# Exploration of the effects of fruit and berry exposure on fruit composition in *Vitis vinifera* cv. Pinot Noir

Mark Robertson, B.Agr.Sc. (Hons)

Submitted in fulfilment of the requirements for the Degree of Master of Science

University of Tasmania

September, 2011

# DECLARATION OF ORIGINALITY

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Mark Robertson

# AUTHORITY OF ACCESS

This thesis is not to be made available for loan or copying for two years following the date this statement was signed. Following that time the thesis may be made available for loan and limited copying in accordance with the *Copyright Act 1968*.

Mark Robertson

Date

# Acknowledgments

This project was funded by an Australian Postgraduate Award and funding from the Grape and Wine Research and Development Corporation. I am very appreciative of this support.

I would like to sincerely thank Dr Steve Wilson, Dr Jo Jones and Dr Dugald Close for their time, patience and input.

I would like to thank all of the vineyards in Tasmania that contributed to this study. In particular I would like to thank Mr Fred Peacock, your input, advice, knowledge and help with this study was greatly appreciated, I would also like to thank Mr Gerald Ellis and Mr Adrian Hallam for their patience and providing great support to this project.

I would like to thank Professor Paul Read for his support, great company and advice.

I would like to thank all the family, friends, summer students and members of staff who have contributed, helped out with mind numbing tasks or provided moral support.

I would like to thank Mr Peter Althaus and Oak Tasmania for their understanding and patience.

I would like to thank David Ratkowsky and Greg Lee for their help with statistical analysis.

Finally I would like to thank Rebecca Jones. The sacrifices you have made to get me over the line and your ongoing love and support are greatly appreciated.

# Abstract

The maintenance and improvement of quality of Pinot Noir table wines has been highlighted as a key factor in the development of the Tasmanian wine industry. This study was designed to further investigate the cultural and environmental impacts on the composition of Pinot Noir fruit in a cool climate.

Over three vintages, 2005, 2006 and 2007, the industry practice of pre-veraison leaf removal in the fruiting zone is investigated. Defoliation was shown to delay ripening and this study provides evidence that the environmental influence over the entire season may have more impact on fruit composition, than increased fruit exposure or source/sink relationships during berry ripening.

The impact of bunch exposure on fruit composition was investigated in 2007 by imposing a range of shading and light exclusion treatments. Results support the conclusions of previous authors that biosynthesis of anthocyanin occurs independently of carbohydrate metabolism.

The impact of bunch compactness on the composition of Pinot Noir is investigated over two growing seasons by manipulation with pre-bloom application of Giberrellic Acid ($GA_3$). Observations suggest that a reduction in bunch compactness, lead to an improvement in grape composition, including higher anthocyanin concentration, in one of two experimental seasons. An argument for a relationship between increasing berry size and reduced total anthocyanin in Pinot Noir is presented.

# Table of Contents

# Chapter 1 - Introduction

## 1.1 The Australian and Tasmanian wine industries

The Australian wine industry in recent times has experienced a phase of limited growth, following the unprecedented growth of the previous decade. Statistics presented in the 2009-2010 Australian Wine and Brandy Corporation (AWBC) annual report (AWBC, 2010) reported a reduction in 3.3% growth in export wine sales, and a drop by 11% in value due to a reduction in the price paid per litre and a strong Australian dollar. A recognised glut on the world wine market, combined with a strong Australian dollar has seen increased pressure on Australian producers of bulk wine products and reduced returns to growers, leading to a reduction in viticultural area, displaying a continued downward trend in both variables during the 2009-2010 financial year. The AWBC highlighted that increasing the price paid per bottle was a key performance indicator for the future development of the Australian wine industry, with the view for Australia to become recognised as a producer of premium wine products.

In spite of the reduction in area seen in other viticultural regions of Australia, the cool climate Tasmanian wine industry has seen sustained growth with continuing investment and increasing area under vine. Though small when compared to the size of the national industry, Tasmania represents 0.4 percent of the national industry which as of 2009 represented 1549 bearing hectares (Ha) with annual crush of up to 9628 tonnes (Wine Industry Tasmania Data 2009). The Tasmanian wine industry does however produce (As of 2009) 1.9 percent of the national total in terms of value at 49 million AUD, which reflects high grape prices (Kerslake, 2011). These figures and growing accolades for wines produced from the region indicate that Tasmanian grapes and wines are increasingly being recognised as a premium product. Though rapidly expanding, the Tasmanian wine industry is often described as “still in its infancy”.

In the 2002-2007 Vineyards Association of Tasmania strategic plan (VAT, 2002) it was identified that for the industry "to be recognised internationally as a world leader and innovative producer of premium cool climate wines", measures would need to be put in place to maintain or ensure the production of grapes and wines of "intrinsic quality". Maintenance of yields and quality have been identified as key factors in the development of the Tasmanian wine industry (Heazlewood, 2005).

## 1.2 Climate, weather and geology of Tasmania

The influence of climate and soil have been demonstrated to have a large influence on fruit composition in particular their relationship to water stress (van Leeuwen et al., 2004). A short growing season, cool weather, and unfavourable precipitation patterns are all factors which may affect the yield and quality of a vintage. (Vasconcelos & Castagnoli, 2001)

The Australian Bureau of Statistics (ABS) describes the Tasmanian climate as mostly a temperate, maritime climate dominated by a prevailing westerly airstream, which leads to variation in cloud cover, rainfall and temperature (ABS, 2005). The western half of Tasmania including the central highland areas, are generally cool, wet and cloudy, while the eastern half and lowlands are milder, drier and subject to less cloud cover (ABS, 2005).

It is the author's opinion that a number of distinct weather patterns are often discussed locally as being of key influence on climate and viticulture. Rainfall events are dominated by two major weather patterns. The first is associated with prevailing westerly winds with embedded cold fronts which often cross the state bringing rainfall to the western mountainous part of the state and north western and northern coastal regions, though significant precipitation rarely extends to eastern districts due to the geological influence of the mountainous west (ABS, 2005; Wilson, 2002). The second rainfall pattern is associated with a "Tasman Low" low pressure system which extends a moist easterly flow across the Tasmania. These events are less numerous and predictable than those associated with westerly flow which accounts for the reduced frequency (ABS, 2005; ACE-CRC, 2010) and lower average (mean) rainfall

(Figure 1-1). During spring (September and October) and coinciding with bud burst and early shoot growth, westerly flow can be interspersed with high pressure systems which bring windless or calm conditions and clear, cloudless skies. This pattern increases the risk of wide spread frost, which is of concern to a large portion of the local industry (Wilson, 2002). Though not unique to the Tasmanian wine industry widespread frost presents significant risks to future development of the industry.

The ABS describe three major influences on average temperatures in Tasmania (ABS, 2005). Proximity to the sea ensures coastal locations experience milder conditions than those further inland. Afternoon sea breezes are common along the coasts of Tasmania where much of the local industry is situated. Average surface temperature decreases as altitude increases, making locations at higher elevations cooler than those situated lower in the landscape. "Cloudiness" in the western part of Tasmania suppresses average daily temperatures as a result of westerly winds. Also of note are the hot northerly and north westerly winds which extend from continental Australia during summer months which lead to warmer than average weather events (ACE-CRC, 2010; Wilson, 2002).

The Tasmanian wine industry is divided over seven regions ranging from approximately 41 to 43.5° S (Figure 1-2) all of which are located in the centre or east of the state at low elevation. Smart and Dry (1980) suggest that Tasmanian viticultural regions fall within the guidelines for cool climate wine regions, though Kerslake, (2011) presents an argument that it may be more appropriate to define Tasmania as "cold climate" region based on mean January temperature (MJT) of less than 18°C.

Comparison of climatic data presented within this study for, Coal River Valley (CRV) and Tamar River (TR) (Kerslake, 2011), allows for two of the seven regions to be compared. Some similarities can be seen in terms of climate for CRV and TR (Table 3-1) for example from MJT (17.4 CRV , 17.2 TR), growing degree days both during the vegetative growth cycle (419.5CRV, 421.2 TR) and growing degree days over the entire season (1178.5 CRV, 1272.5TR) . Large differences however can be observed for annual rainfall (Figure 1-1) and rainfall distribution (Figure 1-3 &

Figure 1-4). Examination of long term averages between maximum, minimum and average temperatures (Figure 1-5 & Figure 1-6) over a 12 month period displays that the southern region is warmer on average during the summer, cooler during the winter and a greater difference between minimum (night) and maximum temperatures. Weather data from the Hobart Airport Australian Bureau of Meteorology (BOM) weather station has been chosen to examine these differences, as it is situated at the south most end of the Coal River Valley and is the closest weather station to many vineyards in the Coal River Valley region, including two of the major trial sites in this study. It was also selected based on the quality and length of the records and taking into the consideration spatial variability in rainfall distribution over relatively short distances (5 kilometres) in this region.

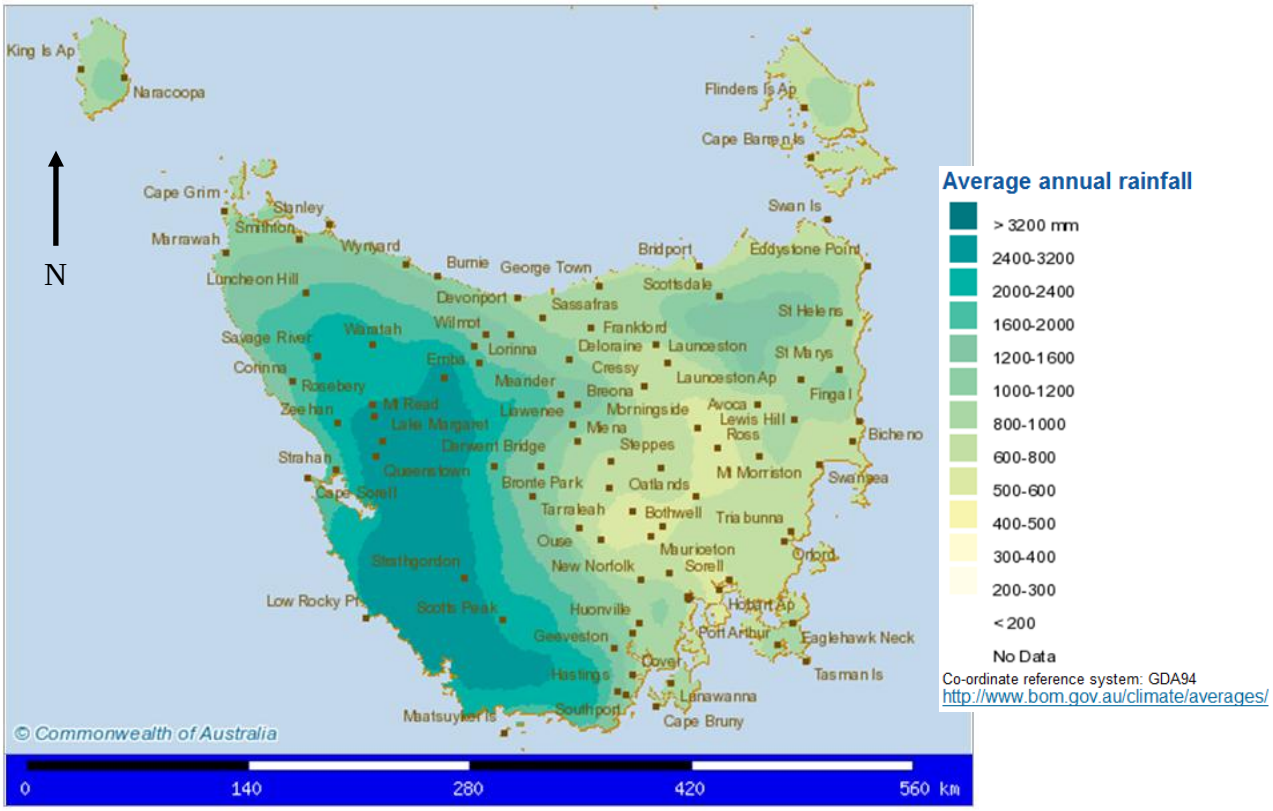


**Figure 1-1, Annual rainfall distribution map of Tasmania generated using "Climate Data Online" using "all available data" (Bureau of Meteorology, 2011).**

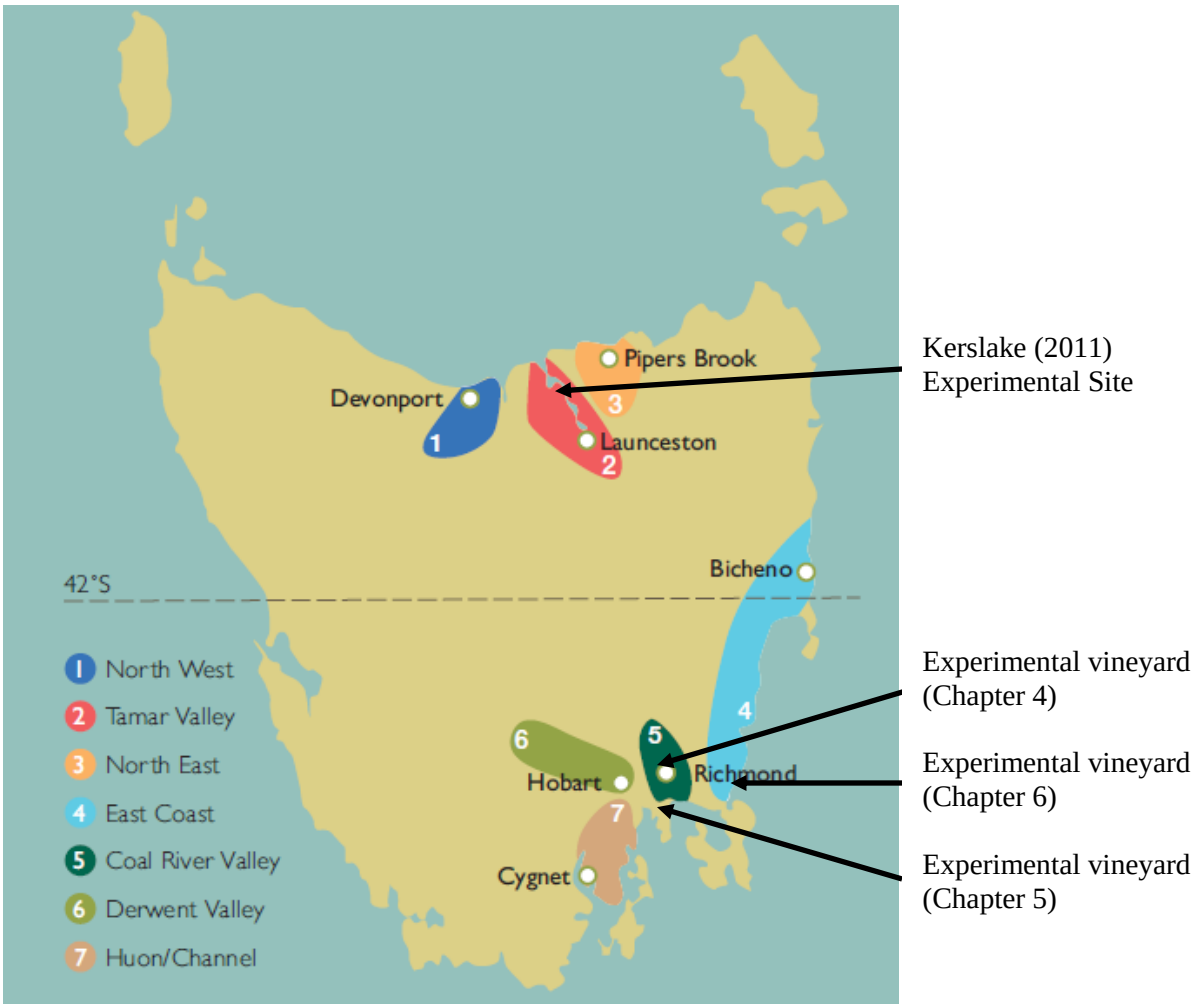


**Figure 1-2, The seven wine regions of Tasmania (WIT, 2009). Experimental vineyards in the current study fall within regions 4 (East Coast) and 5 (Coal River Valley)**

