLC vial. The root pressure was held for two hours at 10 bar and nearly 0.5 mL of xylem sap was collected from each vine. The collected sap was analyzed for sugar concentration by HPLC as described in 2.3.2.

Figure 11: Photosynthetic rate measured by the Portable Photosynthesis System CIRAS-2.

2.4 Dye feeding experiments

Three dye feeding experiments were done to trace xylem connections between the berries and the rest of the plant and to attempt to quantify the xylem pressure inside of the berry using the xylem-mobile dye basic fuchsin (Sigma-Aldrich, St. Louis, MO, USA).

2.4.1 Dye feeding without pressure

The first dye feeding experiment was conducted on three clusters of Merlot and Concord at veraison without pressure. Clusters of dark-skinned cultivars undergoing veraison have a population of berries ranging from green to blue with soluble solids between 7 to 18 Brix. The clusters were cut off by a sharp razor blade and the peduncles were immediately placed in a centrifuge tube containing 30 mL of the xylem-mobile dye basic fuchsin (0.1 % aqueous solution) (Figure 12). The tubes were sealed around the peduncle with parafilm TM to prevent evaporation. Clusters were kept in dye for 24 hours in the glasshouse at a temperature of ~21 ˚C and 65% humidity. Twenty berries for each cluster were then visually rated for the progression of skin pigmentation (green, blush, pink, red, purple and blue) before being sectioned longitudinally for light microscopy. The extent of dye movement into the berry was rated visually and assigned a number from 1 (in brush only) through 6 (continuous throughout entire vascular network) (Figure 13). Following microscopy, the berry juice was expressed and soluble solids concentrations were measured with refractometer.

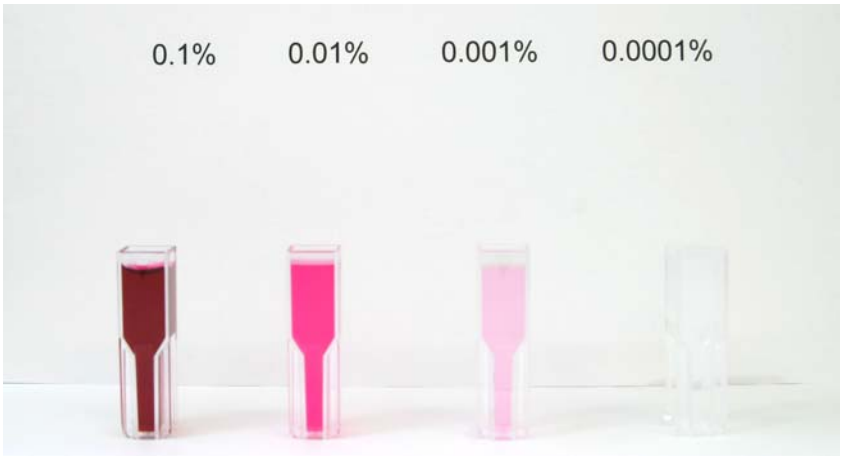

Figure 12: Correlation between dye basic fuchsin dilution and color intensity.

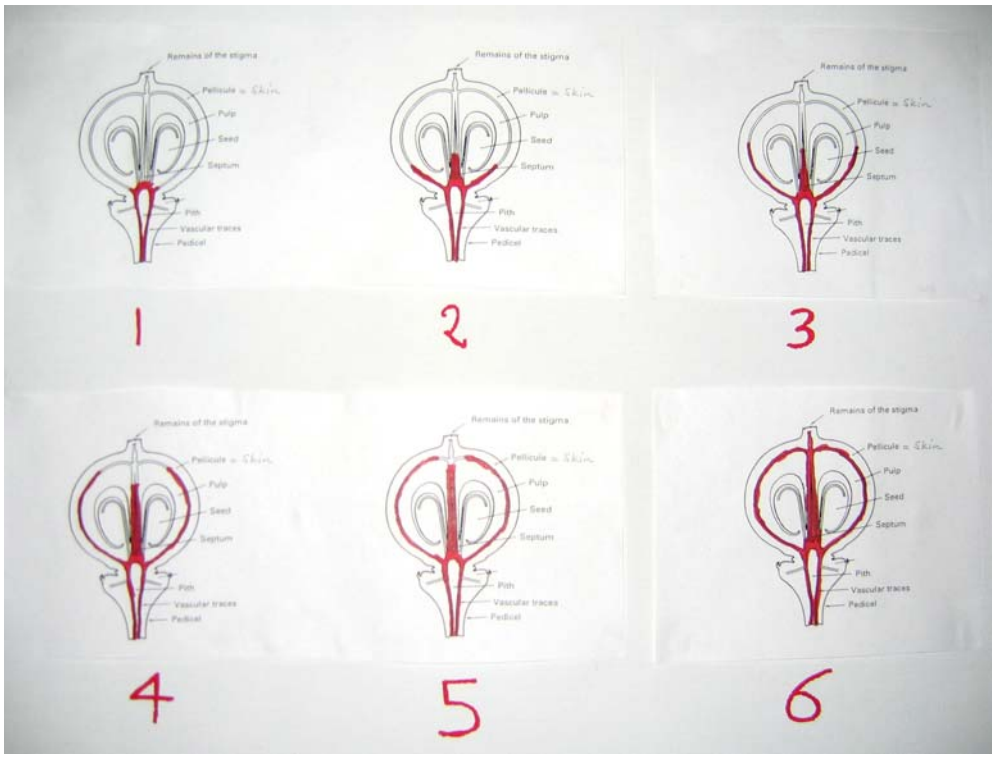

Figure 13: The extent of dye movement into the berry rated visually and assigned a number from 1, when the dye stops in the brush region, through 6, when the dye continues throughout the entire vascular bundles.

2.4.2 Dye feeding / shoot pressure chamber

Dye feeding experiments with pressure applied were conducted in two different stages of berry development. The first set of experiments was performed using Concord, Merlot, and Chardonnay at veraison and the second was conducted later in the season using Merlot, Muscat Blanc and Chardonnay at ~ 22 Brix. Shoots with mature leaves and one cluster were collected from field-grown grapevines in nearby vineyards for both experiments. The freshly cut base of the shoot was inserted through the gland and dipped into the dye container which was placed inside the Model 615 Pressure Bomb. The gland was then tightened around the shoot enough to assure that no gas and dye would escape during the pressurization (Figure 14). The dye was then pushed from the chamber into the cut shoot by pressure. In the first set of experiments, leaves of the shoot were cut off, and cut surfaces were burned and sealed with parafilm to avoid pressure dissipation (Figure 14-A) while in second set of experiments leaves where left on the shoot (Figure 14-B). During veraison, four different experiments were conducted. The first consisted in pressurizing Concord and Merlot clusters to 1 bar for 30 and 60 minutes and 60 berries were sampled. The second consisted in pressurizing Concord and Chardonnay clusters to 2 bar for 30 minutes and 100 berries were sampled. The third consisted in pressurizing Concord clusters to 1, 2 and 4 bar for 30 minutes and 50 berries for each treatment were sampled. The last experiment consisted in pressurizing Concord clusters to 2 bar for 30, 45 and 60 minutes and 50 berries for each treatment were sampled. For all the berries sampled, the extent of dye movement inside of the berry was rated as described in 2.4.1 (Figure 13). Selected berries were also recorded for their skin color, and soluble solids content was measured with refractometer. In the post-veraison study, the freshly cut base

of the shoot was pressurized to 0, 2 and 4 bar for 45 minutes. Each 15 minutes of pressurization, 5 berries were sampled, sectioned longitudinally to determine dye location and soluble solids content measured. The experiment was repeated three times for each variety and for each pressure.

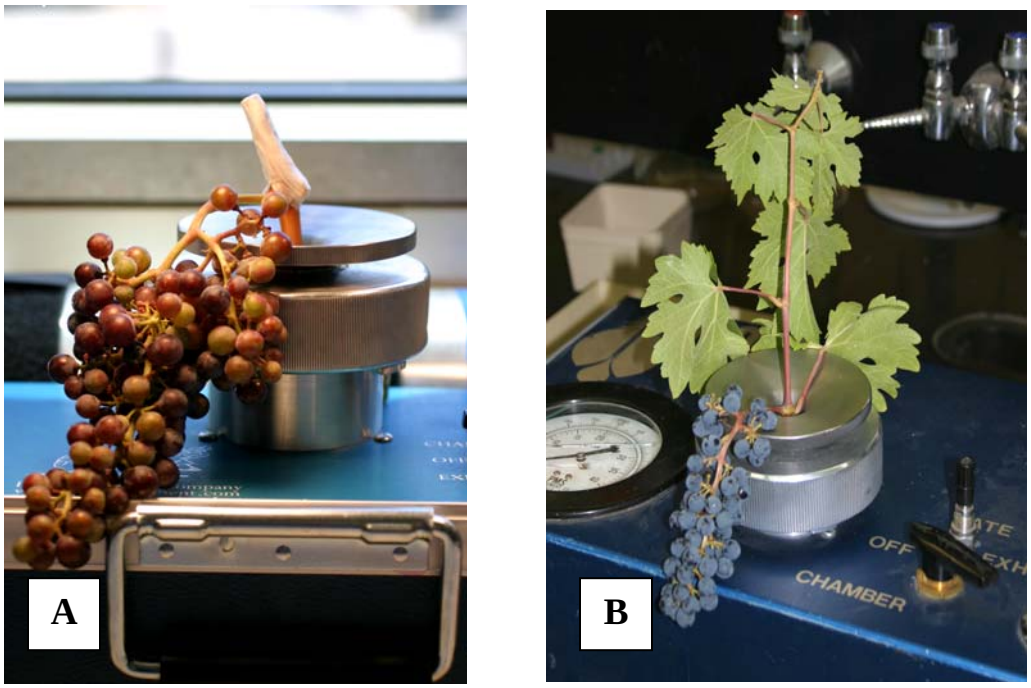

Figure 14: Both figures show a freshly cut shoot inserted through the gland and placed inside the chamber. Merlot shoot with a cluster during veraison and no leaves with cut surfaces sealed by parafilm (A). Merlot shoot with ripe cluster and leaves (B).

2.4.3 Dye feeding reverse / root pressure chamber

In the final dye feeding experiment, three potted post-veraison Chardonnay vines (soluble solids ~22 Brix), were placed in the root pressure chamber (Figure 15-A). The vine was brought to full hydration by a pressure around 5 bar for 30 minutes and a leaf next to the selected cluster was cut off with a sharp razor blade. When water came out of the petiole of the cut leaf, two berries were cut at the stylar end to expose the peripheral (dorsal) and axial (ventral) vascular bundles. The cut end of each berry was then immersed in 0.1% dye solution while still attached to the rest of the plant (Figure 15-B). The roots were pressurized at 5 bar for 5 hours, then 2 bar for 5 hours, then 1 bar for 5 hours and, finally no pressurization for 5 hours. Additionally, three potted post-veraison Chardonnay vines were fed dye without pressurization. Clusters were removed and cross-sections of the pedicel (proximal) end of the treated berry, the pedicel, the rachis, other berries on the same cluster, the peduncle, the leaves and shoot above and below the cluster were examined for dye movement using light microscopy.

Figure 15: Dye basic fuchsion fed to the berry by using a stand placed next to the pressure chamber (A). A Falcon tube used to feed the berry (B).

2.5 Berry weight response to immersion experiments

The following experiments were conducted to understand whether irrigation or rainfall affects berry weight at different maturation stages and whether water could enter in the berry through the skin or pedicel. The study was also conducted to determine whether xylem backflow was an overflow mechanism to balance the amount of water inside the berry and whether sugar could leach out from it.

2.5.1 Berry weight response to immersion

Post-veraison Concord and Merlot berries at ~18 and ~22 Brix respectively were immersed in 10 mL distilled water and six different treatments were applied to ten berries for each variety. The treatments were: entire berry immersed; pedicel immersed with berry protruding from the water (Figure 16-A); berry immersed after sealing the pedicel with petroleum jelly and the pedicel protruding from the water (Figure 16-B); berry immersed with unsealed pedicel protruding from the water (Figure 16-C); berry entirely sealed or skin only sealed and fully immersed. The experiment was done to observe whether water was taken up through the skin or/and pedicel and whether sugar leaches out from it. All the berries were kept in water for five days and water uptake was measured by weighing the berries before and after the test. At the beginning of each treatment, berries with similar color to the berries immersed in water were collected and soluble solids were measured with refractometer. Berry cracking was monitored daily. At the end of the experiment, measurements of soluble solids were taken in the water in which berries were immersed and in the berries after immersion. The amount of sugar lost from each berry was calculated considering that the refractometer calculates sugar

concentration in g 100 mL^{-1} (Brix) and that the berries were immersed in 10 mL of water, so 0.1 Brix in the water is equal to 10 mg of sugar lost.

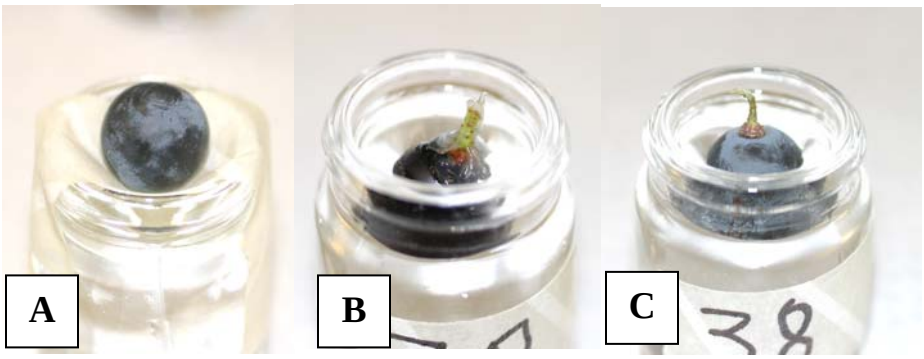

Figure 16: Pedicel immersed with the berry protruding from the water (A). Berry immersed with the sealed pedicel protruding from the water (B). Berry immersed with the pedicel protruding from the water (C).

2.5.2 Early post-veraison berries / response to immersion

One hundred Concord berries at a pink color stage (about 11 Brix) were collected and immersed in small beakers containing 10 mL water (Figure 17). Previously, a correlation between color and soluble solids was calculated after analyzing hundreds of berries so the color-Brix correlation was an assumption used to estimate soluble solids content during veraison. Fifty berries were immersed in distilled water with pH 5.93 similar to rain water. The pH was measured using Mettler-Toledo Mp 255 pH meter (Mettler-Toledo, Schwerzenbach, Switzerland). The other fifty berries were immersed in tap water with pH 7.91 similar irrigation water. All the berries were left in water for five days and their initial and final weight were recorded. Also, berry cracking was monitored daily. At the end of the experiment, soluble solids were measured in the water used for immersion and in the berries after immersion.

