Several effects of xylem discontinuity in Pinot noir and Merlot grape berries were studied. There was a reduction in the amount of apoplastic dye (Eosin Y or azosulfamide) uptake through cut pedicels into soft versus firm berries, suggesting a reduction in maximal xylem flow at that time. Both greenhouse and field grown Pinot noir berries took up less dye after softening. Merlot berries, collected on one date from the field and separated by hand into four developmental categories, took up different amounts of azosulfamide dye according to category. Soft green and just colored berries took up 30%, and fully colored berries 70%, less dye than firm green berries. The reduction in xylem conductivity was related to the developmental stage of each individual berry and not the cluster as a whole.
The accumulation of K\(^+\) (primarily a phloem transported element) and Ca\(^{2+}\) (primarily a xylem transported element) differed in field grown Pinot noir berries during maturation. On a per berry basis K\(^+\) increased after veraison, suggesting greater phloem activity, but Ca\(^{2+}\) content remained stable after veraison, suggesting little xylem activity.

Berry diameters on pre-veraison clusters on well watered vines increased slightly; those on unwatered vines decreased, losing 0.87 mm in diameter during day 3 of the experiment. Pre-veraison berry deformabilities were 380% higher in unwatered versus watered vines on day 4. Bagging pre-veraison clusters to slow transpiration reduced the rate of berry diameter loss and softness only slightly. Pre-veraison berry shrivelling occurred before vine wilting. Post-veraison Pinot noir berry diameters and deformabilities in the greenhouse were not significantly affected by short term vine water stress.

Heat girdling cluster peduncles to block phloem flow reduced pre-veraison berry growth rates to near zero and increased the rate of diameter loss significantly in post-veraison berries. Girdling had little effect on pre-veraison berry deformabilities, but a large effect on those of post-veraison berries.

The different berry responses to vine water stress and peduncle girdling before and after veraison suggested
a change in xylem activity. Prior to veraison there was rapid water loss from the berry to a stressed vine, but after veraison berries were more isolated, showing little response to vine water stress. Blocking phloem transport in the cluster peduncle prior to veraison reduced berry growth to almost zero but affected deformability little, which suggested that xylem was maintaining berry size and firmness. Under the same conditions post-veraison berries lost both size and firmness rapidly, which suggested little xylem activity.
Xylem Discontinuity in *Vitis vinifera* L. Berries

by

Glen L. Creasy

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Typed by Glen L. Creasy for Glen L. Creasy
For my parents,

to whom I owe everything
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Xylem Discontinuity in *Vitis vinifera* L. Berries

**Introduction**

**Chapter 1**

Winegrapes are one of the most widely grown fruit crops in the world, and thus are of great economic consequence (Winkler et al., 1974). Research concerning viticulture is done in an effort to better understand the intricacies of grape production.

In Oregon there is a small but growing winegrape industry producing about 3.2 metric tons of fruit annually. The industry is concentrated in western Oregon's Willamette Valley, which is between the Coastal and Cascade mountain ranges. Pinot noir, Chardonnay, and Riesling are the primary grape varieties planted (Miles, 1987), but fruit maturation in these varieties is often jeopardized by rainfall near harvest.

Grape berry maturation is one of the more intensely studied aspects of grape physiology because of its complexity. Grape berries are unusual in that they can accumulate high concentrations of sugars, and do so in a relatively short period of time (Winkler et al., 1974). They also begin to ripen earlier in the growing season than other fruits. The initiation of ripening is near
veraison, a time during which there are major chemical and physical changes in the berry (Coombe, 1976). One of the more recently discovered physical changes is in the berry vasculature. Two research groups (During et al., 1987; Findlay et al., 1987) have found that flow through pedicel xylem into berry decreases sharply near veraison. In these studies with Riesling and Muscat Gordo Blanco berries, physical breaks in xylem vessels of the berry vasculature were evident post- but not pre-veraison.

A reduction in xylem contribution to fruit development at this time indicates that the berry depends primarily on phloem inputs during ripening. Indeed, blockage of the xylem pathway between the vine and berry may contribute to the fruit achieving such high sugar concentrations at maturity by redirecting photoassimilate flow and preventing water from moving into or out of the berry (Lang and Thorpe, 1989). If a xylem discontinuity exists, berry responses to vine water stress will be different in pre- versus post-veraison berries, since the xylem is a largely unregulated pathway for water movement. Also, because there is less xylem flow from the vine to berry after veraison, and a corresponding decrease in delivery of mineral elements carried in the xylem, certain post-veraison disorders, such as waterberry, may occur (Christensen and Boggero, 1985; Morrison and Iodi, 1990).
In order to clarify the effects of xylem discontinuity on berry maturation, the objectives of this research were: 1) determine if a decrease in dye uptake occurs in Pinot noir and Merlot grapes and if so, 2) determine when during berry development the decrease takes place in relation to external, measurable factor(s) (e.g. berry diameter change, color change, and softening); 3) measure berry mineral compositional changes before and after veraison; and 4) measure pre- and post-veraison berry responses to water stress and blockage of phloem transport. Data gathered should be consistent with the hypothesized reduction in xylem flow after veraison.
Grape Berry Development

Grape berry maturation is a much studied topic, with veraison (Fr. véraison, meaning first color change and berry softening (Winkler et al., 1974)) being the most interesting aspect, physiologically. The grape berry has a double-sigmoidal growth curve (Considine and Knox, 1979; Coombe, 1976; Harris et al., 1968; Nii and Coombe, 1983; Winkler et al., 1974) and is non-climacteric (Biale, 1960; Kader et al., 1985; Peynaud and Ribereau-Gayon, 1971). There are three commonly described phases of growth: anthesis and first stage of rapid growth (cell divisions, enlargement, and pedicel growth), a period of no or slow growth (seed maturation and testa hardening), and a final rapid growth (cell enlargement), which are commonly described as Stage I, Stage II, and Stage III, respectively (Nakagawa and Nanjo, 1965; 1966; Winkler and Williams, 1935; Winkler et al., 1974). An over-ripe stage can also be described, where sugars stop accumulating, but acids continue to decline (Peynaud and Ribereau-Gayon, 1971; Winkler et al., 1974). Veraison is associated with the start of Stage III, and the time of
transition from Stage II to Stage III is of most interest in this study.

Within a period of a few days, a berry goes from a stage of quiescence (lag phase = Stage II) to one of rapid growth, sugar accumulation, acid metabolism, and, for a colored variety like Pinot noir, anthocyanin synthesis. Varieties without anthocyanins (e.g. Chardonnay) become translucent, losing green color through chlorophyll degradation (Harris et al., 1968).

**Berry Structure**

The fruit of *Vitis* spp. is a true berry: a multi-seeded fruit derived from a single ovary. The grape ovary has four ovules, and thus a maximum of four seeds (Nelson, 1985; Winkler and Williams, 1935; Winkler et al., 1974). The majority of the flesh of a mature fruit is made up of mesocarp (Harris et al., 1968; Nakagawa and Nanjo, 1965).

There are two categories of vascular bundles in the berry: peripheral (= dorsal), which lie below the sub-epidermis, and axial (= ventral), which pass through the axis of the berry, branch off to the seed(s), and eventually spread out at the stylar end of the berry (Nelson, 1985).

The pedicel attaches the berry to the cluster framework, the rachis, and enlarges at its junction with
the berry, a region called the torus (Nelson, 1985). The normal abscission zone between a berry and pedicel forms in the torus. If a berry is pulled from its pedicel before the abscission layer forms, the berry brush (portions of the vascular strands and pulp from inside the berry) remains attached to the pedicel (Coombe, 1987; Findlay et al., 1987; Nelson, 1985).

The outer surface of the berry has important function to berry development. The first six to eight layers of epidermal cells contain most of the chlorophyll and anthocyanins and is covered by the cuticle, a thin wax-like layer on the epidermis that protects the berry against water loss and pathogen attack (Nelson, 1985; Peynaud and Ribereau-Gayon, 1971; Radler, 1965a; Winkler et al., 1974). Berries chemically stripped of cuticular wax have a significantly higher rate of water loss than untreated berries, while berries dipped in liquid paraffin (to reduce transpiration) lose water at a rate similar to that of untreated berries (During and Oggionni, 1986; Radler, 1965a). Thus, the natural berry coating is very effective at insulating the berry from desiccation.

**Berry Growth**

During Stage I, berry growth is due to both cell divisions and cell enlargement (Coombe, 1960; Harris et
The embryo increases in size and fresh weight during this stage, but may continue to develop throughout berry maturation (Coombe, 1960; Nakagawa and Nanjo, 1965; 1966). In general, the inner cells of the berry pericarp stop dividing before the outer cells (Nakagawa and Nanjo, 1965). The duration of Stage I seems to be similar for all grape varieties (Coombe, 1976; Nakagawa and Nanjo, 1966).

