A MECHANISTIC VIEW OF SHOOT VIGOR IN GRAPEVINES:

XYLEM ANATOMY, HYDRAULICS, AND REPRODUCTIVE GROWTH

By

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A thesis submitted in partial fulfillment of
the requirements for the degree of
MASTER OF SCIENCE IN HORTICULTURE

WASHINGTON STATE UNIVERSITY
Department of Horticulture and Landscape Architecture
May 2011
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ACKNOWLEDGEMENTS

I would like to express my sincere thanks to all the people that helped me through the Master’s study. Thank you, Dr. Bhaskar Bondada. I’m so grateful for the chance you gave me to pursue my dream in WSU, for your great knowledge and teaching which helped me to grow in the academic area, for your example of being positive and hardworking. Thank you, Dr. Markus Keller. Thanks for your generous support and suggestions, for your great example of being a great scientist and professor. Thank you, Dr. Amit Dhingra, for your passion in scientific research and teaching, for your generous support and encouragement.

I would also like to thank the Department of Horticulture and Landscape Architecture, WSU and professors and students in the department for the excellent education and research atmosphere. Thank the North West Center for Small Fruits Research for supporting this project. Special thanks to Laura Deyermond for being a great partner, to Xiaoyue Wang for helping me with the experiment.
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ABSTRACT

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May 2011

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To gain an understanding of the mechanistic basis of vigor phenomenon in grapevines, the Merlot cultivar was induced to develop different shoot lengths, an index of vigor by pruning the vines to a range of bud numbers. Physiological and anatomical measurements were recorded at specific phenological events in all vigor levels. In a given shoot, the successive internode lengths and the corresponding leaf area increased linearly at the proximal end, whereas opposite occurred at the distal end (shoot tip). Shoot structure exhibited modular characteristics, in which internode without tendril at the bottom tended to be shorter and bear more lateral branches. Significant relationship was found between internode length and leaf size above or beneath that internode. Vessel lumen diameter, vessel number, hydraulic conductivity, the number of radial sectors (RS) bordered by xylem parenchyma and vessel number per RS were smaller at the distal ends than at proximal ends, while vessel density followed the opposite trend. Average shoot length exhibited a negative relationship with bud number. Shoot fresh weight, internode number, total leaf area, cluster weight, stomatal conductance, soluble solids and nutrient levels increased with shoot length, whereas soluble solids decreased with cluster number per shoot. pH increased
with shoot length in September 2010 and did not show significant relationship with shoot length in 2009 or October 2010. Leaf area/fruit weight (LA/F) ratio increased with shoot length in 2010. However, no significant relationship between LA/F ratio and shoot length in 2009 was detected, neither the relationship between LA/F ratio and soluble solids, LA/F ratio and pH. Vessel number per cross-sectional area, sapwood area, vessel lumen diameter, total vessel area per cross-section and hydraulic conductance increased with shoot length, whereas vessel density decreased with shoot length. This study showed that when shoot length was used as a vigor indicator, shoot vigor was strongly related with hydraulic conductivity of the shoot which was determined by xylem anatomy.
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CHAPTER ONE

INTRODUCTION
1.1 Grape production---- the Washington state perspective

The special aromatic taste of wines makes them one of the most popular alcoholic beverages in the world. Wines are also found to benefit health by reducing rate of diseases such as heart disease (Bisson et al., 2002), cancer (Damianaki et al., 2000). Possible reason for these health benefits are due to contained alcohol and phenolic compounds (Soleas et al., 1997). From the earliest record of wine making which is around 5400 to 5000 BC (Soleas et al., 1997), wines had been spread all over the world through human activities. From 2006 to 2009, world total wine production decreased from 28729000 to 26759900 (liters 000), however wine production in United States increased from 2438300 to 2777200 (liters 000) (the wine institute: http://www.wineinstitute.org/files/WorldWineProductionbyCountry.pdf). Washington state is the second largest premium wine producer in the United States only after California and total economic impact of WA wine industry on US economy was $4.7 billion in 2006 (Washington association of wine grape growers: (http://www.wawgg.org/index.php?page_id=31). In order to promote this rapid increase of wine production in Washington state, a better understanding of viticulture is essential in order to promote and sustain fruit quality and yield. One of the thorny problems in viticulture is that vines are inclined to have excessive vigor which affects fruit quality and sustainability of the vineyard management. Thus, the major thrust of this research is focused on the biological mechanism of vigor and the factors that govern vigor in order to gain a better understanding of the balance between vegetative and reproductive growth for optimizing grape composition and vine performance. To address this issue more clearly, it requires an understanding of the origin, habitat and growth characteristics of grapevine.
1.2 Origin and growth characteristics of grapevine

The evolution of wine grapes (Vitis vinifera, L.ssp. vinifera) is closely related with wine making history. On the other hand, a better understanding of the biology of wine grapes is the foundation of viticulture improvement and in turn the prosperity of wine industry. Wine grapes (V. vinifera) belong to the Vitaceae family, which includes more than a thousand species, most of which live in tropical and subtropical regions with extension to the temperate regions (Galet, 2000). Plants of the Vitaceae family are generally woody shrubs that climb like lianas by means of their leaf-opposed tendrils (Galet, 2000). About 19 genera of vitaceae have been identified, in which Vitis is of the most agronomical importance. Vitis genus mostly exists in Northern Hemisphere, including up to 60 inter-fertile species distributed in Asia, North America and Europe (This et al., 2006; Jackson, 2008; Terral et al., 2010). The wild grapes (Vitis vinifera ssp. silvestris), which are believed to be the ancestor of cultivated grapes, grow in the temperate regions of Asia and Europe (Galet, 2000; This et al., 2006).

The cultivation and domestication of the grapevines seem to occur between the seventh and fourth millennia BC, between the Black Sea and Iran (McGovern et al., 2005). Over centuries, grapevines have been spread by human activities throughout the temperate regions of Europe, Northern and Southern Africa, North and South America (from Southern Canada to Chile), several Asian countries and Oceania (Australia and New Zealand) (Rives, 2000). During domestication, the biology of grapes underwent several dramatic changes. One of the major changes is the shift from sexual reproduction (in the wild) to vegetative propagation (under domestication).The other crucial change is from dioecious plant (separate male and female individuals) into a hermaphroditic crop (be able to pollinate itself). Besides, cultivated grapes also have higher yield, more regular production, bigger and more juicy and/or less acid berries
compared to wild forms (McGovern et al., 2005; This et al., 2006; Terral et al., 2010). Changes in seed morphology also occurred, which provides important information for the study of the taxonomic status and geographical origin of grapevines (Terral et al. 2010; This et al., 2006).

Although some characteristics of cultivated vines changed through the evolution in order to produce more fruits with higher quality, some habits such as vigorous growth, fond of light and small requirement of mineral nutrient still remain in the cultivated vines. Thus, a good understanding of the nature and biology of grapevines is important to solve problems related to grape production, such as vigor control, irrigation, pruning methods, etc.

1.3 The growth cycles of wine grapes

There are two separate, however, interactive aspects in vine growth cycle, which are vegetative and reproductive growths. Vegetative cycle starts in spring with bleeding, which is the phenomenon that xylem sap flows from wounds in late winter or early spring because of root hydraulic pressure (Andersen and Brodbeck, 1989). Bleeding is followed by bud swelling which means buds begin to expand inside the bud scales (Lorenz et al. 1995). Bud break happens after bud swelling and indicates the onset of vegetative growth. Only the primary bud becomes active during bud break while the secondary or tertiary buds remain inactive unless the primary bud is killed or severely damaged (Mullins et al, 1992). The vegetative growth of shoots is a combination of fixed growth and free growth. Fixed growth indicates the expansion of pre-formed leaves and internodes, while free growth refers to the production of new leaf primordia and internodes (Mullins et al, 1992). During the vegetative growth, new buds arise in the leaf axils, some of which may give rise to lateral shoots (Jackson, 2008). Leaf fall in the autumn is the end of the vegetative cycle and vines enter dormancy after leaf fall. The function of vegetative growth is to produce herbaceous shoots, root system and main stem in order to
maintain the vine growth and also produce nutrients for fruit ripening (Galet, 2000; Mullins et al., 1992). Cane maturation happens in the late season, whose function is to accumulate food reserves inside the tissues of roots, trunks, arms and canes in order to provide nutrients for next year’s growth (Lorenz et al. 1995). Reproductive growth starts with the initiation of the inflorescence, which is followed by flower initiation, and flower differentiation (Keller, 2010; Pratt C, 1971). Flower development is followed by blooming, fertilization, embryo and berry development (Coombe 1995; Lorenz et al. 1995; Jackson, 2008). The growth or increase in fresh weight of seeded berries traces a double-sigmoid pattern (Matthews and Anderson, 1989). There are three stages of berry growth. In the first stage, seeds and pericarp rapidly increase in size for the first time. Berries are still green, chlorophyll-containing and playing the same physiological role as leaves (Coombe, 1995). The second stage is a lag phase in which the pericarp grows insignificantly, however embryo grows rapidly and the seed enters its maturation stage. The third stage (the ripening period) starts from veraison to berry ripening. Veraison (the berry color changes from green to their final color) signals a fundamental shift from partly photosynthetic to wholly heterotrophic metabolism (Coombe, 1995; Keller, 2010). Overall, the objective of managing the growth cycle is to balance the accumulation of food reserves in the vegetative organs and the movement of sugars and reserves to the berries and the seeds (Galet, 2000).
1.4 Morphology, growth and development of grapevine shoot system

![Diagram of grapevine shoot system]

**Figure 1**: Morphology of one year-old Merlot shoot

The primary shoot consists of a shoot tip, nodes, internodes, leaves, buds, and are usually with lateral shoots in the summer (Bondada, 2011) (Figure 1). The intermittent swellings are called nodes, the interval between two consecutive nodes is called an internode. Nodes bear leaves, buds (lateral buds and compound buds as mentioned before) tendrils and clusters. A leaf consists of leaf blade which is the major part of photosynthesis, and petiole which is responsible for supporting and conduction to the blade. Buds are born in the axils of the foliage leaves at the nodes, which consequently are classified as axillary buds. An axillary bud complex includes...
lateral bud and compound bud (also called dormant bud/eye). Lateral bud is located close to the dorsal side of the shoot, and may develop into lateral shoots in the current season. Compound bud includes primary, secondary and tertiary buds, all of which normally remain inactive in the current season and produce new shoot in the next season. Normally, primary buds generate primary shoots while the secondary and tertiary buds remain inactive (Galet, 2000). A tendril is born on the opposite side to the leaf and compound bud except on every third node in Vitis vinifera (Rives, 2000). Flower clusters usually develop opposite the third and fourth, fourth and fifth, or fifth and sixth leaves on a shoot (Jackson, 2008). In one cluster, the complete branch is called the rachis, which includes the basal stem connected to shoot (peduncle), inner and outer arms. Pedicel gives rise to individual flowers (Jackson, 2008).

Primary shoots develops from the compound buds and the onset of shoot growth is indicated by bud break. The compound buds already developed six to ten leaf primordia, up to three inflorescence primordia and lateral buds primordia before entering into dormancy in the last season (Mullins et al., 1992). Shoot development starts with the enlargement of primordial leaves, tendrils and inflorescence clusters. As growth continues, the shoot differentiates new leaves and tendrils, which is mainly attributed to the activity of shoot apical meristem. In the meantime, new buds arise in the leaf axils and may give rise to lateral shoots (Jackson, 2008).

The stem of Vitis vinifera has dorsiventral structure, which means “having two distinct surfaces or planes” (Pratt, 1974). Dorsal side is usually with larger stipule and the prophyll of lateral shoots (Pratt, 1974). Galet (2000) described ventral side as on which side “all of the latent buds (compound buds) are formed”; while all of the prompt buds (lateral buds) are formed on the dorsal side.
Another characteristic of grapevine shoots is the modular structure, the most common pattern has three phytomers (P0-P1-P2) in each modular (Lebon et al., 2004). P0 phytomer is without tendril, while P1 and P2 are with tendrils. It was noticed that lateral growth rate and duration was higher in P0 phytomers compared with P1 and P2 phytomers (Lebon et al., 2004).

1.5 Stem anatomy of vines

The structure of grapevine stem (Figure 2) goes through primary growth and secondary growth. Stems during the end of primary growth usually have ribs, which disappeared during aging (Esau, 1948). In a cross-section under primary growth, the tissues from the outside to inside are epidermis, cortex and central cylinder. Epidermis consists of a layer of polygonal cells that are arranged in rows, and always covered by an external cuticle to protect the plant. Epidermis may also have stomata, hair, and pearls (Galet 2000; Keller 2010). Cortex is differentiated into collenchymas cells close to the surface and cortical parenchyma cells (Esau, 1948). Cortical parenchyma cells contain chloroplasts, starch, calcium oxalate crystals, and phenolic compounds (Esau, 1948; Keller, 2010). Inside of cortex is the vascular region which consists of vascular bundles and primary rays (Esau, 1948; Galet 2000). There are primary-phloem fiber caps outside each vascular bundle. Vascular region contains the primary phloem, the primary xylem, which are all given rise by precambium (Mullins, Bouquet et al. 1992). Primary rays separate vascular ring into wedges. Pith is in the center of the stem and is composed of parenchyma cells with thin, cellulose-containing walls.

Secondary growth follows primary growth. The first step in secondary growth is that the procambium develops into vascular cambium consisting of interfascicular cambium and intrafascicular cambium. Intrafascicular cambium gives rise to secondary phloem in the direction of surface and secondary xylem in the direction of pith. The secondary phloem includes soft
phloem which contains phloem parenchyma, sieve elements, companion cells, and hard phloem which consists of thick-walled fibers (Esau, 1948; Mullins et al., 1992; Galet 2000). Hard phloem and soft phloem are formed alternatively and hard phloem forms when the stem starts to ripen. The secondary xylem is diffuse-porous and includes large-diameter xylem vessels, and wood fibers (Jackson 2008). The secondary xylem vessels have scalariform or ladder-like thickening (Sun et al., 2006). Ray tissues expand from phloem to pith, which are responsible for the storage of starch, as well as lateral transportation. Xylem is the main pathway of water, nutrients and hormonal signals (Lovisolo, Schubert et al. 2002). A cork cambium (phellogen) is formed in the phloem region, which produces cork toward the outside and phelloderm toward the inside (Esau, 1948; Stevenson et al., 2005). All tissues outside the vascular cambium are collectively referred to as the bark.

As a liana, vines have pronounced wide vessels and low wood density (Putz and Mooney 1991) because of its climbing and light-demanding habits inherited from wild vines which have to climb on other plants in order to compete with them for light and nutrients (Poorter et al., 2009). Besides, the length of vessels is also relatively greater in lianas (Ewers and Fisher, 1989). Explanations for this phenomenon were based on the basic functions of xylem: mechanical support and the transportation of water and minerals (Gartner, 1991). Because vines are not self-supporting, the supportive requirement of xylem is considerably lower than self-supported plants, thus vines can have less fibers and narrower stem than other woody plants (Putz and Mooney, 1991) as while as larger leaf area/ stem cross-section (HV) value and a comparatively higher specific hydraulic conductivity (Ks) (Gartner 1991), for the reason that wider vessels compensate the narrow stem diameter (Ewers et al., 1990).
Figure 2: Basal (A) and top (B) internode cross-sections of a young stem of Merlot collected in June 2010. Scale bars: 0.2 mm. C: cortex; FC: fiber cap; P: pith; Ph: phloem; PX: primary xylem; R: rays; X: xylem; V: vessel.
1.6 The concept and parameters of vigor

The primary goal of vineyard management is to achieve maximum yield with desired fruit quality. In order to achieve this goal, it is important to keep the balance between vegetative and reproductive growths. However, the question in vine balance is how to quantify vegetative and reproductive growths, and how to find out the relationship between them. A lot of emphasis has been put on vigor control management as viticulturists believe that this is an essential way to modify and improve the yield and fruit quality. However, because traditional vineyard management is mostly based on experience, and also because of the complexity of factors involved in the expression of vigor, questions such as what is vigor, how to quantify vigor, and how vigor affects fruit production still confuse viticulturists. Thus, the major purpose of this research is to explore the nature of vigor and to find a practical way of measuring vigor.