Figure 17: A pink Concord berry totally immersed in water.

2.5.3 Late post-veraison berries / response to immersion

One hundred Concord berries at approximation soluble solids content of 18 Brix were collected and immersed in small beakers containing 10 mL water. Fifty berries were immersed in distilled water with pH 5.93 similar to rain and the other fifty berries were immersed in tap water with pH 7.91 similar to irrigation water as described in 2.5.2. All the berries were held in water for five days and their initial and final weight were recorded. Berry cracking was monitored daily. At the end of the experiment soluble solids were measured in the water used for immersion and in the berries after immersion.

2.6 Heat-girdling experiment

A girdling experiment was conducted on Merlot to understand whether sugar can be exported between berries. Ten clusters were selected on the plant during veraison and the selection was based on the main part of the cluster having a population of berries from green to blue with a shoulder of only green berries (Figure 18-A). The green shoulder had berries with a range of sugar concentration from 5 to 7 Brix. Girdling was performed on the peduncle near the shoot in order to stop the sugar influx from the leaves to the berries through the phloem (Figure 18-B). Five clusters also were girdled on the lateral branch of the cluster to avoid sugar import from the main cluster to the green shoulder. The peduncle was heat-girdled by applying 12 V to a 24-cm length of 60% Ni, 16% Cr resistance wire for 4 seconds (Figure 19). The heat time was reduced to 2 seconds for the shoulder of the cluster because of the smaller diameter. Five to ten hard green berries on the shoulder with estimated soluble solids content of 5.5 Brix were tagged with cable ties which are designed to help identify cables, wires and pipes (Partex Marking System, Birmingham, United Kingdom). Other hard green berries were tagged on non girdled clusters as control. Half of the tagged berries were collected after 5 days and the remaining berries after 10 days following the girdling. Berries collected were analyzed for soluble solids content with refractometer.

Figure 18: A Merlot cluster during veraison with a green shoulder (A). The heat-girdling applied to the peduncle of the cluster (B).

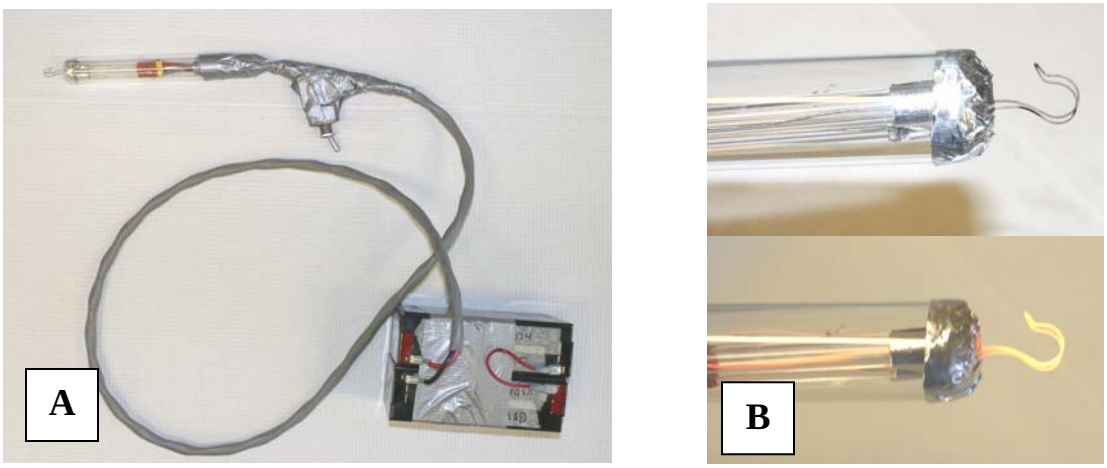

Figure 19: The heat-girdling instrument used (A). The top of resistance wire without (top) or with (bottom) a current of 12 V switched on (B).

Figure 20: The heat-girdling damage on the peduncle.

Moreover ten clusters of Merlot and Concord with a population of berries from green to blue were cut from the vine and hung on the wind wire next to where they had been removed. The excised clusters were left on the wires for five days and then the visible color was observed. At the end of the experiment soluble solids content was measured for the 30 berries with similar color for attached and unattached clusters for each variety. This treatment was conducted to see whether the berries could change color when detached from the vine. At the same time other clusters undergoing veraison were also detached from the vine and immersed in distilled water for five days to see whether berry skin color would change.

2.7 Statistical analyses

Data were subjected to analysis of variance, linear regression, and post-hoc comparisons of treatment means which were performed using Duncan's new multiple range test, using Statistica (version 7.1, StatSoft, Tulsa, OK, USA). Correlations between berries response variables were assessed using Pearson's correlation coefficient (r).

CHAPTER THREE

RESULTS

3.1 Dry-down and re-watering

The three experiments gave similar results so data of one are shown. The average solute concentration at the end of the experiment was 12.5 Brix for the Concord and 12.1 Brix for the Merlot. Pre-veraison berries started shriveling 16 and 18 days for Concord and Merlot respectively after the irrigation was stopped. At the beginning of veraison (solute concentration for Concord was estimated at ~9 Brix and for Merlot at ~10 Brix) berries started to increase in size even though water was not available in the soil. Green berries in both cultivars were visibly shrinking while the blush-pink berries were expanding (Figure 21-A and B). Data recorded from the FI-XSM linear sensors (Figure 22) confirmed that berries stopped shrinking during color change.

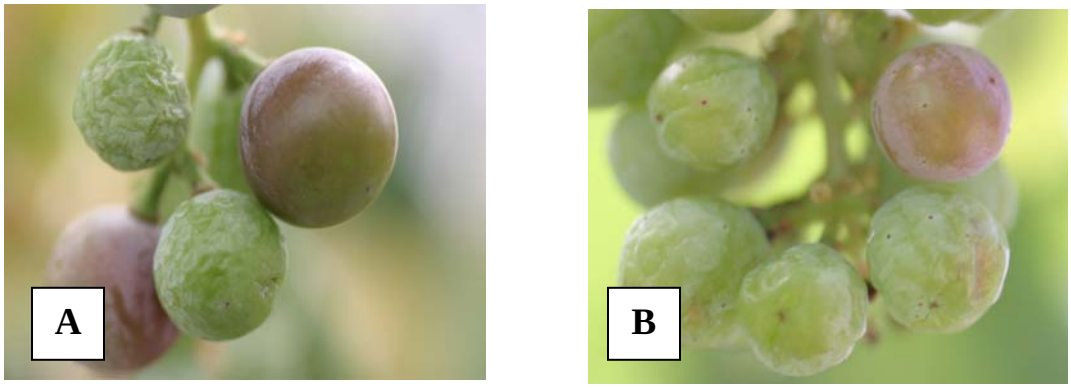

Figure 21: Concord (A) and Merlot (B) berries on water-stressed vine started to expand as soon as color changed from green to blush-pink while the green berries kept shriveling due to low soil moisture.

Transpiration was low due to the long water stress period before berry shriveling started and increased drastically after watering. The increase in berry size during veraison was accentuated by re-watering for both varieties (Figure 22). In Concord berry size could increase up to 30% of initial size (Figure 22-A) while in Merlot increased up to 7% of the initial size (Figure 22-B).

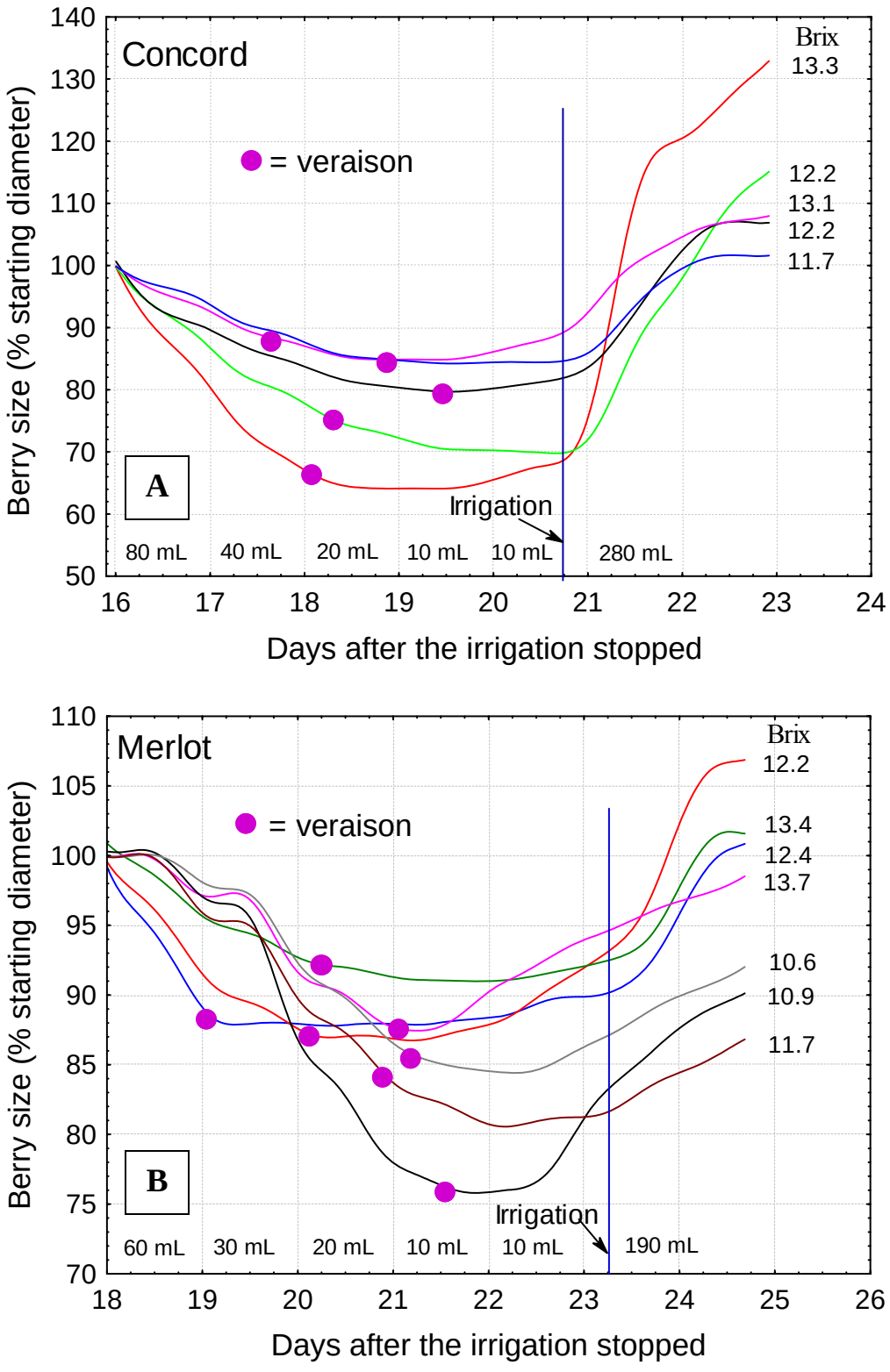

Figure 22: Change in diameter of Concord (A) and Merlot (B) grape berries at veraison during a soil dry down and rewatering cycle. Solids circle indicates the beginning of color change while the vertical line indicates rewatering. Transpiration is reported on the x axis.

3.2 Root pressurization

During veraison Concord berries responded within 20 minutes to changes in plant water status imposed by the application of up to 10 bar pressure to the root system (Figure 23). The berries below 9 Brix soluble solids showing a green color increased up to 10% their size under pressure. Moreover after three hours of pressurization pre-veraison berries stopped expanding and maintained their size without cracking. When berries were at a soluble solids content at least of 9 Brix with a blush color, their diameter no longer responded to root pressure. However, some berries without sensors on the same plant cracked under pressure when their soluble solids was > 11 Brix and their skin color was blue (Table 1). Pink and red berries never cracked.

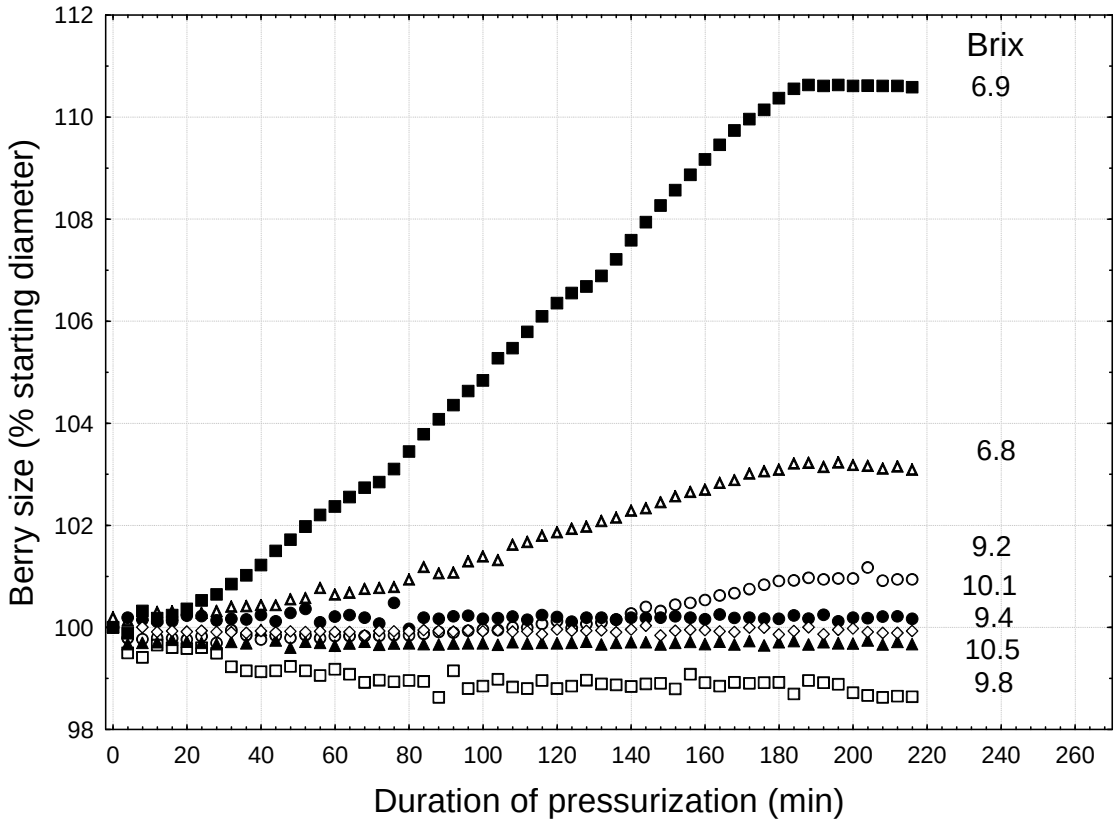

Figure 23: Effect of pneumatic pressure applied to the Concord root system at veraison. Changes in diameter of seven berries on the same vine recorded with electronic transducers.