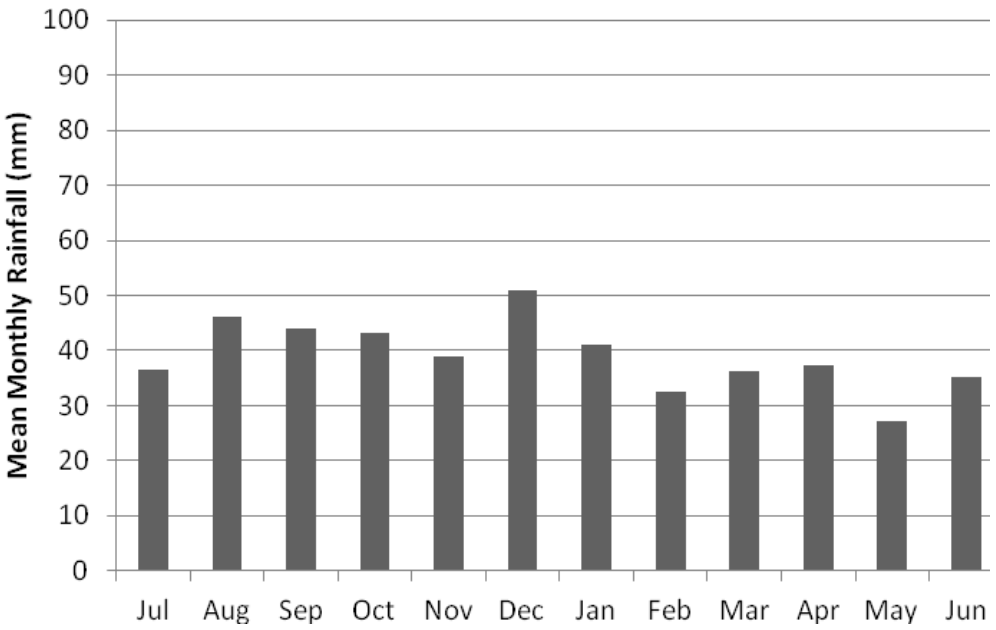


**Figure 1-3, Mean monthly rainfall for BOM (Australian Bureau of Meteorology) Hobart Airport weather station (Station number 094008) from 1981 – 2010.**

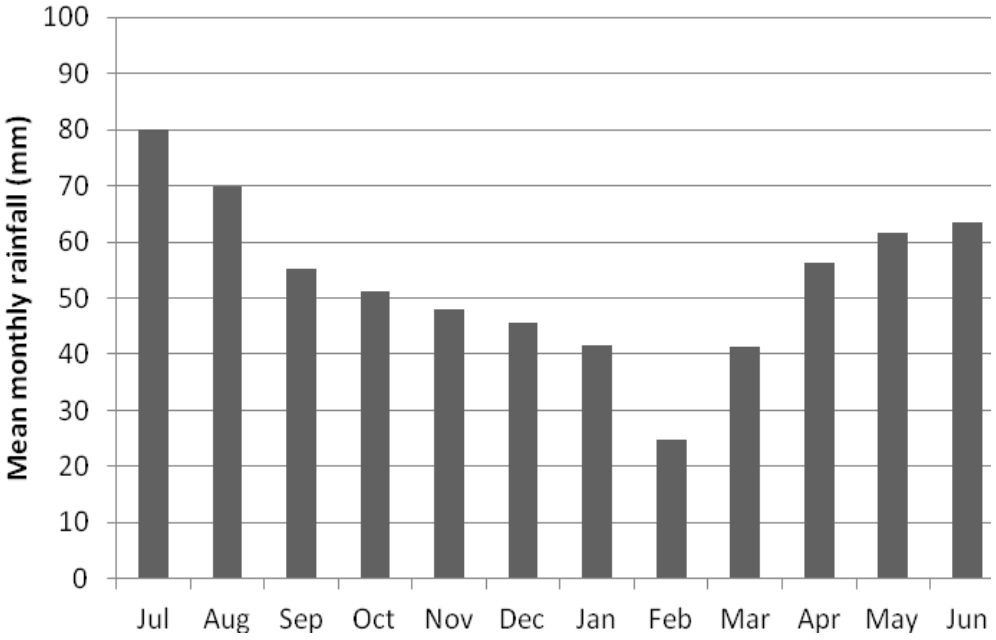


**Figure 1-4, Mean monthly rainfall for BOM (Australian Bureau of Meteorology) Low Head weather station (Station number 091923) from 1981-2010.**

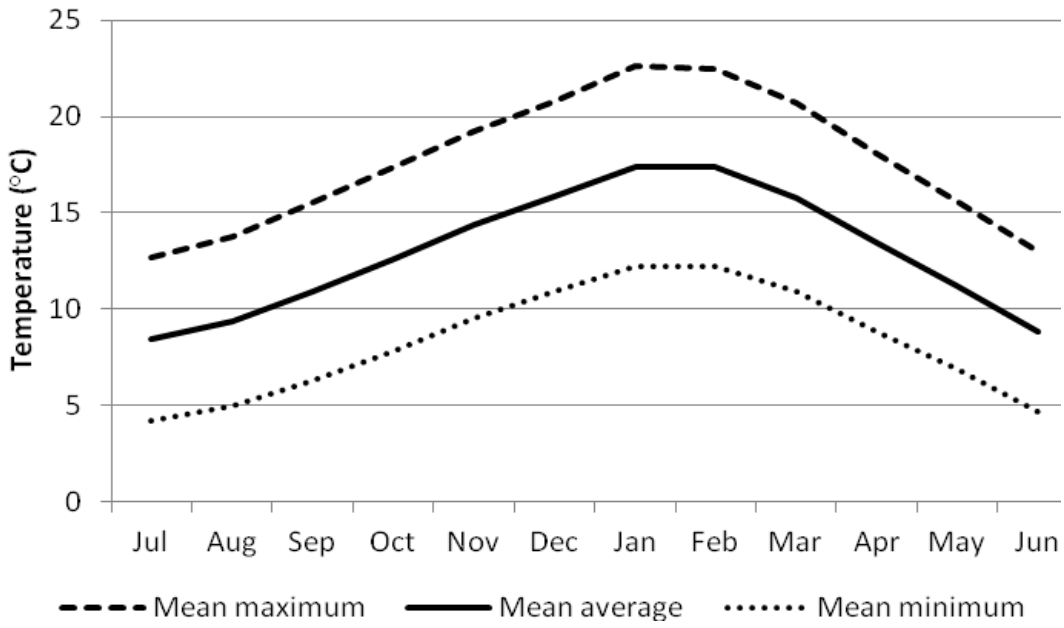


**Figure 1-5, Mean monthly average, maximum and minimum rainfall for BOM (Australian Bureau of Meteorology) Hobart Airport weather station (Station number 094008) from 1981 – 2010.**

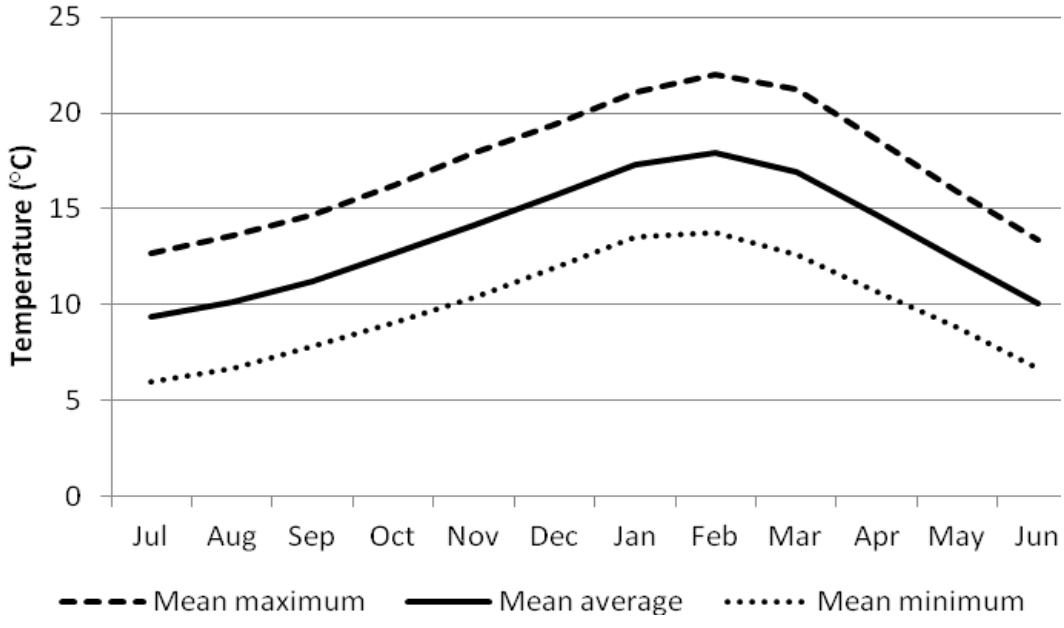


**Figure 1-6, Mean monthly average, maximum and minimum rainfall for BOM (Australian Bureau of Meteorology) Low Head weather station (Station 091293) from 1981 – 2010.**

Geologically Tasmania's landscape is diverse (Figure 1-7). The soils under greatest viticultural development in Tasmania are brown and red soils developed from both Jurassic dolerite and Tertiary basalt, though vineyards have been planted on a diverse range of soils (Doyle & Farquhar, 2011). The variation described by Doyle and Farquhar (2011) in vineyard soil type is present between and within regions and within individual sites. For example the Tamar Valley region has a wealth of different soil types ranging from Vertisols; medium to heavy "shrink swell" clay soils formed on Jurassic Dolerite, silty clay Acidic Grey Kandosol and duplex, Bleached Grey Kurosols formed on Permian mudstone, duplex Brown Kurosols such as that described in (Kerslake, 2011) and duplex Bleached Brown Chromosol formed on tertiary sediments (Doyle & Farquhar, 2011). Conversely Ferosols formed on Tertiary Basalt predominate in the Pipers Brook region in the north east of the state. A similar diversity in soil type is seen in southern and eastern Tasmania where the current study was carried out. Viticulture in the four regions however is predominately situated on the north to eastern facing slopes of Jurrasic Dolerite features which dominate the southern and eastern parts of the Tasmanian landscape (Figure 1-7).

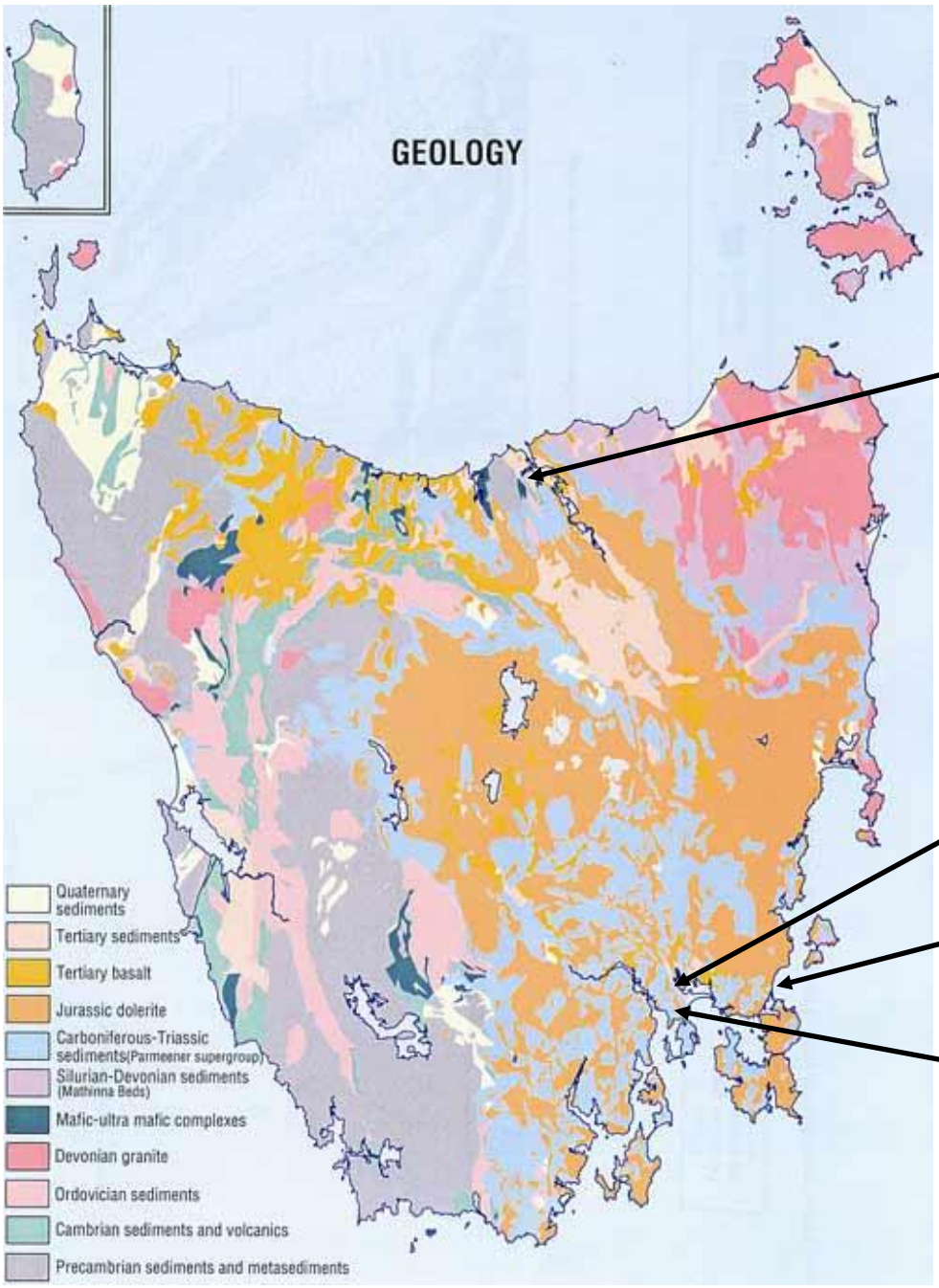


**Figure 1-7 Geology of Tasmania, displaying a diverse range of parent material (Forest education foundation, 2010).**

## 1.3 Pinot Noir

*Vitis vinifera* cv. Pinot Noir is a key industry focus for Tasmanian and southern Australian cool climate wine production. Production of Pinot Noir in the Tasmanian

wine industry accounts for 46 percent of production, the next closest in Chardonnay which accounts for 26 percent (Wine Industry Tasmania data, 2009). Pinot Noir is also the seventh most planted variety on a national scale and the fourth most planted red wine grape variety behind Shiraz (Syrah), Cabernet Sauvignon and Merlot (AWBC, 2010) constituting 9% of the national plantings.