Stage II is a period of reduced or no growth, the length of which is highly variable (Coombe, 1973; 1976; Nakagawa and Nanjo, 1966). The slowing of growth may be caused by competition for photoassimilates between the seed and berry flesh, since during this time period the testa hardens and the seed becomes mature and viable (Cawthon and Morris, 1982; Nakagawa and Nanjo, 1965). However, arguments that neither fruit to vine nor seeds to flesh competition is the cause of periodicity in berry growth have been presented (Matthews et al., 1987; Winkler and Williams, 1935). Berries from seedless varieties usually have a shorter Stage II and weaker double sigmoid shape in their growth curves, perhaps due to the lack of competition between seed and fruit (Coombe, 1960; Winkler and Williams, 1935). Still, several researchers have found that seeds are always mature and viable before berries enter Stage III, so some
interaction is likely (Cawthon and Morris, 1982; Winkler and Williams, 1935).

Stage III starts near veraison and is a period of rapid cell expansion (Harris et al., 1968; Winkler et al., 1974). Harris et al. (1968) reported an apparent decrease in berry cell number during the latter part of Stage III, but dismissed it as a procedural artifact. Jona et al. (1983) reported similar results, but suggested that this may have been the result of cell fusion or compaction rather than an artifact.

Berry size is influenced by seed number, with a greater number of seeds associated with larger berries (Cawthon and Morris, 1982; Winkler and Williams, 1935). Placental tissue produced to replace the volume that a seed would normally take up may be insufficient to do so, resulting in a smaller berry (Nakagawa and Nanjo, 1965). Cheng (1985) reports that seeds influence berry growth through hormonal action, rather than competition.

Jona et al. (1983) found that cell number in two cultivars was the same, yet berry sizes were different. They then concluded that berry size was determined by cell volume and not cell number. Certainly, cell expansion has a great influence on final berry size (Coombe, 1976; Harris et al., 1968).
Berry Expansion and Skin Extensibility

Berry expansion results from a net flow of materials into the fruit (Lang and Thorpe, 1988). Due to modification of pectocellulose in cell walls, berries can expand to accommodate volume increases (Peynaud and Ribereau-Gayon, 1971). Berry skin extensibility may be one measure of the ability of a berry to increase in volume, since the expansion rate may be limited by the skin (Considine and Kriedemann, 1972; Matthews et al., 1987). As expected, in early Stage III skin extensibility increases greatly, but however logical it might be for increased pressure to cause berry expansion, Matthews et al. (1987) found low berry turgor associated with rapid expansion. The rate of assimilate flow into the berry may also be a factor determining the rate of expansion.

Chemical Changes

Berry pH tends to rise after veraison (Al-Kaisy et al., 1981; Carroll and Marcy, 1982; Hrazdina et al., 1984). Berry acidity, which contributes to berry pH, is mainly influenced by tartaric and malic acid content (Hrazdina et al., 1984; Kliewer, 1966; Winkler et al., 1974). When expressed on a percent basis, both rise during Stage I (malic much more so) and fall during Stage III (Coombe, 1987; Hrazdina et al., 1984; Winkler et al.,
1974). Expressed on a per berry basis after veraison, malic acid content falls while that of tartaric remains constant (Carroll and Marcy, 1982).

Glucose and fructose are the predominant sugars, with their ratio being close to one for most of berry development (Hrazdina et al., 1984; Kliewer, 1966; Winkler et al., 1974). More fructose than glucose is present in over-ripe fruit (Winkler et al., 1974).

Sucrose in V. vinifera berries is present in only small amounts, though it does accumulate to a limited extent late in Stage III ((Coombe, 1987; Hrazdina et al., 1984; Kliewer, 1966; Nelson, 1985). However, in V. rotundifolia (the muscadine grape) fruit has a significant amount of sucrose (Carroll and Marcy, 1982).

Starch is virtually non-existent in mature grape berries, like most fleshy fruits (Coombe, 1976; Nelson, 1985).

**Veraison**

Originally, French viticulturalists used the term véraison to denote first color change in clusters (Winkler et al., 1974). For some, the term has come to describe the entire initial process of berry ripening, which occurs near the beginning of Stage III (Coombe, 1973; 1980; Coombe and Bishop, 1980; Findlay et al., 1987; Nii and Coombe, 1983). This includes color change, sugar accumulation, acid metabolism, decreased
respiration, berry softening, and rapid berry expansion (Coombe, 1973; 1980; Coombe and Bishop, 1980; Harris et al., 1968; Peynaud and Ribereau-Gayon, 1971; Winkler et al., 1974). For the purposes of this discussion, veraison will refer to the time of first color change.

**Sequences of Events Near Veraison**

The initiation of different events associated with veraison is not always consistent, and may or may not be cultivar dependent. Findlay et al. (1987) found that berry volume increases about one week after the inception of rapid sugar accumulation, softening, and color development, and Considine and Knox (1979) observed that sugar increase occurs before diameter increase. Coombe (1960; 1980) and Winkler and Williams (1935) found diameter and sugar concentration changes to occur coincidentally. Hrazdina et al. (1984) found that sugar accumulation starts before color development. Coombe and Bishop (1980) found that berry deformability usually, but not always, increases before sugar accumulation and the second rapid diameter increase characteristic of Stage III.

Berry expansion is an integral part of sugar accumulation. Berries kept from expanding by placing them in plastic enclosures did not accumulate sugar, but when released from confinement expanded rapidly and
imported sugar (Coombe, 1973). Similarly, berry skin may be the primary factor limiting berry expansion, since peeled berries absorb much more water without cracking than unpeeled berries (Considine and Kriedemann, 1972). However, Matthews et al. (1987) found little evidence that skin extensibility limits berry expansion.

The sudden weight increase after veraison has been attributed to water and not accumulated sugars (i.e. fresh weight increased before dry weight) (Cheng, 1985; Coombe and Bishop, 1980). This suggests that the vascular tissue brings in larger amounts of water than sugar. However, over the course of Stage III total berry water content decreases (Carroll and Marcy, 1982; Harris et al., 1968).

Sugar accumulation can be a result of both transport into and synthesis within the berry (Coombe, 1987), but concentration is also affected by dehydration and dilution (Coombe, 1976). Peynaud and Ribereau-Gayon (1971) report that in situ sugar synthesis is not the cause of rapid solute rise in grape berries at veraison, thus import must be the major cause.

**Berry Softening**

Fruit softening is used in many fruit crops to determine picking dates (Kader et al., 1985; Westwood, 1988), but not in grape, since it occurs many weeks
before harvest. However, softening can be used as an indicator of imminent sugar and color accumulation, and also diameter increase. Softening may also be implicated in the inception of sugar accumulation in the berry (Coombe and Phillips, 1982).

Berry firmness may decline as a result of a reduction in cell turgor (Matthews et al., 1987) caused by water stress or physiological tissue changes, and is linearly and positively related to (artificially induced) turgor pressure (Bernstein and Lustig, 1985). Temperature may also affect berry pressure (and thus firmness) significantly (Lang and During, 1990).

Vascular Changes

Findlay et al. (1987) and During et al. (1987) discovered independently that major changes occur in the apoplastic pathway entering the berry near veraison. The amount of apoplastic dyes that were taken up through cut berry pedicels or cluster peduncles decreased greatly at that stage of development. Anatomical studies of berry tissue showed that mechanical breaks in xylem vessels at the basal end of the berry (near the berry brush) were much more prevalent in post-veraison berries, and an increase in their frequency corresponded with the decrease in dye uptake and other factors associated with veraison. Both groups of authors postulated that breaks
in the xylem were caused by rapid berry expansion following veraison. The relatively inextensible xylem vessels in the berry brush were stretched beyond the breaking point, while the more extensible phloem elements accommodated the change (During et al., 1987; Findlay et al., 1987).

Peduncle, rachis, or pedicel vascular tissue have ceased growth by veraison, so vascular strands in these organs should not have been stressed enough to break at veraison (Nii and Coombe, 1983; Theiler and Coombe, 1985; Winkler et al., 1974). Therefore, vascular tissue inside the berry must be the site of the discontinuities, since only the berry is rapidly growing. After veraison berries tend to grow faster in girth than length, in opposition to pre-veraison growth (Harris et al., 1968). Thus the peripheral strands experience different stresses after veraison, which may contribute to breakage.