Table 1: Skin color, weight and soluble solids of cracked Concord berries due to application of pneumatic pressure to the root system.

Color	Weight (mg)	Soluble solids (Brix)
Blue	1007	**13.2**
Blue	1265	**12.4**
Blue	1164	**11.7**
Blue	1602	**11.4**
Blue	1164	**12.3**
Blue	1641	**14.6**
Blue	1256	**13.2**
Blue	1387	**12.3**
Blue	1190	**11.9**
Blue	1290	**13.5**
Blue	1228	**14.5**
Blue	1167	**12.8**
Blue	1502	**12.4**
Blue	1345	**11.6**

Sap collected from Concord vines under water stress (Ψ_{leaf} = -2 MPa) was analyzed for sugar (glucose, fructose and sucrose) concentration by HPLC (Figure 24). The total sugar concentration was found to be 0.1 -0.3 g L^{-1} for the three vines tested.

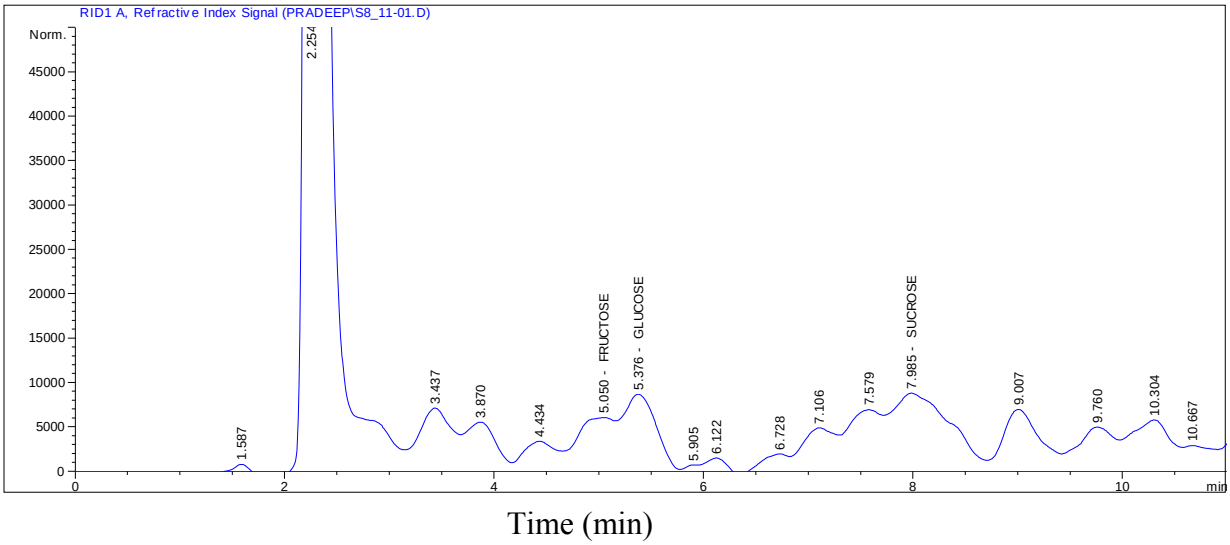

Figure 24: HPLC chromatogram of sugar in a xylem sap sample from a Concord vine.

In the other experiment, no sugar was detected in the sixteen sap samples collected from Merlot and Concord vines during veraison. This may be due to the higher Ψ_{leaf} than the previous experiment where Concord reached -2 MPa while in this case Ψ_{leaf} never dropped below -1.5 MPa and -1.7 MPa in Concord and Merlot respectively since vines were rewatered before sap collection. It was observed that the photosynthetic rate and stomatal conductance increased when the vines were irrigated with 500 mL of water regardless of water stress or phenological stage (Table 2). The photosynthetic rate increased up 270% in Merlot and 320% in Concord and the increase was observed after 2 hours from watering. At the pre-veraison and veraison phenological stages, photosynthesis is closely correlated with severe of water-stress ($r = -0.92$, $p<0.001$).

Table 2: Photosynthetic rate (A) and stomatal conductance (g_s) compared to Ψ_{leaf} at different phenological stage for Merlot and Concord.

Variety	Phenological stage	Before irrigation			After irrigation	
		Ψ_{leaf} (MPa)	A (μmol CO_2 $m^{-2}s^{-1}$)	g_s (mmol m^{-2} s^{-1})	A (μmol CO_2 $m^{-2}s^{-1}$)	g_s (mmol m^{-2} s^{-1})
Concord	pre-veraison	-0.60	**5.1**	40	**6.8**	64
Merlot	pre-veraison	-0.45	**4.7**	42	**6.8**	78
Concord	pre-veraison	-1.10	**0.8**	14	**1.8**	22
Merlot	veraison	-1.25	**n.d.**	n.d.	**1.4**	13
Concord	veraison	-1.45	**1.0**	23	**3.2**	38
Merlot	veraison	-1.50	**1**	8	**2.7**	26
Concord	post-veraison	-1.50	**1**	40	**1.9**	50
Merlot	post-veraison	-1.70	**1.8**	56	**3.6**	79

n.d. = not detected.

3.3 Dye feeding

Basic fuchsin clearly behaved as a xylem lumen-mobile dye and the dye always remained confined to xylem vessels and tracheids, regardless of whether it was fed via the shoot base (forward infusion) or via the berries (reverse infusion). For both Merlot and Concord, feeding dye to shoots without pressure resulted in a decrease in dye penetration through the berry's vascular system as the berry changed color from green to blue (Figure 25). Once the berries reached 14-16 Brix (purple-blue color) the dye was confined to the brush region. However there are significant differences between Concord and Merlot dye uptake (Figure 25).

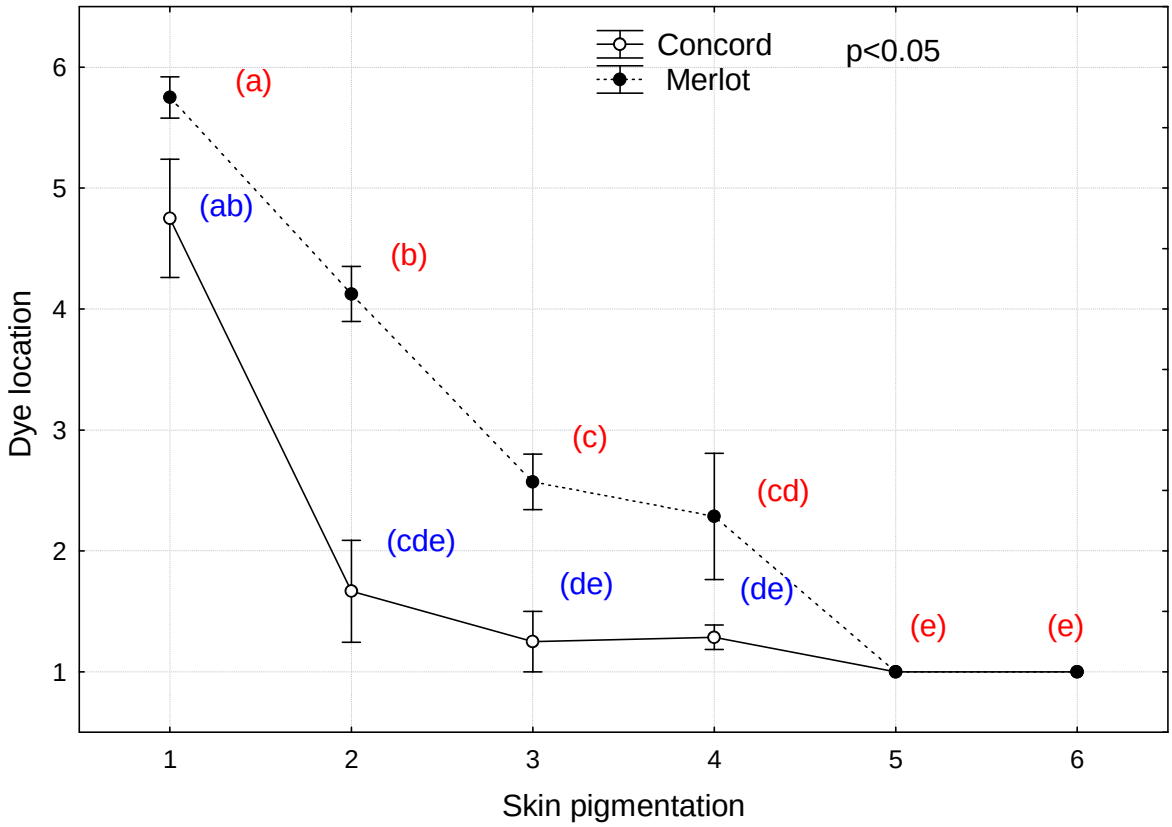

Figure 25: Correlation between dye location (1 in the brush, 6 continuous throughout entire vascular network) and skin color (from green 1 to blue 6) of grape berries during veraison (error bars represent standard error of the mean, $40 \leq n \geq 55$)

As soon as Concord reached a sugar concentration of 9-10 Brix at the blush color stage (Figure 26), the dye was likely to be near the brush region whereas in Merlot dye was located in position 4 at a similar skin color (Figure25). The dye in pre-veraison Merlot and Concord berries at ~ 5.5 Brix (hard green berry), was found to be continuous throughout the entire vascular network. In contrast when berries started softening (7-8 Brix in Concord, 8-9 Brix in Merlot) just before blush color stage, the dye movement became discontinuous in the stylar end as seen in several different stages linked with color, softening and dye location at veraison (Figure 26).

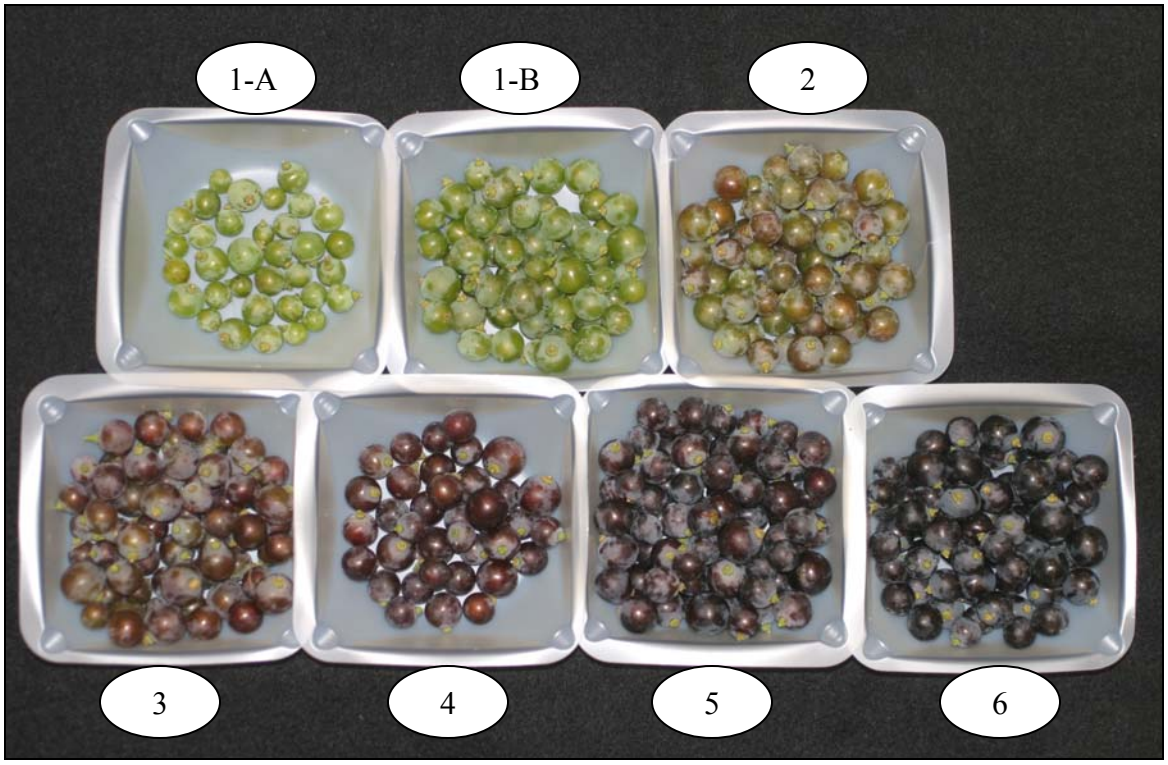

Figure 26: The seven stages of berry skin pigmentation linked with softening and dye location during veraison from hard green to blue color: 1-A hard green, 1-B soft green, 2-blush, 3-pink, 4-red, 5-purple, 6-blue.

The first experiment during veraison in which Merlot and Concord shoots were pressurized to 1 bar for 30 and 60 minutes, showed that dye location was near the brush when berries reached 14 Brix. No significant differences were noted between varieties and time of pressurization on dye location. Moreover when dye was pressurized into the shoot for five hours at 1 bar no dye was found beyond the brush region above ≥ 14 Brix (data not shown). However, pressurization changed the extent of dye penetration at ≤ 11 Brix (Figure 27).