Tasmania is recognised for producing both premium sparkling and table wines from Pinot Noir. The focus of this thesis will be for table wine production in both discussion and interpretation of results. One of the "Noble" varieties, Pinot Noir is planted in many locations around the world. Cooler climates tend to dominate the regions recognised for producing the most esteemed wines made from this variety. Pinot Noir is often described as fickle, challenging to produce and make and difficult to "get right" (Robinson, 2006). The red wines of Burgundy provide the benchmark to which all other Pinot Noir wines are compared and are those that most producers aim to emulate in premium and super premium styles. There is a need for localised research on Pinot Noir, due to the lack of literature pertinent to viticultural management of the variety in a cool maritime climate (Kerslake, 2011). As highlighted by Heazlewood (2005) from which this study follows on, variation in climate from vintage to vintage may also have an impact on both yield and fruit composition. Specific reference is made to the influence of bunch size to impact grape composition as a function of fluctuating berry number and for further investigation of bunch "compactness" to influence grape composition and therefore quality.

## 1.4 Objective

The purpose for study was to provide information and recommendations to industry that assist in the maintenance and improvement of the 'intrinsic quality' in Pinot Noir table wines. It follows on from several key areas identified as requiring further study by Heazlewood (2005).

The objectives of this study were to:

- Investigate the influence of the industry practice of pre-veraison leaf removal in the fruiting zone on a vertically shoot positioned canopy of Pinot Noir to make recommendations to industry based on:
  - The impact on yield components within current and subsequent seasons.
  - The potential to manipulate fruit composition for winemaking.
  - For the assessment of bunch morphological factors over multiple seasons for use in yield prediction.
- Investigate the influence of bunch exposure in isolation of source and sink impacts during berry ripening to optimise fruit composition.
- Investigate the potential for fruit composition of Pinot Noir to be influenced by bunch compactness.

The hypotheses of this thesis were:

- Does fruiting zone defoliation lead to an improvement in fruit composition?
- Does increasing severity of fruiting zone defoliation lead to a reduction in yield components?
- Does measuring bunch length or primary branch number of the rachis predict bunch size at harvest?
- Does increased shading of bunches lead to a negative influences on fruit quality?
- Does increased shading within the bunch lead to a negative impact on grape composition?

# Chapter 2 - Background

## 2.1 Phenology

The growth cycle of the grapevine is extended over two seasons (Figure 2-1) with development of reproductive and vegetative organs spread over a two year cycle (Pearce & Coombe, 2004). Development of vegetative shoots occurs in latent buds in the previous spring and followed by a period of dormancy from late summer through to budburst in the following spring. Following budburst rapid shoot extension occurs. Cessation of shoot growth may be observed between bunch closure and veraison (Vasconcelos & Castagnoli, 2000). In Tasmania cessation of shoot growth regularly occurs following berry set and often coincides with the lag phase of berry growth. It has been observed by the author that leaf fall in Tasmania may begin during berry ripening particularly in stressed vines and complete fall often occurs in the period immediately following harvest.

Induction and initiation of inflorescence primordia occur during spring and summer of the previous year (Pearce & Coombe, 2004). Immediately following bud burst in spring, rapid inflorescence growth occurs (Mullins et al., 1992), with the most rapid growth immediately preceding flowering (Shavrukov et al., 2004). In Tasmania flowering in Pinot Noir commonly occurs through the month of December and may proceed over an extended period (Heazlewood, 2005).

Following fruit set, development of the berry can be divided into two major sigmoidal growth phases (Figure 2-2), separated be a lag phase in berry growth (Coombe and McCarthy, 2000, Ollat et al., 2002, Pearce and Coombe, 2004). While in the past it has been common to divide berry development into three phases based on berry growth (Percival et al., 1994b), there is a growing trend in literature for a 2 stage description of berry growth; stage 1, berry formation, and stage 2, berry ripening (Coombe, B. G. & McCarthy, 2000; Ollat et al., 2002) (Figure 2-2). Berry formation begins with rapid cell division in pericarp tissue, the amount and direction of which determines berry shape and size to a large degree (Coombe, B. G. & McCarthy, 2000).

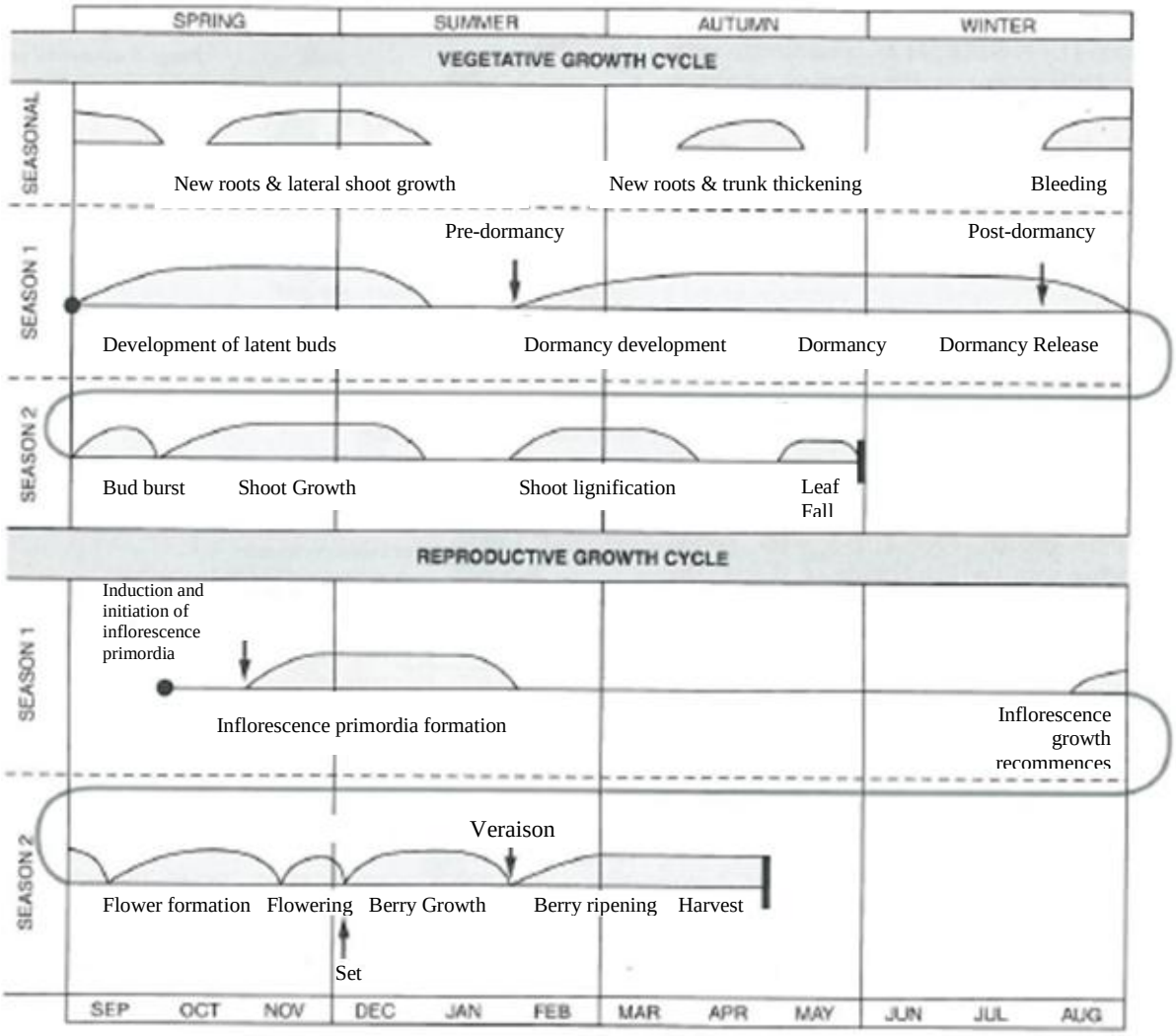


**Figure 2-1 Biennial growth cycle of grapevines displaying both vegetative and reproductive growth cycles differentiated in relation to the months of the year in the Southern Hemisphere. Cycles begin at the circle and end at the rectangle. (Modified diagram from Pearce and Coombe, 2004)**

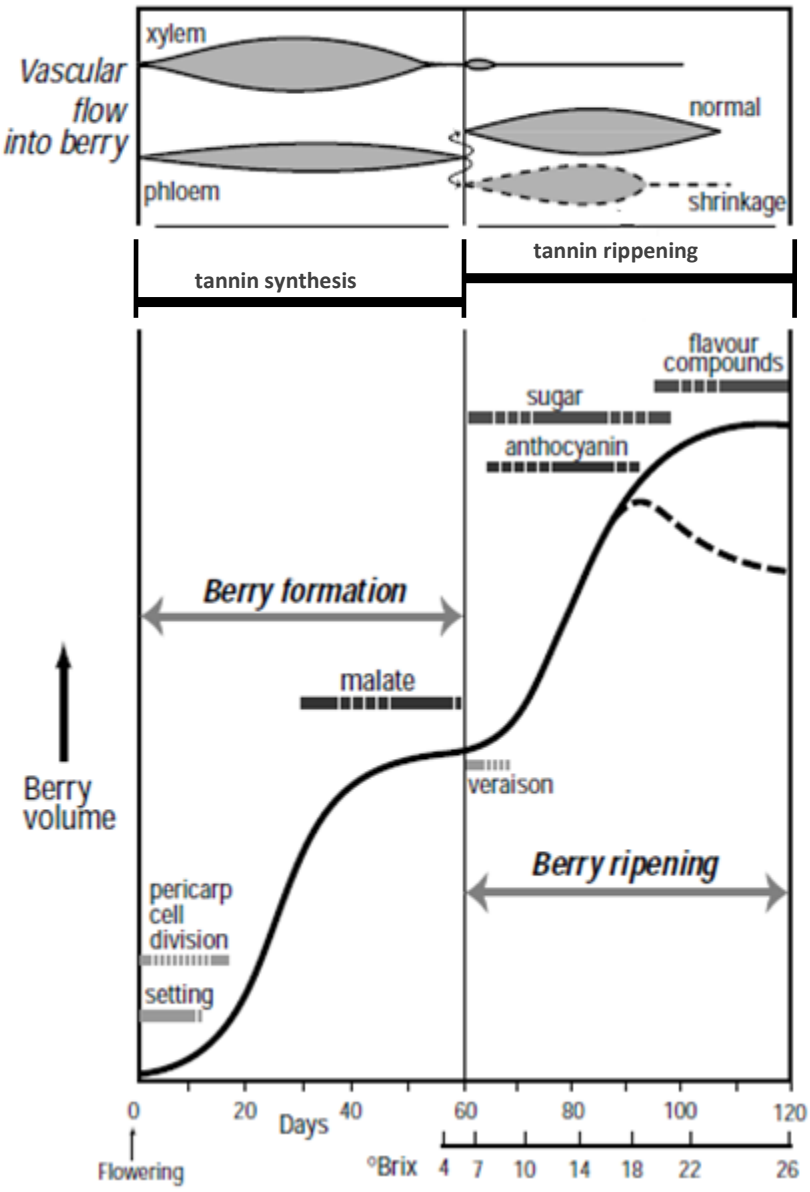


**Figure 2-2, Modified notional diagram describing the development of berries from flowering to harvest. Displaying differences in development for Muscat (solid lines) and Shiraz (dashed lines). The berry volume is an idealised curve against days after flowering and juice °Brix and shows the berry shrinkage and phloem blockage of Shiraz starting at about 18-20°Brix; Accumulation of key berry compositional parameters are included and modified to include tannin synthesis and ripening (Coombe, B. G. & McCarthy, 2000).**

Pericarp cell division slowly changes to cell enlargement, which later slows during the lag phase of development (Coombe, B. G. & McCarthy, 2000). During berry formation tartrate and later malate accumulate (Jackson, DI & Lombard, 1993) and tannin biosynthesis occurs (Kennedy et al., 2006). Water influx during berry formation is derived from both the xylem and phloem, xylem function of the berry is interrupted and discontinued shortly after the beginning of veraison solute accumulation (Creasy et al., 1993).

The start of the second phase (berry ripening) is termed veraison, which constitutes the collective onset of sugar accumulation, berry softening and berry colouring (Coombe, B. G. & McCarthy, 2000). Anthocyanin accumulation and tannin ripening occurs (Kennedy et al., 2006), malate is metabolised (Lakso & Kliewer, 1975), sugar accumulates in the skin and flesh and potassium accumulates in the skin (Coombe, B. G. & McCarthy, 2000). The accumulation of flavour and aroma compounds is believed to occur during the latter stages of berry ripening and has been termed Engustement (Coombe, B.G. & McCarthy, 1997).

## 2.2 Yield, quality and vine balance

The concept of vine balance is a key consideration for the viticulturist. Due to the biennial cycle of growth (Figure 2-1) a balance between vegetative and reproductive phases of growth need to be achieved in order to maintain yield and quality of wine grapes (Howell, 2001).

The relationship between yield and fruit and wine composition has been studied extensively in *Vitis vinfera*. A far greater wealth of literature exists for warm climates (Chapman et al., 2004; Hummell & Ferree, 1998; Keller et al., 2008; Kliewer & Dokoozlian, 2005; Kliewer et al., 2000; McCarthy et al., 1987; Smart, R. E., 1985; Smart, R. E. et al., 1990) than does for cool climates (Heazlewood, 2005; Kerslake, 2011; Petrie et al., 2000a, 2000b, 2000c).The present study follows on from the work of Heazlewood (2005), examining the potential to stabilize fluctuating yields of Pinot Noir in Tasmania. This study reported an increase in anthocyanin concentration paralleled by an increase in yield in vines pruned to 20, 30 and 40 nodes compared

vines pruned to 10 nodes. The observation that anthocyanin concentration increased at yields greater than 6 tonnes per hectare, and therefore fruit quality, was described as being contrary to the industry recommendation and benchmark yield of six tonnes per hectare. It has however been suggested that independently, yield is not a good indicator of wine quality and the ratio of fruit yield to pruning weight (Y:P) was a far better indicator of wine quality (Bravdo et al., 1985).

The relationship between yield and vegetative growth was first investigated during the early 1900's (Ravaz, 1903). The "Ravaz Index" was established which compared yield and pruning weight of one year old wood (Ravaz, 1903, 1906). Practically a retrospective view of balance in a particular season is less appropriate than one which enables us to predict or set a yield target in the following vintage (Howell, 2001). Partridge (1925) suggested that yield in a particular season was a function of the pruning weight from the previous season, which the author termed "The Growth-Yield Relationship" or yield to pruning weight ratio (Y:P). This was then expanded upon by numerous pruning trials between the 1940's to 1960's which suggested the amount of cane growth of the previous season, determined the yield capacity in the following season, with increasing vegetative growth leading to increasing yield capacity (Shaulis et al., 1966). It has been suggested that for varieties with small bunches such as Pinot Noir a Y:P ratio between of 3-6 is optimal (Kliewer & Dokoozlian, 2005) and that this ratio should possibly be lower in cool climates (Dry, PR et al., 2004).