Contrary to the findings of Findlay et al. (1987) and During et al. (1987), Kriedemann (1969) found that $^3$H-glucose applied through Stage III cut Thompson Seedless berry pedicels was distributed in both peripheral and axial vascular bundles. Findlay et al. (1987) reported similar results using $^{14}$C-sucrose perfused through cut pedicels into pre-veraison, but not post-veraison berries. In these Stage III berries most of the radioactivity was found in the brush region.
With regard to the $^3$H-glucose uptake through post-veraison berry pedicels (Kriedemann, 1969), it seems unlikely that phloem could have transported so much material into the berry so quickly, since by 1 hour radioactivity was found throughout the berry. A possible explanation for the seemingly high amount of flow through the xylem of these post-veraison berries was that there was rapid movement of water from the bathing solution through the pedicel apoplast (excluding the xylem) and into the berry, bringing labelled glucose with it. However, in pedicels dipped in dye solutions for less than 2 hours, only the vascular bundles were stained, suggesting that materials must have moved within the pedicel xylem to enter the berry (personal observation), and that the pedicel apoplast (excluding the xylem) was a poor pathway for water movement. A post-veraison xylem discontinuity was not consistent with the findings of Kriedemann (1969). Because seedless grape berries have slightly less pronounced double sigmoid growth curve shape than seeded berries (Coombe, 1960; Winkler and Williams, 1935), xylem elements may not have been stretched to the point of breaking during growth after Stage II. In this case, the Stage III berries of Thompson Seedless used by Kriedemann may have had functional xylem.
There is additional evidence for a drop in xylem conductivity in that accumulation of xylem borne elements (e.g. Ca\(^{2+}\)) tends to level off following veraison, whereas phloem transported elements (e.g. K\(^+\)) continue to accumulate (During and Oggionni, 1986; Hrazdina et al., 1984; Lang and Thorpe, 1989; Morrison and Iodi, 1990; Possner and Kliewer, 1985).

Evidence supporting the hypothesis that the cause of the xylem discontinuity is physical rather than physiological, is that mechanical shocks (such as dropping a berry a short distance) decreases xylem conductivity as measured by dye uptake (Findlay et al., 1987). Large water potential gradients found between the leaves and fruit of grapevine may also suggest an increased resistance to flow into the berry (Cheng, 1985; Greenspan and Matthews, 1991; van Zyl, 1987).

**Xylem:**

**Structure and Function**

The main pathway for water movement in a plant is via the xylem, in which water and dissolved materials may pass with little resistance (Galston et al., 1980). In general, xylem sap moves passively from regions of less negative water potential to those of more negative water potential (i.e. down the water potential gradient) (Canny, 1990; Galston et al., 1980).
A majority of the water supplied to the fruit passes through the xylem. These supplies may be incorporated into the fruit, lost through transpiration, or shunted back into the parent plant by reverse flow (Jones and Higgs, 1982). With the water carried to the fruit are minerals necessary for cell growth (Tromp and Oele, 1972). Very little Ca\(^{2+}\) is carried in the phloem, thus xylem is the primary source of this element (Hanger, 1979; Kirkby and Pilbeam, 1984; Pate, 1975; Tromp and Oele, 1972; Wiersum, 1966).

The calcium ion is required for cell wall, middle lamella, and pectin production and maintenance (Hrazdina et al., 1984; Poovaiah, 1979; Poovaiah et al., 1988). A change in the K\(^+\)/Ca\(^{2+}\) ratio during maturation may affect cell membrane permeability and be involved with the ripening process (Dvorak and Cernohorska, 1972; Poovaiah, 1979; Sacher, 1973). A change in K\(^+\)/Ca\(^{2+}\) ratio may also be associated with berry softening (Lang and Thorpe, 1989; Poovaiah et al., 1988).

Positive xylem pressures may be important in delivering Ca\(^{2+}\) to plant organs with low transpiration rates (Guttridge et al., 1981; Tachibana, 1991). In strawberry and tomato, Ca\(^{2+}\) import into developing leaves and fruits, respectively, is favored during the night cycle, when xylem pressure is positive (Guttridge et al., 1981; Tachibana, 1991). Grape berries have a very low
transpiration rate due to the effectiveness of the cuticle (During and Ogginni, 1986), but water potential gradients within grapevines are not conducive to producing positive root pressures during Stage III (Zimmermann, 1983). In opposition to inward movement, Ca\(^{2+}\) may move out of organs through the xylem in reverse-flow, as dictated by water potential gradients (Hanger, 1979).

Calcium may also get into organs with low transpiration rates through exchange movement, where there is slow transfer of calcium ions along the negatively charged xylem vessel walls (Armstrong and Kirkby, 1979). At least one researcher (Hanger, 1979) states that all Ca\(^{2+}\) movement is the result of exchange reactions, and not mass flow. But if this is true there would be little relationship between transpiration and fruit Ca\(^{2+}\) content. In grape, there is a positive correlation between berry transpiration rate and Ca\(^{2+}\) accumulation within it (During and Oggionni, 1986).

**Contribution to Wine Quality**

Grape berry mineral composition is an important wine making consideration. High K\(^{+}\) concentrations in the must are associated with high wine pH, resulting in color and storage problems (Cox, 1988; Ilard et al., 1988; Winkler et al., 1974). A high juice pH can also result in
spoilage and fermentation problems (Winkler et al., 1974). Both K\(^+\) and Ca\(^{2+}\) can form salts of malate and tartrate that can lower wine titratable acidity; something that may or may not be desirable (Winkler et al., 1974). Both K\(^+\) and Ca\(^{2+}\) have been implicated in the development of waterberry, an affliction that results in flaccid, low sugar fruit (Branas, 1974; Fregoni et al., 1979; Ureta et al., 1981).

**Flow Reversal - Physical Evidence**

There are measurable variations in both fruit and stem diameters that may be related to xylem flow reversal. Apple tree stems and fruits, grape berries, pears, and lemons have all been found to have daily changes in volume or diameter, depending on light, heat, or degree of water stress (Coombe and Bishop, 1980; Huguet, 1985; Jones and Higgs, 1982; Klepper, 1968; Lang, 1990; Shimomura, 1967; Tukey, 1964). Klepper (1968) suggested that fruit could be used as a xylem water potential gauge, since fruit size changes depending on the water status of the tree.

Ca\(^{2+}\) has also been used to follow the movement of water in and out of fruits. Tachibana (1991) found that Ca\(^{2+}\) moved diurnally in tomato fruit, and Wilkinson (1968) found evidence that Ca\(^{2+}\) moved out of apple fruits.
Discontinuity in Grape

Breaks in xylem vessels renders each such vessel inactive, since a column of tension can no longer be maintained (Findlay et al., 1987; Galston et al., 1980; Zimmermann, 1983). This change in vine to berry connections has major implications on our understanding of the maturation process (Lang and Thorpe, 1989). Despite a major pathway for ions and water being cut off, sugar is transported into the berry at a rapid pace; fruit soluble solids can increase as much as 0.8% per day in hotter areas (Al-Kaisy et al., 1981). The rate of sugar accumulation in a berry may be affected by vacuolar solute concentration inside a cell, and since solute concentration is partially regulated by the amount of water entering the berry, a xylem discontinuity can affect sugar accumulation (Coombe, 1980; Coombe and Phillips, 1982).

The inability of xylem to serve as a direct, largely unregulated pathway from the vine to the berry means water relations between the vine and the fruit change after the discontinuity occurs.

Discontinuities in Other Fruits

In wheat and other grasses, xylem continuity is interrupted by modified tracheary elements, leading to
reduced apoplastic flow to the grain (Zee and O'Brien, 1970). Zee and O'Brien (1970) speculate that this may be a device to aid solute transfer to the phloem. However, despite this, Martin (1982) found that both xylem mobile only and phloem mobile only elements minerals were translocated from senescing leaves to the grain.

In some legumes, xylem has been demonstrated to be a two-way conduit for transport of materials to and from fruit (Clements, 1940; Pate et al., 1977; 1985; Peoples et al., 1985). In most model systems, more water enters via the phloem than is required for transpiration or expansion, with the excess leaving through the xylem (Pate et al., 1985; Peoples et al., 1985). This has been suggested for other fruits as well (Clements, 1940; Ziegler, 1963). Others speculate that phloem supplies the balance of water that is not supplied by the xylem but is necessary to meet the needs of the fruit (Pate et al., 1977; van Die and Willemse, 1980).

Pate et al. (1985) suggests a slightly more detailed model in which flow through the xylem is toward the fruit at night, when the phloem cannot supply enough water via mass flow to satisfy fruit transpirational requirements, and outward during the day, when water supplied by mass flow exceeds that needed for fruit transpiration. Peoples et al. (1985) proposes that xylem undergoes periodic flow reversals, with up to 70% of the total
water brought in by both the xylem and phloem being recycled back to the plant through the xylem. All xylem strands may reverse flow for a time, or some strands may continue inward flow while others flow out.

Apoplastic movement of assimilates is not always under strict plant control. Hamilton and Davies (1988a) found that $^{14}$C-sucrose was exported out of heat girdled pea peduncles. Subsequently (1988b) they found that material was exported through xylem in response to water potential gradients established from rapid leaf transpiration. Thus, the phenomenon is a physical response that non-selectively distributes materials.