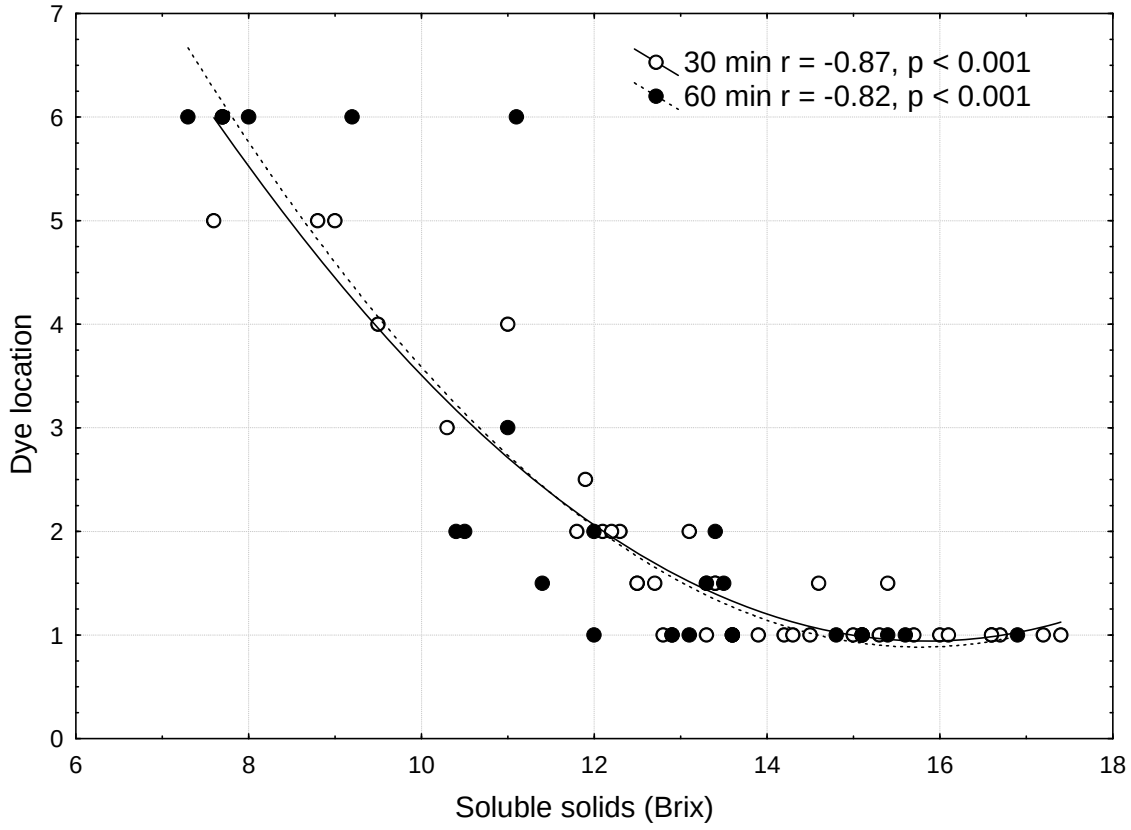

Figure 27: Correlation between berry soluble solids and dye location (1 in the brush, 6 continuous throughout entire vascular network) when berries were pressurized to 1 bar for 30 and 60 minutes.

The pneumatic pressure applied at the base of the shoots in the following experiments was able to push the dye into the berry through the vascular bundles during veraison (Figure 28, 29 30, and 31).

The second experiment which consisted of pressurizing Chardonnay and Concord shoots to 2 bar for 30 minutes showed that the pressure was able to move the dye beyond the brush even when berries were ≥ 14 Brix. However there were not significant differences between varieties (Figure 28).

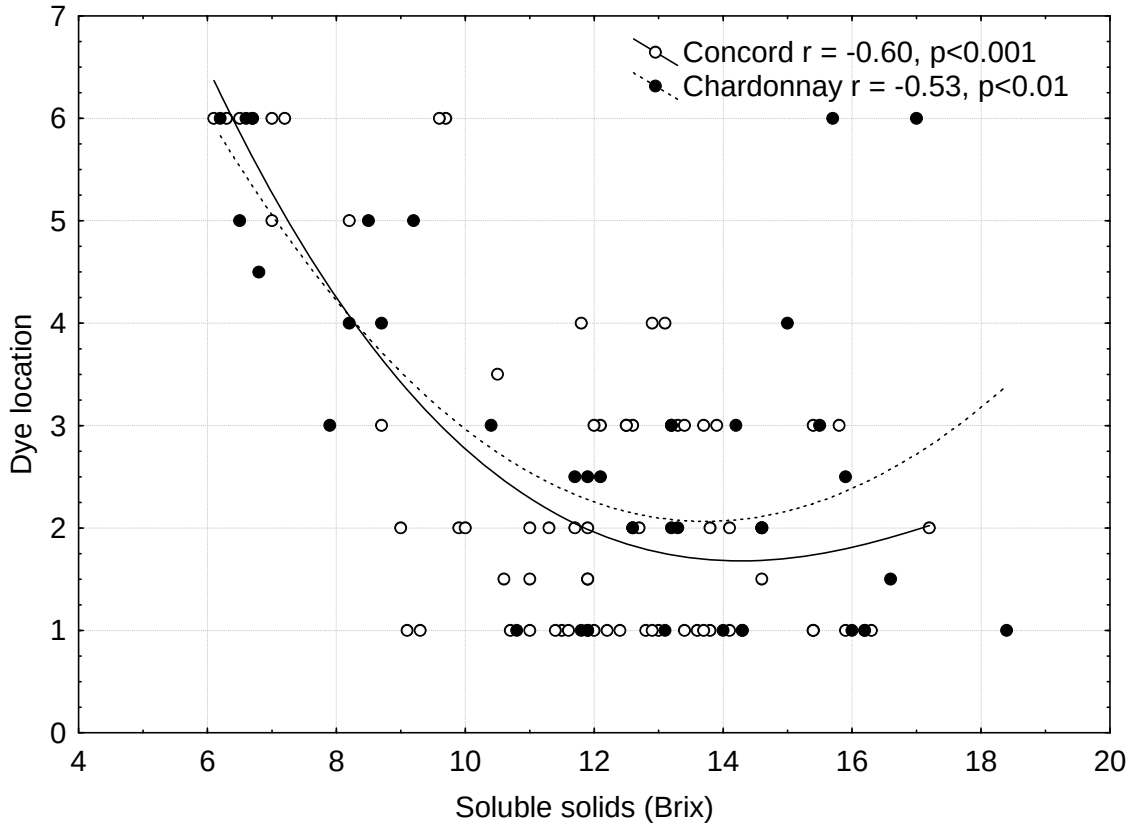

Figure 28: Correlation between dye location (1 in the brush, 6 continuous throughout entire vascular network) and soluble solids for Concord and Chardonnay cluster upon veraison when shoots were pressurized to 2 bars for 30 minutes.

The third experiment at veraison consisted of applying different pressures such as 1, 2 and 4 bar for 30 minutes to Concord and Merlot shoots. The result confirmed that dye could move through the xylem inside the berry when pressure was applied, although there were no significant differences between pressures (Figure 29).

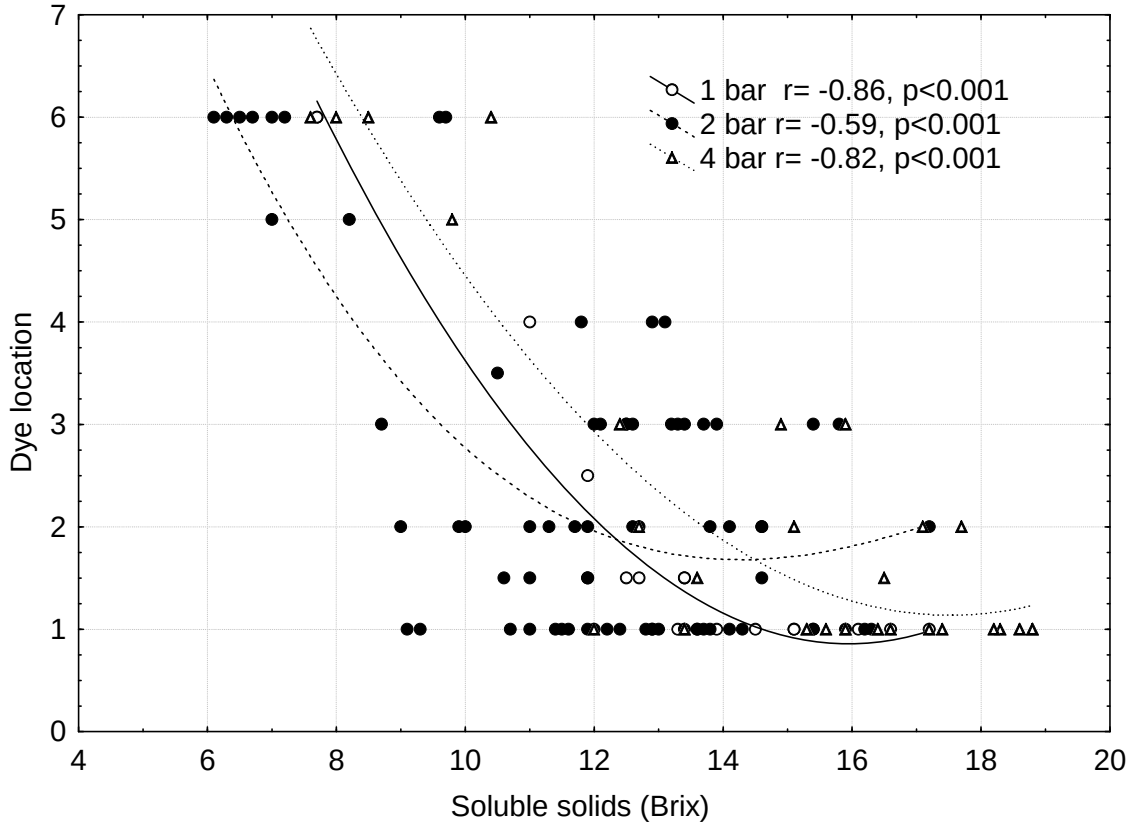

Figure 29: Correlation between dye location (1 in the brush, 6 continuous throughout entire vascular network) and soluble solids under three different pressures applied to the shoot base (1, 2 and 4 bars) at veraison in Concord and Merlot berries.

For the last experiment during veraison, Concord shoots were pressurized to 2 bar for 30, 45, and 60 minutes. The low correlation between soluble solids and dye location confirmed that pressure was able to push the dye into 14 Brix berries. However there were no significant differences between duration of pressurization (Figure 30).

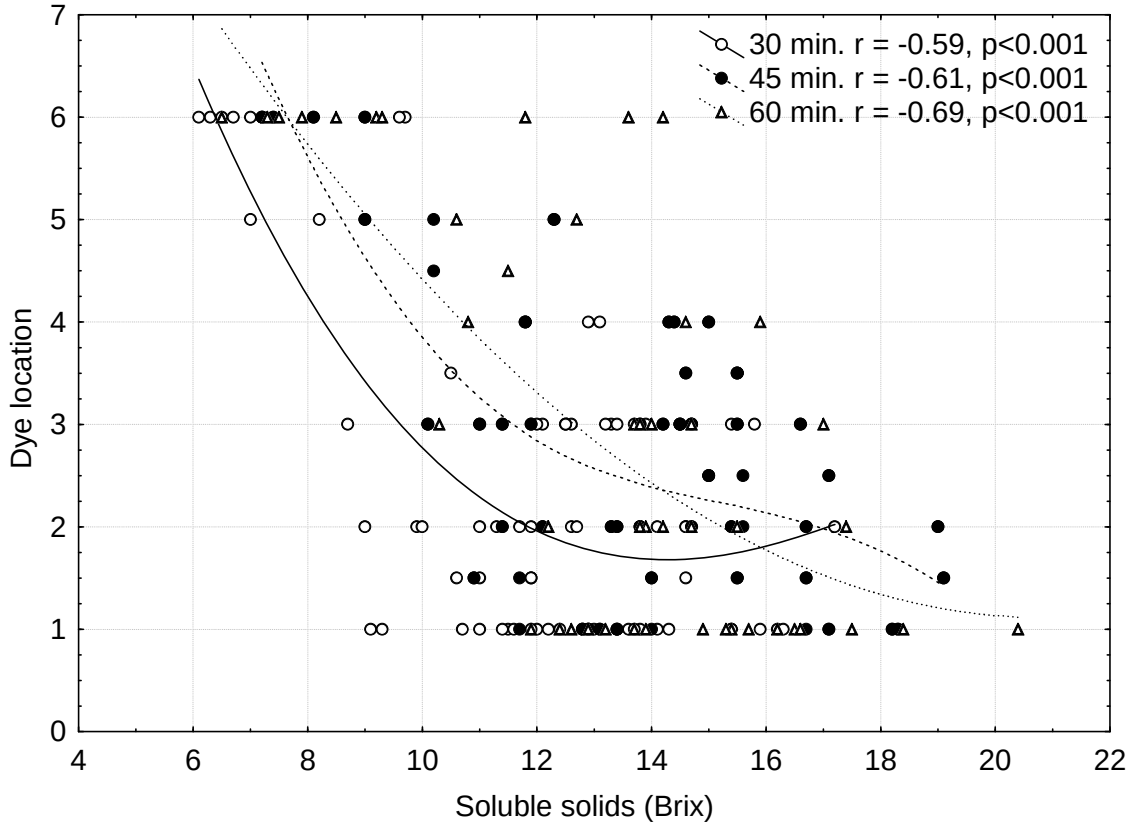

Figure 30: Correlation between dye location (1 in the brush, 6 continuous throughout entire vascular network) and soluble solids under different times of pressurization at 2 bars in Concord clusters.

Chardonnay, Muscat Blanc and Merlot berries behaved identically to shoot base pressure when they were at ~22 Brix. When pressure was applied, dye could move into the post-veraison berries, confirming the integrity of xylem vessels in post-veraison berries. In addition, dye could move farther into the berries when shoots were pressurized to higher pressure for longer time (Figure 31).

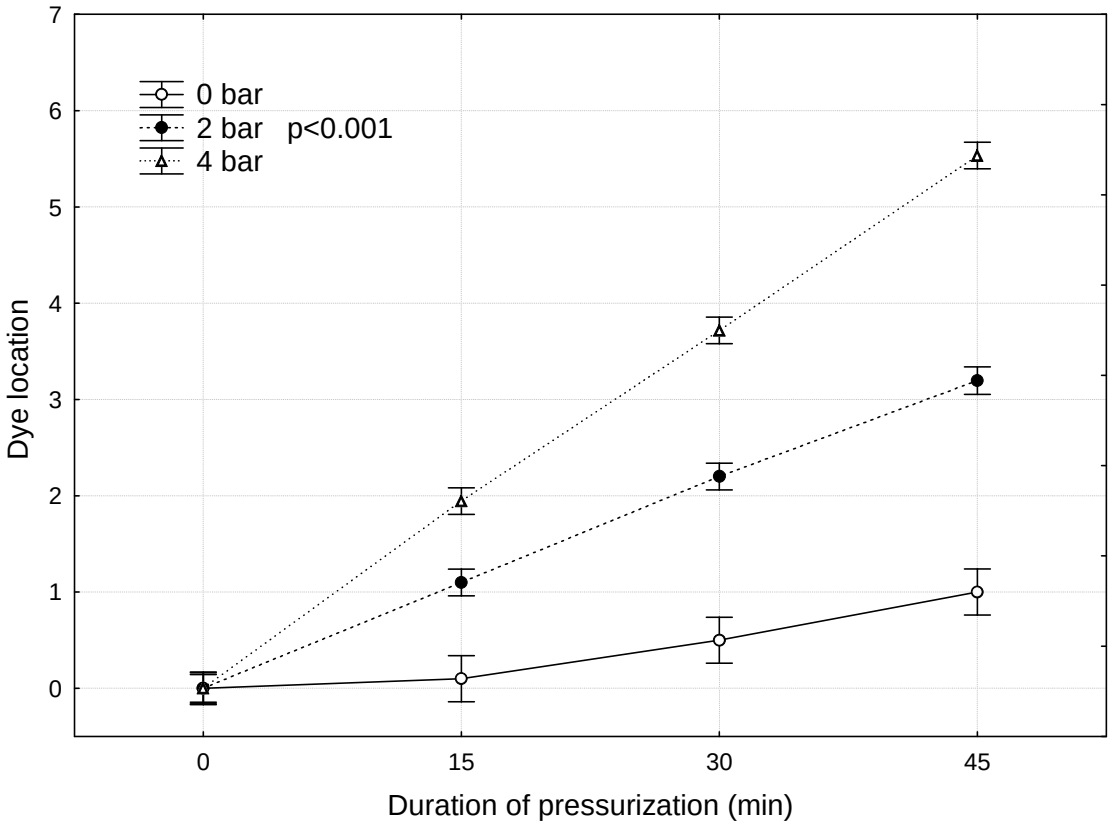

Figure 31: Shoots of Chardonnay, Muscat Blanc and Merlot pressurized by the pressure bomb at 0, 2 and 4 bar for 15, 30 and 45 minutes. The extent of dye movement inside of the berry was rated visually and assigned a number from 0 (no dye) through 6 (continuous throughout entire vascular network). Error bars represent standard error of the mean ($15 \leq n \geq 45$).