The leaf area to yield ratio (LA:Y) has also been highlighted for use in describing the balance between vegetative and reproductive growth (Jackson, DI & Lombard, 1993). LA:Y is the ratio between exposed leaf area (source) and bunches (sink) (Kerslake, 2011). LA:Y is inversely related to Y:P due to the close correlation of pruning weight and leaf area (Bravdo et al., 1985; Gal et al., 1996). Typical ratios of balanced canopies range between 5 to 15 $cm^2/g$ (Dry, PR et al., 2004). Within the Tasmanian industry it has been suggested by growers that 1 $m^2$ of foliage will ripen 1 kg of crop, which equates to a LA:Y 10 $cm^2$ per gram of fruit which is well within the range suggested by Dry et al., (2004). LA:Y is however less time efficient and less practical to measure than Y:P (Kerslake, 2011).

In Tasmania, yield tends to fluctuate from year to year based on seasonal weather patterns around flowering, which may influence fruit set (Heazlewood, 2005). As a result of the fluctuation in seasonal weather events, the effectiveness of using Y:P as a tool to benchmark yields and quality is reduced in the Tasmanian climate (Kerslake, 2011). It has been suggested that pruning to higher bud numbers and adjusting yields following fruit set may be a suitable way of achieving yield benchmarks (Kerslake, 2011).

Practically vine balance may be achieved by the viticulturist by taking into account climatic (Heazlewood, 2005) and geological influence (Deloire et al., 2004) and by interventions during the growing season through pruning, yield manipulation, trimming, leaf removal, vine nutrition and irrigation (Howell, 2001).

## 2.3 Yield Components

Yield in *Vitis sp.* can be expressed as a function of the number of vines per hectare, the number of nodes per vine, the number shoots per node, the number of bunches per shoot, the number of berries per bunch and the weight of berries per bunch (Kerslake, 2011). More simplistically yield can be expressed as "the product of the number of berries present at harvest and their average size" (Dunn & Martin, 2000).

As identified by Heazlewood (2005) the key determinate of yield variation in cool climate Pinot Noir is variability in bunch size as a function of berry number. Variability in mean berry number is greater than berry size, from bunch-to-bunch, from vine-to-vine and from season-to-season and hence, berry number explains more of the vine-to-vine variation in yield. Berry number is determined by (i) the number of flowers present at anthesis, (ii) the proportion of these that set successfully and, (iii) the percentage of berries that remain attached until maturity (Dunn & Martin, 2000).

## 2.4 Fruit composition

The composition of the grape berry varies at any point during berry development up until the point of harvest. Developmental changes which occur during berry formation and during ripening need to be considered when discussing the composition of harvested fruit. During berry formation the principal organic compound being accumulated is malic acid. Grape berry ripening is characterised by the accumulation in the berry flesh of hexose sugars, predominately glucose and fructose (approximately 99%), (Hamilton & Coombe, 2004) and in the skin of sugars, potassium and phenolics (Coombe, B. G. & McCarthy, 2000).

Over 700 compounds are known to exist in grape juice (Hamilton & Coombe, 2004). Water and sugar are the main components of grape berries at harvest (Jackson, DI & Lombard, 1993). The percentage of water in the berry at harvest is largely determined by transpiration of berries (Hamilton & Coombe, 2004) as a function of Xylem discontinuity during ripening (Creasy et al., 1993), and during the later stages of ripening, due to the impedance phloem sap flow (Coombe, B. G. & McCarthy, 2000). The aroma compounds found in Pinot Noir grapes and wines are not well understood (Fang & Qian, 2005), but are thought to develop late during berry ripening in red wine varieties such as Shiraz (Coombe, B. G. & McCarthy, 2000).

Up to ninety percent of the soluble solids in grape juice are hexose sugars, the remainder consists of organic acids, phenolics, polysaccharides, pectins, potassium, proteins and other compounds (Hamilton & Coombe, 2004).

The major acids found in grapes are tartaric and malic acids, with citric acid and a number of others making up the balance (Hamilton & Coombe, 2004). During early berry growth tartrate is accumulated in high concentrations, while malate accumulation occurs most rapidly in the period immediately preceding the beginning of veraison. Titratable acidity declines following veraison, due to decreases in malate as a result of berry respiration (Lakso & Kliewer, 1975), and dilution by water, as berries expand and acids are converted to salts (Jackson, DI & Lombard, 1993).

Phenolics found in grapes, and in turn in wine, contribute to the flavour, colour and health benefits of red wine (Mazza, G. & Miniati, 1993). Phenolics are an important consideration for red wines as they contribute to elegance, softness and depth (Jackson, DI & Schuster, 1987). The phenolic composition of grapes has been shown to vary as a result of both seasonal and viticultural influence (Kennedy, 2008; Kennedy et al., 2006).

There are two main groups of phenolics, flavanoids and nonflavanoids (Allen, 1997; Kennedy et al., 2006). Flavanoids (Figure 2-3) are found in solid parts of the grape and in the bunch stem and non-flavanoids are found in the juice and pulp of grapes (Kennedy et al., 2006). The most important flavanoids in terms of sensory consequence for fruit and wines are anthocyanins, flavan-3-ol monomers and proanthoynadins, all of which exhibit the same base chemical structure (Souquet et al., 1996). Hyroxycinnamic acids are the most abundant non-flavanoids found in grapes, they are important to the colour of white wines and are found in similar amounts in both white and red wines (Kennedy et al., 2006).

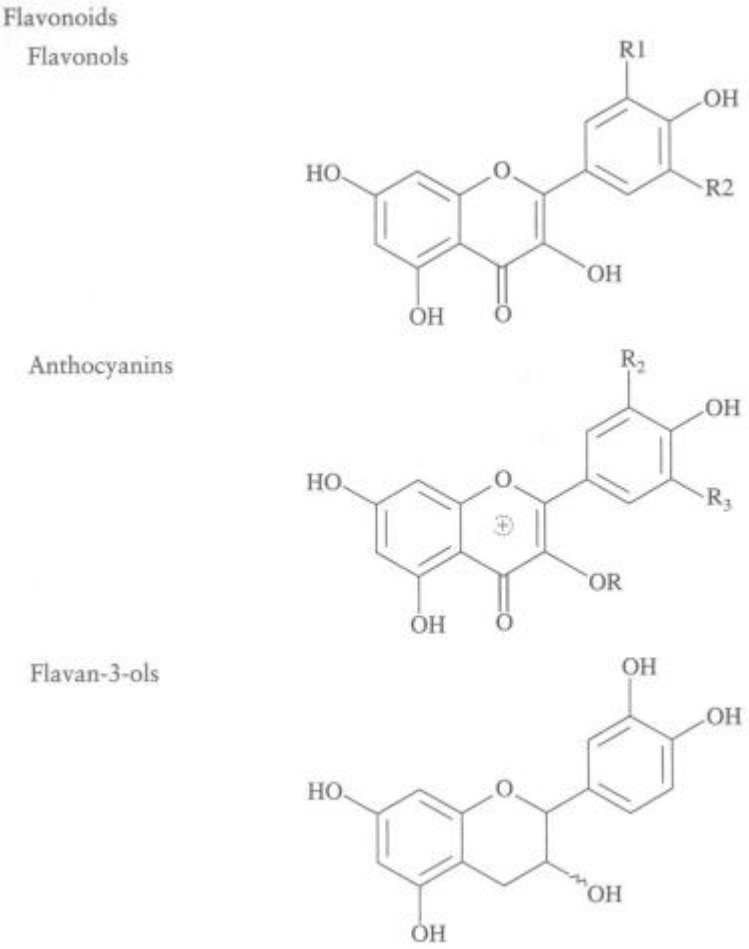


**Figure 2-3, Flavanoid ring structure (Jackson, RS, 2000) cited in (Kerslake, 2011).**

Anthocyanins are largely responsible for the red colouration of grapes and contribute to the red colour of wine (Allen, 1997; Boulton, 2001; Kennedy et al., 2006). Most commonly located in the skins of grapes (Kennedy et al., 2006) they may also be located in the pulp as well as in the flesh of some varieties (He et al., 2010). Anthocyanin biosynthesis occurs during berry ripening, stage 2 of berry growth (Figure 2-2), following the commencement of veraison (Kennedy et al., 2006). It has been established that Pinot Noir contains only non-acylated anthocyanins (Cortell & Kennedy, 2006; Heazlewood, 2005; Mazza, G. et al., 1999) which is unique in *Vitis vinifera*.

Two pathways have been proposed for the biosynthesis of anthocyanins which are enzyme moderated (Boss et al., 1996a, 1996b). The F3'H pathway results in the formation of cyanidin-3-glucoside and peonidin-3-glucoside and the F3'5'H pathway results in the formation of petunidin-3-glucoside, delphinidin-3-gluoside and malvidin-3-glucoside (Boss et al., 1996a).

The flavan-3-ol monomers are thought to contribute to the bitterness and possibly astringency of red wine (Kennedy et al., 2001). Synthesised predominately in the seed coat (Downey et al., 2004) but also in grape skins during berry development (Figure 2-2), compounds such as (+) catechin and (-) epicatechin are the subunits that polymerise to form tannins.

Proanthocynadins (condensed tannins) are flavanoid based compounds which are formed be the polymerisation of the above flavan-3-ol monomers. Found in skins, seeds and bunch stems at harvest, it is believed that proanthocynadins are formed during stage 1 of berry growth and that "ripening" of this group of compounds occurs during stage 2 following veraison (Figure 2-2) (Kennedy et al., 2006).

# Chapter 3 - General methods

## 3.1 Climate

Climate description is based on observations taken from the "Hobart Airport" Bureau of Meteorology (BOM) station: 09400, 42°50'24"S, 147°30'34"E, elevation 2m. The three trial sites of chapters three, four and five were located at 7.4 km, 8 km and 27 km from this location respectively. This weather station was concluded to be the most appropriate for data collection due to proximity to trial sites, rainfall distribution as discussed in chapter one and also as a factor of the length and quality of observation.

There is a need to provide recommendations for management which are directly applicable to the local environment. 30 year average rainfall observations suggest that the southern sites described in the present study have an annual rainfall of approximately 469 mm per year. Rainfall is distributed evenly throughout the year with the highest averages recorded for the month of January (Figure 1-3). As a result of the unpredictable nature and frequency of rainfall events (chapter one), observed daily totals for Hobart Airport can vary significantly and account for large proportions of the annual total rainfall. For example the highest daily total (64.2mm) was observed on the 27$^{th}$ of December 1993 with other high daily totals distributed randomly throughout the year. January and February have the lowest number of mean days of rain with 9.4 days and 7.8 days respectively. The highest mean days of rain occurs during September at 14.3 days. Below average rainfall and growing season rainfall was recorded for the 2005, 2007 and 2008 vintages and above average rainfall was recorded in 2006. Similar trends in rainfall were observed within years and between the north and the south (Table 3-1).

Smart and Dry (Dry, PR & Smart, 1988) suggest that GDD in the viticultural regions of Australia range between 3136 in Roma, 2084 in the Riverland, 1715 in the Barossa, 1432 in Coonawarra and 1020 in Launceston (Tamar River). These figures do not include the month of September, which is included in the present study. Different cool climate viticulture growing regions can exhibit large differences of up to 400

growing season degree days (GDD) (Howell, 2001). Summation data was calculated between the months of September to December, degree days to flowering (FDD) and between the months September to May, growing degree days (GDD).

Day degrees were calculated as follows

$$\text{Day Degree} = \frac{\text{Temp}_{max}+\text{Temp}_{min}}{2} - \text{Temp}_{base}$$

Where $T_{base} = 10$ as for (Dry, PR & Smart, 1988)

Data presented in Table 3-1 suggests heat accumulation in the CRV region was warmer than the long term average in all experimental seasons and warmer than the TR in two of the three experimental seasons reported in Kerslake (2011). Deviation from the average in both regions was not necessarily consistent between regions in particular years.

**Table 3-1, Climatic records over the four experimental seasons of the current study for Hobart Airport (BOM station 094008). Blue and red denote deviation from the long term average.**

| | 2005 | 2006 | 2007 | 2008 | Hobart Airport (1981-2010) |
|---|---|---|---|---|---|
| Mean January temperature (°C) | 17.7 (+0.3) | 17.6 (+0.2) | 18.2 (+0.8) | 19.0 (+1.6) | 17.4 |
| Mean February temperature (°C) | 16.7 (-0.7) | 17.8 (+0.4) | 18.9 (+1.5) | 16.2 (-1.2) | 17.4 |
| Rainfall (mm, Sept – May) | 285.8 (-65.2) | 417.4 (+66.4) | 323.4 (-27.6) | 270.2 (-80.8) | 351.0 |
| Rainfall (mm, Jul – Jun) | 413.6 (-55.7) | 480.4(+11.1) | 391.0 (-78.3) | 357.4 (-1.2) | 469.3 |
| Degree Days (Sep-May GDD, $T_{base}$ =10°C) | 1260.1 (+81.6) | 1285.3 (+106.7) | 1373.9 (+195.4) | 1325.3 (+146.7) | 1178.5 |
| Degree Days (Sep-Dec, FDD, $T_{base}$ =10°C) | 472.6 (+53.1) | 561.7 (+142.3) | 448.5 (+29.1) | 538.5 (+119.0) | 419.5 |

Field sampling, bunch structural measurement and fruit compositional measurement were consistent across the entire study except where specified in Chapter 4. It was a conscious decision not to destructively sample treated vines before commercial harvest because of issues associated with within bunch variability (May, 2000) and the potential to influence source sink interactions of the vine. All experiments were conducted in commercial vineyards which also contributed to the chosen sampling and measurements methods.

## 3.2 Field sampling

In all experiments bunch number was recorded at the time of harvest. Harvest was carried out by hand and timed to correspond with commercial harvest as per the recommendations of the management of each site. A random sub sample of 10 bunches was taken from each experimental plot (defined as an individual treatment within a replicate) using a list of random numbers. The fruit was then placed in re-sealable plastic bags and then on ice for transportation. Samples were frozen at -18°C until processing was carried out.

## 3.3 Bunch structural measurement

Sub-samples were processed while frozen. Bunches were individually weighed and the bunch weight was recorded. The length and width of bunches were measured to the widest possible point along both horizontal and vertical axis. Berries were then removed from the rachis by hand and counted for each bunch of the sub-sample; recording berry number for six of the ten bunches which were randomly selected. Where bunches were damaged or not intact, only sound bunches were chosen to record berry number per bunch.

The number of primary branches from the rachis was recorded after visual examination. Primary branch number was defined as the number of branches in the main stem of the rachis (Plate 3-1) which lead to further braches in the bunch structure and bearing two or more berries (May, 2000).