Vigor is originated from Middle English vigour, from Latin vigor, from vigēre to be vigorous. It is described as “physical strength and good health” in Oxford dictionary and “active healthy well-balanced growth especially of plants” in Merriam-Webster. With reference to grapevine, it is defined as “the rate and extent of vegetative growth” (Bell and Robson, 1999; Jackson, 2008). Vigor was also defined as “the rate of shoot growth or shoot elongation over time” (Bell and Robson, 1999; Rives, 2000; Keller et al., 2008; Keller, 2010). Galet (2000) did not give definition of vigor but described vigor in the following way: “vigor reflects the metabolic activity of growing organs and is manifested most notably by the intensity of respiration, protein synthesis, the functioning of meristems and the growth rate of the plant”. Deshmukh (1975) defined vigor as “the energetic or active state of growth” (Deshmukh et al.,
1975). The varied definitions of vigor indicate the complex nature of plant growth, as well as the necessity to have a better understanding of this old concept.

Obscure definition of vigor also leads to divided vigor measurement methods. Leaf area (Reynolds et al., 2005; Chaves et al., 2007; Drissi et al., 2009), shoot length (van Leeuwen et al., 2004), stem diameter (Rives, 2000), internode length (Balachandra et al., 2009), pruning weight per vine (Percival et al., 1994; Rives, 2000; van Leeuwen et al., 2004) were commonly used vigor parameters. Furthermore, Cortell et al. (2005) used the average of shoot length, leaf chlorophyll content, and trunk area to calculate vigor index. Galet (2000) described a vegetative expression (Ev), which is proposed by Branas (1946) to quantify vigor. Ev is calculated from crop weight, pruning weight and probable alcohol content. Deshmukh (1975) reported use of stem growth (in apple, mango, trifoliate orange, rough lemon and other citrus), shoot: root and bark: wood ratios (in apple and mango), the number of stomata per unit area of dorsal surface of leaf (in mango), electrical resistance, the amount of absorption of mineral nutrients as indicators of vigor. Along with the progress in imaging technology, vigor was also measured by aerial multispectral imaging (Zerihun et al., 2010; Drissi et al., 2009). Cell density has been used as a vigor parameter by Zerihun (2010), whereas the vertical leaf area index (LAI) and gap fraction of the canopy were used as vigor parameters by Rachid (2009). Leaf petiole nitrogen content has also been used as a vigor indicator by Balachandra et al. (2009).

1.7 The influence of water on plant growth and hydraulic structure

Cell growth includes short-term growth and long-term growth. A basic model for short-term cell growth is the turgor-driven theory. In this theory irreversible enlargement of plant cells is believed to be resulted from water absorption and wall yielding. Water absorption increases the volume of growing cells and induces cell wall yielding, which in turn generates the driving
force for water uptake (Cosgrove, 1986). In the long-term growth, assimilation of CO$_2$, absorption of nutrients and synthesis of proteins and other cellular components attribute to cell growth indirectly. Although there is no accurate model encompassing all the factors to describe cell growth, the important role of water in plant growth is significant.

Water in plants is transferred through the soil-plant-atmosphere continuum (SPAC) which contains a series of interrelated and interdependent processes (Fiscus et al., 1983; Kramer, 1983; Tyree and Ewers, 1991). In this continuum, water is firstly absorbed by root hair from the soil. The water is then transported from root hair to vascular bundles through both cell wall (apoplast) pathway and cell to cell pathway (Cosgrove, 1986). In the cell wall pathway, water moves through wall layers between cells or along surface films lining intercellular air spaces in the cell wall pathway. Whereas in the cell to cell pathway, water may move from vacuole to vacuole through cytoplasm and the cell wall, or symplastically through plasmodesmata without crossing membranes. In the long distance transportation, water is transported through the lumens of tracheary elements in the xylem, which provide a much lower resistance compared to living cells (Tyree and Ewers, 1991). Water is then uploaded to living cells from xylem through apoplast and symplast pathways. Instead of being used by the plant itself, most of the water is ultimately evaporated into the atmosphere through stomata, which are the microscopic pores in the leaf epidermis surrounded by a pair of guard cells (Tyree and Ewers, 1991).

The driving force for water movement is the difference between water potentials ($\Psi$), which is the difference in free energy (J mol$^{-1}$ or J m$^{-3}$) between the water under consideration and that of pure water at sea level (Crawley, 1986). Water always moves from high value to low value. Because $\Psi_{air} < \Psi_{leaf} < \Psi_{root} < \Psi_{soil}$, in which $\Psi_{soil}$ is close to zero and $\Psi_{air}$, $\Psi_{leaf}$, $\Psi_{root}$ are negative, water flux is able to flow from soil to the air through SPAC. Transpiration (E) in the
leaves provides the power of water ascent, which is related with the concentration of water in the internal and external atmosphere as well as stomatal conductance ($g_s$) (Crawley, 1986). Stomatal conductance is a measure of the ease with which water molecules can diffuse through the stomata (Crawley, 1986). Changes in $g_s$ can influence transpiration rate and consequently change leaf water potential (Farquhar and Sharkey, 1982).

Cohesion theory is the most widely accepted theory for the mechanism of water ascent in plants. According to cohesion theory, the driving force is generated by surface tension created by transpiration which is transmitted through water transport system into roots and other organs (Holtta et al., 2006). The metastable state of water pathway is achieved by cohesion of water to water and adhesion of water to xylem conduits walls (Crawley, 1986; Tyree, 1997; Holtta et al., 2006). Air bubbles in the xylem sap would destroy this metastable state in different extent according to hydraulic architecture. Small pores in tracheary elements reduce this chance and vessel elements are important structures to constrain this block in that element instead of the whole vessel (Tyree, 1997).

According to the analogy of Ohm’s law developed by Huber in 1924, water transport can be described by the following equation (Kramer, 1983; Tyree and Ewers, 1991):

$$\text{Water flux} = \frac{\text{difference in water potential} (\Delta \Psi)}{\text{resistance} (r)}$$

This formula was adjusted by Kaufmann and Hall (1974) as follows:

$$\text{Water flux} = \frac{\psi_{soil} - \psi_{leaf}}{r_{soil to leaf}}$$
Hydraulic conductance describes the ease with which water can move through pore spaces. Hydraulic conductance ($k_{AB}$) through a discrete region from A to B is calculated by dividing water flux ($F_{AB}$, kg s$^{-1}$) with the water potential drop across the structure ($\Psi_A-\Psi_B$, MPa), $k_{AB} = F_{AB} / (\Psi_A-\Psi_B)$. Hydraulic conductance is the inverse of resistance ($r$). Hydraulic conductivity ($k_h$) equals to the ratio between water flux ($F$, kg s$^{-1}$) through an excised stem segment and the pressure gradient ($dP/dx$, MPa m$^{-1}$) causing the flow (Tyree and Ewers, 1991): $k_h = F / (dP/dx)$. Besides $k_h$, specific conductivity ($k_s$) and leaf specific conductivity (LSC) are also important conductivity parameters. $k_s$ is a measure of the porosity of the stem segment and it is calculated by dividing $k_h$ with sapwood (functional xylem) cross-section area ($A_w$, m$^2$): $K_s = K_h / A_w$ (Tyree and Ewers, 1991). LSC is $k_h$ divided by the leaf area distal to the segment ($A_L$, m$^2$): $LSC = k_h / A_L$. It shows the hydraulic sufficiency of water supplied to leaves from stem (Tyree and Ewers, 1991).

1.8 Xylem anatomy, hydraulic conductivity, and vigor

Xylem is the most important water transport organ in most of the plants, and tracheary elements (TEs) are the most distinctive xylem cells for water transport (Turner et al., 2007). There are two types of tracheary elements: tracheids and vessels, both of which are dead cells formed when TEs become mature by losing their nuclei and cell contents (Turner et al., 2007). Tracheids, which are present in both angiosperms and gymnosperm (Tyree and Ewers, 1991), are elongated, spindle-shaped cells that are arranged in overlapping vertical files interconnected by pits (Taiz, 2008). Vessels, which are only found in angiosperms (grapevines belong to angiosperms) (Tyree and Ewers, 1991; Kirkham, 2005) are composed of a series of vessel elements which are stack end-to-end by perforation plates at the end walls (Tyree and Ewers, 1991). Similar with tracheids, vessel elements at the end of a vessel lack perforation plates and
connected with other vessels through pit pairs (Taiz, 2008). The open end walls and wider diameter of vessel elements allow vessel to have a more specified and efficient capability of water conducting (Tyree and Zimmermann, 2002; Taiz, 2008).

Both experiments and theoretical analysis show that the structure and distribution of tracheary elements determine xylem hydraulic conductivity to a great extent (Tyree and Ewers, 1991). Theoretical hydraulic conductivity is calculated from Poiseuille’s law: the laminar flow rate of an incompressible fluid along a pipe is proportional to the fourth power of the pipe's radius (Oka, 1964; Tyree and Ewers, 1991). The equation is as follow:

$$k_h = \frac{\pi \rho}{128 \eta} \sum_{i=1}^{n} (d_i^4)$$

Where $\rho$ is the density of the fluid in kg m$^{-3}$, $\eta$ is the dynamic viscosity of the fluid in MPa s$^{-1}$, $d$ is the diameter (m) of the pipe, $n$ is the number of pipes in the bundle (Tyree and Ewers, 1991). The assumption of this equation is that: (1) the flow is laminar viscous and incompressible, (2) the flow is through a straight circular tube of uniform cross-section, and the tube is substantially longer than its diameter (Oka, 1964). When this equation is applied to the calculation of stem hydraulic conductivity, tracheary elements are considered to be cylindrical pipes, $d$ stands for the diameter of xylem vessels, $n$ is the number of vessel/ tracheid in the cross-section. This theoretical hydraulic conductance ($k_h$) is proportional to the sum of the forth power of tracheary elements’ diameter. According to Poiseuille’s law, $k_s$ and LSC can be calculated in the following way (Tyree and Ewers, 1991):

$$k_s = k_h / A_w = \frac{\pi \rho}{128 \eta A_w} \sum_{i=1}^{n} (d_i^4)$$

$$LSC = k_h / A_l = \frac{\pi \rho}{128 \eta A_L} \sum_{i=1}^{n} (d_i^4)$$
From an experimental aspect, the close relationship between the anatomy of tracheary elements and hydraulic conductivity was proved in many studies. For instance, peach rootstock with higher vigor showed higher hydraulic conductivity, larger vessels whereas lower vessel number per unit field compared to rootstocks with low vigor and low hydraulic conductivity (Tombesi et al., 2010). However, theoretical $k_h$ according to Poiseuille’s law is usually higher than measured hydraulic conductivity, for example, calculated resistance was 70% of measured resistance in chrysanthemum stems (Nijsse et al., 2001). This could be explained in following aspects: 1) xylem vessel elements or tracheids are not necessarily circular (Tyree and Ewers, 1991); 2) the vessel element’s or tracheids wall is not straight or smooth (Tyree and Zimmermann, 2002); 3) vessel length also has an impact on hydraulic conductivity (Tyree and Zimmermann, 2002); 4) the small error from inaccurate measurement can cause big difference in the result. 5) the resistance arising from inter-conduit connections (Nijsse, van der Heijden et al. 2001).

Several other studies have shown that xylem anatomy features have tremendous influence on vigor through their effect on hydraulic conductance and transpiration rates, for instance in citrus (Rodriguez-Gamir et al., 2010), avocado rootstocks (Fassio et al., 2009), rubber clones (Sangsing et al., 2004), numerous rainforest trees (Poorter et al., 2010), peach rootstocks (Tombesi et al., 2010). Xylem anatomy features also had an influence on transpiration rate, stomatal conductance by influencing hydraulic conductance (Sangsing et al., 2004; Rodriguez-Gamir et al., 2010). Xylem conductivity was positively related with xylem area and leaf area (Sangsing et al., 2004), and negatively related with wood density (Poorter et al., 2010).
1.9 Factors influencing xylem vessel characteristics

Xylem vessel characteristics are related with following factors: 1) CO₂ concentration: oak seedlings were noticed to have increased vessel size and number with elevated CO₂ concentration (Atkinson and Taylor, 1996); 2) shoot orientation: downwardly-growing grapevines tend to have smaller vessels with higher density and lower hydraulic conductivity than upwardly-growing vines (Schubert et al., 1995; Schubert, Lovisolo et al., 1999; Lovisolo et al., 2002), vessel diameter and the sum of vessel cross-sectional areas were smaller in downwardly-growing vines (Schubert et al., 1999); 3) the position of the xylem vessels in a shoot (Sellin et al., 2008): a study of xylem hydraulic conductivity along chrysanthemum stems showed that conduit length, hydraulic conductivity decreased from base to top of the shoot (Nijsse et al. 2001); 4) hormones: high auxin production lead to a higher vessel number and smaller vessel size (Lovisolo et al., 2002; Turner et al., 2007), and the endogenous auxins appeared to be a determining factor of vessel size and number; 5) water stress: vessels of water stressed plants had lower cross-sectional areas, hydraulic conductivity, shoot specific conductivity and leaf specific conductivity (Lovisolo and Schubert, 1998).

1.10 Influence of excess vigor

Grapevines have lower plant food demand and thus less nutrient deficiency problems than other crop plants (Conradie and Saayman, 1989). Instead, tenacious vitality makes vines intend to have excessive vigor, which is the major issue in vineyard management. Excessive vigor leads to lateral shoot growth, which may cause winter damage (Byrne and Howell, 1978). Excessive vigor also causes dense canopy which in turn lead to shading problems and shade-induced reduction in fruit initiation and cold resistance (Lider et al., 1965; Byrne and Howell,
1978). Besides, dense canopy caused by excessive vigor also results in bunch stem necrosis (Dry and Loveys, 1998).

Dry and Loveys (1998) reported that fruit from vines with excess vigor inclined to have lower sugar and tartrate concentration, higher malate, higher pH, less phenolic and flavor compounds, which together reduced fruit quality. In a study conducted by Chaves and Santos (2007), vines suffering water stress exhibited higher concentration of berry skin anthocyanins and total phenols than fully irrigated vines because of a better light interception caused by reduced vigor. In terms of management, excess vigor causes more elaborate system to support heavier shoots and to avoid canopy shading (Dry and Loveys, 1998), higher cost of winter and summer pruning, hedging, etc.

1.11 Vigor control methods

1.11.1 Chemical methods

Maleic hydrazide, cycocel (CCC), ethephone and paclobutrazol (Dry and Loveys 1998; Wample et al., 1987) has been shown to reduce vigor. However, there are some side-effects of chemical control such as reduction in yield, residual on the fruit limited the widely use of chemical control (Dry and Loveys 1998).

1.11.2 Rootstocks

Rootstocks have been successfully used in fruit crops such as apple (Verheij and Verwer, 1973), cherry (Feucht and Gebhardt, 1982), citrus (Rodriguez-Gamir et al., 2010), avocado (Fassio et al., 2009), etc. to control vigor. Although rootstocks are widely used in grape production, they are not used for the purpose of vigor control but primarily for preventing soil-
borne pests, phylloxera and nematodes (Dry and Loveys, 1998). However, certain rootstocks have a higher tendency to lead to excess vigor especially on fertile locations (Lider et al., 1965) and thus should be careful in the selection of rootstocks.