During the dye feeding experiments at veraison a large number of berries were analyzed for their color and soluble solids concentration. A correlation between color and soluble solids was calculated for Merlot and Concord (Figure 32). Also a threshold for

the beginning of skin pigmentation has been noted at 9 Brix for Concord and 10 Brix for Merlot. In addition, blue berries always had >15 Brix.

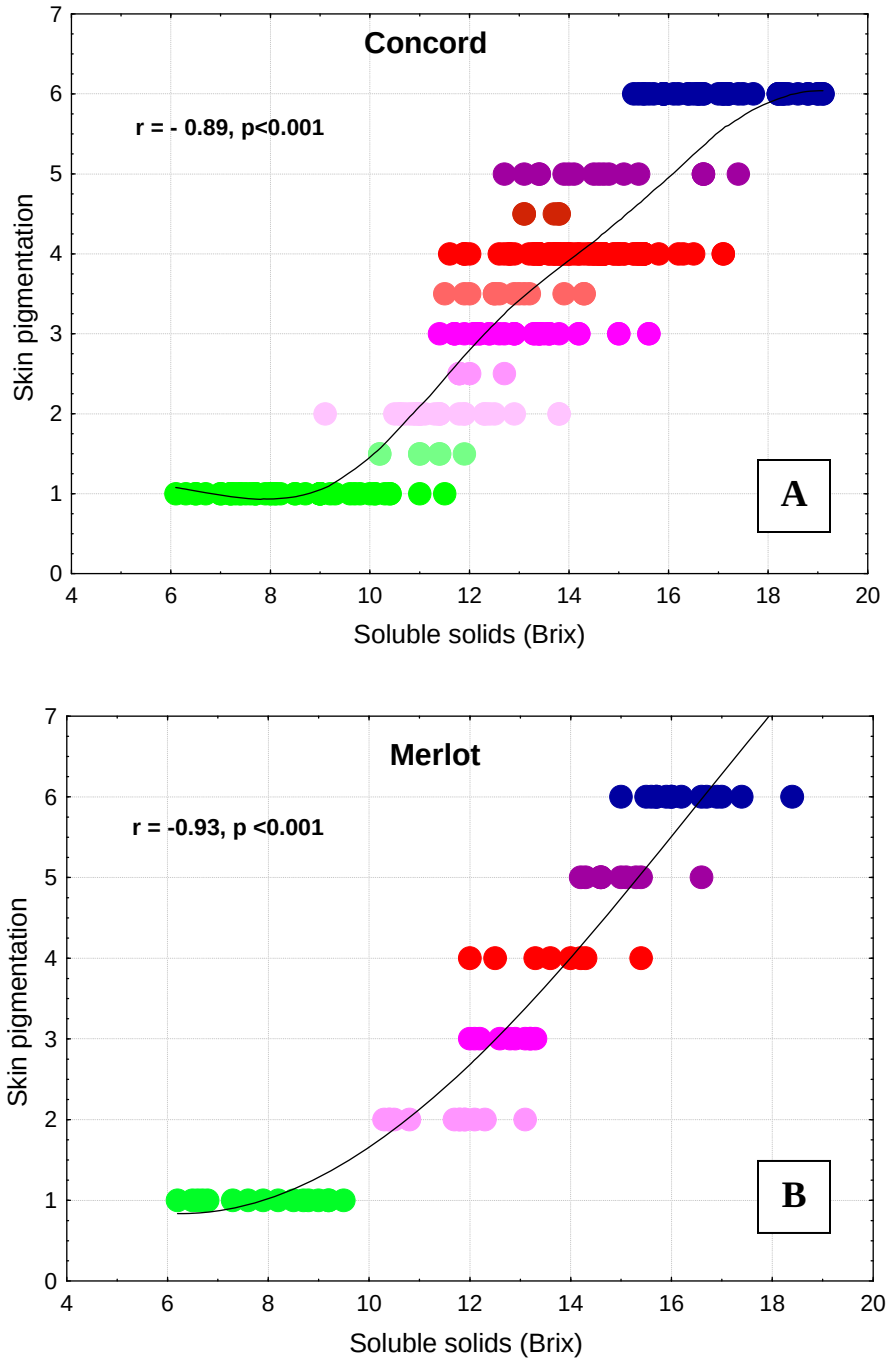

Figure 32: Correlation of the development of skin pigmentation (from green 1 to blue 6) and the soluble solids concentration inside the berry for Concord (A) and Merlot (B).

The reverse dye infusion to Chardonnay berries at ~22 Brix indicated that the dye fed from the berry did not move back to the vine when pressure was applied to the root system. Also, the dye did not move back to the vine even when the pressure applied was 1 bar for 5 hours at the root system. However the dye always moved back through the berry tracheids and through the pedicel when pressure was released. Also the dye movement could be traced throughout the peduncle of the dye fed berry's cluster (Figure 33), nearby leaves, beyond the basal leaf, and towards the trunk of the vine. Although dye also moved to other berries on the same cluster, it stopped in the brush region. The same behavior was observed for the control treatment without pressure.

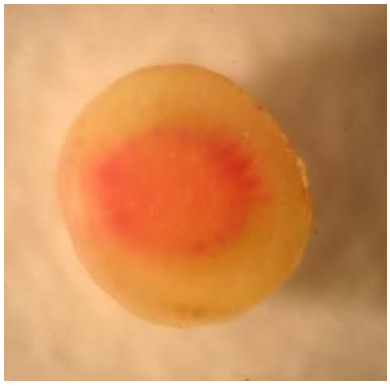

Figure 33: Movement of the xylem-mobile dye basic fuchsin infused from the stylar end of attached Chardonnay grape berries. The photo shows the radial section of the peduncle of cluster where the berry was infused.

3.4 Berry weight response to immersion

The immersion experiment with different treatments applied to post-veraison Merlot and Concord berries at a concentration of soluble solids ~ 22 and ~18 Brix respectively showed that berries gained weight. In addition, Concord berries were more likely to crack than Merlot. There was no difference in water uptake between varieties due to a big variation in volume of cracked berries (Figure 34). The variation was due to

the inability to weigh all berries at the end of the experiment, because some of the cracked berries had become heavily infected with mold.

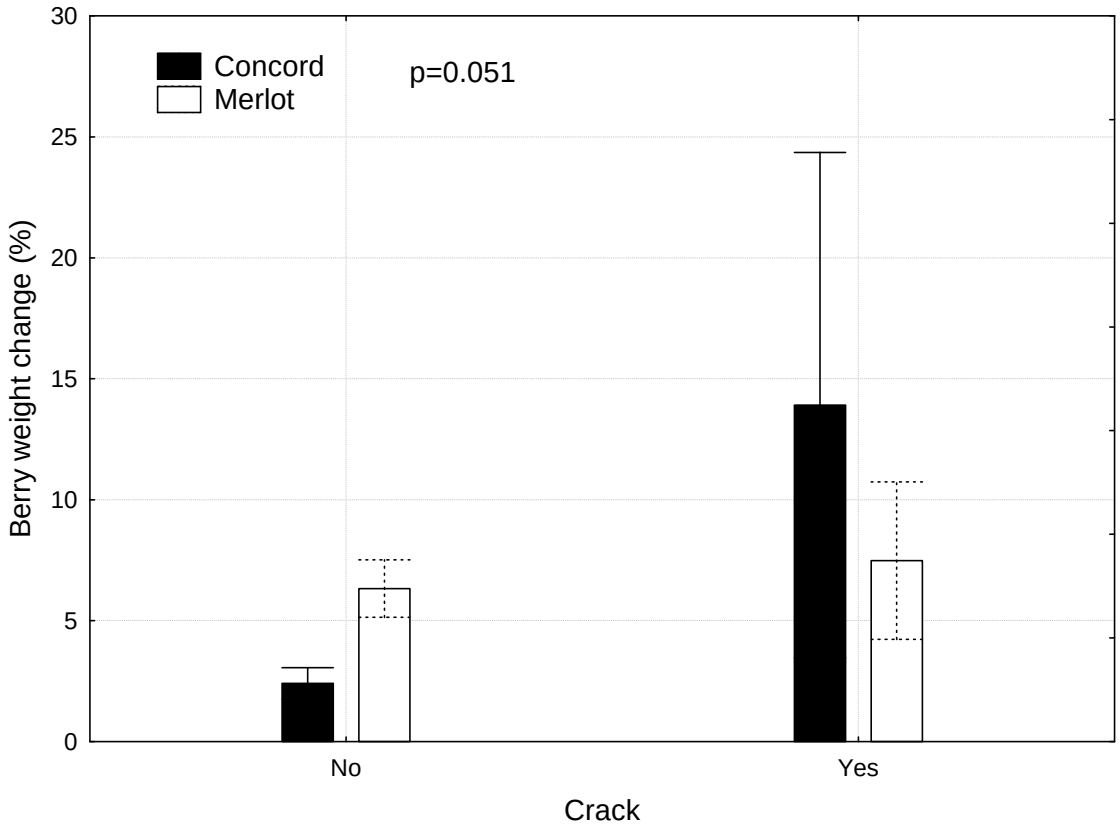

Figure 34: Weight gained by Merlot and Concord berries immersed in distilled water in correlation with cracking. (Error bars represent standard error of the mean, $5 \leq n \geq 51$).

However there were differences among treatments regardless of varieties and berry cracking (Figure 35). In fact, berries entirely immersed with the skin sealed (C) gained the same weight as the berries entirely immersed without sealing (E) showing that water may move inside the berry through the pedicel. Water uptake was the same when the entire berry (F) or just the pedicel (D) was sealed but the weight gain was similar to treatment C and E. Moreover, berries entirely sealed gained weight (F) evidencing that the sealant was not able to avoid water influx into the berry. In addition, the weight gain was significantly lower when the berry protruded from the water with pedicel immersed

(A) and when the berry skin was immersed with the pedicel protruding from the water (B). Also, the immersion of the pedicel with the entire berry out and the totally immersed berry showed that berries for both varieties lose up to 10 mg of solutes. On the other hand, no soluble solids were found in water when berry skin was unsealed and the pedicel was sealed. This shows that solutes can be lost from the berries through the pedicel when berries did not crack.

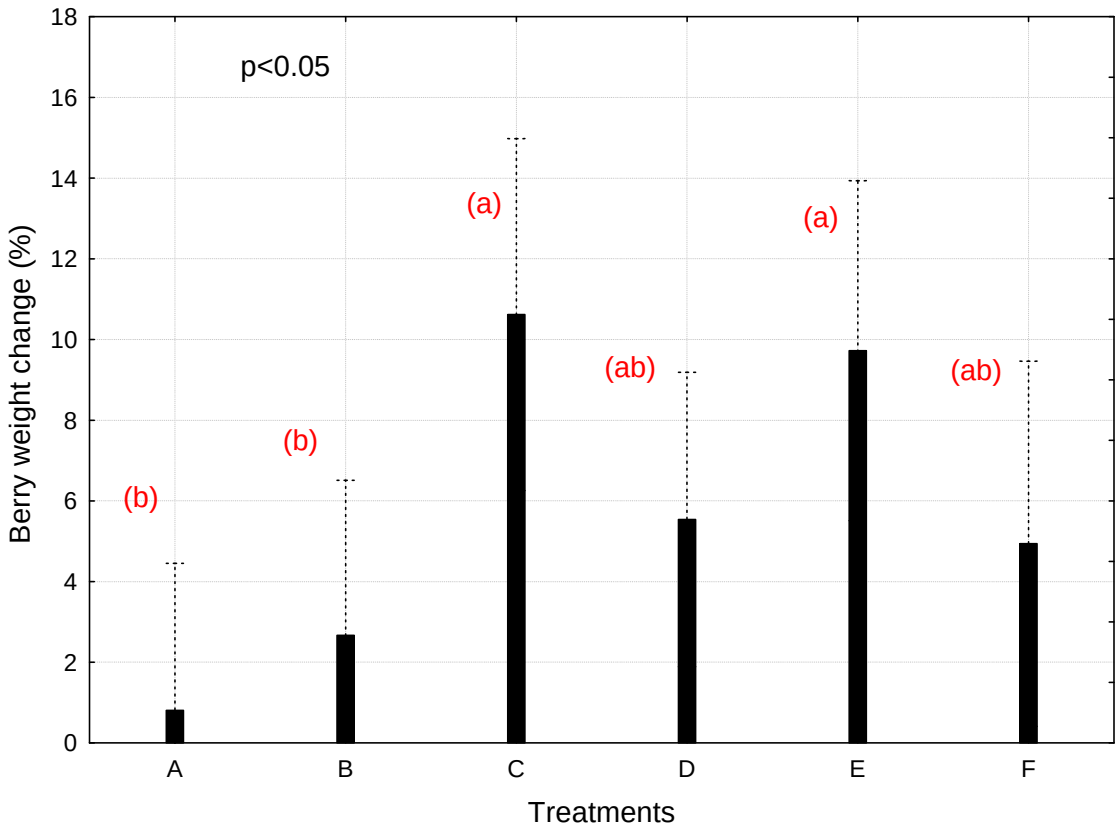

Figure 35: Six different treatments applied to post-veraison Merlot and Concord berries Treatments: A=berry protruding from the water, pedicel immersed; B=pedicel protruding from the water, skin immersed; C=berry entirely immersed with only skin sealed; D= berry entirely immersed with only pedicel sealed; E= berry entirely immersed without sealing; F= berry entirely immersed and sealed. Treatments with the same letter are not significantly different at $p < 0.05$. (Error bars represent standard error of the mean, $13 \leq n \geq 20$)

As soon as the berries cracked their soluble solids dropped from 16 to 2 Brix for Concord and from ~22 to ~8 Brix for Merlot (Figure 36). Measuring soluble solids in the immersion water showed that the berries lost between 100 and 300 mg of sugar. So assuming for Concord, a planting distance of 2.7 m between the rows and 1.8 m within the row, 30 berries per cluster, 200 clusters per vine, and if each single berry lost 100 mg in soluble solids, up to ~ 1.2 tons per ha of soluble solids could be lost on the ground just before harvest due to rainfall or sprinkler irrigation.