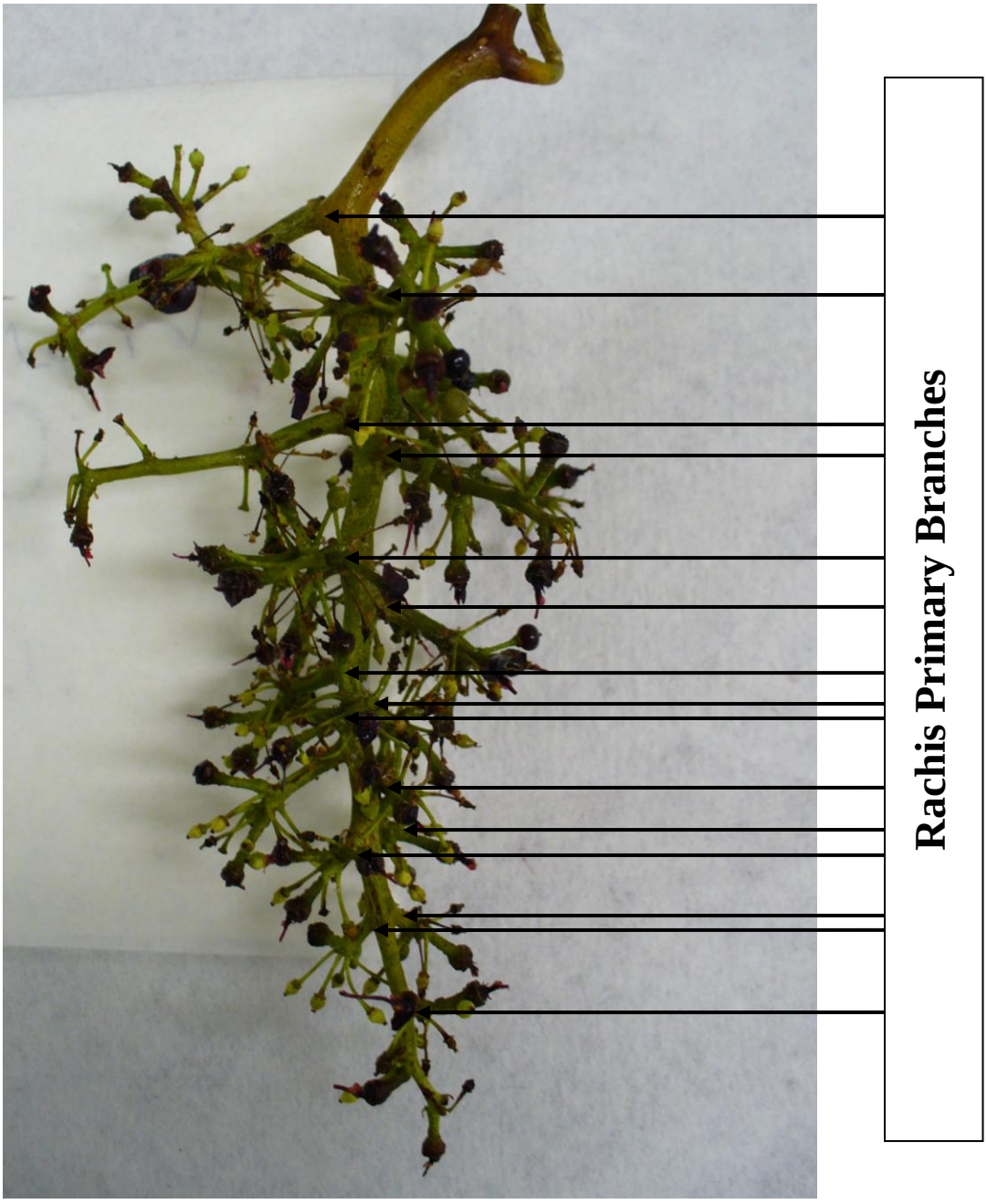


**Plate 3-1, Typical primary branching of the rachis *Vitis vinifera* cv. Pinot Noir.**

## 3.4 Fruit compositional measurement

Total anthocyanin concentration of samples was measured according to AWRI industry standard methods, CRCV (2006).

One of the two, 100 g subsamples was homogenised using an Ultra Turrax RT25 basic and an IKA S25N-18G Dispersing Tool. Samples were macerated for 30 seconds in a 200 ml plastic beaker. The homogeniser was then stopped, the macerated tissue scrapped from the outside of the head, returned to the sample which was then macerated for a further 30 seconds, ensuring that all seeds were macerated and that all homogenate was scraped from the shaft and head on completion and returned to the sample.

One gram of this homogenate was then transferred to a 10 ml plastic centrifuge tube and the weight of the homogenate (homogenate weight) was recorded to two decimal places. 10 ml of 50% v/v aqueous ethanol was then added to the tubes and mixed by inverting every 10 minutes for a period of one hour. The mixture was then centrifuged at 1800 g for 10 minutes using a Beckman Coulter Avanti J-301 Centrifuge. The supernatant is now termed the extract. Its final volume was estimated as 10.5ml, as all extract sample weights weighed between 0.95 and 1.05g as per the standard method.

200 µL of the extract was then transferred to a 4ml acrylic cuvette of 10 mm path length. 3.8 ml of 1.0M HCl was then added to the extract. Cuvettes were then covered with *Parafilm* and mixed by inverting several times and allowed to incubate for between 3 and 24 hours. The absorbance of the acidified diluted extract was then measured at 520 nm (A520) using a 1.0 M HCl blank and S2000 WDA Lightwave, diode-array UV/Vis spectrophotometer.

Duplicates were run every 10 samples to estimate the laboratory accuracy for the method. The accuracy of the laboratory was determined to be ±2.01%.

Total anthocyanin and anthocyanin per berry were then calculated using the following method:

$$\text{Total anthocyanin per gram (mg/g)} = \frac{\text{A520 x *DF x final extract volume (mL) x 1000}}{\text{500 x 100 x homogenate weight (g)}}$$

*Dilution factor (DF) is the level of dilution upon addition of 3.8 ml of 1.0 M HCl which in this case is 20.

Total anthocyanins per berry were calculated by multiplying total anthocyanin per gram by mean bunch weight and dividing by the number of berries.

Total anthocyanin calculations are based on the absorbance of a 1% w/v (1 g/100 mL) solution of malvidin-3-glucoside, 10 mm path length (Somers & Evans, 1974). As per industry standards (CRCV, 2006). Malvidin-3-glucoside is one of the five major anthocyanins in *Vitis vinifera* Cv. Pinot Noir grapes (Cortell & Kennedy, 2006; Heazlewood, 2005; Mazza, G. et al., 1999). Malvidin-3-glucoside has been shown to be the major anthocyanin accumulated in Pinot Noir (Cortell & Kennedy, 2006) and hence it is appropriate results are expressed in malvidin-equivalents for comparative purposes only as per (CRCV, 2006).

The second sub-sample was allowed to thaw to room temperature and berries were then pressed by hand and the free run juice separated through a gauze for determination of soluble solids, pH and titratable acidity which is expressed as Tartaric acid equivalents (Jackson, DI & Lombard, 1993).

Soluble solids were measured using an optical hand held refractometer. pH and Titratable acidity (TA) were measured using a Metrohm SM Titrino 702, autotitrator, titrating against a 10ml volume of 0.1 M NaOH standrard.

Mean berry weight was calculated as bunch weight divided by berry number for selected bunches from the subsample.

Yield was calculated by multiplying bunch weight and bunch number to give yield per vine which was then multiplied by the number of vines per hectare and the units converted to tons per hectare (t/Ha). Average treatment yields for all experiments across all years ranged between 5.24 and 10.11 tonnes per hectare.

Statistical design and method is detailed in individual chapters.

# Chapter 4 - The influence of fruiting zone defoliation on fruit composition and yield

## 4.1 Introduction

Studies have described the effects of defoliation on the vegetative biology of grapevines with particular focus on photosynthetic capacity and partitioning of carbohydrates to vegetative and reproductive organs. Examination of the literature indicates that the vegetative response of the *Vitis* sp. to defoliation is dependent on a number of factors, the most relevant being the timing, level and environmental stimulus.

Removal of leaf area directly affects the energy produced by source leaves of the vine and therefore may influence growth of both reproductive and vegetative organs and changes the carbohydrate metabolism of the plant (Heuvel et al., 2005). Sugars are the main source of energy in the grapevine (Caspari et al., 1998). Sink organs are those which attract sugars, for example the growing shoot, developing leaves, inflorescences or bunches (Lebon et al., 2008). Source organs are those that synthesise and export sugars, namely from converted carbohydrate stored in the roots and wood early in growth and later from the leaves (Lebon et al., 2008). Developing leaves and inflorescences act as sinks up until the point of fruit set, whereby developing bunches become the dominant sink (Mullins et al., 1992). Factors which influence the number of sinks or sources directly impact on sugar transport around the plant for example defoliation (source removal), shoot trimming (source removal) or bunch removal (sink removal) (Williams, 1996).

Defoliation has the effect of removing leaf area available for production of photosynthate and may therefore reduce sink strength. A leaf area of between 7-14 $cm^2$ is required to adequately ripen 1 gram of fruit (Jackson, DI & Lombard, 1993). Delayed ripening has been observed as a consequence of defoliation by shoot trimming of field grown Sauvignon blanc over a range of 17-21 $cm^2/g$ (Petrie et al.,

2003) which supports the conclusion, that this ratio may vary between varieties or due to climatic influence (Dry, PR et al., 2004; Kliewer & Dokoozlian, 2005).

Defoliation has been shown to affect root growth and accumulation of root dry matter. Partial defoliation across the whole vine has been shown to increase root growth by increasing root density, in particular when the treatment is applied at pea size (Hunter & Le Roux, 1992). Pre-Harvest defoliation has also been shown to increase the sugar concentration of roots. Petrie et al., (2000b) found that various levels of defoliation in potted Pinot Noir vines lead to a proportional decrease in root weight but caused no difference in partitioning of dry matter between above and below ground parts of the vine. The author reported a "highly conserved relationship" for dry matter partitioning between above and below ground parts of the vine.

It has been described that the grape vine may compensate for the photosynthetic area removed by defoliation by either subsequent growth resulting in increased leaf area, or as a result of increased photosynthetic rates in response to altered source sink relationships. In potted Pinot Noir vines, Petrie et al., (2000b) observed no effect on shoot extension except in cases of extreme defoliation in both bearing and non-bearing vines. New leaf area across bearing and non-bearing vines was reduced by defoliation indicating that vines were unable to compensate for defoliation. Total leaf area was less on cropped, than uncropped vines. Non-bearing vines responded to defoliation by reducing internode length which resulted in the production of more leaves and a larger leaf area (Petrie et al., 2000b). In field experiments, various authors have commented on the ability of the grapevine to produce additional leaf growth in order to compensate for defoliation. Soil water availability is likely to have large influence on the ability for additional growth (Candolfivasconcelos et al., 1994). In field grown Cabernet Sauvignon, Hunter and Le Roux, (1992) observed no difference in shoot extension in response to defoliation. Both studies however observed a reduction in dry matter accumulation in response to defoliation that was attributed to the observed smaller, thinner shoot growth. Lateral shoot length has been reported to increase in response to partial defoliation (Vasconcelos & Castagnoli, 2000), especially when defoliation is carried out earlier in the season (Weaver & McCune, 1959a).

There has been a focus in past research as to the effect of defoliation on remaining leaves, particularly on photosynthetic rates. Hunter et al., (1995) found that defoliation at fruit set in Cabernet Sauvignon/99 Richter increased the photosynthetic activity of old leaves lower in the canopy and lead to a reduction in leaf sucrose and fructose concentration. Poni and Intrieri, (1990) suggested that leaves higher in the canopy may contribute more to total assimilation than leaves lower in the canopy, however when leaf size was taken into account, photosynthetic rates appear to be similar across the whole vine. Candolfivasconcelos et al., (1994) found that in Pinot Noir, photosynthetic rates were similar in defoliated and non-defoliated vines. Their observations also indicated that defoliation had little effect on the rate of transpiration and water use efficiency of vines. Leaves opposite clusters were however found to have a decline in photosynthetic rate during fruit maturation and these leaves also had lower transpiration and water use efficiency. This observation is similar to that of Intrieri et al., (1992), where it was suggested that following the lag phase of berry growth, photosynthetic rates of leaves directly adjacent to the developing clusters declined.

The experiments of Intrieri et al.,(1997) using bush and cordon trained spur pruned Chardonnay vines found that total assimilation was not impacted by removal of up to 27% of leaf area. In these experiments total assimilation was greatest in bush grown vines at 6-6.5 $m^2$/m of canopy whereas in vines trained to a fixed cordon, rates were not saturated and a linear relationship was recorded between total assimilation and leaf area. These experiments highlight the potential interaction of trellis/training system on the total assimilation of defoliated vines. Vines grafted to differing rootstocks have also been shown to have different photosynthetic rates (Candolfivasconcelos et al., 1994).

Petrie et al., (2003) measured whole vine photosynthetic response of Sauvignon Blanc to defoliation during the lag phase of berry growth by removing leaf area from both the top and bottom of the canopy. The author concluded that defoliation in the lower part of the canopy had the largest effect on photosynthesis per unit leaf area immediately following defoliation, suggesting that the lower part of the canopy contributes more to whole vine photosynthesis. This contradicts the arguments

outlined by other authors. Later measurements of whole vine photosynthesis in this experiment suggest that while some compensation for defoliation, through increased photosynthetic rate, may occur, this increase could not fully compensate for the leaf area removed by defoliation.

In the experiments of Petrie et al., (2000b) increased leaf area, as a result of defoliation in non-bearing vines, did not result in increased dry matter production. The author concluded that these vines were likely to be carbohydrate sink limited, which in previous studies had been linked to a declining photosynthetic rate. In the same experiment the author concluded that cropped vines were likely to be source limited, due to an observed delay in fruit maturity (Petrie et al., 2000a). Similar dry matter accumulation between bearing and non-bearing vines in the presence of defoliation appears to have implications for previous studies where leaf age has often been attributed to declining photosynthetic rates of older leaves and their importance in crop ripening questioned. The degree of sink limitation may also affect the ability of the canopy to increase photosynthetic rate in response to defoliation a conclusion which supported by the results of Petrie et al., (2003).

The ability of the vine to compensate for significant leaf removal either through increased photosynthetic rate or vegetative growth would therefore seem to be moderated by:

- The degree of sink limitation following defoliation.
- The ability of the vine to establish new leaf area through environmental limitation.
- The previous carbohydrate status of the vine.

Decisions made in the vineyard in one season have been shown to have impacts both within the season and in subsequent seasons (Howell et al., 1994). In experiments conducted by Percival et al., (1994a, 1994b) in Riesling, yield was shown to both increase and decrease with defoliation. The direction of the response was impacted by the timing of the defoliation. When vines were severely defoliated earlier in the season, a reduction in yield due to inhibited berry growth lead to a reduction in

cluster weights (Percival et al., 1994b). Defoliation applied later in the season has lead to an increase in berry size and a resultant increase in cluster weight in Riesling (Percival et al., 1994a). This observation has also been reported by a number of other authors (Ezzahouani & Williams, 2003; Hunter et al., 1995; Petrie et al., 2003). The author also reported that no reduction in bud fertility in subsequent seasons was observed. It is worth noting that these experiments were conducted on vigorous grapevines. Experiments on Cabernet Sauvignon have demonstrated that leaf removal following fruit set strongly reduced berry growth by reducing the cell size of the berry pericarp, so that berry size at the lag phase of berry growth, size was half that of the control (Ollat & Gaudillere, 1998). In the same experiment restoration of leaf area, as a result of further vegetative growth, allowed berries to grow at the same relative rate during ripening. Growth rate compensation did not occur during ripening, meaning berry size was proportional to size of the fruit at the beginning of berry ripening. Kliewer and Antcliff (1970) in Sultana found that berry weight was the variable most affected by defoliation, stating that the earlier the treatments were applied, the greater the impact. Many of the major effects of defoliation on yield and yield components observed are likely to be the result of source limitation following defoliation. Defoliation has been shown to significantly reduce the sucrose and fructose concentration of leaves (Heuvel et al., 2005). It has also been observed that in some cases defoliation, though not extreme, had no effect on yield components (Bledsoe et al., 1988).