1.11.3 Root volume restriction

Root volume has a direct influence on the size of root system and therefore on shoot vigor (Dry and Loveys, 1998; Wang et al., 2001) and restricting rooting volume is used to control vigor in many crops including grapevines. Root pruning is one way to control root volume and has showed a devigorating effect in apple (Ferree et al., 1997), cherry (Webster et al., 1997), etc. However, the research of root pruning in grape production is limited (Dry and Loveys, 1998). Permanent grass cover has been shown to decrease vine vigor via the competition between grass rooting and the vine root system (Byrne and Howell, 1978; Morlat and Jacquet, 2003). However, weed cover can also cause reduced yield to a large extent (Byrne and Howell, 1978), Morlat R and Jacquet A (2003) reported that grass cover decreased the number of vine roots in the interrow, but and increase close to the row compare to vineyard without grass cover. The technique of rooting-zone restriction by container or raised bed has also been used as a vigor control method in apple, mandarin, peach, cherry, etc (Wang et al., 2001). In grapevines, rooting-zone restriction also restricts root growth and leads to lower vigor: smaller trunk, shorter shoot, smaller leaf area, lower photosynthetic rate (Wang et al., 2001).

1.11.4 Soil conditions

As an essential factor in terroir, soil type highly influences vine vigor (van Leeuwen et al., 2004) and for this reason site selection affects vigor to some extent. This influence of soil on
vigor is related with soil depth, fertility and water distribution (Dry and Loveys, 1998; Balachandra et al., 2009).

### 1.11.5 Fertilization

Moderate rates of nitrogen fertilization stimulated vine growth and vigor resulting in an increase in canopy density (Bell and Robson, 1999; Balachandra et al., 2009). Generally, nitrogen fertilization increases fruit yield when it fulfills the demand of plant growth, however, decreases fruit yield when nitrogen supply is excessive. And this yield reduction might be due to poor canopy microclimate (Bell and Robson, 1999). On the other hand, reduced nitrogen could decrease vine vigor, increase carbohydrate levels, fruit sugar concentration, yield and cold resistance (Byrne and Howell, 1978).

### 1.11.6 Trellis system

Vines have upright-growth habit (Howell et al., 1991), upward growth increases vigor while downward or bended growth decreases vigor (Schubert et al., 1995; Schubert et al., 1999; Lovisolo et al., 2002). Thus, trellis system can influence vigor by mediating shoot directions, bud number, canopy structure, etc.

### 1.11.7 Bud number

Shoot vigor is inversely proportional to shoot number per vine (Byrne and Howell, 1978; Dry and Loveys, 1998; Keller et al., 2008). This knowledge has been used as the foundation of pruning, however, the mechanistic basis is not clear. One explanation of this relationship is that lower vigor is caused by inter-shoot competition for assimilation (Dry and Loveys, 1998). It is noteworthy that by pruning, reserves contained in canes are also removed with the pruned sections (Rives, 2000), however, this does not the main factor that influence yield and vigor.
because roots and trunk play an more important role in providing nutrients. Minimal pruning is a good example of controlling vigor by retaining a high amount of buds (Dry and Loveys, 1998). On the contrary, severe pruning increases shoot vigor, yield per shoot and decreases yield, number of cluster per vine and total sugar per vine (Byrne and Howell, 1978, Lider et al., 1965). The influence of pruning on yield is mainly caused by the number of compound buds retained in pruning that will bear fruit in the following season. Rives (2000) reported that crop load is theoretically determined in proportion to the size of the vine. In other words, less pruning severity induces higher load and lower vigor.

1.11.8 Irrigation

Irrigation increases vegetative growth to a large extent, it also has a positive effect on weights of berries, clusters, and number of berries per cluster (Kliwer et al., 1983). Thus, irrigation is an important vigor control method especially in areas with less precipitation. A mild water stress in certain growth period effectively controls vigor and improves fruit quality (Dry and Loveys, 1998; Rives, 2000; Chaves et al., 2007; Acevedo-Opazo et al., 2010). This is mainly caused by lower turgor pressure which is impaired by low xylem pressure potential. Early water deficit induced shorter shoot growth period, less maximum growth rate, accelerated periderm formation and slower fruit development (Matthews et al., 1987).

Two widely used irrigation methods for water regulation is regulated deficit irrigation (RDI) which is to remove or reduce water input during certain growth period especially after fruit set, and partial root-zone drying (PRD), which is to maintain half of the root system in a drying state and the remainder of the root system irrigated (Chaves et al., 2007). Studies on RDI showed that it decreased berry size (Ojeda et al., 2001), yield (Matthews et al., 1987; Acevedo-Opazo et al., 2010) and increased the concentration of phenolic compounds, soluble solids and
anthocyanins (Acevedo-Opazo et al., 2010). The effect of deficit irrigation on fruit quality may be due to higher distribution of photoassimilates in producing reproductive tissues and secondary metabolites by reducing vegetative growth (Chaves et al., 2007).
CHAPTER TWO

MATERIALS AND METHODS
2.1 Plant materials

Vines used in this research were field-grown grapevines (*Vitis vinifera* L. cv. Merlot) planted on their own roots in 1999 in the vineyard (46°17'49"N; 119°44'07"W; elevation 364 m) at WSU’s Irrigated Agriculture Research and Extension Center in Prosser, WA (Figure 3). They were grown in north-south-oriented rows on a ~2% south-facing slope and spaced at 1.8 m (within rows) by 2.7 m (between rows) (Keller and Mills, 2007). Vines were trained to loose vertical shoot positioning (VSP) system and spur pruned. Standard cultural practices were used to maintain healthy vines throughout the growing season. The vineyard was drip-irrigated using regulated deficit irrigation after bloom. The grapevines experienced repeated dry down and re-watering cycles in order to determine the effect of shoot number on vine’s response to water deficit in a complimentary project. Irrigation water was applied at the first sign of leaf wilting following each dry-down cycle at a rate of 2L water per hour.

In 2009 and 2010, shoots were maintained with one cluster per shoot after bloom in order to reduce the variation derived from different cluster number per shoot, however, in 2009 there were several samples were missed in the cluster thinning and had two or three clusters. Other regular vineyard managements such as pest and weed control were done according to the vineyard condition.
2.2. Pruning

In 2009, 40 vines with one or two trunks were randomly pruned in spring into bud number ranging from 45 to 90 per vine. In 2010, 100 healthy vines with two trunks were randomly pruned into bud number ranging from 18 to 124 on March 12th, before the growing season started (Figure 4).
Figure 4: Merlot vines (A) before pruning and (B) after pruning (March 12, 2010).

2.3 Measurement of growth characteristics

2.3.1 Shoot length

In 2009, 16 shoots with bud number ranging from 45 to 105 were collected in the end of the season for shoot length measurement. In 2010, 45 vines with bud number ranging from 18 to 124 were chosen for regular shoot length measurement. On each vine, three randomly chosen vertically positioned shoots were tagged and the shoot length of these shoots was measured every 7 to 10 days. The shoot length measurement started on May 24th, 2010 when shoots had 4 to 8
internodes and ended on August 16\textsuperscript{th}, a month after the shoot stopped growing. Temperature data in Roza vineyard 2010 was provided by The Washington Agricultural Weather Network (Version 2.0, WSU Prosser). Growing degree days (GDD) is an expression of heat units. GDD=\[\frac{(T_{\text{MAX}} + T_{\text{MIN}})}{2}\]−\(T_{\text{BASE}}\) (McMaster GS and Wihelm WW, 1997). In this experiment GDD was calculated by adding the average temperature greater than 10 °C from bud break (April 22/ Day of the year 112).

2.3.2 \textit{Shoot morphology}

One group of 16 shoots with different vigor levels were cut and measured on September 15\textsuperscript{th}, 2009. Four groups of totally 70 shoots with different vigor levels were collected on June 15\textsuperscript{th}, July 20\textsuperscript{th}, August 20\textsuperscript{th}, and October 18\textsuperscript{th}, respectively in 2010 and immediately brought to laboratory for recording both shoot morphological and anatomical measurement (Figure 5).

\textbf{Figure 5:} Photographs of shoots with different vigor levels (July 20\textsuperscript{th}, 2010)
Fresh weights of whole shoots and thereafter individual clusters were measured immediately after shoots were excised using an electronic balance (Mettler PM1200, Mettler-Toledo Inc., Columbus, OH). Total number of nodes and leaves were counted. Shoot length and leaf dimensions (length and width) were measured to the nearest 1 mm with a ruler. Shoot diameter was measured with a digimatic caliper (Mitutoyo Corporation, Kanagawa, Japan). Leaf area was measured with leaf area meter (LI-3100C; LI-COR, Lincoln, NE). Berry number per cluster was counted manually and berry weight was measured with an electronic balance (PA313; Ohaus, Pine Brook, NJ).

2.4 Measurement of stomatal conductance

Stomatal conductance was measured on July 20th (19 shoots) and on August 20th (17 shoots). In each shoot, fully exposed fourth to fifth leaf from the shoot tip was measured using a leaf porometer (SC-1, Decagon Devices, Pullman, WA) between 12 and 2pm.

2.5 Leaf nutrient analysis

19 shoots with different vigor in July 2010 were used for leaf nutrient analysis. All of the leaves from each shoot were collected with petiole. Leaves were dried by being placed in an oven at 70 °C for one week. Dried leaves were ground to 20-mesh size and then determined elemental composition of leaves by a commercial laboratory using inductively coupled plasma spectroscopy (ICP (Soltanpour et al., 1996)).
2.6 Yield and fruit composition

2.6.1 Yield and cluster number per vine

Vines were harvested on October 20th manually and their cluster numbers per vine were determined. Yield was measured by weighing clusters using an electronic balance scale (PM1200, Mettler-Toledo, Columbus, OH).

2.6.2 Total soluble solids (°Brix) and pH

From each vine, about 1kg of fresh berries was randomly picked from all the clusters for fruit quality measurements. Berries were manually crushed, and clear juice was used for °Brix and pH measurement. Total soluble solids (°Brix) were measured with a digital refractometer (300035, Sper Scientific, Scottsdale, AZ, USA). pH was measured with a pH Meter (Seven Multi series, Mettler- Toledo, Columbus, OH).
2.7 Light microscopy and morphometric analysis

After morphological measurement, each shoot was equally divided into top, middle and bottom parts (Figure 6). One internode from each portion was chosen for fresh sectioning. Fresh cross sections (Figure 6) were made by using a sharp razor blade, and observed with stereomicroscope (Stemi 2000-C, Carl Zeiss, Thornwood, NY) attached with a digital camera (DXM 1200C, Nikon Instruments, Melville, NY), which was used for capturing digital images. Digital images were then modified with ImageJ software (1.42q, National Institute of Health,

**Figure 6:** A photograph of (A) 1-year old Merlot shoot showing top, middle, and bottom parts and (B) fresh handmade cross sections from top, middle, and bottom parts of the internode showing internal organization of their transport systems.
Bethesda, Maryland, USA) and Adobe Photoshop (version 7.0, Adobe Inc., San Jose, CA). The ImageJ software was used to draw xylem vessels and perform morphometric analysis (Figure 7).

In addition to fresh sectioning, stem tissues from top, middle, and bottom parts were fixed and preserved in formalin-acetic acid-alcohol as per the procedure described by Bondada (2011). Briefly, the fixed tissues were dehydrated using the tertiary butyl alcohol series, infiltrated and embedded in paraffin, sectioned at ~10 \( \mu \)m thickness with a microtome (MT 990; Boeckeler Instruments, Tucson, AZ), affixed to glass slides (8×3 cm), and stained with Johansen’s safranin (1% (w/v) dissolved in 50% EtOH) and fast green (0.2% (w/v) dissolved in 95% EtOH) protocol (Ruzin, 1999). The staining procedure involved rehydration in descending strengths of alcohol, staining with safranin, dehydration in ascending strengths of alcohol, and counter staining with fast green. When staining was complete, a drop of Permount (Fisher Scientific, Fair Lawn, NJ) mounting medium was used to affix coverslips to the slides. Slides were placed under a compound microscope (Axioskop 2 plus; Carl Zeiss, Thornwood, NY) attached with a digital camera (DXM 1200C; Nikon Instruments, Melville, NY), which was used for capturing digital images. Steps involved in performing morphometric analysis were illustrated in Figure 7. The first step is to redraw xylem vessels manually with ImageJ, after that, the redrawn vessels were collected and copied to form a new picture with white background. The new picture was analyzed with ImageJ to calculate vessel area and vessel number. Vessel diameter was calculated according to the formula to calculate the area of a circle: \( \text{essel area} = \pi (\text{vessel diameter}/2)^2 \). The diameter of pith, xylem, and cross section were also measured with ImageJ. Pith area, sapwood (functional xylem) area and cross section area were calculated according to the formula of the area of a circle.
2.8 Scanning Electron Microscopy (SEM)

One-year-old shoot sections were cut using a razor blade and fixed in 3% glutaradehyde overnight, rinsed with 0.1 M potassium phosphate butter at pH 7.2, and post-fixed in 2% osmium tetroxide overnight. The samples were subsequently dehydrated in a 30-95% alcohol series each for 10 minutes and 100% alcohol for 3 times with 10 minutes each time. Samples were then critical point-dried, coated with gold and viewed with a Hitachi S-570 scanning electron microscope (Franceschi Microscopy and Imaging Center, Pullman, WA, USA) using an accelerated voltage of 20 kV.

Figure 7: Stereomicrograph of (A) stem cross section showing xylem vessels, (B) after iterative deconvolution with ImageJ, (C) the number of xylem vessels was quantified in ImageJ, (D) number of rays and cross sectional area were quantified in ImageJ.
2.9 Hydraulic conductivity features

2.9.1 Hydraulic conductivity ($k_h; \text{kg m MPa}^{-1}\text{s}^{-1}$)

Theoretical hydraulic conductivity ($K_h; \text{kg m MPa}^{-1}\text{s}^{-1}$) was calculated according to Tyree and Ewers (1991) based on Hagen-Poiseuille’s law:

$$k_h = \left(\frac{\pi \rho}{128\eta}\right) \sum_{i=1}^{n} (d_i)^4$$

Where $d$ is the diameter of vessel in meters, $\rho$ is the fluid density (assumed of water at 20°C (998.2 kg m$^{-3}$ at 20°C) and $\eta$ is the viscosity of water at 20°C (1.002 x 10$^{-9}$ MPa s at 20°C)

2.9.2 The specific conductivity ($k_s; \text{kg MPa}^{-1}\text{m}^{-1}\text{s}^{-1}$)

$k_s$ is a measure of the porosity of the stem segment. It is obtained by dividing $k_h$ with the sapwood cross-section area ($A_w, \text{m}^2$) (Tyree and Ewers, 1991). The equation is:

$$k_s = k_h/A_w$$

2.9.3 The leaf specific conductivity ($LSC; \text{kg MPa}^{-1}\text{m}^{-1}\text{s}^{-1}$)

$LSC$ is obtained by dividing $k_h$ with the leaf area distal to the segment ($A_L, \text{m}^2$) (Tyree and Ewers, 1991), the equation is:

$$LSC = k_h/A_L$$

2.10 Statistical analysis

Data were subjected to one-way and two-way analysis of variance, one-way or two-way linear regression, non-linear regression, using SigmaPlot (version 11.0; SPSS Inc., Chicago, IL). Relationship between shoot weight, diameter, internode number, leaf area, cluster weight, etc
with shoot length in both 2009 and 2010 were analyzed with one-way linear regression analysis using SigmaPlot (version 11.0; SPSS Inc., Chicago, IL). Relationship between bud number and shoot length in 2009 was analyzed with one-way linear regression analysis, while factory analysis of variance was performed for the shoot length in 2010 versus day of the year and bud number groups. In 2010, the data of shoot length, days of the year and bud number were also subjected to multiple linear regression analysis in order to detect the influence of bud number and days of growth on shoot vigor without the influence of variance brought by grouping. Data of soluble solids in 2009, shoot length and cluster number were also analyzed with multiple linear regression. Relationship between stomatal conductance in 2009, August 2010 with shoot length were analyzed with linear regression analysis, while in July 2010, nonlinear regression was performed, and the model with the highest $r^2$ value was chosen to describe the relationship. Comparison of bottom vessel features among different vigors as well as leaf nutrient levels among different vigors were conducted with either linear regression or non-linear regression according to the highest $r^2$ value given by SigmaPlot.
CHAPTER THREE

RESULTS
3.1 The indicators of vigor

3.1.1-(1) Shoot fresh weight and shoot length

Shoot fresh weight showed significant positive linear relationship with shoot length in July ($r^2=0.93$), August ($r^2=0.97$), and October in 2010 ($r^2=0.89$) (Figure 8).