The changing role of xylem is likely one way for a plant to balance the water economy of a developing fruit (Pate, 1975). Pate et al. (1977) working with lupin fruit found that xylem contributes less and less to fruit development as the fruit becomes a stronger sink. In tomato, Ho et al. (1987) found that xylem contribution to fruit development gradually falls from three weeks after pollination to maturity. Ninety percent of the water for fruit growth comes from the phloem, possibly due to the low transpiration rate of the tomato fruit. These researchers also speculate that reverse flow out of tomato fruit via the xylem may have been restricted, an idea similar to that previously suggested by Jones and Higgs (1982) for apple fruits. Neither group of
researchers proposed a mechanism for the increased xylem resistance.

Diurnal changes in fruit volume are well documented, and may suggest liquid flow out of fruits (Coombe and Bishop, 1980; Greenspan and Matthews, 1991; Jones and Higgs, 1982; Klepper, 1968; Lang, 1990; Shimomura, 1967; Tukey, 1964). In apple, Lang (1990) found that xylem flows to the fruit reverses, especially during times of high plant transpirational stress, suggesting that this is a part of the tree's overall water economy. The fruit acts as a water buffer from which the parent plant draws water during times of high transpirational stress: an idea supported by the data of Huguet (1985), Greenspan and Matthews (1991), Klepper (1968), Shimomura (1967), Tukey (1964), and van Zyl (1987). Lang (1990) also found that xylem contributes less and less material as an apple fruit matures, resulting in most Ca\(^{2+}\) accumulating in the early stages of fruit growth. Other researchers have reported similar Ca\(^{2+}\) accumulation patterns (Wiersum, 1966; Wilkinson, 1968). These factors combined with xylem flow reversal, which may result in solute export (Lang, 1990), make up the mineral and water economy of the apple fruit.
Xylem and Water Relations in *Vitis* spp.

According to Lang and Thorpe (1988) there are four water flows in a "nearly mature" grape berry that is no longer enlarging: 1) out through the xylem, 2) in through the phloem, 3) out via transpiration, and 4) in via osmotic absorption. During rapid sugar accumulation in the berry, Lang and Thorpe (1989) speculate that xylem backflow may be necessary to balance excess water brought in with sugar by the phloem but not lost through transpiration. However, Greenspan and Matthews (1991) find that while xylem is an important contributor to pre-veraison grape berry development, it is not during post-veraison berry maturation. In fact water delivery through the xylem after veraison may not be necessary: Findlay et al. (1987) calculated that phloem could supply all of the berry water requirements. However, the researchers did not speculate as to whether excess water would be brought in, or where it would go if it was.

van Zyl (1987), on research on vine and cluster water potentials, postulated that berries act as a water reservoir, supplying water to the vine when the leaves were under high transpirational stress and being recharged from the soil at night. Greenspan and Matthews (1991) and Shimomura (1967) found that diurnal changes in berry size were exacerbated under higher vine water stress, but that post-veraison berries has a lesser
response to the same conditions, supporting the idea of increased resistance to xylem flow after veraison.

**Xylem Discontinuity and Sugar Accumulation**

Xylem discontinuity may enhance sugar unloading in the berry. Lang and Thorpe (1986) and Lang et al. (1986) theorize that translocation favors regions within the plant that have a more negative water potential. Solute storage in the berry apoplast (as Coombe (1976) suggests) would contribute toward decreasing the water potential inside the berry. Reduced xylem flow would also contribute to a more negative water potential because transpirational water loss from the berry will increase the solute concentration (Coombe, 1976). Lang and Thorpe (1989) and Matthews et al. (1987) found significantly lower water potentials in Stage III than in Stage I or II berries. Smith and Milburn (1980) found in *Ricinus communis* that as xylem water potential (which approximates that in the apoplast) decreases, solute flux through the phloem increases.

Earlier theories concerning the rapid increase in fruit soluble solids after veraison included those of Coombe (1960), who suggested that sugars were imported into the berry first, which then attracted water into the berry osmotically. Later (1980) Coombe speculated that the sugar accumulation rate was influenced by solute
concentration in cell vacuoles, since an unloading mechanism based on concentration would explain the similar sugar accumulation rates in berries of different sizes. However, Coombe (1976; 1980) also observed that berries continued to accumulate sugar on a per berry basis after volume increases had stopped, so size increases were not linked exclusively to sugar accumulation. Since berry water potentials were found to be very negative after veraison (Cheng, 1985; Matthews et al., 1987), in order for phloem sap to be translocated into that sink, the sap must have a higher solute concentration than that already present in the fruit (Lang and Thorpe, 1989). The high potential fruit pressures that would be associated with high osmotic potentials could be compensated for in grape through the restriction of water flow to and from the fruit (Cheng, 1985; Considine and Brown, 1981).

**Xylem Reversal Revisited**

The reduction in xylem conductivity may also prevent the movement of berry solutes, including sugar, out of the berry (Findlay et al., 1987; Lang and Thorpe, 1989). Lang and Thorpe (1989) found that exudate forced from intact post-veraison berry pedicels was identical to expressed berry juice, supporting their idea that a post-
veraison grape berry is better described as a "bag of sugary water" than as an organized plant tissue.

Cell wall degradation at veraison (Considine and Knox, 1979) coincides with berry softening and sudden loss in turgor; a disruption in membrane function may be partly responsible for these happenings (Lang and Thorpe, 1989). These drops in berry turgor have been associated with rapid berry expansion during Stage III (Matthews et al., 1991). The "breakdown of apoplast:symplast compartmentation" (proposed by Lang and Thorpe (1989)) allows a large negative osmotic potential difference to build up between the vine and the berry: a potential gradient that may help direct assimilate partitioning (Lang and Thorpe, 1986; 1989; Lang et al., 1986; Lee, 1986). This forms the causal link between softening and sugar import (Lang and Thorpe, 1989), but still does not explain what triggers the process.

Berry Cuticle, Transpiration, and Mineral Content

Grape berries have a surface coating ten times as thick as that on a leaf, and are thus more resistant to water loss (Radler, 1965b). Most grape berry transpiration occurs during the day, and as berries mature they have significantly lower transpiration rates (Lang and Thorpe, 1989; During and Oggionni, 1986). However, net cuticle wax per unit area remains relatively
constant after veraison (Considine and Knox, 1979; Radler, 1965b; Rosenquist and Morrison, 1988). Berry transpiration rate is affected by turgor, with higher turgor associated with more water loss (Lang and Thorpe, 1988). Post-veraison berries, however, have low turgors (Matthews et al., 1987), possibly explaining the transpiration rate decrease.

Pathways for direct water loss through the berry skin are limited, increasing the fruit's resistance to desiccation. Grape berries may have stomata (Peynaud and Ribereau-Gayon, 1971) or may not (Nakagawa and Nanjo, 1965; 1966; Pratt, 1971). Lenticels have also been reported to occur on grape berries (Nelson, 1985; Pratt, 1971).

A decrease in transpirationally driven xylem flow after veraison should preclude rapid and large changes in berry solutes normally associated with that flow (such as Ca\(^{2+}\)). This view is supported by the frequent observation that Ca\(^{2+}\) does not accumulate significantly after veraison (During and Oggionni, 1986; Hrazdina et al., 1984; Lang and Thorpe, 1989; Morrison and Iodi, 1990; Possner and Kliwer, 1985).

However, in one experiment (During and Oggionni, 1986) cuticle-stripped grape berries accumulated Ca\(^{2+}\) after veraison in much the same pattern as that of K\(^{+}\), a finding that runs contrary to most. No explanation for
this observation has been offered. Nevertheless, with few discrepancies, this trend of decreased Ca\textsuperscript{2+} accumulation following veraison is well established for healthy grape berries.

The Ca\textsuperscript{2+} accumulation rates in tomato and apple fruits also decrease during maturation, but Ca\textsuperscript{2+} does move into these fruits later in maturation (Ho et al., 1987; Tromp and Oele, 1972). Lang (1990) reasons that the reduced role of xylem later in apple maturity causes fruit Ca\textsuperscript{2+} accumulation to stop. In apple, this phenomenon is unrelated to transpiration, since transpirational water loss remains relatively constant throughout fruit development.

Alternatively, the decrease in Ca\textsuperscript{2+} accumulation rate in ripening berries could be due to the increased phloem flux to the berry. Ca\textsuperscript{2+} levels may fail to rise simply because increased phloem activity decreases the relative xylem flux, irrespective of any possible increase in resistance to flow through the xylem (Van de Geijn and Smeulders, 1981).

**Waterberry (implications of recent findings)**

Waterberry (also known as Stiellähme, desséchement de la rafle, palo negro, bunch stem necrosis, and shanking) is a serious problem in many viticultural areas (Christensen and Boggero, 1985), and a recent study has
elucidated the differences between affected and normal berries (Morrison and Iodi, 1990). Affected clusters will have dark or necrotic spots on the peduncle, rachis, and/or pedicel at any time after veraison, which, in effect girdle the portion apical to the necrosis. Affected berries are "watery, soft and flabby," and their colors will be "metallic, opaque, or dull green in white grapes and red to dark blue in black grapes" (Branas, 1974; Fregoni et al., 1979; Ureta et al., 1981; Winkler et al., 1974).