While contradictory observations exist, a general consensus of the effects of the severity and timing of defoliation on yield and yield components can be suggested.

- Defoliation during berry formation generally corresponds to reduced yield as a result of a reduction in berry size. This effect increases with severity of the treatment. In cases where this is not observed the change in temperature imposed by the exposure of fruit, or the proportion of the canopy removed, may not have been sufficient to alter normal tissue growth. This observation seems more likely to be observed in cool climates or vigorous canopies.

- Defoliation during the lag phase or berry ripening generally has little or no impact on berry growth to harvest, except in the case where removal from dense canopies may stimulate growth through increased exposure and a resultant increase in evapotranspiration, sugar loading and bunch size as a function of larger berries, is observed (Dreier et al., 2000).

Changes to fruit composition have been shown to occur as a result of defoliation though they may not be consistent between vintages (Main & Morris, 2004; Reynolds et al., 1996). Soluble solids in response to defoliation have been shown to increase (Main & Morris, 2004; Petrie et al., 2003), show no response (Hunter et al., 1995; Main & Morris, 2004), or decrease (Koblet et al., 1994; Petrie et al., 2000a; Reynolds et al., 1996; Vasconcelos & Castagnoli, 2000). It has been reported that excessive removal of leaves in Pinot Noir may lead to a reduction in soluble solid accumulation possibly as a result of source limitation (Bledsoe et al., 1988). While increases in soluble solids are likely to be the result of increased sink strength as a result of increased berry size, increased evapotranspiration of the berry through increasing exposure or a combination of the two (Dreier et al., 2000).

Similarly both increases (Hunter et al., 1995; Petrie et al., 2003) and decreases (Main & Morris, 2004; Reynolds et al., 1996) have been observed in titratable acidity in response to defoliation while pH has been observed to decrease (Hunter et al., 1995; Koblet et al., 1994; Petrie et al., 2003).

Maturation of grape berries following veraison is characterised by a decline in acid levels and an increase in pH (Jackson, DI & Lombard, 1993). It is accepted that the reduction in titratable acidity during berry ripening is related to the respiration rate of the berry and is a function of temperature (Jackson, DI & Lombard, 1993). Malic acid and tartaric acid are the most important organic acids contributing to the titratable acidity and pH of grape berries. Experiments by Kliewer & Lider (1968) found that increasing exposure of Thompson seedless grapes led to lower titratable acidity and higher pH than shaded fruits, with malate being 2-3 times greater in shaded fruits, whereas tartaric acid levels remained relatively constant. Kliewer (1971) reported that malic acid metabolism was temperature dependent and increased at higher

temperatures, while later experiments have suggested that accumulation was at its greatest between 20-25°C (Lakso & Kliewer, 1975). Diurnal fluctuations in temperature have also been discussed as being important in maintaining acid and lowering pH for longer during maturation with high day temperatures and low night temperatures being the most favourable (Kliewer & Torres, 1972). It has also been highlighted that increases in pH and decreases in malate proceed more slowly in cool climates than in warm climates (Jackson, DI & Lombard, 1993).

Increased incident light has been observed to both increase (Ezzahouani & Williams, 2003; Heuvel et al., 2005; Hunter et al., 1995; Mazza, G. et al., 1999; Staff et al., 1997), inconsistently increase (Zoecklein et al., 1997) and have no effect on anthocyanin accumulation in red grape varieties such as Pinot Noir (Vasconcelos & Castagnoli, 2000) in response to leaf removal. Leaf removal has been shown to lead to improved colour, aroma and palatability of both Optima and Cabernet franc wines (Staff et al., 1997) and lead to improved colour in wines made from Cynthiana (*Vitis aestivalis* Michx.) (Main & Morris, 2004).

The effects of increasing exposure through leaf removal on flavour and aroma are probably best described in white wine varieties. Leaf removal has been shown to increase flavour precursors in Chardonay and Riesling (Zoecklein, Wolf, Duncan, et al., 1998; Zoecklein, Wolf, Marcy, et al., 1998) and increase flavour compounds in Gewurztraminer which carried through to sensory differences (Reynolds et al., 1996).

As a result of changes to the fruiting zone microclimate, defoliation has been shown to significantly decrease the incidence of economically important diseases powdery mildew (Chellemi & Marois, 1992; Stapleton et al., 1995) and botrytis bunch rot (Duncan et al., 1995; English et al., 1993; Ferree et al., 2003; Gubler et al., 1991; Percival et al., 1994b; Staff et al., 1997; Stapleton & Grant, 1992; Zoecklein et al., 1992). Whilst the management of disease remains an important practical consideration for the local industry, this study was conducted to measure changes in grape composition and yield components in response to varying degrees of defoliation.

## 4.2 Materials and Methods

### *4.2.1 Site characteristics*

The trial was carried out at a commercial vineyard, Cambridge, Tasmania, at 42°48'39"S, 147°25'39"E and an elevation of between 55 and 60 metres above sea level. Vines were cane pruned to 24 buds, consisting of two horizontally trained, ten bud, cordons, and two, two bud spurs located in the head of the vine to provide replacement shoots. Shoots were positioned vertically. Vine spacing was 1500 mm and row spacing was 2100 mm, 3175 vines per ha. Fruiting wire height 900 mm and hedging height 2000 mm. Row orientation for four replicates was in a north-south direction and four replicates were orientated North-north-west and south-south-east.

### *4.2.2 Treatment design*

Four defoliation treatments were applied to eight replicates in a randomised complete block design. Blocks were distributed over three clones of *Vitis vinifera* Cv. Pinot Noir (MV6 replicates 2 and 5, D2V5 (8104) replicates 3 and 6, D5V12, (2051) replicates 1, 4, 7 and 8). Each trellis panel of four vines was designated a plot. The centre two vines were used to measure bunch number and weight, berry number and weight, pruning weight, trunk circumference and fruit compositional analysis at harvest. The outside two vines of each plot were used as buffer vines. Treatments were applied in the final week of January during 2005, 2006 and 2007. Four defoliation treatments were carried out 10 to 14 days before the beginning of veraison denoted as EL31-32 using the modified EL system as for Coombe (1995). This timing was considered to be in line with current industry practice. The treatments aimed to manipulate leaf area within the fruiting zone, denoted as the area between the fruiting wire and extending 300 mm directly above it. Defoliation was carried out by hand. Treatments aimed to remove 0% of leaf area in the fruiting zone (control), Treatment 1 (Plate 4-1), 40% of leaf area in the fruiting zone, Treatment 2 (Plate 4-2), 70% of leaf area in the fruiting zone, Treatment 3 (Plate 4-3) and 100% of leaf area in the fruiting zone, Treatment 4 (Plate 4-4). This experiment was designed as a

longitudinal study, treatments were imposed annually on the same vines for three consecutive years.

**Plate 4-1. Treatment 1, control or 0% leaf area removal in the fruiting zone**

**Plate 4-2. Treatment 2, 40% leaf area removal in the fruiting zone**

**Plate 4-3. Treatment C, 70% leaf area removal in the fruiting zone**

**Plate 4-4. Treatment D, 100% leaf area removal in the fruiting zone**

Climate data from Hobart Airport (BOM station 094008) were calculated to be compared to seasonal averages for yield and fruit compositional parameters relationships. Sunshine hours were obtained from the Campbell-Stokes sunshine recorder as per BOM methods. Biological significance was denoted as relationships which had a P-value less than or equal to (P=0.05).

Hour degrees were calculated as the sum of degrees above 10°C over the entire vintage July – June.

Harvest and fruit processing was carried out as per the general methods.

Data from all years was pooled for analysis by univariate linear regression.

### *4.2.3 Estimation in timing of veraison*

During veraison in 2007 progression through veraison was estimated. All bunches were scored from replicates 1, 4, 7 and 8. All bunches were individually divided into 4 categories. Category 1, 0% of berries coloured, category 2, less than 50% of berries coloured, category 3, greater than 50% of berries coloured and category 4, all berries coloured. A mean bunch veraison score was then calculated by multiplying the category number, by the number of bunches in that category and creating a sum value of all categories. This number was then divided by the total number of bunches for the sample. This number was denoted as the veraison score for each plot.

### *4.2.4 Pruning weights*

Pruning weights were recorded in the field following the 2005 and 2006 vintages. Yield to Pruning (Y:P) ratio was calculated where Y:P = Yield (g) / Pruning weight (g). Bunch density was calculated by dividing bunch length by bunch weight as in Ferree et al., (2003).

### *4.2.5 Statistical analysis*

This experiment presented significant statistical difficulties as a result of inadequate and unbalanced experimental design. The experiment was conducted over a three year period and though design issues were highlighted immediately after the initial field trial setup, it was deemed more important to continue than abandon the

experiment, as a full season of data would have been lost given that the critical stage for treatment application had passed. The design issues highlighted for statistical analysis are further exacerbated by missing data, the result of the grower harvesting multiple and differing replicates across years combined with significant bird damage. It was deemed that the following analysis was most appropriate given the constraints imposed by poor design and missing data.

All results were normally distributed and untransformed data were analysed using an ANOVA in SAS 9.1 using a type III sum of squares analysis, both for individual years and across years. Means were compared using Fischers Least Significant Difference (LSD) calculated at P=0.05 after the method of Steele and Torrie (Steel & Torrie, 1980). As no significant interactions were found between replicates and treatments, row orientation and clonal difference were treated as natural variability in the final analysis.

Correlation analysis was carried out using the univariate linear regression models package in SPSS version 19 to assess independent variables.

## 4.3 Results

### *4.3.1 Fruit composition and yield parameters*

For the 2005, 2006 and 2007 vintages there was no significant effect ($P<0.05$) of treatment on fruit compositional parameters at harvest. In 2005 there was a marginally significant treatment effect on bunch number ($P = 0.046$). The control treatment with a mean of 30.57 was not significantly different to the 40 and 70% treatments with bunch numbers of 29.06 and 25.00 respectively but was significantly different to the 100 % defoliation treatment, 24.50 bunches, which was also not significantly different from 40 or 70% defoliation. In the following vintages there was no significant effect of treatment on bunch number (Table 4-1). Pruning weights were not significantly different for either vintage. Y:P ratio was significantly lower in the 40% and 70% treatments in the 2007 vintage. No other significant treatment effects on yield components were recorded for yield or individual yield components within a single vintage ($P>0.05$). There were no significant treatment by replicate interactions ($P>0.05$).

When data from all three vintages were combined, increasing levels of defoliation were shown to have a significant effect on bunch number, yield and soluble solids (

Table 4-2). More severe levels of defoliation were linked to a reduction in bunch number yield and soluble solid accumulation. There were no other significant effects of treatment on any of the measured or calculated variables ($P>0.05$).

**Table 4-1, The effect of increasing fruiting zone defoliation on bunch number, yield and soluble solids separated by vintage. (Superscript denote significantly different groupings based on Fischers Least Significant Difference (LSD) calculated at P=0.05)**

| | Bunch number | | | Yield (t/ha) | | | Soluble solids (°Brix) | | | Y:P | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Level of Defoliation** | **2005** | **2006** | **2007** | **2005** | **2006** | **2007** | **2005** | **2006** | **2007** | **2006** | **2007** |
| **0%** | 30.57 $^{A}$ | 24.64 | 31.83 | 10.11 | 6.85 | 9.68 | 24.12 | 23.79 | 22.24 | 2.79 | 2.65$^{A}$ |
| **40%** | 29.06 $^{AB}$ | 24.21 | 27.58 | 10.03 | 6.44 | 7.19 | 23.54 | 23.37 | 22.40 | 2.81 | 2.05$^{B}$ |
| **70%** | 25.00 $^{B}$ | 24.43 | 29.50 | 8.89 | 5.63 | 8.73 | 23.14 | 23.41 | 21.98 | 3.35 | 2.75$^{A}$ |
| **100%** | 24.50 $^{B}$ | 22.14 | 24.92 | 8.18 | 5.24 | 6.88 | 23.49 | 22.70 | 22.02 | 2.45 | 1.98$^{B}$ |
| LSD (p=0.05) | 4.63 | ns | ns | ns | ns | ns | ns | ns | ns | ns | 0.54 |

**Table 4-2, The effect of increasing fruiting zone defoliation on bunch number, yield and soluble solids concentration across combined vintages 2005, 2006 and 2007, (Superscript denote significantly different groupings based on Fischers Least Significant Difference (LSD) calculated at P=0.05)**

| **Treatment** | **Bunch number** | **Yield (t/ha)** | **Soluble solids (°Brix)** |
|---|---|---|---|
| **0%** | 28.87 $^{A}$ | 8.91 $^{A}$ | 23.46 $^{A}$ |
| **40%** | 27.02 $^{AB}$ | 8.05 $^{AB}$ | 23.12 $^{AB}$ |
| **70%** | 26.15 $^{AB}$ | 7.81 $^{AB}$ | 22.89 $^{AB}$ |
| **100%** | 23.83 $^{B}$ | 6.90 $^{B}$ | 22.77 $^{B}$ |
| LSD (p=0.05) | 3.325 | 1.30 | 0.627 |

There were marked differences recorded between different vintages. Significant differences were recorded across all fruit compositional parameters (Table 4-3). pH was significantly different in all vintages being the highest in 2005 (3.62), lowest in 2007 (3.40). The mean pH was 3.53 in 2006. Titratable acidity was significantly lower in 2006 (4.09) than 2005 (4.64) which was also significantly lower than 2007 (5.25). Soluble solid concentration was significantly lower in 2007 (22.15), than vintage 2005 (23.55) and vintage 2006 (23.31), which were not significantly different. Anthocyanin concentration (mg/g) was significantly different across all vintages, being highest in 2006 (0.882) and lowest in 2005 (0.619). Anthocyanin concentration (mg/g) was 0.755 in 2007. When converted to a per berry basis, anthocyanin concentration per berry was significantly higher in 2007 (0.863), than in both 2005 (0.673) and 2006 (0.740) which did not differ significantly.

Marked differences were also recorded across all yield and bunch structural components across years (Table 4-4). Bunch number was significantly lower in 2006 (23.86) than in 2005 (27.25) and 2007 (3.40), which were not significantly different. Bunch weight was significantly different in all vintages, being lowest in 2006 (80.46) and highest in 2005 (109.06), bunch weight was 90.80g in 2007. Berry number was significantly lower in 2007 (74.56) than in 2005 (93.03) and 2006 (88.29), which did not differ significantly. Berry weight was significantly different in all three vintages, being lowest in 2006 and highest in 2007. Yield was also significantly different in all three vintages and was highest in 2007 (8.12) and lowest in 2006 (6.06). Yield was 8.12 t/Ha in 2007. Primary branch number was significantly lower in 2006 (12.75), than in 2005 (14.07) and 2007 (14.25), which were not significantly different. Bunch length was significantly different in all vintages, being highest in 2005 (93.09) and lowest in 2007 (80.75). Bunch length in 2006 had a mean of 88.18. Bunches were significantly wider in 2005 (63.85) than both 2006 (56.75) and 2007 (54.75), which were not significantly different. Timing of veraison was significantly affected by treatment. In 2007 Veraison score was significantly lower in the 100% defoliated treatment than all other treatments (Table 4-5).