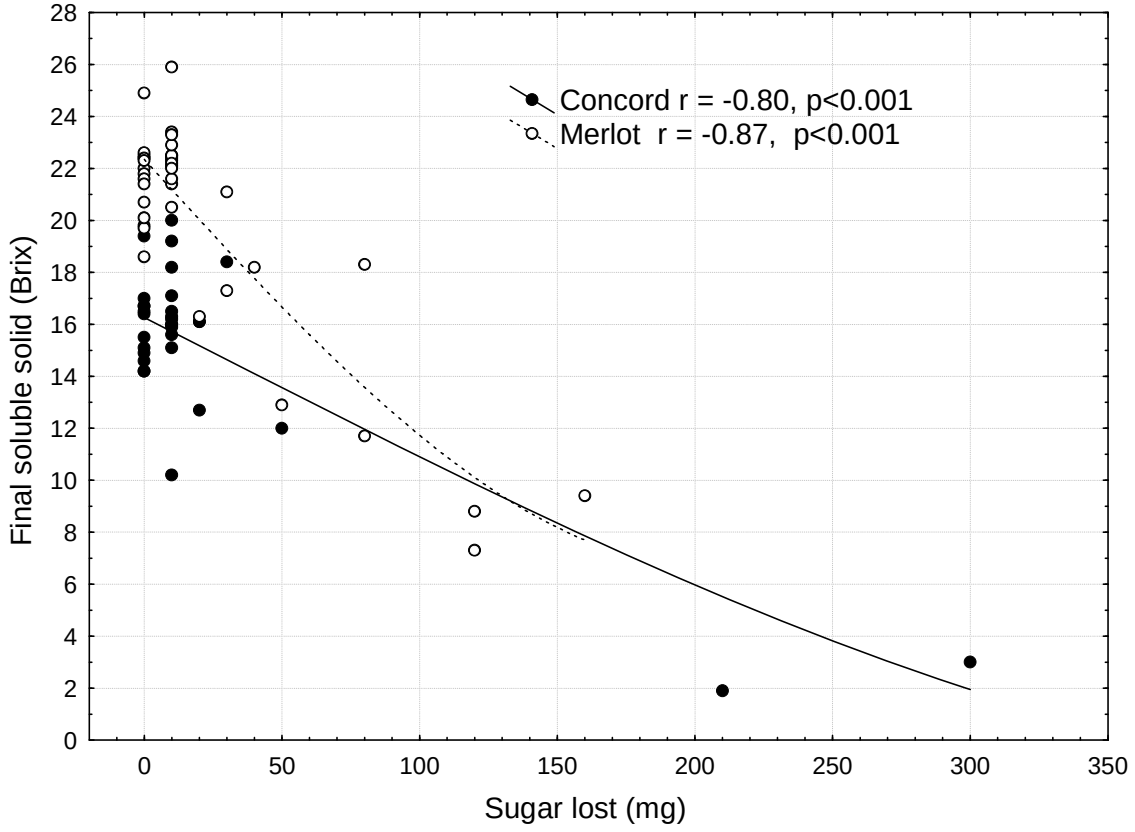

Figure 36: Correlation between the final soluble solids and sugar lost for Merlot and Concord berries immersed in distilled water.

The early post-veraison experiment, which consisted of immersing Concord berries in two different water sources, showed that berries took up water and increased their weight. However, there was no significant difference between water pH when the berries did not crack (Figure 37-A). When berries did crack, berries immersed in distilled water increased their weight more ($p<0.05$) than berries immersed in tap water (Figure 37-B). About 10 % of berries cracked and there was no significant difference between water pH. Moreover, no soluble solids were found in the water used for immersion when berries did not crack. Intact berries gained from 100 to 300 mg in weight which dropped the soluble solids concentration from a range of 10-12 Brix before the immersion to 7-9 Brix after the immersion.

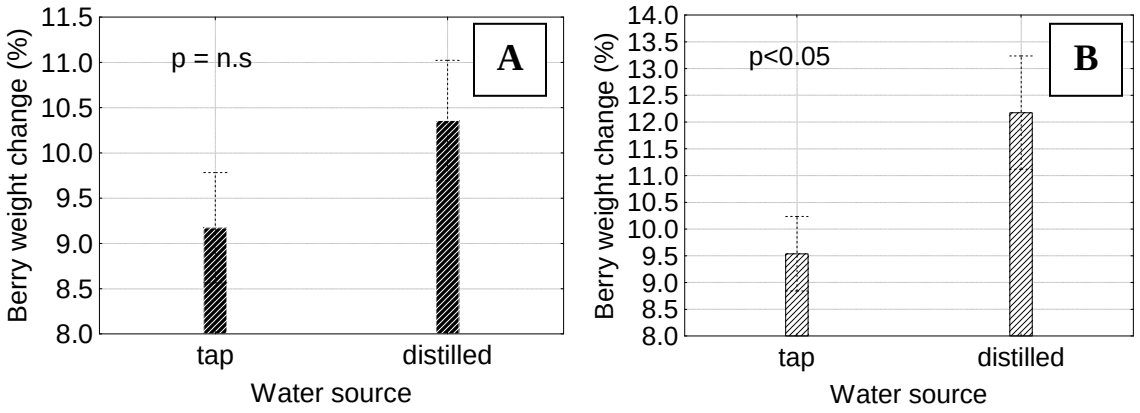

Figure 37: Concord berries immersed in distilled water gained more weight than in tap water. No significant difference between water pH when the berries did not crack (A). Significant difference in gained weight between water sources when the berries did crack (B). (Error bars represent standard error of the mean, $n = 40$ in Figure A and $1 \leq n$ in Figure (B)).

The immersion experiment later in the season did not show significant differences between water uptake and water pH or between early and late post-veraison berries when berries did not crack. When the berries did crack, the difference in water uptake became significant similar to the early post-veraison immersion experiment (Figure 38). However, the more mature berries were more likely ($p<0.05$) to crack than the early post

veraison berries. Also there was a significant difference between water pH. In fact, 54 % of Concord berries immersed in distilled water at ~18 Brix cracked and 59% of them cracked within 24 hours. On the other hand, 28% of berries cracked when immersed in tap water and 35% of them cracked within 24 hours (Table 3). Moreover, berries lost up to 10 mg of solutes regardless of water pH when the berries were intact. When the berries cracked, they lost between 100 and 300 mg of sugar.

Table 3: Effect of different pH of water on cracking for Concord berries at ~18 Brix.

Water source	Total berries	No. of cracked berries	Cracked within 24 hours	Weight change of intact berries (%)
Distilled water (pH 5.93)	**50**	**27**	**16**	**8.2**
Tap water (pH 7.91)	**50**	**14**	**5**	**6.6**

3.5 Heat-girdling

The heat girdling experiment in Merlot showed that berries started shriveling and changing color within 5 days following the girdling treatment, even though they were hard and green with ~5.5 Brix at the time of girdling (Figures 38, 39). Also no visible color change differences were observed between girdling the peduncle of the cluster or girdling the peduncle and lateral shoulder. The soluble solids concentration of hard green berries, which were tagged, moved from 5.5 Brix before the girdling to ~9 Brix 5 days later at the same time their skin color changed to red. Ten days after girdling they had moved to ~12 Brix and were visibly shrinking (Figure 38-B, 39-B). No significant differences were found in the soluble solids content between the two treatments (Figure 40). Although the average of soluble solids measurement for the girdled clusters was

higher ($p<0.05$) 5 days following the girdling than in the control. This difference disappeared when the soluble solids was measured 10 days after girdling (Figure 40). Also noted, when the vine was water-stressed during veraison, the berries changing color expanded (Figure 21 and 22), but when clusters were girdled at the same time, berries shrank (Figure 38 and 39).

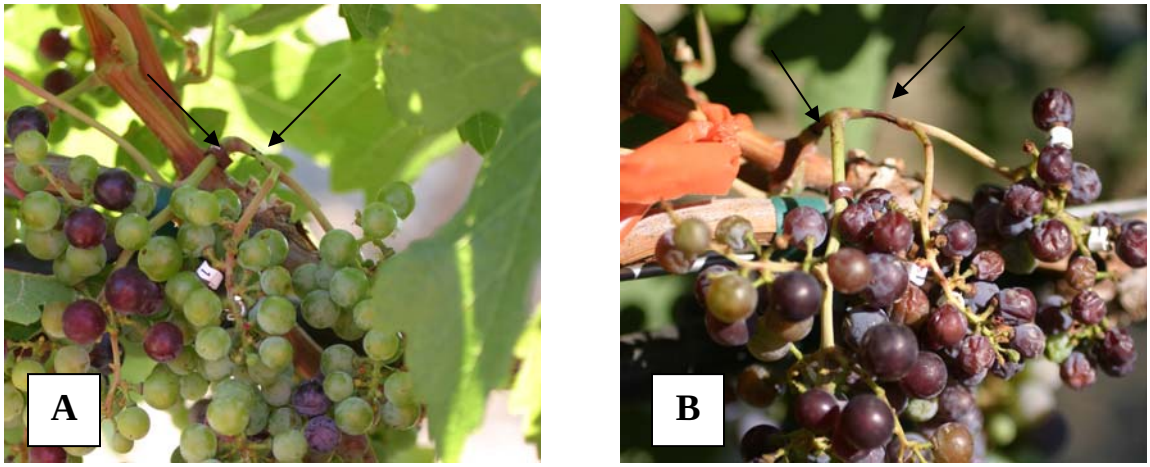

Figure 38: Merlot cluster. The heat-girdling done on the peduncle of the cluster and on the lateral shoulder indicate by the arrows. Figure A shows a girdled cluster at the early stage of ripening while Figure B shows the same cluster 5 days after girdling.

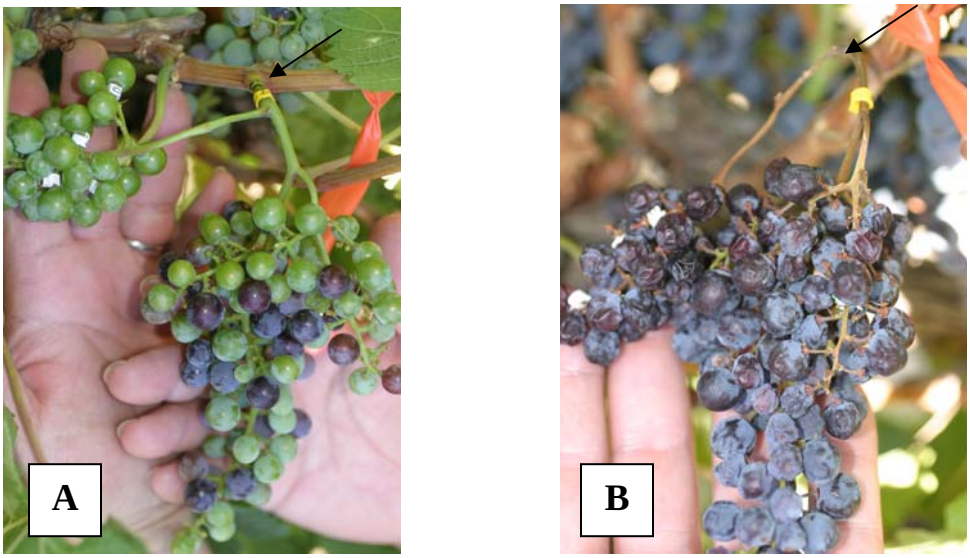

Figure 39: Merlot cluster. The heat-girdling done only on the peduncle of the cluster indicated by the arrows. Figure A shows a cluster undergoing veraison while Figure B shows the same cluster 10 days after girdling.

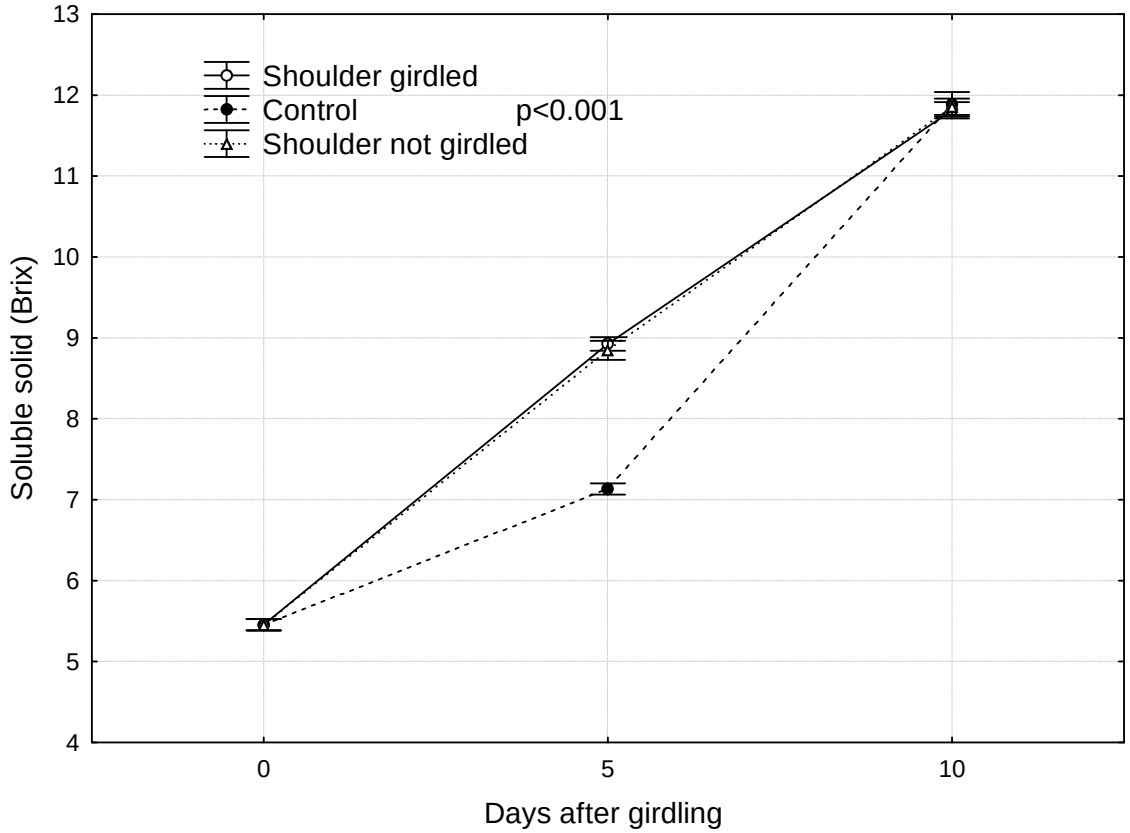

Figure 40 Correlation between days after girdling and soluble solids for the girdled and control Merlot clusters. Girdling done on the peduncle and the lateral shoulder of the cluster (shoulder girdled) and only on the peduncle of the cluster (shoulder not girdled) were the treatments. Soluble solids concentration of the berries measured for the girdled and control clusters at the time of girdling, after 5 and 10 days. (Error bars represent standard error of the mean, $53 \leq n \geq 73$).