Morrison and Iodi (1990) speculate that the phloem activity in affected berries slows, resulting in greatly reduced berry growth at veraison. Compared to unaffected berries, waterberry weight increases much more slowly after veraison. $\text{Ca}^{2+}$ (expressed on a concentration and per berry basis) continues to increase in waterberries, unlike in healthy berries, whose rapid size increase is associated with no net $\text{Ca}^{2+}$ gain. To explain this the authors speculate that xylem connections between the waterberry and vine remain continuous, unlike in normal berries, where xylem interruptions are postulated to occur (During et al., 1987; Findlay et al., 1987). Thus the berries may be growing under pre-veraison xylem and phloem inputs, which are responsible for slow and incomplete berry maturation. Morrison and Iodi (1990) suggest waterberry results in a modified ripening pattern
rather than the absence of ripening, since affected berries still soften and their malic acid content falls.

Kasimatis (1957) reported that tyloses were plugging xylem in pedicels of waterberries, restricting solute movement to and from the fruit, and causing the disorder. However, recent work by Morrison and Iodi (1990) suggests that the xylem is still functioning in affected berries.
Xylem Discontinuity in Pinot noir and Merlot Grapes:
Effect on Dye Uptake and Mineral Composition
During Berry Maturation

Chapter 3

Abstract

To estimate changes in xylem conductivity during greenhouse and field grape berry maturation, apoplastic dye (aqueous Eosin Y or azosulfamide) was allowed to perfuse through cut pedicel ends for a fixed amount of time. Dye uptake in soft but not yet colored Pinot noir berries was significantly less than that in firm green berries. Merlot clusters, collected from the field near veraison and the berries separated by deformability and color, showed a similar decrease in dye uptake. Pinot noir and Merlot berry deformability increased sharply before veraison (3.5 ±1.3 SD days before for Pinot noir), and preceded the second rapid berry expansion. In Pinot noir, total $K^+$ per berry increased rapidly after veraison, but total $Ca^{2+}$ per berry did not. The decrease in dye uptake and lack of $Ca^{2+}$ accumulation after veraison suggest a xylem discontinuity and coincide with berry softening and initiation of rapid growth.
Introduction

Grape berries are one of the few fruits to accumulate sugars to such high concentrations, >25% soluble solids (SS), and with such rapidity, >0.8% SS per day in hotter areas (Al-Kaisy et al., 1981). Much research has focused on berry maturation in an attempt to understand why berries are such strong assimilate sinks after veraison, yet many aspects of the ripening process are still not well understood.

That xylem connections between the berry and the vine are severed near veraison further complicates our understanding of the ripening process. There is direct evidence for xylem flow interruption in that physical gaps are found in post- but not pre-veraison berry brush xylem, and that apoplastic dyes do not traverse this area in post-veraison berries as readily (During et al., 1987; Findlay et al., 1987).

Indirect evidence for a change in xylem conductivity can be seen in berry to vine water relations. Pre-veraison berries are much more susceptible to drought stress than post-veraison berries, showing greater diurnal diameter changes, or shrinking under more severe stress (Greenspan and Matthews, 1991; Shimomura, 1967). After veraison, diurnal berry diameter changes are less apparent, possibly due to restricted water flow to and
from the fruit (Coombe and Bishop, 1980; Greenspan and Matthews, 1991).

A decrease in calcium accumulation after veraison also suggests a change in xylem conductivity. Movement of Ca\(^{2+}\) into berries is a passive process, being brought in with the transpiration stream, and has been used as an indicator of cumulative xylem flow (During and Oggionni, 1986; Hrazdina et al., 1984; Lang and Thorpe, 1989; Morrison and Iodi, 1990; Possner and Kliewer, 1985).

Xylem discontinuity may also affect photoassimilate partitioning within the vine. A low sink water potential may increase assimilate partitioning to that sink (Lang and Thorpe, 1986; 1989; Lang et al., 1986; Lee, 1986). Xylem discontinuity can lower berry water potential indirectly, because the primary route for supplying water for transpiration is cut off. The berry will lose water through the skin, concentrating the solutes inside, decreasing the fruit water potential, and causing more assimilates to be directed to that sink. Thus, the discovery of a major drop in xylem conductivity may be an important key to understanding grape berry ripening.

While a reduction in dye uptake and physical breaks in the berry xylem have been reported in Riesling and Muscat Gordo Blanco berries near veraison (During et al., 1987; Findlay et al., 1987), the exact timing of this phenomenon in relation to other berry attributes has not
been reported. Determining the relative timing of xylem discontinuity and quantifying related effects in two red wine grape cultivars are the objectives of this study. Decreases in apoplastic dye conductivity into berries were compared with changes in berry %SS, size, deformability (a measure of berry softness), color, and K⁺ and Ca²⁺ content.
Materials and Methods

Greenhouse Pinot noir Perfusion (Expt. 1): Established greenhouse Pinot noir vines in 3 liter pots were trained to one upright shoot and fertilized with half-strength Hoagland’s solution twice weekly. One or two clusters were permitted to develop on each shoot, and berries thinned from them to reduce cluster compactness at maturity and allow access for diameter measurements. First bloom date for each cluster was recorded and used to calculate approximate age when perfused. Diameters of selected berries were measured with a micrometer and recorded as a reference for comparing dye uptake and berry growth stage. Data were collected between 10 May and 11 July, 1990; greenhouse temperatures varied from 17°C to 26°C during this time.

One or two clusters of the same age were collected at 3 to 4 day intervals throughout berry development. All clusters were harvested before 0900 to minimize temperature and transpiration effects on vines. Fifteen berries from these clusters were used in each dye uptake experiment. If most berries on a cluster were soft (as determined by increased berry deformability and flesh translucency), only soft berries were used in that perfusion experiment. This same selection procedure was followed for berries at veraison. Berry deformability
was defined as the difference between berry diameter with and without external pressure. A micrometer supplied the compression force through its friction stop, which was modified to reduce pressure applied at the contact points.

Pedicels with berries still attached were cut from the rachis with a razor blade under distilled water. Pedicel ends were suspended in either a 5% (w/v) aqueous Eosin Y (an apoplastic dye (Findlay et al., 1987)) solution (ten berries - perfused treatment), or distilled water (five berries - controls) for 6 hr. Total time for dye uptake (= perfusion) was determined by running an experiment on extracted dye versus time perfused.

After perfusion, berries were rinsed with distilled water, blotted dry, their pedicels removed, and weights recorded. Berries were ground with a mortar and pestle and the dye extracted with 2.0 ml 30% glacial acetic acid in a centrifuge tube. Ground berries and solvent were agitated for 10 sec, then centrifuged at 2400 rpm for 10 minutes to settle particulates. Part of the supernatent (1.6 ml) was pipetted into test tubes containing 20 μl 30% H₂O₂ to bleach anthocyanins. After 30 minutes 1.5 ml of the extract was pipetted into semi-micro disposable polystyrene cuvettes and absorbance at 520 nm (peak dye absorbance) and 580 nm (background absorbance) determined with a Shimadzu UV-160 spectrophotometer. Absorbance
values for control berries were averaged, and data from perfused berries expressed as extracted dye absorbance:

\[(\text{perfused } A_{520} - A_{580}) - (\text{control } A_{520} - A_{580}).\]

**Field Pinot noir Perfusion (Expt. 2):** Mature Pinot noir vines used in this study were located in the southern Willamette Valley (Woodhall III vineyard, Alpine, Oregon). Five primary clusters were sampled from one vineyard block at 2 to 5 day intervals (starting at 49 days from first bloom) during the 1990 growing season. Diameters of eight berries on thinned clusters in the block were measured before 0800 at 2 to 3 day intervals throughout the season. Softening date (determined as in Expt. 1) and first coloring for each of these berries was also noted. From the five clusters collected, 15 berries were used for perfusion.

An experiment was run on field collected berries to determine an appropriate length of time for perfusion in 1% (w/v) aqueous azosulfamide solution (4-sulfanyl phenyl-2-azo-7-acetamido-1-hydroxy naphthalene 3,6-disulfonic acid, disodium salt; an apoplastic dye (Ashworth, 1982)). Berries were perfused in azosulfamide or water for 5 hr and the dye extracted in the same manner as in Expt. 1. Absorbance of extracted dye was read at 505 nm (peak dye absorbance) and 700 nm (background absorbance), and the data expressed on a
relative absorbance (RA) basis (data from control berries averaged first),

\[ RA = \frac{[(\text{perfused } A_{505} - A_{700}) - (\text{control } A_{505} - A_{700})]}{(\text{control } A_{505} - A_{700})}, \]

where "\( A_{505} - A_{700} \)" values were calculated from raw absorbance data first, and the result expressed on a per gram fresh weight basis.