**Table 4-3, The influence of vintage on fruit compositional parameters across vintages 2005, 2006 and 2007. (Superscript denote significantly different groupings based on Fischers Least Significant Difference (LSD) calculated at P=0.05)**

| Vintage | pH | Titratable acidity (g/L) | Soluble solids (°Brix) | Anthocyanin concentration (mg/g) | Anthocyanin concentration (mg/berry) |
|---|---|---|---|---|---|
| **2005** | 3.62 $^{A}$ | 4.64 $^{B}$ | 23.55 $^{A}$ | 0.619 $^{C}$ | 0.673 $^{B}$ |
| **2006** | 3.53 $^{B}$ | 4.09 $^{C}$ | 23.31 $^{A}$ | 0.882 $^{A}$ | 0.740 $^{B}$ |
| **2007** | 3.40 $^{C}$ | 5.25 $^{A}$ | 22.15 $^{B}$ | 0.755 $^{B}$ | 0.863 $^{A}$ |
| **LSD (p<0.05)** | **0.07** | **0.19** | **0.54** | **0.073** | **0.073** |

**Table 4-4, The influence of vintage on yield and bunch structural components across vintages 2005, 2006 and 2007. (Superscript denote significantly different groupings based on Fischers Least Significant Difference (LSD) calculated at P=0.05)**

| Vintage | Bunch number | Bunch weight (g) | Berry number | Berry weight (g) | Yield (t/ha) | Primary branch number | Length (mm) | Width (mm) |
|---|---|---|---|---|---|---|---|---|
| **2005** | 27.25 $^{A}$ | 109.06 $^{A}$ | 93.03 $^{A}$ | 1.10 $^{B}$ | 9.29 $^{A}$ | 14.07 $^{A}$ | 93.09 $^{A}$ | 63.85 $^{A}$ |
| **2006** | 23.86 $^{B}$ | 80.46 $^{B}$ | 88.29 $^{A}$ | 0.85 $^{C}$ | 6.06 $^{C}$ | 12.75 $^{B}$ | 88.18 $^{B}$ | 56.75 $^{B}$ |
| **2007** | 28.46 $^{A}$ | 90.80 $^{C}$ | 74.56 $^{B}$ | 1.15 $^{A}$ | 8.12 $^{B}$ | 14.25 $^{A}$ | 80.75 $^{C}$ | 54.75 $^{B}$ |
| **LSD (p<0.05)** | **2.89** | **8.66** | **7.21** | **0.055** | **1.13** | **0.82** | **4.59** | **4.32** |

**Table 4-5, The effect of defoliation treatment on the level of progression through veraison in 2007. (Superscript denote significantly different groupings based on Fischers Least Significant Difference (LSD) calculated at P=0.05)**

| Level of defoliation | Veraison Score |
|---|---|
| **0%** | $2.891^{A}$ |
| **40%** | $2.876^{A}$ |
| **70%** | $2.664^{A}$ |
| **100%** | $2.165^{B}$ |
| **LSD (P<0.05)** | **0.464** |

### *4.3.2 Comparison of yield, structural and fruit compositional components*

Yield components were compared by linear regression analysis (Table 4-6). There was a strong relationship between berry number and bunch weight (Figure 4-5). Primary branch number displayed moderate to weak relationships, with berry number and weight, bunch weight and length (Table 4-6). No correlation was recorded between primary branch number and bunch width or bunch number.

**Table 4-6, Significant (P≤0.05) positive linear relationships between yield components and bunch morphology comparing pooled data across the 2005, 2006 and 2007 vintages (Values presented represent the value for the correlation coefficient, $R^2$)**

| | **Yield** (T/Ha) | **Primary branch number** | **Bunch weight (g)** | **Berry Weight (g)** |
|---|---|---|---|---|
| **Berry number** | 0.2079 | 0.3347 | 0.6386 | ns |
| **Berry weight (g)** | 0.3241 | 0.2865 | 0.3807 | |
| **Bunch number** | 0.3294 | ns | | |
| **Bunch weight (g)** | 0.4987 | 0.4987 | | |
| **Length (mm)** | 0.1983 | 0.5218 | | |
| **Width (mm)** | 0.2147 | ns | | |
| **Primary branch number** | 0.2363 | | | |

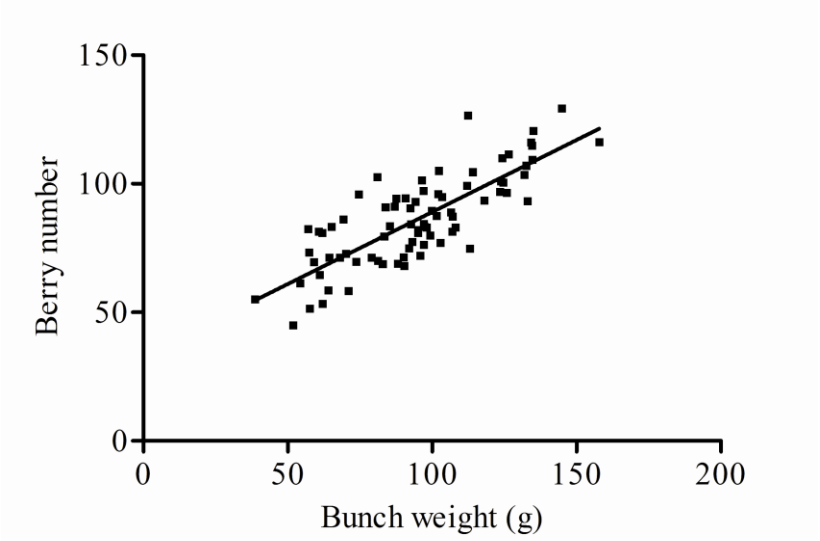


**Figure 4-5, The positive relationship between berry number and bunch weight (g) using combined data across the 2005, 2006 and 2007 vintages. Linear regression where y = 0.5600 x - 58.95, and $R^2$ 0.6386.**

Berry weight was shown to be significantly correlated with TA (Figure 4-6) and anthocyanin concentration (mg/g) (Figure 4-7). Anthocyanin concentration displayed significant relationships with yield (Figure 4-8) and compactness of the bunch (Figure 4-9). Significant correlations were displayed when comparing vintage averages of anthocyanin (mg/berry) and pH (Table 4-7), to climatic variables associated with temperature. Though not significant, pH displayed a correlation coefficient ($R^2$ = 0.9826) with average minimum temperature.

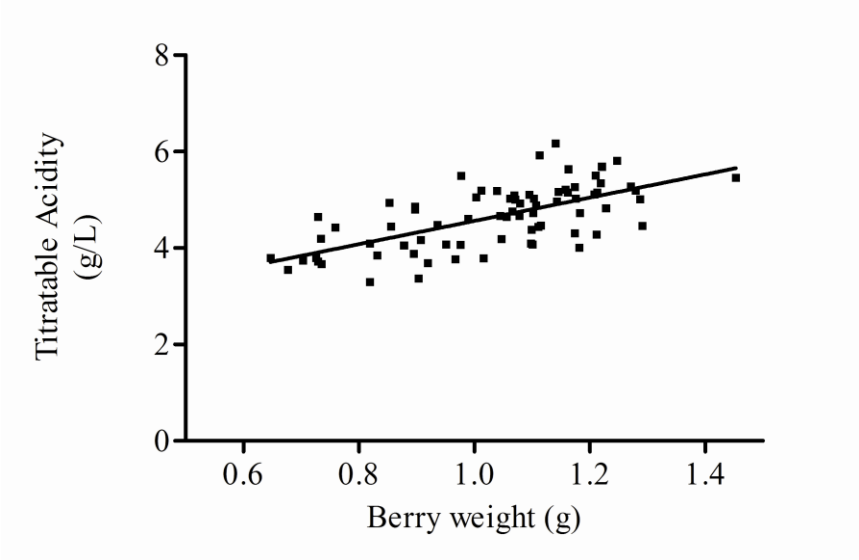


**Figure 4-6, The positive relationship between titratable acidity (g/L) and berry weight (g) using combined data across the 2005, 2006 and 2007 vintages. Linear regression where y = 2.414x + 2.149, and $R^2$ 0.4228.**

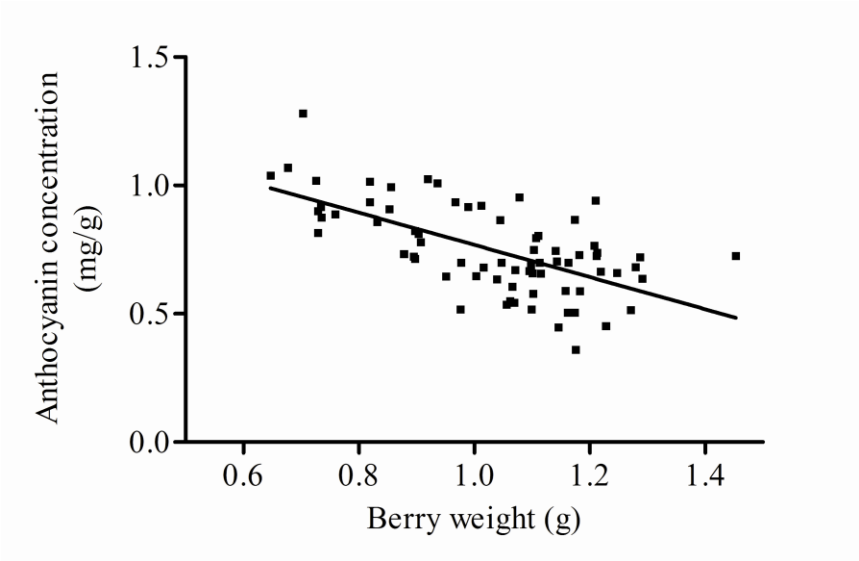


**Figure 4-7, The negative relationship between anthocyanin concentration (mg/g) and berry weight (g) using combined data across the 2005, 2006 and 2007 vintages. Linear regression where y = -0.6270x + 1.396, and $R^2$ 0.3981.**

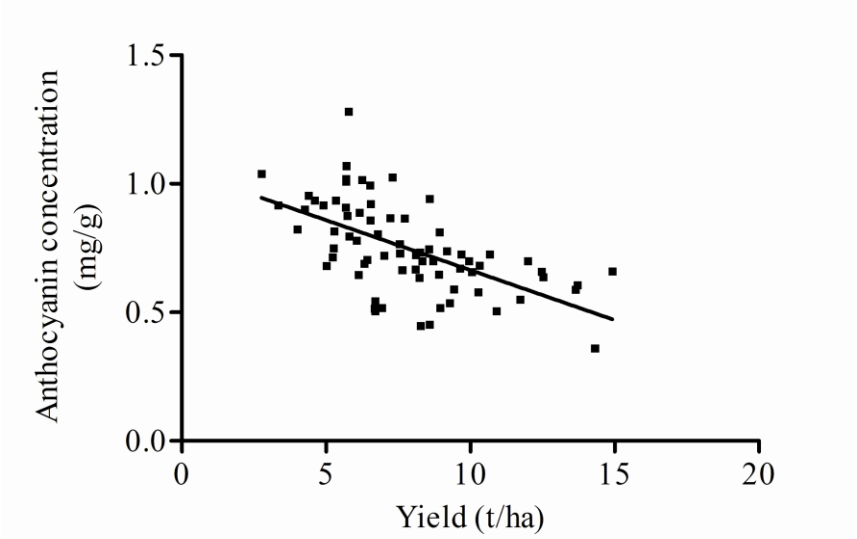


**Figure 4-8, The relationship between anthocyanin concentration (mg/g) and yield (t/Ha) using combined data across the 2005, 2006 and 2007 vintages. Linear regression where y = -0.03886x + 1.053, and $R^2$ 0.3447.**

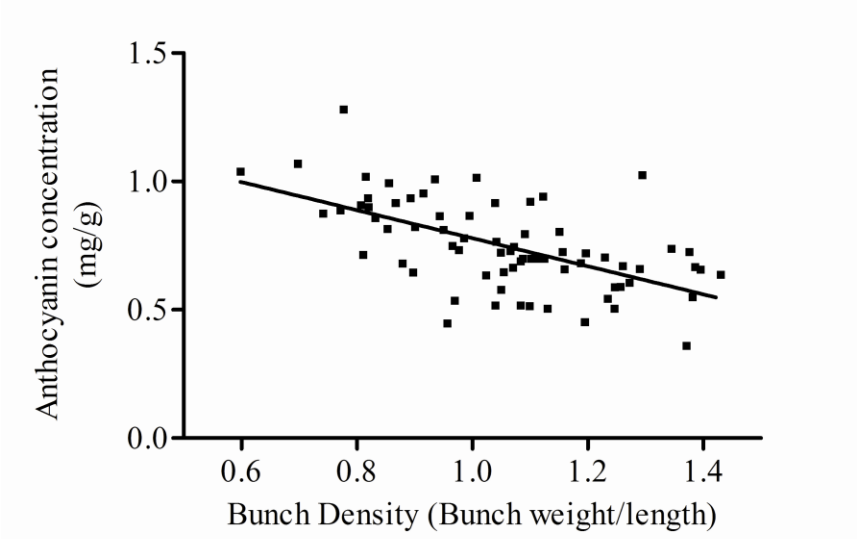


**Figure 4-9, The relationship between anthocyanin concentration (mg/g) and bunch density (Bunch weight/bunch length) using combined data across the 2005, 2006 and 2007 vintages. Linear regression where y = -0.5464x + 2.426, and $R^2$ 0.3469.**

**Table 4-7, Displaying average climatic data for Hobart Airport (BOM station 094008) and significant and non significant (ns) linear regression relationships between anthocyanin per berry and pH comparing average across years, the direction of the correlation is displayed in brackets.**

| Vintage | Accumulated hour degrees above 10°C | Average daily temperature | Number of hours of bright sunshine |
|---|---|---|---|
| **2005** | 10583 | 16.31 | 7.50 |
| **2006** | 11056 | 16.59 | 7.33 |
| **2007** | 11991 | 17.04 | 7.61 |
| **Linear regression** | | | |
| **Anthocyanin per berry (mg/berry) ($R^2$)** | 0.9996 (+ ve) | 0.9984 (+ ve) | ns |
| **pH ($R^2$)** | 0.9931 ( - ve) | 0.9994 ( - ve) | ns |
| **Berry weight** | ns | ns | ns |

## 4.4 Discussion

Interpretation of the results of this experiment must be made within the limitations of the experimental design. This does not allow for conclusions to be drawn for the response of a specific clone of Pinot Noir or of the entire Pinot Noir variety in response to defoliation. The following discusses the observations and trends recorded across several common clones and several years which provide interesting background for discussion and further study.

### *4.4.1 The impact of defoliation*

Fruit composition was not influenced by defoliation, except for soluble solids when data was combined from all three seasons. This observation would suggest that for Pinot Noir in a southern Tasmanian vineyard, anthocyanin concentration, pH or TA was not affected in response to increased exposure from fruiting zone defoliation.

Leaf removal was observed to both increase (Ezzahouani & Williams, 2003; Hunter et al., 1995; Kerslake, 2011; Staff et al., 1997) and have no effect on anthocyanin accumulation in red grape varieties (Vasconcelos & Castagnoli, 2000). This increase is however not consistent between years (Kerslake, 2011; Zoecklein et al., 1997). The observation of inconsistent increases in particular seasons may point to interaction between exposure and source sink relationships of the vine which are extremely difficult to separate (Downey et al., 2004). Source limitation of energy within the grape vine may have limited anthocyanin biosynthesis (Downey et al., 2004). It is also expected that canopies of lower vigour, such as in the present study, are less likely to exhibit increases in anthocyanin, as exposure in non-defoliated vines may be adequate to enable maximal anthocyanin biosynthesis. The relationship between exposure and anthocyanin development in isolation of source limitation will be explored further in chapter 5 of this thesis.