![Figure 8: Relationship between shoot fresh weight and shoot length in 2010.](image)

**Figure 8:** Relationship between shoot fresh weight shoot length in 2010. $r^2=0.93$, n=19, P<0.001, • -July 2010; $r^2=0.96$, n=17, P<0.001, ○-August 2010; $r^2=0.89$, n=18, P<0.001, ▼-October 2010.

3.1.1-(2) Node number and shoot length

Node number equates with internode number. Node number (or internode number) showed a significant positive linear relationship with shoot length in both in 2009 ($r^2=0.76$) and 2010 ($r^2=0.77$, 0.95, 0.83 in June, July, August, respectively) (Figure 9).
3.1.1-(3) Internode and shoot lengths

Average internode length showed significant positive linear relationship with shoot length in 2009 ($r^2=0.93$) and 2010 ($r^2=0.79$, June 2010; $r^2=0.90$, July 2010; $r^2=0.85$, August 2010) (Figure 10).
3.1.1-(4) Stem diameter and shoot length

The diameter of basal ends of the stem and shoot length showed significant positive linear relationship in 2009 ($r^2 = 0.40$) and 2010 ($r^2 = 0.77, 0.85, 0.92, 0.92$ in June, July, August, October, respectively). Regressional lines showed similar slopes in July, August, and October in 2010, whereas in June the slope was steeper (Figure 11).

Figure 10: Relationship between average internode length and shoot length in 2009 (A) and 2010 (B). $r^2=0.93$, n=16, P<0.001 (September 2009); $r^2=0.79$, n=16, P<0.001, ●-June 2010; $r^2=0.90$, n=19, P<0.001, ○-July 2010; $r^2=0.85$, n=17, P<0.001, ▼-August
Total leaf area (main leaves and lateral leaves) per shoot exhibited strong positive linear relationship with shoot length in both 2009 ($r^2=0.83$) and 2010 (June, July, August, and October, $r^2=0.83, 0.84, 0.96, 0.90$, respectively). Leaf area: shoot length ratio increased in the beginning of the growing season when the main leaves and lateral leaves continued to grow, whereas it decreased during the end of the season when leaf senescence occurred (Figure 12).

**Figure 11**: Relationship between stem diameter and shoot length in 2009 (A) and 2010 (B). $r^2=0.40$, n=16, P<0.001 (September 2009); $r^2=0.77$, n=16, P<0.001, ●-June 2010; $r^2=0.85$, n=19, P<0.001, ○-July 2010; $r^2=0.92$, n=17, P<0.001, ▼-August 2010; $r^2=0.92$, n=18, P<0.001, △-October 2010.

**3.1.1-(5) Total leaf area and shoot length**

Total leaf area (main leaves and lateral leaves) per shoot exhibited strong positive linear relationship with shoot length in both 2009 ($r^2=0.83$) and 2010 (June, July, August, and October, $r^2=0.83, 0.84, 0.96, 0.90$, respectively). Leaf area: shoot length ratio increased in the beginning of the growing season when the main leaves and lateral leaves continued to grow, whereas it decreased during the end of the season when leaf senescence occurred (Figure 12).
Figure 12: Relationship between total leaf area per shoot and shoot length in 2009 (A) and 2010 (B). $r^2=0.83$, n=16, P<0.001, Sep 2009; $r^2=0.83$, n=16, P<0.001, ●-June 2010; $r^2=0.84$, n=19, P<0.001, ○-July 2010; $r^2=0.96$, n=17, P<0.001, ▼-August 2010; $r^2=0.90$, n=18, P<0.001, △-October 2010.

3.1.1-(6) Main leaf area and shoot length

Average main leaf area exhibited significant positive linear relationship with shoot length in 2009 ($r^2=0.30$) and 2010 ($r^2=0.57$, June 2010; $r^2=0.89$, July 2010; $r^2=0.90$, August 2010) (Figure 13). $r^2$ was small in 2009, and it increased from June to August in 2010.
Figure 13: Relationship between main leaf area and shoot length in 2009 (A) and 2010 (B). $r^2=0.68$, n=16, $P<0.001$, September 2009; $r^2=0.57$, n=16, $P<0.001$, ●-June 2010; $r^2=0.89$, n=19, $P<0.001$, ○-July 2010; $r^2=0.90$, n=17, $P<0.001$, ▼-August 2010.

3.1.1-(7) Lateral : main leaf area ratio (%)  

The percent lateral leaf area to main leaf area ratio increased linearly with an increase in shoot length both in 2009 ($r^2=0.38$) and 2010 ($r^2=0.30$, June; $r^2=0.77$, July; $r^2=0.91$, August; $r^2=0.69$, October) (Figure 14).
3.1.1-(8) Stomatal conductance (gs) and shoot length

Stomatal conductance showed significant positive linear relationship with shoot length in September 2009 ($r^2=0.35$) and August 2010 ($r^2=0.62$). It exhibited sigmoidal relationship with shoot length in July 2010 ($r^2=0.94$) (Figure 15) in which the increase in $g_s$ was slower when shoots are short but became steeper when the shoot length increased.
3.1.1-(9) Cane maturation and shoot length

Cane maturation as measured by the extent of bark encasing along the stem following periderm formation showed significant positive linear relationship with shoot length both in 2009 ($r^2=0.52$) and 2010 ($r^2=0.96$) (Figure 16).
The relationship between vigor, yield components and fruit composition

3.1.2 The relationship between vigor, yield components and fruit composition

3.1.2-(1) Cluster weight and shoot length

Cluster fresh weight increased linearly with an increase in shoot length both in 2009 ($r^2=0.42$) and 2010 ($r^2=0.78, 0.91, 0.82$, July, August, October) (Figure 17). Cluster weight increased in 2010 from July to October and cluster weight: shoot length ratio increased during the season: $0.59$ (July), $0.60$ (August), $1.48$ (October).

In 2010, average rachis length was measured on June 17th before bloom. Average rachis length exhibited positive linear relationship with shoot length ($r^2=0.52$) (Figure 17).

**Figure 16**: Relationship between cane maturation and shoot length in 2009 (A) and 2010 (B). $r^2=0.52$, n=16, P=0.020, September 2009; $r^2=0.96$, n=18, P<0.001, October 2010.
Cluster/ shoot fresh weight (%) and shoot length

Cluster/ shoot fresh weight (%) exhibited exponential decay relationship with shoot length in October 2010 ($r^2=0.68$, $n=18$, $P=0.0002$) (Figure 18).
Figure 18: Relationship between cluster/ shoot fresh weight (%) and shoot length in October 2010 showed exponential relationship. \( r^2 = 0.68, \ n=18, \ P=0.0002 \).

### 3.1.2-(3) Interrelationship between berry weight, berry number and cluster weight

Both berry weight (\( r^2 = 0.84, 0.71, \) August, October, respectively) and berry number per cluster (\( r^2 = 0.82, 0.94, \) August, October, respectively) in August and October 2010 showed significant positive linear relationship with cluster weight (Figure 19).
Figure 19: Relationship between berry weight and cluster weight in August 2010 (A), $r^2=0.84$, n=17, P<0.001; October 2010 (C), $r^2=0.71$, n=18, P<0.001. Berry number as a function of cluster weight in August 2010 (B), October 2010 (D), $r^2=0.82$, n=17, P<0.001, August 2010; $r^2=0.94$, n=18, P<0.001, October 2010.
3.1.2-(4) pH, soluble solids (°Brix), leaf area/fruit (LA/F) ratio and shoot length

In 2009, pH did not show significant multiple linear relationship with shoot length and cluster number (P=0.302). No significant relationship between LA/F ratio and pH was detected (P=0.581).

Shoots in 2009 had variant cluster numbers ranging from 1 to 3 clusters per shoot. Multiple linear regression analysis was performed in order to determine how shoot length and cluster number affected soluble solids (Figure 20). The result showed that soluble solids tended to decrease with an increase in shoot length and an increase in cluster number ($r^2 = 0.43$, n=16, $P=0.026$). However, because $P_{\text{shoot length}}=0.13$, $P_{\text{cluster number}}=0.025$, it was not sufficient to estimate soluble solids by shoot length, while cluster number had significant negative influence on soluble solids. Multiple linear regression analysis of soluble solids as a function of shoot length and LA/F ratio did not show significant relationship (P=0.092). Multiple linear regression analysis of LA/F ratio as a function of shoot length and cluster number did not show significant relationship (P=0.092).

In 2010, pH and soluble solids were measured on September 28 (three clusters were collected per vine) and October 18 (one day before harvest). pH and soluble solids both significantly increased with shoot length in September ($r_{\text{pH}}^2=0.19$, $r_{\text{Brix}}^2=0.34$) (Figure 21). In October, pH did not show significant relationship with shoot length (P=0.715) or LA/F ratio (P=0.257). Soluble solids and sugar content per shoot (calculated by multiplying soluble solids with cluster weight× 100) both showed significant positive linear relationship with shoot length ($r_{\text{Brix}}^2 = 0.300$, $r_{\text{sugar}}^2 =0.830$) (Figure 23). pH and soluble solids in October were significantly
higher than those in September ($P_{\text{pH}}=0.027$, $P_{\text{Brix}}<0.001$, $\alpha=0.05$). LA/F ratio showed a significant positive linear relationship with shoot length ($r^2 = 0.331$) (Figure 23), however, no significant linear relationship between soluble solids and LA/F ratio was detected ($P=0.198$).

![Figure 20: Relationship between soluble solids, cluster number and shoot length in 2009. Soluble solids = 25.944 - (0.0185 × shoot length) - (1.447 × Cluster number), $r^2=0.43$, n=16, P=0.026. $P_{\text{cluster number}}=0.025$, $P_{\text{shoot length}}=0.13.$](image-url)
Figure 21: Relationship between pH (A) and shoot length, soluble solids (B) and shoot length on September 28, 2010: $r^2=0.19$, n=35, P<0.001 (A); $r^2=0.34$, n=35, P<0.001 (B)
Figure 22: relationship between soluble solids (A), sugar content per shoot (B) and LA/F ratio (C) and shoot length: $r^2 = 0.30$, n=18, P=0.019 (A); $r^2 = 0.83$, n=18, P<0.001 (B); $r^2 = 0.33$, n=18, P=0.012 (C).
3.2 The influence of bud number on vigor

Vines were classified into four groups according to bud number (A: 18-45 buds, B: 45-70 buds, C: 70-95 buds and D: 95-120 buds) (Figure 23). According to the relationship between shoot length and vegetative growth found in the previous experiment, shoot length was used as an indicator of vigor in this study. The difference in shoot length among the groups with different bud number was not significant during the beginning of the season. However, it became significant with the shoot growth and development. Growth rate was higher in the beginning and gradually slowed down in July and August (day of year around 200). In each group, average shoot length showed a large variation. Two way ANOVA test of shoot length versus bud number group and days of the year indicated that shoot length decreased significantly from group A to B and C and group B and C had significant longer shoots than group D. However, there was no significant difference of shoot length between group B and C (\(P_{\text{group}}<0.001, \alpha=0.05\)). In order to detect the influence of bud number on vigor without the influence of variation, multiple linear regression analysis of shoot length as a function of growing days (start from bud break) and bud number was performed (Figure 24). It showed that both bud number and growing days appeared to contribute to predicting shoot length (\(r^2=0.50, n=495, P<0.001, P_{\text{bud number}}<0.001, P_{\text{date}}<0.001\)) and shoot length increased with growing days and decreased with bud number. In July 2010 (shortly after bloom), some shoot tips became very weak or even died, this phenomenon became very frequent during August (a month after bloom, a month before veraison). On August 16\(^{th}\) 2010, 69 out of 135 shoots had no tips or very weak tips. As per t-test of shoot length of shoots with and without tips on Aug 16\(^{th}\) showed that shoots with tips were significantly longer than that without tips (Figure 25). As per t-test, shoots with more than 66\% tips tended to have lower bud number than shoots with less than 34\% tips.
In order to explore how bud number affected shoot growth, regression analysis was performed for the average shoot length on July 20th (Figure 26), when shoots stopped growing and differences of vigor among groups were most detectable. The analysis showed that there was a significant negative relationship between shoot length and bud number ($r^2 = 0.27$, $n=45$, $P<0.001$). Total shoot length per vine was calculated by multiplying average shoot length with bud number. It showed polynomial cubic relationship with bud number ($r^2=0.50$), total shoot length per vine increased with bud number when bud number per vine was smaller than 100, and tended to decrease when bud number exceeded 100 (per vine). Around 100 bud per vine, total shoot length varied tremendously.

**Figure 23:** Average shoot length in different bud number groups and GDD in 2010 (A: 18-45 buds, B: 45-70 buds, C: 70-95 buds, D: 95-120 buds). Error bars indicate the standard errors of the mean.
Figure 24: Multiple regression analysis of shoot length, day of the year and bud number. Shoot length (cm) = -32.445 - (0.298 × Bud number) + (0.585 × Julian date), $R^2 = 0.50$, $n = 495$, $P < 0.001$. $P_{bud\,number} < 0.001$, $P_{date} < 0.001$. 
Figure 25: t-test for shoots with tips and without tips in August 2010 (A) showed that shoots with tips were significantly longer than shoots without tip. t-test for vines with more than 66% shoots having shoot tips and vines with less than 34% shoots having shoot tips in August 2010 (B) showed that vines with more tips had less bud number than vines retained less tips ($\alpha=0.05$).

Figure 26: Average shoot length (A) and total shoot length (B) as a function of bud number (July 20, 2010). Linear relationship, $r^2=0.27$, n=45, P<0.001 (A); Polynomial cubic relationship, $r^2=0.50$, n=45, P<0.0001.
3.3 The influence of bud number on yield and fruit quality

Regression analysis between yield and bud number showed that yield had a significant positive relationship with bud number ($r^2=0.43$) (Figure 27). Meanwhile, cluster number showed a significant positive relationship with bud number ($r^2=0.74$), while average cluster weight did not show significant relationship with bud number ($P=0.109$) (Figure 27).