**Field Merlot Perfusion (Expt. 3):** Merlot clusters from Woodhall III vineyard were collected on one date near veraison (18 Sept., 1990) and berries separated by degree of softness (as determined by touch) and color into four categories (firm-green, soft-green, just colored, and fully colored). Ten berries were treated as controls and 20 perfused with dye for 5 hr within each group. Berry deformability and weight without pedicel were measured after perfusion but before storage at -80°C. Approximately 3 weeks after collection berries were removed from storage and ground frozen with a mortar and pestle. After the tissue was ground, %SS of each control berry was measured with a hand-held refractometer. Dye was extracted and its amount measured following the procedures described in Expt. 2.
Field Pinot noir Mineral Analysis (Expt. 4): During the 1989 and 1990 growing seasons random samples of 50 primary clusters were taken (from the same block used in Expt. 2) during fruit development and frozen at -80°C. A subsample of 200 berries from both years was hand sorted to remove seedless and multi-seeded berries, weighed, and then ground to a frozen powder using liquid nitrogen and a Waring blender. A 0.5 g to 0.9 g portion of the resulting powder was mixed with 1.0 ml 10% HNO₃ and ashed at 500°C for 8 hr. The ashed samples were diluted with 10 ml 5% HNO₃ one day before K⁺ and Ca²⁺ content was determined by atomic absorption spectrophotometry. Data were expressed on a per berry basis.
Results

Greenhouse Pinot noir Perfusion (Expt. 1): Greenhouse berry growth curves were double sigmoidal in shape, with softening just after the lag phase, 56 days from first bloom, and veraison 4 days later (data not shown).

The absorbance of Eosin Y extracted from pre-veraison berries in the time series experiment is shown in Fig. 3.1. Dye uptake was linear for up to 6 hr and resulted in sufficiently high absorbance values after extraction.

Pre-softening berries took up a relatively large, but variable, amount of Eosin Y (Fig. 3.2). There was a large drop in the amount of dye extracted from soft versus firm berries, and another drop in that extracted from berries at veraison. Pre-softening berries in all experiments took dye up evenly in their peripheral vascular strands (Fig. 3.3), while post-softening berries (both uncolored and at veraison) did not (Fig. 3.4). Dye distribution within the berry also differed, with less dye visible in the axial and peripheral vascular strands of a post-softening berry as compared to a pre-softening one (Fig. 3.5).
Field Pinot noir Perfusion (Expt. 2): Seasonal changes in berry diameters were double sigmoidal in shape. Growth curves of eight Pinot noir berries are shown in Fig. 3.6. Seven of these berries softened before or on the day that rapid berry growth resumed; veraison for all berries occurred after the start of Stage III. On average, Pinot noir berries softened 3.5 (±1.3 SD) days prior to veraison.

Azosulfamide dye uptake through cut pre-veraison berry pedicels was approximately linear for up to 12 hr (Fig. 3.7), but to avoid problems with pedicel end browning and berry softening that occurred during longer perfusion times, berries in future experiments were perfused for only 5 hr.

Dye uptake before veraison was variable, but relative uptake dropped significantly at veraison (Fig. 3.8). There was no significant difference between dye uptake in soft but uncolored berries (days 68 and 70) and dye uptake in earlier samples. However, there was a visible difference in dye distribution within the berry, as noted in Expt. 1.

Field Merlot Perfusion (Expt. 3): Merlot berries that were soft or colored took up significantly less dye than firm green berries (Fig. 3.9). The pattern of dye uptake in pre- and post-softening berries was similar to that in
Pinot noir. Berry deformability, which was higher in more developed berry categories, was inversely related to dye uptake. Berry %SS was lowest in pre-softening berries (5.5%) and highest in fully colored ones (11%), but there was no difference in the amount of %SS rise between the categories. There was also no significant difference in berry weights between categories.

Field Pinot noir Mineral Analysis (Expt. 4): Per berry Ca$^{2+}$ content increased only slightly during the time surveyed in 1989, but per berry K$^+$ levels rose rapidly post-veraison (68 days from first bloom) (Fig. 3.10a). A similar post-veraison pattern of mineral accumulation occurred in berries sampled from the same block in 1990, where veraison occurred 71 days from first bloom (Fig. 3.10b). There was no clear trend for accumulation of Ca$^{2+}$ per berry before veraison in the 1990 data.
Discussion

Dye conductivity through the berry xylem decreased before veraison in all perfusion experiments. Data from the greenhouse (Expt. 1) and Merlot (Expt. 3) studies suggest that this decrease occurs as the berries soften, but data from the field Pinot noir study (Expt. 2) suggest that the decrease occurs later, at veraison. However, a distinct change in dye distribution within the berry after softening occurred in all the perfusion experiments.

There is a possible explanation for the high dye uptake in soft field Pinot noir berries, Expt 2. (Fig 3.8). A newly mixed batch of azosulfamide dye was used on day 65 and later perfusions, and the fresh dye may have perfused into berries more rapidly than the older dye. After repeated use during the season, the dye solution would develop darker gel-like aggregations, which would settle out upon standing. If the dye were polymerizing, the resulting larger molecules may not have entered or moved through the xylem as readily (Zimmermann, 1983). If the older dye solution did not perfuse in the same manner as that of freshly mixed dye, the unexpectedly high amounts of dye uptake at berry softening could be explained. If the relative absorbance on day 65 is taken as a new average for pre-softening
berries, then there is a significant drop in relative absorbance between day 65 and 68, and another significant drop when berries colored on day 71 (Tukey HSD, p=0.01).

Peripheral strands are outlined by dye more completely in pre- versus post-softening berries. The pattern of dye visible in the peripheral strands of post-softening berries is less regular than that of pre-softening berries. Although dye enters post-softening berries, fewer peripheral xylem strands are visibly stained, resulting in a more uneven distribution pattern. Also, dye in peripheral strands of post-softening berries diffused outward, away from the bundle, more than dye in pre-softening berries (see Fig. 3.4), suggesting cellular breakdown. Consistent with this, Lang and Thorpe (1989) theorize that during softening cell membranes are disrupted within the berry, leading to a mixing of the contents of the apoplast and symplast.

Axial vascular strands also stained less predictably after softening. Unlike in firm berries, dye in soft ones rarely reached the stylar end (see Fig. 3.5). Contrary to the findings of Findlay et al. (1987), there was no evidence of more dye accumulating in berry pedicels before versus after veraison; the amounts of dye in both categories of tissue did not differ significantly.
The Merlot experiment, in which fruit was harvested on the same day, demonstrated that the stage of berry development, and not that of the cluster as a whole, was the critical factor in the dye uptake decrease. There was significantly higher %SS in soft-green versus firm-green berries, which suggested that sugar accumulation coincided with the drop in dye uptake. Both berry softening and sugar accumulation occurred before veraison, as has been reported by other researchers (Coombe and Bishop, 1980; Hrazdina et al., 1984).

Per berry Ca\(^{2+}\) content has been reported to remain relatively constant after veraison (During and Oggionni, 1986; Hrazdina et al., 1984; Lang and Thorpe, 1989; Morrison and Iodi, 1990; Possner and Kliewer, 1985). However, a pre-veraison rise in per berry Ca\(^{2+}\) content was not found here, unlike in other reports (Lang and Thorpe, 1989; Possner and Kliewer, 1985). This result may have been due to the berry sorting procedure before mineral analysis. There was very poor Pinot noir set in 1990, which resulted in high numbers of seedless berries. To reduce the effect of berry size and seed number on the analysis, berries from both years were visually sorted by size to retain only one-seeded berries. This may have artificially selected larger pre-veraison berries, leading to higher Ca\(^{2+}\) levels per berry. The average berry weight curve for samples used in mineral analysis
was double sigmoidal, but it was possible that pre-veraison berry weights were still unrepresentatively high.

The trend toward gradual post-veraison $\text{Ca}^{2+}$ accumulation in 1989 and 1990, as seen in Fig. 3.10 and in the research of During and Oggionni (1986) may be due to cation exchange movement along xylem vessel wall surfaces (Armstrong and Kirkby, 1979). The amount of $\text{Ca}^{2+}$ brought into berries by exchange before veraison would be masked by the relatively massive transport of $\text{Ca}^{2+}$ via the transpirational stream. Alternatively, the limited xylem connection that exists between the vine and fruit after veraison may still supply small amounts of water (and thus $\text{Ca}^{2+}$) to the berry.