When Merlot and Concord clusters undergoing veraison were detached from the vine, a change of color from green to red-purple was observed (Figure 41 and 42). Also, the soluble solids concentration in the detached clusters was not significantly different from that of the attached clusters. Detached Concord and especially Merlot berries were visibly shriveling (Figure 41-B and Figure 42-B) so this result indicates the soluble solids concentration in the berries increased due to water loss from the berry. In addition, it was noted that some Concord clusters were still undergoing veraison on the vines while the detached clusters had totally turned color.

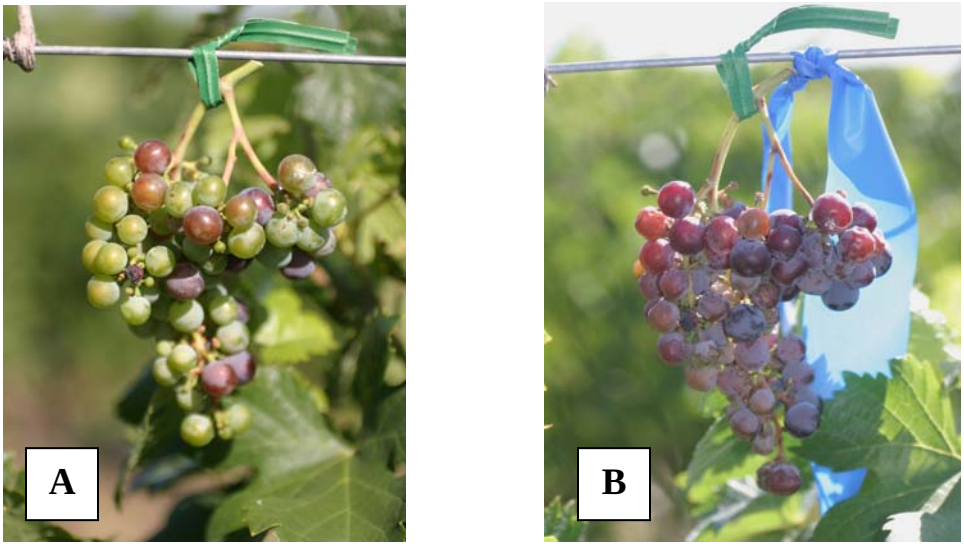

Figure 41: A cluster of Merlot detached from the vine during the changing of color. A Merlot cluster detached from vine (A). The same cluster 5 days later (B).

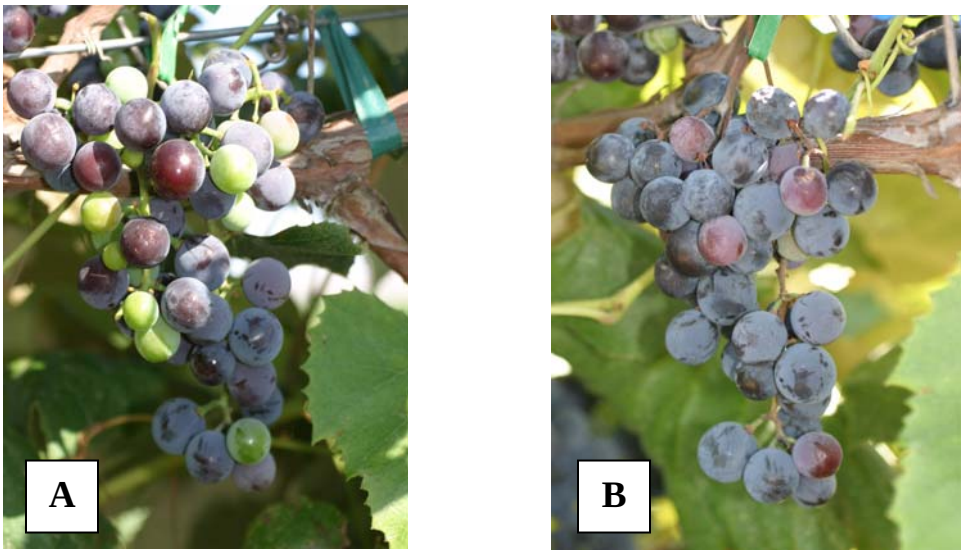

Figure 42: A cluster of Concord detached from the vine during the changing of color. A Concord cluster detached from vine (A). The same cluster 5 days later (B).

Other clusters that had been collected on the same day and immersed in distilled water for the same time period did not show any color change. In fact, the green berries on the cluster were still green after 5, 10 and 15 days of immersion for both Merlot and Concord (Figure 44). Soluble solids were not measured for this experiment but results from 3.4 showed that intact berries were increasing their weight from 100 to 300 mg while their soluble solids concentration was decreasing.

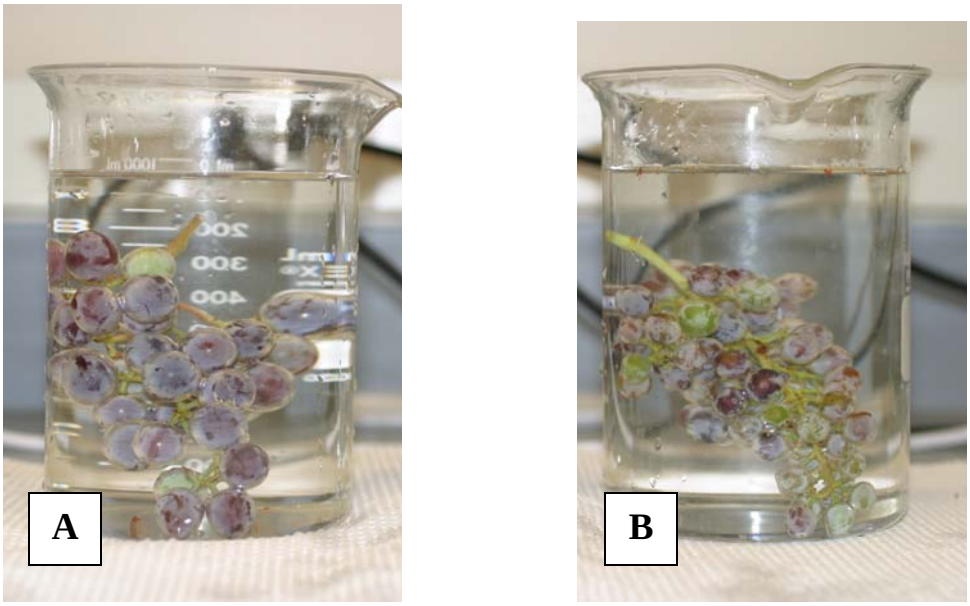

Figure 43: A Concord cluster (A) and a Merlot cluster (B) collected from the field during veraison and immersed in distilled water.

CHAPTER FOUR

DISCUSSION

The response of grape berry diameter to changes in soil moisture during veraison was similar for the two genetically distinct cultivars Merlot and Concord. The shrinking berries undergoing veraison during dry-down period began to re-expand before rewatering while other green berries on the same cluster continued to shrink. This suggests the mechanism responsible for this increase during dry-down is the phloem influx. Since no water was available in the soil, phloem influx may be sufficient to sustain pericarp cell expansion in ripening berries. This theory is supported by Coombe (1992) who observed the resumption of growth followed after a few days of measurable increase in hexose concentrations in berries at veraison, suggesting an increase of phloem flow. Düring *et al.* (1987) and Findlay *et al.* (1987) have independently shown using dye perfusion that there is no water influx through the xylem into ripening berries. Other studies have found that water flow via the xylem in grape berries decreases markedly after veraison (reviewed by Ollat *et al.,* 2002). Whereas xylem sap is the main source of water for the berries before veraison, phloem sap becomes the primary source during ripening (Greenspan *et al.,* 1994). At the same time fruit transpiration declines during development, implying that evaporative water loss ceases to function as the driving force for water influx (Rogiers *et al.,* 2004). So, the suggestion is that, during veraison, water flow to the berry switches from xylem influx to phloem influx. Phloem flow is caused by the differences in turgor pressure resulting from the loading and unloading of sugars in source and sink regions (Lalonde *et al.,* 2003). Further, my observations have shown that berries began to expand even when the vines were facing a heavy water stress (-2 MPa). However, berries undergoing color change increased in size due to the change in water influx from xylem to phloem which was accentuated by re-watering. Berry volume

recovered totally and increased within the 24 hours after re-watering. Keller *et al.* (2006) showed that, pre-veraison berries rapidly rehydrated and resumed growth upon rewatering, whereas no such response was found in post-veraison berries. Water deficits imposed during berry growth period have reduced berry size, with deficits imposed during phases I and II achieving the largest effect (Matthews and Anderson, 1988; McCarthy, 1997). My results suggest that during veraison berry growth is still affected by soil water status and berry skin can still be elastic at this stage.

Further observations noted that berry growth and color change was anticipated by softening as reported by Coombe *et al.* (1987) showing that the increase in glucose and fructose began on the same day of softening. Creasy *et al.* (1993) reported the dye distribution in soft-berries was uneven in comparison to that in hard-green berries. Keller and Shrestha (unpublished results) observed that the apoplast solute concentration increased in the berry during ripening concomitantly with the cell (vacuole) solute concentration creating equal water potential between the cell wall and the cell. Apoplast phloem unloading in the berry (Zhang *et al.,* 2006) coupled with solute accumulation in the apoplast may be a requisite for a decline of xylem water influx in post-veraison berries.

Pneumatic pressure applied to the root system was rapidly transmitted to xylem pressure (Wei *et al.,* 2000). At veraison, Concord berries below 9 Brix showing a green color increased their size under pressure, confirming results by Keller *et al.* (2006). This suggests that a change in grape berry diameter before veraison is due to skin elasticity

and a direct water influx and efflux. When berries reach soluble solids of 9 Brix showing a blush color, they no longer respond to root pressure. This may suggest that the water may continue to flow through the berry xylem during veraison but rather than bringing water into the berry, the xylem may recycle excess phloem water back to the shoot as suggested by Keller *et al.* (2006). Water recycling via the xylem would make fruits less responsive to plant water status (Van Ieperen *et al.,* 2003) and could decrease their vulnerability to cracking by serving as an 'overflow' mechanism. Moreover, berries >11 Brix were likely to crack when pressure was applied to the roots. The cracked Concord berries had a soluble solids concentration between 11.4 to 14.6 Brix showing a blue skin color. On the other hand, the correlation found in normal condition among skin color and soluble solids concentration showed blue berries at >15 Brix. One suggestion is the pneumatic pressure applied to the root system pushed solutes and water (Wei *et al.,* 2000) into the berries diluting the soluble solids content or maybe the pressure prevented xylem efflux from the berries. In addition, the cracking of post-veraison Concord berries under root pressurization without any increase in diameter confirms that fruit expansion is limited by the elastic properties of the skin cell walls (Considine and Kriedemann, 1972; Matthews *et al.,* 1987). Effective wall thickness accounts for about 56% of the variance of resistance to pressure applied (Considine, 1981).

Hexose sugar was found to be 0.1-0.3 g L^{-1} in the xylem sap collected from water stressed Concord vines (-2 MPa). The xylem sap of many vines contains sugars, and the osmotic pressure developed by the sugars has been considered the driving force to explain the flow of the sap from tapped xylem in spring or to explain water uptake (Canny, 1995).

However, no sugar was detected in the sap samples collected from Merlot and Concord vines during veraison. This may be due to the higher Ψ_{leaf} than the post-veraison vines where Ψ_{leaf} reached -2 MPa while during veraison Ψ_{leaf} never dropped below -1.5 MPa and -1.7 MPa in Merlot and Concord respectively. The result of this experiment suggests that vines remobilize and pump carbohydrates in the xylem vessel in the root to generate an osmotic gradient between the xylem vessel and the soil at any time during ripening if heavily water stressed. So, water stress during late ripening might result in depletion of starch reserves in root causing poor cold hardiness or budbreak (Mullins *et al.,* 2003).

The photosynthetic rate and stomatal conductance increased when the water-stressed vines were irrigated with 500 mL of water for both genetically different cultivars. The observed increase was within 2 hours regardless of the intensity of water stress or the phenological stage. Düring (1988) reported that the rate of photosynthesis of stressed vines was lower compared to irrigated vine. The overwhelming limitation on plant productivity is leaf water supply (Sperry *et al.,* 2002). Also, the present results show that stomatal conductance increased with the photosynthetic rate after rewatering. The function of stomata is like a pressure regulator. As a pressure regulator which limits pressure changes by controlling flow rate, stomata limit the variation in plant water potential with soil moisture and evaporative demand by controlling transpiration (Poni *et al*., 1993). Also the stomatal regulation of leaf gas exchange is directly influenced by the water status and hydraulic structure of the whole plant (Hubbard *et al.,* 2001; Franks, 2004). In water stress conditions, the prime role of stomata might be to avoid damaging xylem cavitations by closing (Jones 1998). However, the active contribution of stomatal

factor to the imitation of photosynthesis rate is still debated (Bota *et al.,* 2004; Downton *et al.,* 1988). My results showed that stomata close during water stress and open wider in two hours after watering resulting in a higher gas exchange rate causing a rapid increase in photosynthesis. An increase in photosynthesis implies an increase in phloem export of solutes from the leaves and therefore an increase of phloem flow to the berries. Ripening grape berries are a very strong sink for dry matter transported from current photosynthesis in green tissues (Petrie *et al.,* 2000). The recovering of berry size after rewatering may be because of an increase in photosynthesis therefore, an increase in phloem flow. The importance of phloem influx for berry expansion after a dry-down and watering cycle is also supported from the root pressure experiment which shows no response in berry size to pneumatic pressure applied to the root system. This could mean that rapid changes in plant water status do not affect berry size due to xylem water influx in post-veraison stage but due to the increase of phloem influx as a consequence of an increase in photosynthetic rate.