Soluble solids showed a significant negative relationship with bud number ($r^2=0.56$). pH did not show significant relationship with bud number ($r^2=0.085$). Total sugar content per cluster decreased linearly with bud number ($r^2=0.26$) while total sugar content per vine increased linearly with bud number ($r^2=0.33$) (Figure 27).
Figure 27: In 2010, linear relationship between yield (A), cluster number (B), soluble solids (°Brix) (C), total sugar per shoot (D) and total sugar per vine (E) and bud number. $r^2=0.43$, $n=35$, $P<0.001$ (A); $r^2=0.74$, $n=23$, $P<0.001$ (B); $r^2=0.56$, $n=23$, $P<0.001$ (C); $r^2=0.26$, $n=23$, $P=0.012$ (D); $r^2=0.33$, $n=23$, $P=0.006$ (E).
3.4 Nutritional composition of total leaves per shoot

The concentration and content of macro-nutrients N, P, K, Ca, Mg, S and micro-nutrients Fe, Mn, Zn, Cu, B, Al were analyzed (Figure 28). The concentration of K increased linearly with the increase in shoot length ($r^2=0.48$), N, S, P showed positive curvilinear relationship with shoot length ($r^2=0.86$ (N), $r^2=0.70$ (S), $r^2=0.62$ (P)). The concentration of Mg decreased with an increase in shoot length ($r^2=0.35$). The concentration of Ca and B did not show significant linear relationship with shoot length ($P=0.30$ (Ca), $P=0.19$ (B)). For the concentration of micro-elements, only Cu showed significant positive linear relationship with shoot length ($r^2=0.54$). The concentration of Fe, Mn, Zn, Al did not show significant relationship with shoot length ($P=0.174$ (Fe), $P=0.139$ (Mn), $P=0.38$ (Zn), $P=0.852$ (Al)).

The contents of macro- and micro-elements in total leaves per shoot all showed significant positive linear relationship with shoot length (Figure 29). ($r_N^2=0.94$, $r_P^2=0.94$, $r_K^2=0.90$, $r_{Cu}^2=0.92$, $r_{Mg}^2=0.91$, $r_S^2=0.90$, $r_B^2=0.91$, $r_{Fe}^2=0.88$, $r_{Mn}^2=0.87$, $r_{Cu}^2=0.93$, $r_{Zn}^2=0.85$, $r_{Al}^2=0.85$, $n=19$, $P<0.001$).
Figure 28: Linear relationship between minerals concentration and shoot length in July 2010. N\% (A) peak relationship, $r^2=0.86$, n=19, $P<0.001$; P\% (B) sigmoid relationship, $r^2=0.62$, n=19, $P=0.002$; K\% (C) positive linear relationship, $r^2=0.48$, n=19, $P<0.001$; Mg\% (D) negative linear relationship, $r^2=0.35$, n=19, $P=0.008$; S\% sigmoid relationship, $r^2=0.63$, n=19, $P=0.0015$; Cu ppm (F) positive linear, $r^2=0.54$, n=19, $P<0.001$. 
3.5 Xylem anatomy and shoot length

3.5.1 Basal xylem vessel number and shoot length

Vessel number from bottom parts of the stem (the axis of the shoot) did not show significant linear relationship with shoot length in September 2009 (P=0.102) and July 2010 (P=0.061), however, showed a significant positive linear relationship with shoot length in August 2010 ($r^2=0.84$) and October 2010 ($r^2=0.81$) (Figure 30).
3.5.2 Basal vessel lumen diameter and shoot length

Average vessel diameter in the bottom part increased in a curvilinear fashion with shoot length in September 2009 ($r^2=0.58$), July 2010 ($r^2=0.77$), August 2010 ($r^2=0.87$) and October 2010 ($r^2=0.94$) (Figure 31). The increase in vessel diameter was faster when shoots were short and became slower when the shoot length reached around 100cm.

Figure 30: Relationship between vessel number and shoot length in August 2010 (A) and October 2010 (B). $r^2=0.84$, $n=17$, $P<0.001$ (A); $r^2=0.81$, $n=18$, $P<0.001$ (B).
Figure 31: Basal vessel diameter of bottom stem section showed sigmoid relationship with shoot length in September 2009 (A), July 2010 (B), August 2010 (C) and October 2010 (D). $r^2=0.58$, $n=16$, $P=0.023$ (A); $r^2=0.77$, $n=19$, $P=0.0002$ (B); $r^2=0.87$, $n=17$, $P<0.0001$ (C); $r^2=0.94$, $n=17$, $P<0.0001$ (D).

3.5.3 Basal total vessel area and shoot length
Total vessel area from bottom cross-sections showed sigmoidal relationship with shoot length in September 2009 ($r^2=0.50$), July 2010 ($r^2=0.78$) and August 2010 ($r^2=0.93$) (Figure 32). Although the figure did not show typical sigmoid trend, the sigmoidal relationship in SigmaPlot provided the highest $r^2$ value. The total vessel area per section peaked at shoot length of 120 cm in October, 2010 ($r^2=0.95$), which was mainly caused by the shoot around 140cm. In July and August 2010, this relationship was close to linear.

**Figure 32:** Relationship between total vessel area per section and shoot length in September 2009 (A), July 2010 (B), August 2010 (C) and October 2010 (D). $r^2=0.50$, n=16, P=0.0112 (A); $r^2=0.78$, n=19, P=0.0002 (B); $r^2=0.93$, n=17, P<0.0001 (C); $r^2=0.95$, n=18, P<0.0001.
3.5.4 Basal theoretical hydraulic conductivity and shoot length

Theoretical hydraulic conductivity ($k_h$) increased with an increase in shoot length in a sigmoid pattern (according to the highest $r^2$ value given by SigmaPlot) in September 2009.
It showed peak relationship with shoot length in October 2010 ($r^2=0.95$) (Figure 33), which was mainly caused by the shoot around 140cm.

### 3.5.5 Basal specific hydraulic conductivity ($k_s$)

![Figure 34: $k_s$ and shoot length showed sigmoid relationship in September 2009 (A), July 2010 (B), August 2010 (C); peak relationship in October 2010 (D). $r^2=0.37$, n=16, P=0.013 (A); $r^2=0.72$, n=19, P<0.0001 (B); $r^2=0.87$, n=17, P<0.0001 (C); $r^2=0.96$, n=18, P<0.0001 (D).]
Specific hydraulic conductivity ($k_s$) in the bottom part increased with an increase in shoot length in both 2009 and 2010. In 2009, the linear relationship was significant however not great due to big variation. In August, 2010 the relationship was close to sigmoid, and in October, it was close to peak (Figure 34).

### 3.5.6 Basal leaf specific hydraulic conductivity (LSC)

Leaf specific hydraulic conductivity (LSC) of the bottom part did not show significant linear relationship with shoot length in 2009 ($P=0.295$), July 2010 ($P=0.227$) or October 2010 ($P=0.06$). However in August 2010, LSC showed significant linear relationship with shoot length ($r^2=0.45$, $n=17$, $P=0.003$) (Figure 35).

![Figure 35: relationship between LSC shoot length (August 2010). $r^2=0.45$, $n=17$, $P=0.003$.](image-url)
3.5.7 Basal sapwood area

Sapwood (functional xylem) area in the bottom part increased close to linearly with the increase of shoot length. This trend showed some exceptions when shoot length reached to 160cm in 2009 and 150 cm in July, 2010 (Figure 36).

![Figure 36: Sapwood area and shoot length showed sigmoid relationship in September 2009 (A) and July 2010 (B), linear relationship in August 2010 (C) and October 2010 (D). $r^2=0.60$, n=16, P=0.0291 (A); $r^2=0.83$, n=19, P<0.0001 (B); $r^2=0.93$, n=17, P<0.001 (C); $r^2=0.91$, n=18, P<0.001 (D).]
3.5.8 Basal sapwood area percentage and shoot length

Sapwood area percentage (sapwood area/ total cross-section area ×100%) did not show significant linear relationship with shoot length in 2009 (P=0.208), however, a weak but significant positive linear relationship in 2010 ($r^2=0.27$, Jul; $r^2=0.27$, Aug; $r^2=0.52$, Oct) (Figure 37).

**Figure 37:** Relationship between sapwood% and shoot length in July (A), August (B), October 2010 (C). $r^2=0.27$, n=19, P=0.023, (A); $r^2=0.27$, n=17, P=0.031, (B); $r^2=0.52$, n=18, P<0.001 (C).
3. 5.9 Basal vessel density

Vessel density (vessel number/ sapwood area (mm$^2$)) of the bottom part as a function of shoot length fitted to polynomial inverse third order equation in 2009 ($r^2 = 0.61$) and 2010 ($r^2=0.78$, Jul; $r^2 = 0.92$, Aug; $r^2=0.97$, Oct) (Figure 38). It decreased with the increase in shoot length, the rate of decreasing slowed down when shoot length reached around 100 cm.

![Figure 38: Relationship between vessel density and shoot length in September 2009 (A), July 2010 (B), August 2010 (C) and October 2010 (D). $r^2=0.61$, n=16, P=0.0083 (A); $r^2=0.78$, n=19, P<0.0001, (B); $r^2 = 0.92$, n= 17, P<0.0001 (C); $r^2=0.97$, n=18, P<0.0001 (D).]
3.6 Anatomy and morphology of Merlot shoot

3.6.1 Xylem anatomy of top, middle, and bottom parts of stem

In Sep 2009 and Aug 2010, vessel number, total vessel area, k_h, sapwood: cross section area (%) significantly increased from top to middle, bottom part (Table 1). Average vessel diameter in 2009 and 2010 in bottom and middle part were not significantly different, but both of them were significantly larger than that in the top part. Vessel density (vessel number per sap wood area) in 2009 decreased significantly from top to middle, middle to bottom part. In 2010, vessel density did not change significantly between bottom and middle sections, but it was significantly bigger in top sections. In both 2009 and 2010, ray number in bottom part was significantly larger than that in middle and top part, however did not show significant difference between middle and top parts. In 2009, there was no significant difference in pith area (%) between middle and bottom parts and it was significantly greater than the top part. In 2010, pith area (%) decreased significantly from top to middle, bottom parts. ANOVA for pith area/ cross section area percentage could not be performed in 2010 because of unequal variance. In 2009, vessel number per radial sector did not change significantly between bottom and middle parts, however, vessel number per radial sector in bottom and middle were significantly larger than that in top part. In 2010, vessel number per radial sector increased significantly from top to middle and from middle to bottom. k_s in 2009 did not show significant difference between bottom and middle parts, but k_s in top section was significant smaller than that in bottom or middle parts. Again, ANOVA test for k_s in 2009 could not be performed because of unequal variance especially between top and bottom parts, t-test of k_s between bottom and middle parts showed no significant difference, k_s in middle sections was significantly larger than that in top sections.
ANOVA for cross section area, sapwood area could not be performed because of unequal variance.

Two-way ANOVA of \( k_s \) in 2010 was performed in order to compare \( k_s \) between bottom, middle, and top parts (Figure 39). Shoots were classified into a and b groups according to bottom \( k_s \) value (a: 5.29-15.42, b: 18.65-35.30 kg MPa m\(^{-1}\)s\(^{-1}\)). In “b” group, \( k_s \) significantly decreased from bottom to middle to top; in “a” group, \( k_s \) did not show significant difference among bottom, middle and top parts. Two-way ANOVA of sapwood area in 2009 showed that in both a (bottom sapwood area from 19.24 to 19.28 mm\(^2\)) and b (bottom sapwood area from 20.73 to 33.01 mm\(^2\)) groups, sapwood area decreased significantly from bottom to middle and from middle to top parts. Images of cross sections from top, middle and bottom parts are shown in Figure 40.

Table 1: Wood features of sections from top, middle, and bottom parts of stems

<table>
<thead>
<tr>
<th>Year</th>
<th>Vessel number per section</th>
<th>Average vessel diameter (mm)</th>
<th>Total vessel area per section (mm(^2))</th>
<th>Pith area %</th>
<th>Sapwood area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>2009 428.31c 2.45c 0.032 0.033b</td>
<td>2010 4.09c 2.45c 0.032 0.033b</td>
<td>2009 *-0.38c 2.45c 0.032 0.033b</td>
<td>2010 *-0.55 c 2.45c 0.032 0.033b</td>
<td>2009 41.1 6a</td>
</tr>
<tr>
<td>Middle</td>
<td>2009 623.13b 2.63b 0.054 0.052a</td>
<td>2010 623.13b 2.63b 0.054 0.052a</td>
<td>2009 *0.24b 2.63b 0.054 0.052a</td>
<td>2010 *0.0033b 2.63b 0.054 0.052a</td>
<td>2009 29.7 b</td>
</tr>
<tr>
<td>Bottom</td>
<td>2009 849.25a 2.91a 0.058a 0.050a</td>
<td>2010 849.25a 2.91a 0.058a 0.050a</td>
<td>2009 *0.42a 2.91a 0.058a 0.050a</td>
<td>2010 *0.28 a 2.91a 0.058a 0.050a</td>
<td>2009 27.6 2b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Ray number per section</th>
<th>Vessel number per radial sector</th>
<th>Vessel density (mm(^2))</th>
<th>( k_b ) (kg m MPa(^{-1})s(^{-1}))</th>
<th>( k_s ) (kg MPa(^{-1})m(^{-1})s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>2009 47.19b 43.83b 9.10b *0.81c</td>
<td>2010 47.19b 43.83b 9.10b *0.81c</td>
<td>2009 2.18a 2.13a</td>
<td>2010 *0.53c 2.13a</td>
<td>2009 *0.53c 2.13a</td>
</tr>
<tr>
<td>Middle</td>
<td>2009 48.88b 43.83b 12.72a *0.99b</td>
<td>2010 48.88b 43.83b 12.72a *0.99b</td>
<td>2009 1.75b 1.71b</td>
<td>2010 *1.51b 1.71b</td>
<td>2009 *1.51b 1.71b</td>
</tr>
<tr>
<td>Bottom</td>
<td>2009 60.44a 54.24a 14.07a *1.18a</td>
<td>2010 60.44a 54.24a 14.07a *1.18a</td>
<td>2009 *1.59c 1.65b</td>
<td>2010 *1.74a 1.65b</td>
<td>2009 *1.74a 1.65b</td>
</tr>
</tbody>
</table>
Shapiro-Wilk Normality test, all pairwise multiple comparison procedures (Holm-Sidak method):
overall significance value=0.05, * log10 was performed transforms because of unequal variance.

**Figure 39:** Two-way ANOVA of sapwood area in 2009 (A) and 2010 (B) and $k_s$ in 2010 (C).  
a: bottom sapwood area from 19.24 to 19.28 mm$^2$, b: from 20.73 to 33.01 mm$^2$. Error bars indicate standard error.
Figure 40: Light micrographs of stem from top (A, B); middle (C, D); bottom (E, F) parts of one-year old cane. Bars: 0.5mm (A), 1mm (C), 0.2 mm (B, D), 1.25 mm (E), 0.1 mm (F)
3.6.2 Xylem anatomical characteristics of one-year old Merlot shoot

Figure 41: Stereomicrographs of (A) mature stem and (B) immature stem of Merlot showing their hydraulic design. V: ventral, D: dorsal, L1: lateral part which has bud attached to the base of that internode; L2: lateral part has no bud attached to the base of that internode but sometimes was with tendril attached to the base.

In Figure 41 (A), xylem cross-sectional area has been divided into four radial wedges. L1 and L2 were lateral parts, which had small vessel, limited vessel number per RS, shorter rays and thinner barks compared with ventral (V) and dorsal (D) parts. This phenomenon occurred mostly in middle and bottom parts, which, however, was not frequently observed in the top sections. Between L1 and part L2, L1 was with bud attached to the base of that internode and had smaller and less vessels than L2, which was on the opposite surface where tendrils occurred.
Figure 42: Stereomicrographs of one-year old stem showing leaf-traces in top (A) and middle (B) sections (arrow indicates a leaf trace). L1, L2: lateral parts; D: dorsal side; V: ventral side; D1, D2: dorsal leaf traces; V1, V2: ventral leaf traces; L: lateral leaf trace. Scale bars: 1 mm (A) and 0.2 mm (B).
There were usually five or more RS with much more vessels, some of which were very small and intense especially in top and middle cross-sections (Figure 42). RS with such characteristics were usually with thicker cortex (ridges). This phenomenon was less common in bottom part because RS in bottom part developed more even and cross-sections were rounder. However, such RS can still be found by observing primary xylem vessels in the bottom sections.