One so far unrepeated result published by During and Oggionni (1986) occurred in post-veraison berries treated with an emulsion to increase their transpiration rate. Despite normal post-veraison expansion of emulsion treated berries, $\text{Ca}^{2+}$ in them accumulated rapidly, and in a pattern very similar to that of $\text{K}^+$. This seems to be contrary to findings here and elsewhere that report a reduction in xylem flow near the initiation of rapid berry expansion (During et al., 1987; Findlay et al., 1987). Any increase in water flux through the berry skin should not result in significant amounts of $\text{Ca}^{2+}$ being delivered to the fruit if the xylem flow capacity is
reduced. Phloem sap contains little or no Ca$^{2+}$, and is not considered to be a medium for Ca$^{2+}$ movement (Hanger, 1979; Kirkby and Pilbeam, 1984; Pate, 1975).

An interesting connection between xylem discontinuity and waterberry has been published recently (Morrison and Iodi, 1990), and supports the hypothesis that rapid berry expansion causes xylem discontinuities in the berry. These researchers found that waterberries expanded at a much slower rate than unaffected berries, and that significant amounts of Ca$^{2+}$ accumulated in these berries after veraison. The accumulation of Ca$^{2+}$ that is associated with reduced growth in waterberries after veraison gives strong proof that xylem connections between the affected berry and vine are still continuous.

Follow-up research on Ca$^{2+}$ accumulation into berries treated to increase transpiration, further study of waterberries, and experiments dealing with the restriction of berry growth during Stage III and extensibility of berry xylem could do much to elucidate the causes of xylem discontinuity and its effects on berry maturation.
Fig. 3.1 Time series for perfusion of 5% (w/v) aqueous Eosin Y into pre-veraison Pinot noir berries collected from the greenhouse. Regression equation (and $r^2$ value) for 0.5 to 6 hours is given in the figure. See text for calculation of extracted dye absorbance. (Expt. 1)
Fig. 3.2  Dye extracted from greenhouse grown Pinot noir berries after perfusion in 5% (w/v) aqueous Eosin Y as a function of fruit development. Dye uptake was through cut berry pedicels for 6 hr. Berry softening occurred 56 days from first bloom and veraison on day 60 (arrows). See text for calculation of extracted dye absorbance. Bars indicate SE. (Expt. 1)
Fig. 3.3 Pre-softening Pinot noir berries perfusing 1% (w/v) aqueous azosulfamide solution. Berries collected from the greenhouse.
Fig. 3.4  Post-softening Pinot noir berries perfused in 1% (w/v) aqueous azosulfamide solution for five hours and cut longitudinally to show the uneven dye uptake. Berries collected from the greenhouse.
Fig. 3.5 Pre- (left) and post- (right) softening Pinot noir berries perfused in 1% (w/v) aqueous azosulfamide solution and cut to show differences in dye uptake. Berries collected from the greenhouse.
Fig. 3.6 Diameters of eight Pinot noir berries grown at Woodhall III vineyard (Alpine, Oregon) in 1990. Softening for all but one occurred before or coincidentally with the start of Stage III. (Expt. 2)
Fig. 3.7 Time series for perfusion of 1% (w/v) aqueous azosulfamide into pre-veraison Pinot noir berries collected from Woodhall III vineyard in 1990. Regression equation (and $r^2$ value) for 1 to 12 hours is given in the figure. See text for calculation of relative absorbance. (Expt. 2)
Fig. 3.8  Relative absorbance of dye extracted from perfused Pinot noir berries collected from Woodhall III vineyard. Softening occurred 68 days from first bloom (upward pointing arrow), and veraison on day 71 (downward pointing arrow). Berries perfused in 1% (w/v) aqueous azosulfamide dye for five hours. See text for calculation of relative absorbance. Bars indicate SE. (Expt. 2)
Fig. 3.9 Berry deformability and relative absorbance of dye extracted from Merlot berries separated into four developmental stages and perfused in 1% (w/v) aqueous azosulfamide for five hours. Berries collected from Woodhall III vineyard on 18 Sept., 1990. See text for calculation of relative absorbance. Bars indicate SE. (Expt. 3)
Fig. 3.10  Developmental changes in Pinot noir $K^+$ and $Ca^{2+}$ content per berry in 1989 (a) and 1990 (b). Veraison in the field occurred 68 days from first bloom in 1989 and 71 days from first bloom in 1990 (arrows). Berries were collected from Woodhall III vineyard. (Expt. 4)
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Heat Girdling and Vine Water Stress Affects Pre- and Post-Veraison Grape Berry Growth and Deformability

Chapter 4

Abstract

To elucidate the effects of developmental xylem discontinuity in post-veraison berries, greenhouse grown Pinot noir vines were subjected to water stress and peduncle girdling or detaching. Berry growth rate (change in diameter per day) and deformability were measured as an indicator of berry water status. Berry diameters on pre-veraison clusters on well watered vines increased slightly; those on unwatered vines decreased, losing diameter at a rate of 0.87 mm/day during day 3. Pre-veraison berry deformabilities were 380% higher in unwatered versus watered vines on day 4. Bagging pre-veraison clusters to slow transpiration had little effect on berry growth rates and deformabilities. Post-veraison berry growth rates and deformabilities were not affected significantly by vine water stress.

Girdling cluster peduncles reduced pre-veraison berry growth (in diameter) from 0.12 to 0.03 mm/day, but had a negligible effect on berry deformabilities. Girdling increased post-veraison berry diameter loss from
0.04 to 0.09 mm/day, and increased berry deformabilities by 50%. The rate of berry shrinkage on a detached cluster was almost three times higher, and berry deformability 20% higher, than the same measurements on a girdled cluster. A xylem discontinuity in the berry near veraison is consistent with these findings.
Introduction

The growth curve of a grape berry has a double sigmoid shape (Coombe, 1976; Coombe and Bishop, 1980). Much research, including that on berry water relations, has focused on the last period of growth (Stage III), where there is rapid sugar accumulation inside an expanding berry (for example: Coombe, 1980; 1987; Coombe and Iland, 1986; During et al., 1987; Findlay et al. 1987; Lang and Thorpe, 1988; 1989). Less research has concentrated on pre-veraison (Stage I and II) grape development, especially with reference to fruit water economy (Greenspan and Matthews, 1991; Matthews et al., 1987).

Reports that xylem flow between the berry and the vine is interrupted prior to veraison (During et al., 1987; Findlay et al., 1987) challenge conventional ideas about vine to berry relationships during ripening (Lang and Thorpe, 1989). The grape berry, like many other fruits, acts as a water reservoir for the vine. A more negative water potential in leaves than in clusters during the day draws water out of berries, and at night a reversed potential gradient replenishes the water (Huguet, 1985; Klepper, 1968; van Zyl, 1987). Diurnal changes in fruit diameter are thought to be a manifestation of this phenomenon, which occurs in many
fruits (Huguet, 1985; Jones and Higgs, 1982; Klepper, 1968; Lang, 1990; Tukey, 1964). Pre-veraison grape berries also exhibit this pattern of diameter change, which is exacerbated by decreasing vine water status (Greenspan and Matthews, 1991; Shimomura, 1967).

Post-veraison grape berries were thought to serve a similar role in acting as a reservoir, with the water coming in through the phloem and exiting via the xylem (Lang and Thorpe, 1988). However, in comparison to pre-veraison grape berries, post-veraison berries show lesser or no diurnal diameter changes under normal and low vine water status, suggesting limited water flow between the vine and fruit (Coombe and Bishop, 1980; Greenspan and Matthews, 1991; Shimomura, 1967). A xylem discontinuity near veraison would explain the pre- versus post-veraison change in diameter fluctuations.

To investigate the effects of xylem discontinuity on pre- and post-veraison berries several treatments were imposed on greenhouse grown Pinot noir vines and clusters. Vines were subjected to water stress, or cluster peduncles girdled or detached. Berry growth rate (change in diameter per day) and deformability, measured before and after application of treatments, were used as a measure of berry water status.
Materials and Methods

Greenhouse grown Pinot noir vines in 3 liter pots were trained to one shoot with one or two clusters per shoot. Shoot height was maintained at 1.2 m by repeated trimming. Berries were thinned after fruit set to reduce cluster compactness at maturity and to allow access for diameter measurements. Vines were fertilized with one-half strength Hoagland’s solution twice weekly; powdery mildew was controlled with alternating wettable sulfur and Bayleton sprays. Greenhouse temperatures ranged from 17 °C (night) to 30 °C (day).

Diameter and deformability were measured between 0700 and 0800 using a 25mm micrometer with its friction stop modified to apply less pressure during deformability measurements. Berry deformability was defined as the difference between berry diameter with and without pressure (as applied by the micrometer’s friction stop).

Water Stress Effect on Berry Growth and Deformability:
Water stress was applied by withholding water, starting on day 0. The small pot volume meant that water availability was extremely limited; after day 4 the vines were visibly wilted and the experiment stopped. Unstressed vines were watered two or three times daily to
ensure adequate moisture. Vine leaf water potential was measured with a pressure bomb on days 0, 2, and 4.