Basic fuchsin clearly behaved as a xylem lumen-mobile dye, and the dye was always confined to xylem vessels and tracheids. When feeding the dye without pressure from the base of the shoot during veraison, the dye was found to be continuous throughout the entire vascular network in hard green berries (5.5 Brix). The extent of dye penetration through a berry's vascular system decreased gradually as the berry changed color from green to blue and began to accumulate hexose sugar as reported by Keller *et al.* (2006). The rise in sugars precedes by a few days the changes in color appearance and the resumption of berry growth (Coombe and Bishop, 1980; Coombe, 1992). A

correlation between color and soluble solids was noted for Merlot and Concord. Also a threshold for the beginning of skin pigmentation has been noted at 9 Brix for Concord and 10 Brix for Merlot and blue berries always had >15 Brix in both varieties. Dye was also found to extend less far into Concord berries at 10 Brix (blushing color) compared with Merlot berries at the same development stage. This may suggest that the stop in xylem flow in Concord berries occurs at lower Brix than in Merlot due maybe to higher xylem pressure built inside Concord berries at the same solute content. Further observations found that as soon as the berry started softening (7-8 Brix in Concord, 8-9 Brix in Merlot), just before the berries began to blush (9 Brix for Concord, 10 Brix for Merlot), the dye movement became discontinuous in the stylar end which is confirmed by Creasy *et al.* (1993). Moreover, dye was confined to the brush region (proximal) for both genetically different cultivars when berries had reached 14-15 Brix soluble solids as found by Keller *et al.* (2006). The decline in dye penetration coincided with the re-growing of berries suggesting a progressive hydraulic isolation as sugar content increased inside the berry. However, this trend could be reversed by pressurizing the shoot base. It suggests that berry-xylem conduits retain their capacity for water and solute transport during ripening confirming recent results by Bondada *et al.* (2005) and Keller *et al.* (2006). Also the inability to push dye to the berries at ≥ 14 Brix when 1 bar pressure was applied for 5 hours suggests that the xylem pressure inside of the berries ≥ 14 Brix is above 0.1 MPa. The reverse dye feeding experiment suggests the xylem is functional in post-veraison berry confirming results from Bondada *et al.* (2005) and Keller *et al.* (2006). Also <0.1 MPa may be the pressure which stops water efflux from the xylem during ripening because with reverse infusion of the dye from the berry did not move

back to the vine when the root system was pressurized to 1 bar. However we can only speculate about this because pressure has never been measured inside the xylem vessels into the berry due to their very small size. Others have measured turgor pressure in the mesocarp cells (Matthews and Shackel, 2005) which was around 0.3 MPa before veraison but dropped to around 0.03 MPa before stabilizing during ripening. Also xylem pressure has been measured in the pedicel (Tyerman *et al.,* 2004) which was -0.2 to -0.1 MPa until veraison and then increased to zero when the juice osmotic potential reached about -3 MPa in Chardonnay and -4 MPa in Shiraz. However, the ability to push the dye into the berry and the reverse movement of the dye from the berry to the vine without root pressure shows xylem functionality in post-veraison berries. This confirms data presented by Bondada *et al.* (2005) and Keller *et al.* (2006), but contrasts with other studies using apoplastic dye perfusion through the berry pedicel which was interpreted as demonstrating a breakdown in xylem functionality in post-veraison berries (Düring *et al.,* 1987; Findlay *et al.,* 1987; Creasy *et al.,* 1993). The implications for irrigation during and after veraison are that watering may increase the photosynthetic rate causing an expansion of fruit volume due to sugar import from leaves. Also, irrigating vines in the end of the season may not interfere with fruit water content, storage reserves and cold acclimation allowing growers to leave the fruits on the vine longer for flavor development without losing yield.

The immersion experiment with different treatments applied to post-veraison Merlot and Concord berries at a concentration of soluble solids ~ 22 and ~18 Brix respectively showed that berries gain weight and that Concord berries were more likely to

crack. Also, berries could take up water through the skin and the pedicel. The cracking of post-veraison Concord and Merlot berries in response to immersion confirms that fruit expansion is limited by the elastic properties of the skin cell walls (Considine and Kriedemann, 1972; Matthews *et al.,* 1987; Lang and Düring, 1990). The epidermal and sub epidermal layers limit berry enlargement while the pericarp tissue is more elastic (Considine and Kriedemann, 1972). However, further investigation noted that the handling of the berry may be a cause of cracking, so a different method of sealing needs to be used for further study.

The immersion of the pedicel with the entire berry out and the totally immersed berry treatments indicated that berries lose solutes in both varieties. On the other hand, no soluble solids were found in water when the berry skin was unsealed and the pedicel sealed. This may suggest that solutes can be lost from the berry through the pedicel in intact berries. About 10 mg of solutes were lost from the intact berries at 18 Brix for Concord and 22 Brix for Merlot. It is possible that the small amount of solutes lost was due to low berry volume occupied by the apoplast (Hardie *et al.,* 1996, Diakou and Carde, 2001). Also leached solutes from the pedicel can be actively reabsorbed by the phloem (Thompson and Holbrook, 2003). In addition, as soon as the berries cracked the soluble solids in the berry dropped for both cultivars due to volume gain and sugar leaching.

The early post-veraison berry immersion shows that berries took up water increasing their weight. About 10 % of the berries cracked, and when this happened,

berries increased weight more when immersed in distilled water (pH 5.93) than in tap water (pH 7.91). This suggests that distilled water can move easily into the berry due to a larger osmotic gradient explained by the difference of mineral concentration in the water source. This implies that rain water may induce increased berry size and cracking more than irrigation. The same trend was noted also in the later immersion experiment. Post-veraison Concord berries cracked more in distilled water than in the tap water suggesting that higher water influx with high berry solute concentration generated enough pressure to crack the skin (Considine and Kriedemann, 1972; Lang A and Thorpe, 1989).

The heat girdling experiment did not show any ability of berries to export soluble solids from one berry to another on the same cluster. However the outcomes indicated that berries start shriveling following girdling suggesting no flow in the vascular bundles. Partial occlusion of xylem conduits due to girdling could increase r_h (hydraulic resistant) (Zwieniecki *et al.,* 2004). This result contrasts other studies on xylem-phloem flow which assumed that girdling eliminates phloem flow without affecting xylem flow (Lang and Thorpe 1989; Greenspan *et al.,* 1994, 1996). Berries shriveling can be partially explained by berry water lost due to transpiration which has been estimated at <100 $\mu L\ d^{-1}$ (Greespan *et. al.,* 1996, Rogiers *et al.,* 2004) and maybe due to xylem efflux. However, Greenspan *et al.* (1994, 1996) claimed that xylem backflow from ripening grape berries was likely to be insignificant. Also berries turn color from green to blue after girdling, even though they were hard and green at ~5.5 Brix at the beginning of the experiment. No differences were observed for color change or soluble solids concentration between girdling the peduncle or girdling the peduncle and lateral shoulder of the cluster. The fact

that berries changing color expand on water stressed vines and berries on girdled clusters shrink confirms that water influx to the berry is through the phloem while water efflux is through the xylem and skin.

Also, berries changed color from green to red in five days after being detached from the vine. In addition, no significant differences were found in the soluble solids concentration between the clusters attached and detached from the vine which may be a consequence of the visible shriveling. The increase of soluble solids concentration inside the berries could occur due to water loss by evapotranspiration of the berry and/or xylem flow out of the berry. Other studies found that pigmentation development is closely related to sugar accumulation (Hrazdina *et. al.,* 1984; Pirie and Mullins, 1977, 1980) and irradiance (Keller and Hrazdina *et al.,* 1996). Color variation of the grape berry conforms to a peculiar pattern of genotype-specific expression of the whole set of anthocyanin genes as reported by Castellarin and Di Gaspero (2007). So, berry shrinking associated with skin color change suggests that the soluble solids concentration may start veraison by inducing the biosynthetic genes for anthocyanins in the berry skin. Further observation identified a threshold at 9 Brix in Concord and 10 Brix in Merlot which induces gene cascade for anthocyanins biosynthesis even when berries were detached from the vine.

CHAPTER FIVE

CONCLUSIONS AND FUTURE STUDIES

Our results prove that the xylem vessels are functional and the berry is not hydraulically isolated from the rest of the plant during ripening. During veraison, fruit volume change in green berries is due to water import from the roots, through the xylem. This switches to sap import from the leaves, through the phloem, when berries change color from green to blush. The results also showed that xylem influx to green berries decrease as soon as the berry became soft green. Further investigations using dye infusion, which moves as water in the xylem vessel, might help in understanding the difference in xylem flow between hard and soft green berries.

After veraison, xylem flow may be responsible for the outflow of water from the berry while phloem flow is responsible for the inflow to the berry. Irrigation during ripening may increase photosynthesis rate, causing an expansion of fruit volume due to sugar import from leaves. Also, watering vines in the end of the season may not interfere with fruit water content, storage reserves and cold acclimation allowing growers to leave the fruits on the vine longer for flavor development without losing yield. On the other hand, water stress during ripening may decrease photosynthesis of leaves resulting in low sugar production. In addition, low soil moisture may reduce storage carbohydrates in the roots because sugar may be needed to generate an osmotic gradient between the soil and the xylem vessels for water influx. This may result in poor cold hardiness and budbreak. Further investigations are needed to investigate the relationship between the increase of photosynthetic rate and berry volume change. One suggestion is to cover vine leaves during veraison to avoid light interception, resulting in no photosynthetic rate, while berry volume is monitored.

Berries take up water through the skin and pedicel regardless of the variety, water source and maturity stage. Late and early season overhead sprinkler irrigation or rainfall might effectively increase berry weight and dilute berry solutes. Post-veraison berries may crack due more likely to late rainfall than overhead sprinkler irrigation resulting in lost sugar by leaching. Also sugar may leach out from the pedicel of intact berries during ripening. Further investigations are necessary to determine if the sugar leaching from the berries may be increased by water stress during ripening. One suggestion is to pressurize water-stressed clusters and collect the sap coming from the pedicel.

Previous studies looked at grape ripening based on days after bloom, missing the development of the single berry. Berries start to soften, change color, and accumulate sugar differently from others berries in the same cluster. The beginning of color change starts at a specific threshold of soluble solids concentration which triggers gene expressions for the biosynthesis of anthocyanins. Berries also complete their color change even when detached from the shoot showing a dependency on soluble solids concentration regardless the connection to the vine. Further investigations on berry color development are necessary and one suggestion is to investigate the changes in gene expression that trigger veraison.

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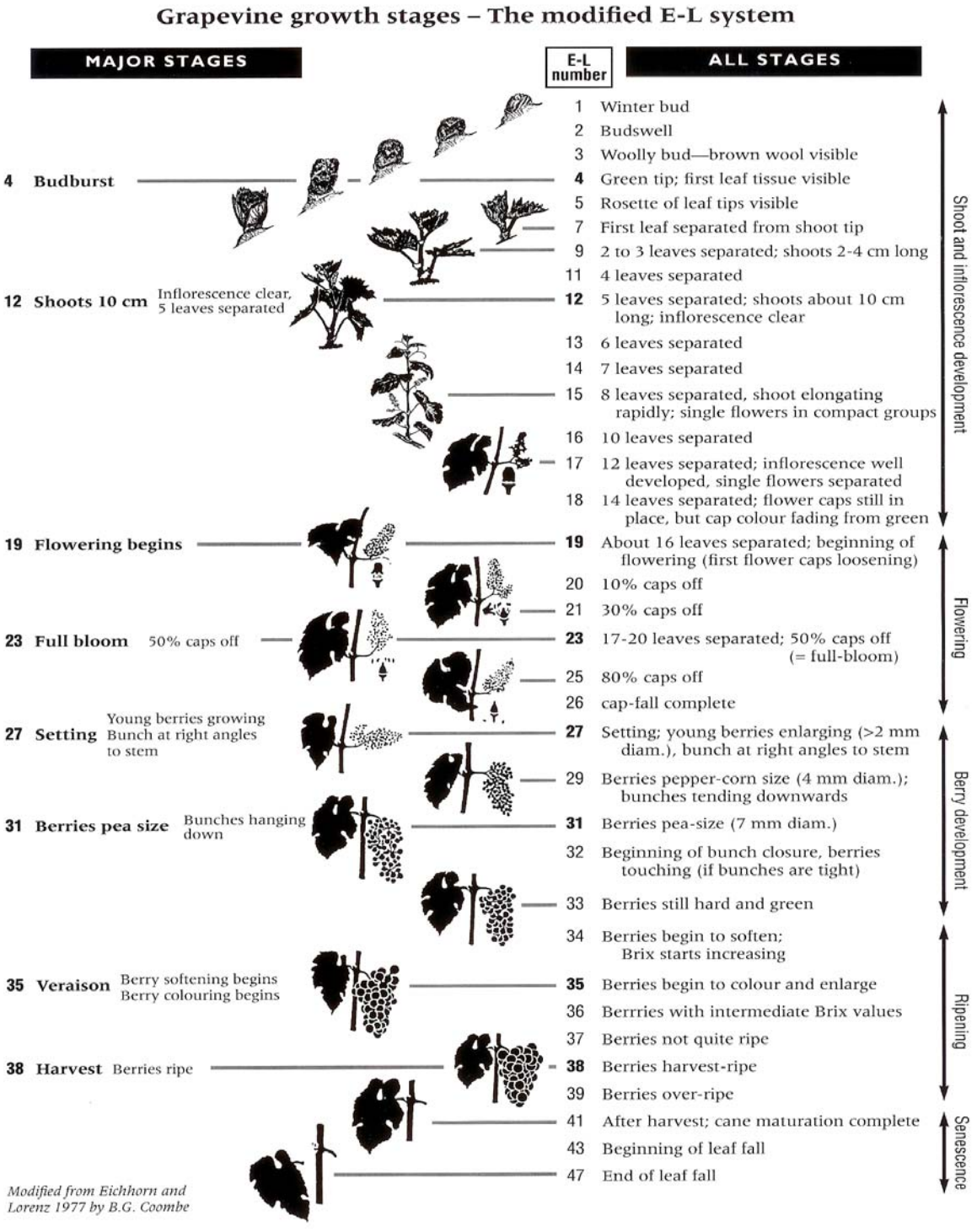
APPENDIX



Appendix 1: Grapevine growth stages according to Eichhorn and Lorenz. Illustration from Coombe (1995).