In SEM pictures, soft and hard phloem were significant especially in bottom and middle sections, while in top sections, few layers could be found. Starch was mainly distributed in ray parenchyma cells in term of amyloplasts (Figure 43).
Internode length and leaf area of one-year-old shoot

Internode length and leaf area data from June, July and August showed similar changes in internode length and leaf area along the one-year-old shoot (Figure 45). The shoot was divided

Figure 43: SEM graphs of one-year-old stem sections. Bottom xylem (A), hard/soft phloem in bottom part (B), amyloplasts in ray parenchyma cells (C), pith, xylem and bark in top part (D). H: hard phloem; S: soft phloem; A: amyloplasts. Bar indicated 0.33 mm (A), 125 μm
into three parts, from bottom to top which are: Part I, II, III, according to the pattern of internode length instead of evenly dividing the shoot into bottom middle and top parts. Part I (usually in the first 3 to 4 internodes counted from the bottom): internode length and leaf area both increased linearly; Part II: internode lengths fluctuate in repeated ternary structure, leaf area also fluctuated in this part, but did not show clear ternary structure; Part III is close to shoot tip, in which both internode length and leaf area decreased linearly, number of internode in this part differs according to different shoot length. It is worthwhile to notice that, in Part II, in every third internodes, one internode is shorter than the two beside it. Because the tendril location in grapevines has a similar pattern: a tendril is born on the nodes except on every third node (Rives, 2000), it is easy to think of correlating this phenomenon with tendril location. It is observed that, those shorter internodes are the ones have no tendril on the bottom of that internode (Figure 44).

When it comes to the leaf area, although main leaf area and total leaf area (main and lateral leaf area) at each node also fluctuated in Part II, there is no clear repeated pattern like the internode. However, it is interesting that on the shorter internode, main leaf area was usually comparatively smaller than that above or below, however, the lateral leaf area was significantly larger than that above or below. This may indicate that the function of those “shorter internodes” might be to produce lateral shoots.

Without considering the fluctuation of internode length and leaf area along the shoot, generally speaking, internode length and leaf area both increase in the first several internodes and decrease afterwards.
Figure 44: The ternary repeated pattern of internode length on the one year-old shoot. The scale bar is 15cm long. The lengths of A, B, C internodes were respectively 8.6 cm, 6.2 cm and 7.2cm. The nodes below A, C were with tendril, and the node below B without tendril. Arrows indicate tendrils.
Figure 45: Internode length and leaf area on one-year-old shoots collected from Jun 2010 (A), Jul 2010 (B) and Aug 2010 (C). N indicates the node below that internode did not have tendril. Arrow (A) means the end of Part I and the start of Part II, Arrow (B) means the end of Part II and the start of Part III.
Figure 46: Two-way regression of main leaf area as the function of internode length and internode number (the first internode starts from bottom). The main leaf above the internode (B, D, F); the main leaf below the internode (A, C, E). $R^2=0.91$, $n=11$, $P<0.001$ (A); $R^2=0.93$, $n=10$, $P<0.001$ (B); $R^2=0.97$, $n=23$, $P<0.001$ (C); $R^2=0.89$, $n=22$, $P<0.001$ (D); $R^2=0.89$, $n=21$, $P<0.001$ (E); $R^2=0.86$, $n=21$, $P<0.001$ (F).
In order to explore the relationship between internode length and main leaf size, multiple regression analysis was performed for main leaf area, internode length and internode number below that leaf or above that leaf. Three shoots samples were collected in June, July and August in 2010 (Figure 46). The bottom internode was numbered as the first internode. The result indicated that main leaf size was highly related with both internode length, internode number above and below that leaf. $r^2$ values were higher between leaf size, internode length and internode number below that leaf than above that leaf except in Jun 2010.
CHAPTER FOUR

DISCUSSION
4.1 Indicators of vigor

Shoot fresh weight, node number, average internode length, stem diameter, and total leaf area all exhibited strong and significant relationship with shoot length in 2009 and 2010. Positive relationship of shoot length with stem diameter and other growth features was in agreement with studies in other woody species (Xiang et al., 2009). The strong relationship among node number, internode length and shoot length indicated that the elongation of a shoot was achieved through an increase of internode length as well as node number. This also suggested that shoot elongation was associated with an increase in leaf number since main leaf number equaled with node number. Besides main leaf number, main leaf area also exhibited strong linear relationship with shoot length in both 2009 and 2010, which suggested that the increase in leaf area was caused by both the enlargement of leaf area and the increase in leaf number. It was observed that $r^2$ between average main leaf area and shoot length in 2010 was lower in June but higher in July and August. This suggested that differences in total leaf area among different vigorous shoots were predominantly due to leaf number in the early season, whereas both leaf area and leaf number played a role in the late season. One possible explanation for this phenomenon may be that leaves were immature in the early season; consequently leaf area had less influence compared with leaf number. Other rationale could be that shoot elongation was slower in June and August which might have lowered the influence of leaf number in causing differences among total leaf area in various vigorous shoots. In 2009 growing season, 2,4-D damage was observed and since 2,4-D retards lamina expansion (Bondada, 2010), the smaller $r^2$ value for leaf area in 2009 was probably due to 2,4-D damage.

The above observation indicated that the development and growth of leaf, internodes and other vegetative organs in the one-year-old shoot were interrelated to each other, and that shoot
length was a good indicator of the growth of other vegetative organs. Thus, when “vigor” is defined as the extent of shoot growth, shoot length can be used as an indicator of vigor. Compared with other vigor indicators, shoot length has following advantages: 1) It has comparatively higher correlations with the growth of other vegetative organs and thus is more accurate than shoot diameter, node number, etc.; 2) The measure of shoot length is non-destructive, therefore has less restrictions of measuring time and sample size compared with destructive indicators such as shoot weight, leaf area, etc. 3) Measurement is simple and easy to apply, and thus is practical for vineyard management. However, considering the complex nature of shoot growth, shoot length has limitations in representing vigor. One limitation is the trade-off between shoot length and stem diameter, e.g. some long shoots may be slimmer than shorter shoots. This trade-off occurred during the late season when shoot elongation stopped whereas shoot thickening continued. As observed in 2010 growing season, stem diameter increased more rapidly with an increase in shoot length in the beginning of the season than at the end of the season because that stem thickening during late season narrowed down the variation between weak shoots and vigorous shoots in the beginning of the season. The trade-off between shoot elongation and stem diameter could also occur because of differences in lateral growth, injury of shoot tips (e.g. by wind, chemicals, water stress, etc.).

When “vigor” is defined as “shoot growth rate” instead of growth “extent”, it becomes more complex to define the vigor parameters. Shoot length as an indicator of vigor had its own disadvantages when shoot elongation stopped in the middle or late season whereas lateral growth, stem thickening and cluster growth continued. Thus, when shoot elongation rate was used to describe vigor, vigor could not be defined once elongation stopped. The same scenario also occurs when lateral growth, shoot diameter or other parameters are used as vigor parameter. In
spite of the strong relationship among different vegetative growth aspects, there is no single vegetative organ that can simply represent the comprehensive growth status of the whole shoot throughout the growing season. On the other hand, shoot weight is a more comprehensive parameter on the shoot level; however, the measurement of shoot weight is destructive which makes it difficult to follow the growth situation with time. Besides the difficulties of describing vigor through different growth periods, different understanding of “rate” would also bring ambiguity to vigor. Since “rate” is the change during certain time, vigor of a certain shoot changes when different time ranges are used. For example, average growth rate of a shoot in a day, a week, a month or the whole season are not same, thus, “vigor” of one shoot at a certain time differed greatly when it is calculated in time span.

Higher lateral leaf area proportion in high vigorous shoots indicated the relationship between vigor and lateral growth. It was reported by Dry (1998) that the degree of lateral shoot development is directly proportional to the vigor of the main shoot. This difference in lateral leaf area proportion among different vigor levels was smaller during the beginning of the season and larger during the late season because lateral growth started later than primary growth and continued after primary growth stopped. It indicated that high vigor triggered a higher lateral growth and thus, extent of lateral growth can be used as an indicator of vigor in the late season.

Linear relationship between shoot length and stomatal conductance ($g_s$) measured in 2010 indicated that higher vigorous shoots tended to have higher stomatal conductance. Stomatal aperture is the pathway for gas exchange, and thus stomatal conductance is related to both transpiration and photosynthesis (Wong et al., 1979; Farquhar and Sharkey, 1982) and thus strongly influences water relations and photosynthesis activity of leaves. For instance, water potential and leaf temperature are influenced by stomatal conductance through transpiration rate.
Partial pressure of CO$_2$, leaf temperature and water potential together influence CO$_2$ assimilation rate by affecting Rubisco activity (Farquhar and Sharkey, 1982). Thus, the difference in stomatal conductance among different vigorous shoots indicated that low vigorous shoots may have lower transpiration and photosynthetic rates.

A positive linear relationship existed between $g_s$ and vigor in 2009 and August 2010, however, it was sigmoidal in July 2010. This difference may have been due to differences in temperature, vapor pressure deficit, ambient CO$_2$ concentration or leaf water potential (Jarvis, 1976).

Periderm (phellem) formation is important for overwintering viability because cold acclimation and cold hardiness is dependent on tissue maturation (Wolf and Pool, 1988). The extent of phellem (periderm) formation increased with vigor in 2009 and 2010 indicating that instead of delaying cane maturation, high vigor induced increased cane maturation based on the progression of browning (phellem formation) along the stem length. This phenomenon contradicts studies of Wolf and Pool (1988), in which they noticed that water deficit induced less vigorous shoots and earlier periderm formation. However, this result is similar to the one reported by Smithyman and Howell (1998), in which they found out that higher bud number (which meant lower vigor) reduced cane maturation (Smithyman et al., 1998). The increased wood maturation of vigorous shoots may be due to an increased activity of phellogen which paralleled with increased growth in vigorous shoots.

4.2 Reproductive growth and vigor

Cluster weight per shoot increased with an increase in vigor indicating that high vigor is beneficial to promoting reproductive growth. The expected decline in cluster weight with vigor
was not observed in this study, however, the ratio of cluster weight to total shoot weight decreased with an increase in shoot length. This suggested that the increase in cluster weight was not paralleled by an increase in vegetative growth. A similar trend of rachis length and shoot weight was observed before blooming. This suggested that high vigor not only increased the size of clusters but also the rate of reproductive growth. This was also noticed in studies by Rives (2000) who reported that rapid shoot growth was positively correlated with inflorescence initiation. Fruitfulness (yield/node retained) increased with high vigorous vines caused by more severe pruning (Byrne and Howell, 1978).

Interrelationship among berry weight, berry number and cluster weight indicated that heavier cluster tended to have more and bigger berries. The higher $r^2$ between berry number and cluster weight compared to those between berry weight and cluster weight revealed that berry number was more effective in affecting cluster weight than berry weight. As per the positive relationship between vigor and cluster weight, cluster weight and berry weight, high vigor tended to induce larger berry size, which is not a preferred character for wine quality. Smaller berries are more desirable because the higher concentration of quality-relevant components which are extracted from skin according to a larger surface to volume ratio (Keller et al., 2008).

A significant negative relationship was observed between soluble solids and cluster number in 2009, whereas shoot vigor did not show significant relationship with soluble solids. Also, crop load (inverse of LA/F ratio) did not show significant relationship either with soluble solids or shoot length. This pattern was different from other studies, which showed that sugar accumulation was delayed by higher crop load (Edson et al., 1995; Keller et al., 2004). The reason for the nonsignificant relationship between soluble solids and shoot length was possibly due to small sample size in 2009. Nonsignificant relations between LA/F ratio and shoot length,
soluble solids was possibly due to severe 2,4-D damage during that season which led to inaccurate leaf area data at the end of the season.

In 2010, soluble solids exhibited weak, however, significant linear relationship with shoot length both before harvest and at harvest, and soluble solids increased significantly from September to October due to fruit ripening. This was identical with other studies which showed that soluble solids had positive relationship with leaf area (Edson et al., 1995, Jackson, 1986). Sugar content per cluster at harvest exhibited strong positive linear relationship with shoot length. This can be explained by availability of more photosynthates ensuing from larger leaf area, which enabled more and accelerated sugar accumulation in the cluster. Increased pruning severity has been known to advance fruit ripening (Byrne and Howell, 1978). However, since grapes were harvested very late in 2010 and soluble solids of some clusters reached to 27, higher ripening speed was a possible but not the only reason for higher soluble solids in more vigorous shoots. LA/F ratio in 2010 was positively related to shoot length. However, no significant relationship between soluble solids and LA/F ratio was detected in 2010. Jackson (1986) pointed out that the increase of soluble solids with LA/F ratio stopped when LA/F reached around 10-15 (cm²/g). In 2010, all LA/F ratios were above 10 (cm²/g) and most of the LA/F ratios were above 15 (cm²/g) because clusters were thinned to one cluster per shoot. This indicated that LA/F ratio values beyond the optimum range (roughly between 7 to 14 cm²/g) (Jackson, 2008) did not have much effect on fruit quality, whereas vigor would have more significant influence on fruit quality.

pH tended to increase with shoot length in September, however, did not show significant relationship with either shoot length or LA/F ratio in October 2010 or 2009. Jackson (1986) mentioned that pH was positively related to leaf area and negatively related to berry number.
Byrne and Howell (1978) also observed that pH increased with severe pruning and higher vigor. The lack of significant relationship between pH and vigor in 2009 and October 2010 maybe due to small sample size because sample size, i.e. 16 shoots as opposed to 35 shoots in September 2010.

These comparisons were based on single shoots without considering the whole vine. However, the fruit quality in a given shoot is influenced by the growth condition of the whole vine. Comparing different vigorous shoots from different vines limited interpreting the variations between shoots from the same vine and the variation in average shoot performance from different vines.