Three berries on each of three clusters were measured in each treatment. On unwatered vines, an additional two clusters on one vine were enclosed with a clear plastic bag containing a damp paper towel to increase humidity and reduce cluster transpiration.

**Heat Girdling Effect on Berry Growth and Deformability:**
Berry growth rate and deformability measurements were taken from six pre-veraison clusters on three vines. Five berries per cluster were monitored for 3 days, after which the peduncle of one cluster on each vine was heat girdled. Data were taken on all clusters for an additional 4 days.

Cluster peduncle heat girdling was accomplished with a bent nail and alcohol burner, a method similar to that used by Dewey et al. (1987) and Hamilton and Davies (1988). The nail was heated to a dull reddish color, then held close to the peduncle for 10 sec. The lower and upper sides of the peduncle were treated in this manner two times each. Metal foil was wrapped around the cluster and shoot to protect them from possible heat damage.

Post-veraison treatments were similar. Berries were in late Stage III and already losing diameter. Berry
growth and deformability were measured on five berries on each of three vines for 6 days. After the day 6 measurements, all cluster peduncles were girdled and one cluster detached. The cut end of the detached cluster was covered with Parafilm and hung in the canopy. Berry measurements were taken for an additional 7 days.
Results

Water Stress Effect on Berry Growth and Deformability:
Pre-veraison berries showed a dramatic response to water stress. By day 2 berries on unwatered vines were visibly shrivelled, but vines were not visibly wilted until day 4. Water potentials of leaves on unwatered vines did not decrease during the experiment, but those on watered vines became less negative (Fig. 4.1).

Pre-veraison berry growth rates on watered vines were positive throughout the experiment (Fig. 4.2a). Pre-veraison berry deformabilities on watered vines were low, and did not differ significantly during this time (Fig. 4.3a).

Water stressed pre-veraison berry growth rates decreased rapidly after cessation of watering (Fig. 4.2a). Bagging pre-veraison clusters resulted in slightly less negative berry diameter changes (Fig. 4.2a) and slightly lower deformabilities by day 3 (Fig. 4.3a). By day 4 pre-veraison berries on all unwatered vines were extremely shrivelled, leading to very high deformability measurements.

Growth rates of post-veraison berries on both watered and unwatered vines decreased slightly during the experiment (Fig 4.2b). Post-veraison berries showed little deformability change during the experiment, with a
slight decrease in watered vines and a slight increase in unwatered vines (Fig 4.3b). On watered vines post-veraison berry deformabilities were about twice those of pre-veraison berries.

**Heat Girdling Effect on Berry Growth and Deformability:**
Immediately following girdling, peduncle tissue became a lighter green and by 8 hr the tissue had collapsed and browned: observations similar to that of Dewey et al. (1987). Heat girdled peduncles retained rigidity and were able to support the weight of the cluster easily.

Girdling pre-veraison cluster peduncles slowed berry growth by 75%, to near zero (4.4a) but deformability changed only slightly (Fig 4.4b). Post-veraison berries on girdled clusters lost 0.05 mm in diameter per day more than those on ungirdled clusters (Fig. 4.4a). Post-veraison berry deformabilities were 50% higher on girdled versus ungirdled clusters (Fig. 4.4b). Girdling had a much larger effect on berry deformabilities post-veraison than pre-veraison. Berry deformabilities on post-veraison ungirdled clusters were 170% of those on pre-veraison clusters.

Berry diameter loss on a detached post-veraison cluster was almost three times as high and berry deformabilities 20% higher (Fig. 4.4) than that on a girdled cluster.
Discussion

Berry growth rates and deformabilities were much more sensitive to vine water status before veraison than after veraison. Pre-veraison berries showed signs of water stress (e.g. reduced growth rates and increased deformabilities) earlier than post-veraison berries, and long before vines began to wilt. Post-veraison berries showed little signs of stress in all watered and unwatered treatments. Similar results were reported by Greenspan and Matthews (1991), where diurnal changes in berry diameter were measured. They showed that diurnal berry contractions were smaller in magnitude on post-versus pre-veraison clusters in both potted and field vines.

Bagging pre-veraison clusters to reduce transpiration did little to prevent berries from shrivelling on unwatered vines, suggesting that water movement from the cluster to the vine, not transpiration from the berries, is the primary mode of water loss. Greenspan and Matthews (1991) found that bagging pre-veraison clusters reduces the magnitude of diurnal berry contractions only 30%, while bagging whole shoots reduces the contractions by 70%. This suggests that shoot transpiration is partly responsible for the diurnal change in berry diameter. For the pre-veraison grape,
the water demands of the vine appear to take precedence over those of the berries. Pre-veraison grapes are highly dependent on the xylem to support growth (Greenspan and Matthews, 1991), so factors that affect the availability of xylem water in the vine will affect berry water status. Post-veraison grapes, however, are more isolated from the vine. In potted and field grown vines bearing post-veraison clusters, diurnal berry diameter changes were not affected by bagging shoots to slow vine transpiration, suggesting that shoot transpiration does not contribute to diurnal berry contractions (Greenspan and Matthews, 1991).

For a berry to accumulate high concentrations of sugars isolation from the water demands of the vegetative organs is almost a necessity (Lang and Thorpe, 1989). Cell wall degradation in the grape berry near veraison results in a breakdown between apoplast and symplast compartmentation (Considine and Knox, 1979; Lang and Thorpe, 1989). The resulting sugary sap that fills the berry could be drawn out in response to vine water stress unless there is a xylem interruption (Findlay et al., 1987; Lang and Thorpe, 1989). Also, if xylem flow is not restricted, water could move from the vine to the berry in response to water and hydrostatic pressure gradients, thus diluting the sap (Lang and Thorpe, 1989; Lang et al., 1986; Lee, 1986). There has been shown to be a
favorable shoot to cluster water potential gradient in ripening berries on both well watered and water stressed vines (Greenspan and Matthews, 1991), but despite this water does not move in and dilute berry contents. In terms of water flow through the xylem, post-veraison berries seem to be isolated from the vine and no longer serve as a water reservoir for the vine. Berry growth during the ripening stage is highly dependent on the phloem and not the xylem (Greenspan and Matthews, 1991; Lang and Thorpe, 1989), so factors affecting phloem transport should have a significant impact on berry development.

Heat girdling has been used extensively to block phloem transport in plants (Clements, 1940; Dewey et al., 1987; Hamilton and Davies, 1988; Lang, 1990; Lang and Thorpe, 1989; Martin, 1982). In grape a heat girdle has two different effects, depending on at what stage the berries are when the girdle is made. This is because the xylem in post-veraison berries is largely non-functioning. In a berry on a girdled pre-veraison cluster the xylem can supply enough water to support limited growth and turgor maintenance (which is responsible, in part, for berry firmness (Matthews et al., 1987)). In a post-veraison berry xylem conductivity is much lower (Creasy, Chap. 3; During et al., 1987; Findlay et al., 1987), so when the phloem is blocked the
berry shrinks and becomes softer. Water lost through berry transpiration cannot be replaced.

However, berries on a detached cluster shrank faster and had higher deformabilities than berries on girdled clusters, suggesting that berries on girdled clusters still had xylem connections to the vine. Studies using dye taken up into berries show that xylem conductivity near veraison decreases but does not stop completely, supporting these findings (Creasy, Chap. 3; During et al., 1987; Findlay et al., 1987). The significant differences in growth rate and deformability between post-veraison berries on girdled and detached clusters may also be due to the extra transpirational surface on the detached cluster. The rachis of the girdled clusters still had a direct xylem connection to the vine, and thus a pathway through which to satisfy its transpirational water requirements. The rachis on the detached vine did not, and so may have drawn water out of the attached berries via the phloem or remaining xylem connections, resulting in rapid berry decline. Cluster rachises and berry pedicels should be coated with an anti-transpirant to reduce this effect, or berries removed from the vine individually and measured.
Fig. 4.1  Leaf water potentials of watered and unwatered potted Pinot noir vines during a water stress experiment. Means of three to five measurements. Bars indicate SE.
Fig. 4.2 Berry growth rates on pre- (a) and post- (b) veraison greenhouse Pinot noir watered or unwatered vines. Means of 9 berries. Bars indicate SE.
Fig. 4.3  Berry deformabilities on pre- (a) and post- (b) veraison greenhouse Pinot noir watered or unwatered vines. Points for unwatered, bagged pre-veraison clusters are means of 6 berries; all others are means of 9 berries. Bars indicate SE.
Fig. 4.4  Growth rates (a) and deformabilities (b) of pre- and post-veraison berries on ungirdled, girdled, and detached greenhouse Pinot noir clusters. Means: pre-veraison ungirdled = mean of 90 measurements on 30 berries; pre-veraison girdled = 60 on 15; post-veraison ungirdled = 90 on 15; post-veraison girdled = 90 on 15; post-veraison detached = 25 on 5. Bars indicate SE.
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