4.3 Bud number and vigor

In 2010, average shoot length was used as vigor indicator to examine the influence of bud number on vigor. Average shoot length per vine exhibited big variation in groups determined by bud number. This variation may have resulted from wide range of bud number in each group; differences in vine capacity; variation in soil type, lack of uniformity in irrigation, etc. Vigor differences among bud groupings were small 30 days after bud break, and increased with shoot elongation and reached the highest level around 100 days after bud break when shoots elongation almost stopped (after bloom before veraison). This cessation in growth was due to inactivity of shoot tip (meristem), which may be caused by regulated deficit irrigation (RDI) after bloom because a great amount of tips were noticed to dry out during RDI. High vigorous shoots and vines with less bud number tended to maintain more active tips after water stress. This indicated that high vigor vines utilized water efficiently and thus were able to withstand
water stress. Similar results were also reported by Zerihun (2010). An interesting question to put forth here is that how the vigorous vines were able to withstand water stress knowing that more vigorous vines have bigger xylem vessels, which are easier to induce embolism (Lovisolo et al., 2002; Poorter et al., 2010). One possible explanation for this contradiction is that water stress resistance depends on different stress level. At a lower water stress level, water stress may only cause lower hydraulic conductivity because of smaller vessel diameter; whereas a high water stress level induces vessel embolisms (Lovisolo and Schubert, 1998). Thus, it is suggested that moderate water stress is beneficial for smaller vessel size and thus reduced vulnerability to vessel embolism (Lovisolo and Schubert, 1998). Although high bud grouping showed large variation, it could be seen that this group tended to have shorter shoots throughout the season and vice versa. Besides, shoot elongation exhibited parallel trend with growing degree days (GDD), which was in agreement with other research that plant growth was closely related with air temperature (Lebon et al., 2004). Two-way linear regression test for shoot length as the function of growth days and bud number showed that shoot length was a function of both the growth days and bud number: shoot length increased with growth days and decreased with bud number. One way regression analysis of shoot length as a function of bud number on July 20, 2010 further approved the negative effect of bud number on vigor. The negative relationship between bud number and vigor had also been mentioned by former study. Summer et al. (1995) noticed that minimal pruning resulted in shorter shoot, shorter internodes and smaller leaves compared with cane pruning. However, the low $r^2$ value indicated the complex nature of shoot growth in which other factors such as vine capability, health, microclimate, soil and water condition, etc also may have played important roles in vine growth. Total shoot length per vine increased with bud number in the beginning and tended to decrease when bud number extended 100 per vine. High
variation in total shoot length beyond 100 buds per vine could be due to differences in vine 
capacity as indicated by trunk diameter. This indicated that total vegetative growth was not only 
related to bud number but also to vine capacity. Higher bud number reduced 

vigor in both large vines and small vines. However, total vegetative growth was 
increased by higher bud number in large vines wherease decreased in small vines. In conclusion, 
bud number had a stunting effect on average shoot length while had a positive effect on total 
shoot growth, this was in agreement with other studies (Byrne and Howell, 1978; Dry and 

Yield, cluster number in 2010 increased with bud number. Higher bud number induced 
more clusters because one cluster was kept on each shoot by cluster thinning after bloom. 
Average cluster weight did not show significant relationship with bud number, thus, higher yield 
caused by higher bud number was mainly because of higher cluster number instead of average 
cluster weight. °Brix decreased with bud number, however, total sugar content increased with 
bud number. The decrease in °Brix with bud number was because lower shoot vigor caused by 
higher bud number. The increase in total sugar content per vine with bud number coordinated 
with an increase in total shoot length per vine with bud number. However, total shoot length per 
vine decreased with bud number when it exceeded 100 buds per vine. However, total sugar 
content did not show decrease when bud number exceeded 100. This indicated that the balance 
between vegetative and reproductive growth tended to incline towards reproductive growth when 
more buds were retained, especially more than 100 buds per vine.
4.4 Nutritional composition:

Nitrogen is an important element for vegetative growth. It is highly contained in RuBP carboxylase (an important enzyme in photosynthesis), nucleic acids, chlorophyll, and several growth regulators. A positive relationship has been found between total N content and vine vigor (Wolf et al., 1983; Balachandra et al., 2009), whereas excessive N concentration had a depressive effect on growth (Wolf et al., 1983). In this study, N concentration increased with shoot length in the beginning and decreased a little when shoots were longer than 150 cm. However, N content per shoot exhibited strong positive linear relationship with vigor. This indicated that N content is strongly related to vigor, while N concentration may decrease a little in very high vigorous shoots due to dilution effect.

Phosphorus is an important element in cell-membrane lipids, nucleic acids, ATP and some proteins, and sugar metabolism. Grapes require small amount of phosphorus and thus phosphorus deficiencies are rare (Conradie and Saayman, 1989). Phosphorus fertilization had been noticed to have a positive effect on yield and shoot growth (Conradie and Saayman, 1989). Similar result was also observed in this study: phosphorus concentration increased with shoot length in a sigmoid trend, in which phosphorus concentration did not increase with shoot length after it reached its maximum concentration around 0.24-0.26%. Conversely, phosphorus content showed a strong positive linear relationship with shoot length.

Potassium is important in cellular osmotic and ionic balance, electrochemical processes, neutralization of organic acids, regulation of stomatal function, cell division, enzyme activation, protein synthesis and synthesis and translocation of sugars. Because of the active transport of K, its uptake is independent of K content in the soil (Conradie and Saayman, 1989), wherein the cation exchange ability and soil depth are important for K absorption (Jackson, 2008). Higher K
content is also associated with higher yield and vigor (Wolf et al., 1983; Conradie and Saayman, 1989). Leaf K content was reported to have a positive relationship with fruit size, especially citrus (Reuther et al., 1958). In this study, K concentration and K content increased linearly with an increase in shoot length, which was in agreement with former studies that K had a positive effect on vigor.

Sulfur is the component of the amino acids cysteine and methionine, and the key function of sulfur is to provide disulfide bonds for protein synthesis (Roubelakis-Angelakis, Amâncio et al. 2009). Thus, higher sulfur concentration and content indicated more protein synthesis in more vigorous shoots.

Mg is a vital element in chlorophyll, and metabolism processes such as activating phosphate-transfer enzymes, stabilizing ribosome, etc. (Jackson, 2008). Mg had positive effect on vegetative growth (Wolf and Haeseler, 1983). Wolf and Haeseler (1983) observed that grapevines without Mg fertilization exhibited less growth than vines with Mg solution; however, different solution concentration did not make difference in plant growth. Thus, the result that leaf Mg concentration decreased with vigor may be caused by dilution effect by larger leaf area. Besides, other studies showed that the increase in potassium concentration in leaves were likely to induce magnesium deficiency in fruit trees (Reuther et al., 1958).

Copper is a cofactor in oxidative reactions and the synthesis of proteins, carbohydrates and chlorophyll. Copper concentration and content both increased with vigor, which indicated positive effect of copper concentration and content in vigor. Excessive copper exposure can negatively affect plant growth (Llorens et al., 2000).
4.5 Xylem anatomy:

Xylem vessel number per section did not show significant relationship with shoot length in 2009. This was associated with small range in shoot length compared to 2010. In 2010, longer shoots tended to have more xylem vessel number per cross section, which may be caused by large sapwood area.

Average vessel diameter increased in a sigmoid pattern with an increase in shoot length, in which average vessel diameter did not increase or increased more slowly when shoot length reached around 100 cm. Total vessel area increased with shoot length in a curvilinear trend, wherein the rate of increase slowed down when shoots got longer, even decreased when shoots achieved a length of 140 cm in Oct 2010. The positive relationship between total vessel cross-sectional area and shoot length was also reported in other studies (Schubert et al., 1999). The slight decrease in vessel diameter and vessel area in the most vigorous shoots may be a safety mechanism: longer shoots need to support heavier tissues and thus needed higher wood density, which was increased by fiber density and decreased by vessel area (Sellin et al., 2008; Poorter et al., 2009; Poorter et al., 2010). $k_h$ also exhibited curvilinear relationship with shoot length, in which the increase of $k_h$ with shoot length slowed down when shoot length exceeded 140 cm, which may also be a safety mechanism. The increase in $k_h$ with vigor (shoot length) was also observed in previous studies. For instance, Tombesi and Johnson (2009) reported that low $k_h$ maybe the main reason for dwarling characteristics in peach rootstocks. Higher $k_h$ was caused by both vessel size (especially large vessels) and vessel number. Other studies also showed that the differences in xylem anatomy appeared to influence hydraulic conductivity directly in peach (Tombesi et al., 2010). Rootstocks of avocado trees with wider and fewer vessels were related to higher sap flow rate (Fassio et al., 2009). Positive relationship between root hydraulic
conductivity and vessel lumen diameter was found in citrus trees (Rodriguez-Gamir et al., 2010). Although vessel number in 2009 was not significantly related to vigor, $k_h$ in 2009 still increased with vigor, which suggested that large vessels had a more important role than vessel number in controlling hydraulic conductivity. Similar results were reported by Sellin et al. (2008).

Compared to $k_h$, whose increase with vigor was partially caused by larger sapwood area, $k_s$ indicated xylem porosity regardless of sapwood area (Tyree and Ewers, 1991). Similar to $k_h$, $k_s$ was a function of shoot length, which mostly increased with shoot length but decreased when shoot length was around 140 cm in Oct 2010. LSC is an indicator of the hydraulic efficiency that supports leaves above the cross section with water (Tyree and Ewers, 1991). LSC did not show significant relationship with vigor except in Aug 2010. This may be caused by an inaccurate leaf area value in the late season. In other studies, smaller vessels and lower hydraulic conductivity were also related to lower shoot specific conductivity and leaf specific conductivity (Lovisolo and Schubert, 1998). Sapwood area increased with shoot length and decreased when shoots achieved a length of around 150 cm in 2009 and July 2010. The relationship between sapwood area and shoot length and hydraulic conductance (Martinez-Vilalta et al., 2009) indicated that sapwood area could be used as a vigor indicator. Except in 2009, sapwood area percentage (sapwood area/cross section area × 100%) increased slightly with shoot length, which indicated that high vigorous shoots may have invested more material in sapwood, or that other stem materials (bark, pith) were crushed by the fast growth of sapwood. Vessel density (vessel number/mm$^2$) decreased with the increase in shoot length in polynomial inverse third equation. The association between bigger vessel and smaller vessel density was observed in other studies (Lovisolo et al., 2002; Sellin et al., 2008). One possible explanation for the decrease in vessel density of large lumens that larger vessels occupied larger area per unit area of the cross section,
which otherwise would have contained an increased number of small vessels. This further corroborated the fact that large vessels and not increased vessel density are more important in affecting hydraulic conductivity.

### 4.6 Xylem anatomy of top, middle, bottom parts

The increase in vessel number, total vessel area, $k_h$, $k_s$, sap wood /cross section area (%) from top to bottom parts indicated that xylem had a larger distribution in bottom part, which was associated with higher hydraulic conductivity in the bottom parts. This was supported by other studies: conduit length, hydraulic conductivity of chrysanthemum stems decreased from base to top of the shoot (Nijssse et al., 2001).

Because the average vessel diameters in bottom and middle parts were not significantly different, the increase in total vessel area from middle to bottom part was due to the increase in vessel number. The decrease in vessel density (vessel number per sap wood area) was in agreement with the trade-off between vessel density and vessel size as reported in previous studies (Lovisolo et al., 2002; Sellin et al., 2008). In 2010, vessel density did not change significantly between bottom and middle sections similar to the trend in vessel size. The increase in ray number from top to bottom parts suggested that the circumferential growth in stem was partially achieved by the increase in rays. Pith area/ cross section area (%) was significantly higher in top part than that in middle and bottom parts, which was partly because xylem took up more portions in the bottom and middle part and xylem growth in some degree crushed pith; it also indicated that pith was more active in top part than middle part, which may be explained by the possible function of young pith as the matrix for vascular tissue development (Mauseth, 1988). Higher vessel number per RS in bottom compared to top parts suggested two directions of
xylem growth: tangential growth by increasing RS number and vessel number, longitudinal growth by increasing xylem vessel number per RS and RS area.

$k_s$ in 2009 did not show significant difference in bottom and middle part, but $k_s$ in top section was significant smaller than that in bottom and middle sections. In 2010, $k_s$ significantly decreased from top to middle, to bottom parts in samples with bottom $k_s$ from 18.65-35.30 kg MPa m$^{-1}$ s$^{-1}$. Similar results were also noticed in silver birch, that $k_s$ and LSC decreased while vessel density and relative area of vessel lumina increased distally along main branches (Sellin et al., 2008).

4.7 Characteristics of Merlot xylem

Two major characteristics were noticed in Merlot xylem: First, different vessel distribution in lateral, dorsal and ventral parts: lateral 1 part had bud and leaves attached to the base while lateral 2 did not. Lateral parts had significantly less, smaller vessels, shorter rays, thinner barks compared with dorsiventral part. Second, frequent and regular appearance of leaf traces especially in middle and top section. The common number of leaf trace was five, with two dorsal leave traces, two ventral traces and one lateral trace. Leaf traces tended to be wider than other RS and had more xylem vessels especially small vessels. Different xylem characteristics in lateral, dorsal and ventral parts described in the results were in agreement with other studies. Stevenson et al. (2004) also mentioned dorsal, ventral and lateral leaf traces as well as different distribution of xylem vessels in lateral and dorsiventral parts. Chatelet and Matthews (2006) approved the existence and function of open conduits between petiole and leaf by observing the movement of paint. It was noticed that connections between stem and petiole occurred in primary xylem and mostly started from three internodes below the leaf. These open vessels between stem and leaves may facilitate the spread of bacteria pathogens (Chatelet et al. 2006). In
the study by Thorne et al. (2006), it was confirmed that both air, beads and bacteria can move unimpeded between stem and leaves without having to cross pit membranes, and this movement happened in the leaves within three internodes above the loaded internode (Thorne et al. 2006).

4.8 Modular pattern in Merlot

It was noticed in this study that internode length increased linearly in the base part of the shoot for four to five internodes, fluctuated every three internodes in the middle session, and decreased linearly in the top part. Internodes without tendrils tended to be shorter than the two adjacent internodes which were with tendrils. Main leaf area tended to be smaller on the internode without tendrils while lateral leaf area was much bigger than leaves attached to internodes with tendrils. This pattern was also mentioned by Lebon et al. (2004). Lebon et al. (2004) divided a shoot into two parts: a proximal part and a distal part. Internode length increased in the proximal part and then fluctuated around 100mm. They also mentioned that the internodes of P0 phytomers (without tendril attached) were shorter and presented more developed lateral shoots (Lebon et al. 2004). Leaf area had strong relationship with the position of the leaf and internode length either below or above that leaf. The strong association between leaf area and internode length was also mentioned by Wample and Schnabel (1987). This may be caused by internode and its attached leaves have similar hormone distribution, meanwhile, larger leaves produce more photosynthate and thus support faster growing internode.
CHAPTER FIVE

CONCLUSIONS
Generally speaking, vigor is the ability of vegetative growth. In grapevines, it is measured as the extent and rate of shoot growth. Our study proved that shoot length had significant linear relationship with shoot components, stomatal conductance, and nutrient levels. Thus, shoot length can be used as an indicator of vigor. Bud number exhibited a negative influence on vigor. The increase in cluster weight and soluble solids with an increase of shoot length indicated that vegetative growth promoted reproductive growth in a single shoot. However, the balance tended to skew toward reproductive growth, as evident from the ratio of cluster weight to shoot weight declining with vigor. pH and LA/F also increased with vigor in 2010, however, did not exhibit significant relationship with vigor in 2009.

When shoot vigor was expressed in term of shoot length, data in 2009 did not show significant relationship between bud number and shoot vigor according to limited sample size. However, both factory ANOVA test and multiple regression analysis for the data in 2010 showed significant relationship between shoot vigor and bud number, in which average shoot length decreased with an increase in bud number. This knowledge is useful in making pruning decision and advancing the understanding of vigor.

For the anatomy and hydraulic conductivity study, this study provided more accurate anatomical and hydraulic conductivity data compared to previous research for the following reasons: First, all the xylem vessels in a cross section were analyzed in this research instead of only few RS as studied in previous research. Second, xylem vessels were redrawn manually, which provided more accurate data than software generated images, whose accuracy may be influenced by picture quality and the incapability of software to separate vessels from other part of the cross section. Since theoretical hydraulic conductivity was calculated by summing up the fourth power of vessel diameter, \( k_h \) gained from this study was more accurate than former studies.
because of the reliable data of vessel features. Vessel number per cross-sectional area, sapwood area, vessel lumen diameter, total vessel area per cross-section and hydraulic conductivity increased with shoot length, whereas vessel density decreased with shoot length. $k_h$ exhibited curvilinear relationship with shoot length in some cases, whereas linear relationship in other cases. The sample size in this study was not enough to conclude whether $k_h$ related with shoot length in a linear or non-linear pattern. Besides, although shoot length is a good indicator of shoot vigor, it may not be the only factor that is related with $k_h$. Shoot hydraulic structure is too complex to be described by shoot length alone. Over all, these results showed that vigor was positively related to hydraulic conductivity, which was caused by variations in xylem anatomical features among different vigor levels. However, the strong relationship between vigor and hydraulic conductivity was not enough to conclude whether high vigor was caused by higher hydraulic conductivity or higher hydraulic conductivity was caused by high vigor.

In a given shoot, vessel lumen size, vessel number, hydraulic conductivity, the number of radial sectors (RS) bordered by xylem parenchyma and vessel number per RS were smaller at the distal ends than at proximal ends, while vessel density followed the opposite trend. Higher hydraulic conductivity at the bottom of the shoot which was caused by corresponding xylem features is reasonable, because the bottom part has to support the water supply for the whole shoot. Shoot structure exhibited modular characteristics, in which internode without tendril at its bottom tended to be shorter and bear more lateral branches than its adjacent internodes which have tendrils attached at the bottom. This pattern has not been well studied in previous studies and thus provided important information for understanding the structure of grapevine shoots. The common appearance of leaf traces and smaller vessel in lateral sides has not been widely studied and is worth examining in future studies.
In future, more attention should be paid to the growth situation of the whole vine instead of only shoot growth because root, trunk, cordon also play important roles in grapevine vigor. Physiological studies such as canopy structures, total photosynthesis, total transpiration, etc. will be important in providing a comprehensive view of grapevine vigor. Furthermore, the anatomical analysis for cordon, trunk and roots will also provide more information of the grapevine hydraulic conductivity. Since both growth and xylem vessel development are closely related to hormones levels, the study of hormones distribution would be another interesting area of research to understand vigor. Current way to calculate theoretical $k_h$ is only based on vessel diameter, however, hydraulic conductivity is also affected by other factors such as vessel length. Thus, a more comprehensive way of calculating theoretical $k_h$ will make the estimation closer to measured $k_h$. 
REFERENCES


## APPENDIX

### Table 2: definition of major symbols used in the text

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>°Brix</td>
<td>total soluble solids</td>
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<tr>
<td>gₘ</td>
<td>stomatal conductance (mmol/m²s)</td>
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<td>Kₜ</td>
<td>water conductivity (kg m Mpa⁻¹s⁻¹)</td>
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<tr>
<td>Kₛ</td>
<td>specific hydraulic conductivity (kg Mpa⁻¹m⁻¹s⁻¹)</td>
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<tr>
<td>LA/F</td>
<td>leaf area/fruit ratio (cm²/g)</td>
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<tr>
<td>LSC</td>
<td>leaf specific hydraulic conductivity (kg Mpa⁻¹m⁻¹s⁻¹)</td>
</tr>
<tr>
<td>Pith area %</td>
<td>pith area/ cross-section area ×100%</td>
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<tr>
<td>RS</td>
<td>radial sectors bordered by xylem parenchyma</td>
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<tr>
<td>Sapwood area %</td>
<td>sapwood area/ cross-section area ×100%</td>
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<tr>
<td>Vessel density</td>
<td>vessel number/ sapwood area (mm⁻²)</td>
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### Table 3: equations for statistical analysis

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<tbody>
<tr>
<td>Average internode length (cm) (A) vs. shoot length (cm) (B)</td>
<td>A = 1.578 + (0.0288 × B)</td>
<td>A = 1.755 + (0.0457 × B)</td>
<td>A = 2.423 + (0.0220 × B)</td>
<td>A = 2.310 + (0.0236 × B)</td>
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<tr>
<td>Shoot diameter (mm) (A) vs. shoot length (cm) (B)</td>
<td>A = 4.000+ (0.0236× B)</td>
<td>A = 2.340+ (0.0816× B)</td>
<td>A = 3.869+ (0.0378×B)</td>
<td>A = 3.523+ (0.0391×B)</td>
<td>A = 2.781 + (0.0472 × B)</td>
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<td>Total leaf area per shoot (cm²) (A) vs. shoot length (cm) (B)</td>
<td>A = 74.641 + (22.234 × B)</td>
<td>A = -339.427+ (24.693× B)</td>
<td>A = -692.122+ (36.482× B)</td>
<td>A = -1364.239+ (43.021× B)</td>
<td>A = -1094.511+ (38.621× B)</td>
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<td>Node number (A) vs. shoot length (cm) (B)</td>
<td>A = 16.446 + (0.0596 × B)</td>
<td>A = 6.373+ (0.115 × B)</td>
<td>A = 7.478+ (0.131 × B)</td>
<td>A = 9.145+ (0.114× B)</td>
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<td>Average main leaf area per shoot</td>
<td>A = 57.483 + (0.362 × B)</td>
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<td>Shoot area (cm²) (A) vs. shoot length (cm) (B)</td>
<td>Jun 2010, A = -17.114 + (7.036 × B)</td>
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<td>Jul 2010, A = 45.351 + (0.544 × B)</td>
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<td>Aug 2010, A = 34.733 + (0.596 × B)</td>
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<td>Proportion of lateral leaf area (A) vs. shoot length (cm) (B)</td>
<td>Sep 2009, A = 6.626 + (0.198 × B)</td>
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<td>Jun 2010, A = -6.009 + (0.190 × B)</td>
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<td>Jul 2010, A = -6.341 + (0.255 × B)</td>
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<td>Aug 2010, A = -9.840 + (0.310 × B)</td>
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<td>Oct 2010, A = -8.482 + (0.371 × B)</td>
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<tr>
<td>gs (mmol/m²s) (A) vs. shoot length (cm) (B)</td>
<td>Sep 2009, A = 23.796 + (0.463 × B)</td>
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<td>Jul 2010, sigmoid five parameter: ( f = y_0 + a/(1 + \exp(-(x-x_0)/b))^c )</td>
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<td>Aug 2010, A = 2.366 + (0.255 × B)</td>
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<td>Phellem length (cm) (A) vs. shoot length (cm) (B)</td>
<td>Sep 2009, A = -14.681 + (0.696 × B)</td>
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<td>Oct 2010, A = -27.778 + (1.022 × B)</td>
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<td>Cluster fresh weight (g) (A) vs. shoot length (cm) (B)</td>
<td>Sep 2009, A = 32.751 + (1.131 × B)</td>
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<td>Jul 2010, A = -12.383 + (0.590 × B)</td>
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<td>Aug 2010, A = -9.693 + (0.603 × B)</td>
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<td>Oct 2010, A = -27.083 + (1.481 × B)</td>
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<td>Average cluster length (cm) (A) vs. shoot length (cm) (B)</td>
<td>Jun 2010, A = 6.327 + (0.118 × B)</td>
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<td>Cluster/shoot fresh weight (%) vs. shoot length (cm)</td>
<td>Exponential Decay, Modified Single, 3 Parameter ( f = a \exp(b/(x+c)) )</td>
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<td>2010 °Brix (A) vs. shoot length (cm) (B)</td>
<td>Sep 2010, A = 16.659 + (0.0554 × B)</td>
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<td>Oct 2010, A = 23.911 + (0.0131 × B)</td>
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<td>2010 sugar content per cluster (g) (A) vs. shoot length (cm) (B)</td>
<td>A = -7.628 + (0.384 × B)</td>
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<td>2010 LA/F ratio (cm²/g) (A) vs. shoot length (cm) (B)</td>
<td>A = 12.173 + (0.101 × B)</td>
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<td>Sep 2010 pH (A) vs. shoot length (cm) (B)</td>
<td>A = 3.136 + (0.00149 × B)</td>
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<td>2010 berry number (A) vs. cluster fresh weight (g) (B)</td>
<td>Aug 2010 A = 45.415 + (1.426 × B)</td>
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<td>Oct 2010 A = 28.743 + (0.828 × B)</td>
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<td>2010 Average berry weight (g) (A) vs. cluster fresh weight (g)</td>
<td>Aug 2010 A = 0.186 + (0.00337 × B)</td>
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<td>Oct 2010 A = 0.567 + (0.00200 × B)</td>
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<td>Shoot fresh weight (g) (A) vs. shoot length (cm) (B)</td>
<td>Jul 2010, A = -58.887 + (2.204 × B)</td>
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<td>Average shoot length (cm) vs. bud number (B)</td>
<td>July 2010, $A = 98.234 - (0.404 \times B)$</td>
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<td>Total shoot length (cm) vs. bud number</td>
<td>July 2010, Polynomial cubic, $f = y_0 + a \times x + b \times x^2 + c \times x^3$</td>
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<td>Yield (g) (A) vs. bud number (B)</td>
<td>2010, $A = 1167.178 + (39.853 \times B)$</td>
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<td>Cluster number (A) vs. bud number (B)</td>
<td>2010, $A = 8.935 + (0.872 \times B)$</td>
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<tr>
<td>°Brix (A) vs. bud number (B)</td>
<td>2010, $A = 27.523 - (0.0461 \times B)$</td>
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<tr>
<td>Total sugar per cluster (g) (A) vs. bud number (B)</td>
<td>2010, $A = 18.876 - (0.0763 \times B)$</td>
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<tr>
<td>Total sugar per vine (g) (A) vs. bud number (B)</td>
<td>2010, $A = 381.343 + (7.538 \times B)$</td>
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<tr>
<td>N % vs. shoot length (cm)</td>
<td>Jul 2010, Peak, Modified Gaussian, 5 Parameter $f = y_0 + a \times \exp(-0.5 \times \text{abs}(x-x_0)/b)^c$</td>
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<td>P% vs. shoot length (cm)</td>
<td>Jul 2010, Sigmoidal, Chapman, 4 Parameter $f = y_0 + a \times (1 - \exp(-b \times x))^c$</td>
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<tr>
<td>K % (A) vs. shoot length (cm) (B)</td>
<td>Jul 2010, $A = 0.574 + (0.00187 \times B)$</td>
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<tr>
<td>Mg% (A) as the function of shoot length (cm) (B)</td>
<td>Jul 2010, $A = 0.622 - (0.00106 \times B)$</td>
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<tr>
<td>S% vs. shoot length (cm)</td>
<td>Jul 2010, Sigmoidal, Chapman, 4 Parameter $f = y_0 + a \times (1 - \exp(-b \times x))^c$</td>
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<tr>
<td>Cu (ppm) (A) vs. shoot length (cm) (B)</td>
<td>Jul 2010, $A = 8.501 + (0.0347 \times B)$</td>
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<tr>
<td>Vessel number per bottom section (A) vs. shoot length (cm) (B)</td>
<td>Aug 2010, $A = 408.101 + (4.678 \times B)$</td>
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<td></td>
<td>Oct 2010, $A = 383.978 + (5.266 \times B)$</td>
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<tr>
<td>Average vessel diameter (mm) vs. shoot length (cm)</td>
<td>Sep 2009, sigmoid 3 parameters $f = a/(1 + \exp((-x-x_0)/b))$</td>
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<td>Jul 2010, sigmoid 5 parameter $f = y_0 + a/(1 + \exp((-x-x_0)/b))^c$</td>
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<td>Oct 2010, sigmoid 4 parameter $f = y_0 + a/(1 + \exp((-x-x_0)/b))$</td>
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<td>Total vessel area per section (mm²) vs. shoot length (cm)</td>
<td>Sep 2009, Sigmoid 3 parameters $f = a/(1 + \exp((-x-x_0)/b))$</td>
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<td>Jul 2010, Sigmoid 5 parameter $f = y_0 + a/(1 + \exp((-x-x_0)/b))^c$</td>
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<td>Aug 2010, Sigmoid 3 $f = a/(1 + \exp((-x-x_0)/b))$</td>
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<td>Oct 2010, Peak 4 $f = y_0 + a \times \exp(-0.5 \times (x-x_0)/b)^2$</td>
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<tr>
<td>$k_h$ (kg m Mpa s⁻¹ × 10⁻⁵) vs.</td>
<td>Sep 2009, sigmoid 5 parameter $f = y_0 + a/(1 + \exp((-x-x_0)/b))$</td>
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<tr>
<td>Shoot length (cm)</td>
<td>x0/(b)^c</td>
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<td>Jul 2010, sigmoid 5 parameter f=y0+a/(1+exp(-(x-x0)/b))^c</td>
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<td>Aug 2010, sigmoid 4 parameter f= y0+a/(1+exp(-(x-x0)/b))</td>
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<tr>
<td>Oct 2010, peak Gaussian 4 parameter f=y0+a<em>exp(-.5</em>((x-x0)/b)^2)</td>
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<tr>
<th>k_s (kg Mpa m^-1 s^-1) (A) vs. shoot length (cm) (B)</th>
<th>Sep 2009, A = 13.222 + (0.103 × B)</th>
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</thead>
<tbody>
<tr>
<td>Jul 2010, A = 6.646 + (0.139 × B)</td>
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<tr>
<td>Aug 2010, sigmoid 5 f=y0+a/(1+exp(-(x-x0)/b))^c</td>
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<tr>
<td>Oct 2010, Peak, Gaussian, 4 Parameter f=y0+a<em>exp(-.5</em>((x-x0)/b)^2)</td>
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| LSC (kg Mpa m^-1 s^-1) (A) vs. shoot length (cm) (B) | Aug 2010, A = 80.757 + (0.882 × B) |

<table>
<thead>
<tr>
<th>Sapwood area (mm^2) (A) vs. shoot length (cm) (B)</th>
<th>Sep 2009, sigmoid 5 parameter, f=y0+a/(1+exp(-(x-x0)/b))^c</th>
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<tbody>
<tr>
<td>Jul 2010, sigmoid 4 f= y0+a/(1+exp(-(x-x0)/b))</td>
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<tr>
<td>Aug 2010, A = -1.612 + (0.243 × B)</td>
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<tr>
<td>Oct 2010, A= -3.214 + (0.310 ×B)</td>
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<tr>
<th>Sapwood area% (A) as the function of shoot length (cm) (B)</th>
<th>Jul 2010, A = 48.464 + (0.0592 × B)</th>
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</thead>
<tbody>
<tr>
<td>Aug 2010, A = 52.186 + (0.0571 × B)</td>
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<tr>
<td>Oct 2010, A = 49.279 + (0.0927 × B)</td>
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<thead>
<tr>
<th>Vessel density (mm^-2) vs. shoot length (cm)</th>
<th>Sep 2009, Polynomial, inverse Third Order, f=y0+(a/x)+(b/x^2)+(c/x^3)</th>
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<tbody>
<tr>
<td>Jul 2010, Polynomial, Inverse Third Order, f=y0+(a/x)+(b/x^2)+(c/x^3)</td>
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<td>Aug 2010, Polynomial, Inverse Third Order, f=y0+(a/x)+(b/x^2)+(c/x^3)</td>
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<td>Oct 2010, Polynomial, Inverse Third Order, f=y0+(a/x)+(b/x^2)+(c/x^3)</td>
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<tr>
<th>Main leaf area (A) as the function of internode length (cm) (B) and internode number (C) below the leaf</th>
<th>Jun 2010, A = 100.337 + (14.832 × B) - (14.755 × C)</th>
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<tbody>
<tr>
<td>Jul 2010, A = 136.474 + (9.307 × B) - (5.998 × C)</td>
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<tr>
<td>Aug 2010, A = 77.387 + (17.926 × B) - (5.144 × C)</td>
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<tr>
<th>Main leaf area (A) as the function of internode length (cm) (B) and internode number (C) above the leaf</th>
<th>Jun 2010, A = 57.474 + (18.637 × B) - (8.711 × C)</th>
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<tbody>
<tr>
<td>Jul 2010, A = 76.184 + (16.432 × B) - (3.625 × C)</td>
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<tr>
<td>Aug 2010, A = 19.749 + (21.934 × B) - (1.873 × C)</td>
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