There was no significant effect of treatment on titratable acidity or pH. The results of this experiment are similar to that of Ollat & Gaudillere (1998) and Vasconcelos & Castagnoli (2000). The impacts of fruiting zone defoliation experiments on acid

metabolism in the developing berries of *Vitis Vinifera* are varied. Hunter et al., (1995) and Reynolds et al., (1996) reported a decrease in titratable acidity and pH in response to defoliation, while Kliewer & Antcliff (1970) reported an increase. Considering the strong influence of temperature on acid metabolism of *Vitis vinifera* fruits, particularly temperatures above 20°C it is expected that the lack of an effect of defoliation on the acid metabolism of fruit in this instance is not entirely unexpected due to the cool local climate and the lack of a vigorous growth and heavily shaded canopy.

When measurements were combined across all vintages soluble solids were significantly decreased (Table 4-2) as a result of defoliation. Only the control and severe levels of defoliation were significantly different in soluble solids accumulation. The results of this experiment are both in agreement with some authors, who observed reduced soluble solids with defoliation (May et al., 1969; Reynolds et al., 1996; Vasconcelos & Castagnoli, 2000), and in disagreement with other authors, who observed increasing or no difference in soluble solids with defoliation (Bledsoe et al., 1988; Main & Morris, 2004; Ollat & Gaudillere, 1998; Percival et al., 1994b). The delayed timing of veraison (Table 4-5) observed in response to the 100% treatment is likely to have caused lower soluble solids at harvest as a result of source limitation of the plant (Petrie et al., 2000a). It has been observed that the leaves of *Vitis vinifera* cv. are able to compensate for defoliation by increased photosynthetic rates (Bledsoe et al., 1988; Hunter et al., 1995). In Pinot Noir photosynthetic rates between defoliated and non-defoliated vines have been observed to be similar (Candolfivasconcelos et al., 1994). Candolfivasconcelos et al., (1994) attributed the lack of compensation of photosynthetic capacity to a later timing of defoliation. The observations of Bledsoe (1988) in Sauvignon Blanc also support this theory. In the current experiment cessation of shoot growth was observed to have occurred in all three years coinciding with, or immediately preceding defoliation. Additional vegetative growth was not observed to have occurred but this was not measured. In the present experiment, the observed delay in ripening and reduced soluble solids, indicate that photosynthetic compensation did not occur. The results of the present study would confirm the

conclusion that anthocyanin accumulation is independent of sugar accumulation (Kerslake, 2011).

Defoliation lead to significant differences for bunch number and yield when data was combined across all vintages (Table 4-2). A reported significant effect on bunch number in the first experimental season (Table 4-1) was dismissed, as natural variability as bunch number was set before treatments were imposed. Fruiting zone defoliation has been reported to reduce yield and yield components through a reduction in bunch number by affecting bud fertility in subsequent seasons (May et al., 1969), a reduction in berry weight (Coombe, B. G., 1959; Kliewer & Antcliff, 1970; May et al., 1969) and reduced fruit set (Coombe, B. G., 1959). Shoot numbers were not measured. It is not known if bunch number reduced as a function of reduced shoot number or reduced bunch number per shoot, making it difficult to propose a mechanism for the observed reduction in yield.

The observation that Y:P ratio was significantly affected by defoliation (Table 4-1) would support an argument that defoliation was impacting vine growth in the subsequent season, and therefore potentially fruit quality, as a result of changes to vine balance. In the experiments of Kerslake (2011), Y:P increased with defoliation in one of the two vintages suggesting vine balance (Y:P, >4) was not necessarily negatively impacted by defoliation through sink limitation. It is the author's opinion that the observations of Kerslake (2011) were associated with a more vigorous canopy, which as a result of environmental influence lead to an increase in berry weight, not observed in the present study, and therefore bunch weight. It has been shown that vigour may influence anthocyanin accumulation of Pinot Noir (Cortell et al., 2007). Canopy vigour and size may therefore explain the lack of consistency observed for fruit composition between regions and between seasons (Kerslake, 2011; Zoecklein et al., 1997). In the present study Y:P ratios for 2006 and 2007 vintages were 2.85 and 2.34, below that suggested to be optimal for Pinot Noir (Kliewer & Dokoozlian, 2005) Y:P is generally lower in cool climates (Dry, PR et al., 2004). The delay in ripening observed in the present study and a decrease in the Y:P ratio in two of the three defoliated treatments, suggest that in low vigour sites the optimal Y:P ratio may be below that of previous recommendations.

### *4.4.2 Seasonal variation*

It has been identified that season or site differences have a significant impact on the direction and magnitude of response of grape composition to defoliation (Kerslake, 2011; Zoecklein et al., 1997). Significant differences were observed in all variables across all years, confirming these observations (Table 4-3 & Table 4-4). Kerslake (2011) suggests that in seasons with high rainfall or lower than average MFT, leaf plucking may be of little or no use for improving grape composition, a major conclusion of this study is that seasonal influences have a far greater impact on fruit composition than management techniques and that a range of climatic variables may be associated with grape and wine composition (Presented in Table 3-1). Averaged fruit quality and yield component data compared to MJT, MFT, Rainfall, (mm, Sept – May), Rainfall (mm, Jul – Jun), GDD or FDD presented in Table 3-1, were shown to have no significant correlation in this study, though principal component analysis was not conducted and may have been more appropriate for examining differences between seasons. Both hour degrees and average temperature were shown to have significant positive relationships with pH and total anthocyanin (Table 4-7). This may suggest the pH and total anthocyanin accumulation were related to temperature accumulation or controlled by the availability of photosynthate. In a complex field environment the underlying mechanisms of this observation are difficult to separate (Downey et al., 2006).

The amount of variability and the size of the data set of this experiment allows for further investigation of relationships between yield components, or between yield components and fruit composition.

Variation in bunch weight was shown to explain approximately 50% of the variability in yield greater than all other components (Table 4-6). Berry number was observed to be strongly correlated with bunch weight (Figure 4-5). This confirms the well established relationship bunch weight as a function of berry number is a key determinate of yield (Dunn & Martin, 2000). Berry number in Pinot Noir is strongly influenced by the effect of weather at flowering (Heazlewood, 2005), this is the likely major underlying factor for differing bunch weights and yields displayed in Table 4-4.

Berry weight was significantly different across all vintages though they were largest in 2007.

Early prediction of bunch weight would significantly aid growers in achieving target yields and further reducing variability. Branch number, width and length are to a large degree determined prior to anthesis (Shavrukov et al., 2004) and, given a suitable level of a association with berry number and weight, would provide further information for early yield estimation and potentially manipulation. There is a logarithmic relationship between branch number and inflorescence number (May, 2000). Correlation analysis (Table 4-7) suggests that primary branching accounts for less than 50% of the variation in yield. The results observed for primary branch number in Table 4-7 confirm the observations of previous authors. Variation in branching within the rachis accounts for less than 50% of variation in bunch size in cool climates, primarily due to the effects of weather pattern on fruit set (May, 2000).

In the present study, increasing berry weight was found to be positively correlated with TA (Figure 4-6) and negatively correlated with anthocyanin concentration mg/g (Figure 4-7). It is proposed that both relationships are based on the surface area to volume ratio of the fruit, but each in a different manner. TA is directly influenced by the respiration rate of the berry which is a direct function of temperature (Kliewer & Lider, 1968), larger berries are likely to heat and cool at a slower rate and therefore have a lower TA (Jackson, DI & Lombard, 1993). Increased total anthocyanin was also shown to be negatively correlated with berry weight. This relationship has been discussed by a number of authors (Roby et al., 2004; Roby & Matthews, 2004) and in greater depth in Chapter six.

A significant relationship was found between yield and total anthocyanin concentration (Figure 4-8). In 2006 small bunch weights were recorded partly as a result of reduced berry weight. Weather, such as unusually cool temperatures or rain, at flowering have been suggested to be implicated in reduction of yield (Heazlewood, 2005), these events are often associated with a disorder known colloquially as "Hen and Chickens" (millerandage) (May, 2000). Significantly lighter berries as a result of the disorder in the 2006 vintage may have driven this relationship through a

significantly higher surface area to volume ratio as outlined above. An alternative hypothesis is that a smaller number of berries in the 2006 vintage, spread over a greater length (Table 4-4), reduced the compactness of the bunch increasing the exposure of individual berries and therefore increasing the total anthocyanin concentration as displayed by the relationship presented in Figure 4-9. The fact that the anthocyanin concentration per berry was higher in the 2007 vintage, would seem to favour the first of the two hypotheses. The relationship between bunch exposure and bunch compactness and their individual or combined influence on fruit composition, are examined in the final two experimental chapters of this thesis.

# Chapter 5 - The influence of fruit exposure on the composition of Pinot Noir

## 5.1 Introduction

In chapter 4 it was reported that defoliation in the fruiting zone of *Vitis vinifera* Pinot Noir grown in Tasmania had a limited effect on basic measures of fruit composition, though seasonal differences and interactions with aspects of natural and cultural influence have previously been shown to significantly impact fruit composition of Pinot Noir (Cortell et al., 2008; Kerslake, 2011; Koblet et al., 1994). Experimentally it has remained difficult to isolate the direct impact of fruit exposure on basic fruit composition without compromising to at least some degree the source sink interactions of the plant (Downey et al., 2006). The results of chapter four suggest that source limitation due to defoliation in Pinot Noir may have been an overarching factor in a lack of a result; field variability may have also been a factor. GDDs were also found to be correlated with total anthocyanin per berry. The possibility of light regulated changes to berry composition have been highlighted by recent research (Shabala & Wilson, 2001), as has the potential for intra-bunch shading to occur (May, 2000). An attempt is made in this chapter to separate the effects of exposure to light and ambient temperature surrounding the bunch without altering the source sink relationships of field grown Pinot Noir vines.

Anthocyanins accumulate steadily during berry ripening, stabilising toward maturation and may reduce during over ripening and berry shrivel (Mazza, G. et al., 1999). Modification of the fruiting microclimate, by defoliation, has been shown to significantly increase anthocyanin biosynthesis of *Vitis* sp. fruits. Heuvel *et al*., (2005). Increased anthocyanin biosynthesis has been reported in the De Chaunac variety and Mazza *et al*., (1999) reported increases in the anthocyanin production of Merlot, Cabernet Franc and Pinot Noir as a result of defoliation. Vasconcelos and Castagnoli (2000) reported that there was no change in the accumulation of anthocyanin in response to defoliation.

The relationship between exposure of fruit of *Vitis* sp. to solar radiation and the development of anthocyanin has been explored to a considerable degree and has been recently reviewed by Downey et al., (2006). The review highlights a number of investigations that suggested that lower levels of light led to reduced colour of Pinot Noir (Kliewer, 1970), Emperor table grapes (Kliewer, 1977), Shiraz (Smart, R. E. et al., 1985) and Cabernet Sauvignon (Dokoozlian & Kliewer, 1996; Hunter et al., 1995; Morrison & Noble, 1990) and the emergence of contradictory studies suggesting that exposure led to no change (Vasconcelos & Castagnoli, 2000), or even lower levels of anthocyanin as a result of overexposure or limited photosynthate (Bergqvist et al., 2001; Hunter et al., 1995; Spayd et al., 2002). Other authors have observed shifts in the levels of various anthocyanins contributing to overall composition (Cortell & Kennedy, 2006; Downey et al., 2004; Haselgrove et al., 2000; Joscelyne et al., 2007; Price et al., 1995).

It was previously thought that anthocyanin biosynthesis seems to be mostly regulated by temperature (Downey et al., 2006). A review by Downey et al., (2006) discusses the attempts of various authors to separate the effects of temperature and light on anthocyanin biosynthesis, highlighting three approaches to separate these variables. Kliewer and Torres (1972) and Dokoozlian and Kliewer (1996) in Pinot Noir and Cabernet Sauvignon respectively, successfully manipulated anthocyanin concentration by differing temperature using potted vines in a phytron. The conditions imposed in the experiments were highly artificial and the result may have been an artefact of either or both using potted vines or the phytron. The second approach discussed were experiments using the vine canopy to impose different levels of exposure. Bergqvist et al., (2001) concluded that in Grenache and Caberenet Sauvignon when monitoring the shaded and sunny sides (east west orientated rows) of the canopy that increasing light up until 100mmol/$m^2$/s increased the accumulation of anthocyanin but beyond this, accumulation began to decrease (Downey et al., 2006). Spayd et al., (2002) used north south orientated rows to expose fruit to the morning sun on the eastern side and the afternoon sun on the western side. The author showed that the temperature of fruit exposed on the western side of the canopy was substantially higher than that on the eastern side of the canopy. Fruit from the

cooler (eastern) side of the canopy was shown to have lower levels of anthocyanin. When fruit of the western side was artificially cooled and when fruit from the eastern side was artificially heated, both treatments lead to in an increase in anthocyanin biosynthesis suggesting that temperature played a key role. The third approach is that used by Downey et al.,(2004) on Shiraz and by Cortell and Kennedy (2006) on Pinot Noir. In these experiments a light exclusion box was used to modify the light environment of bunches without modifying temperature or humidity. Downey et al., (2004) observed that Shiraz was able to accumulate anthocyanin, in the absence of light, at similar levels to that of exposed fruit, and anthocyanin accumulation of Shiraz was not influenced by fruit exposure to light. In Pinot Noir total anthocyanin content was not observed to be impacted by exposure, while ratios of individual anthocyanins were shown to differ (Cortell & Kennedy, 2006).

The issue of temperature and light is further complicated by the suggestion that the optimal temperature for biosynthesis is likely to differ for individual varieties and that diurnal temperature fluctuations may influence anthocyanin biosynthesis (Mori et al., 2005).

## 5.2 Materials and Methods

### *5.2.1 Site Characteristics*

The experiment was carried out in a commercial vineyard located in southern Tasmania, (42°52'30 "S 147°25'21"E), on a north facing slope with an elevation of 75 metres above sea level. Vine spacing was 1m between vines and a row spacing of 2 m. The fruiting wire height was 90 cm. Vines were eight years old and cane pruned consisting of two, ten bud, horizontally trained cordons, and two, two bud spurs located in the head of the vine to provide replacement shoots. Shoots were positioned vertically. Management was carried out in line with normal commercial practice and was uniform across all treatments. Rows were orientated in a north south direction. The variety was Pinot Noir clone D5V12 (2051).

### *5.2.2 Treatment design*

The experiment was a 5 replicate by 6 treatment random block design. Replicates were blocked to rows with treatments randomly assigned within the block. Treatments were applied at the first sign of berry colouration, January 23$^{rd}$ 2007. Treatments were applied to eight basal bunches on a single vine and kept in place until harvest, April 2$^{nd}$ 2007.

Treatment 1 was designed to exclude all light from the bunch (Plate 5-1). Polyethylene sheeting was cut and shaped into two separate dish shaped structures. Each dish measured 200 mm deep and 120 mm wide at the base. The sides were folded and glued into place so as the opening of the wider dish measured 200 mm deep and 190 mm wide and the narrower dish 200 mm deep and 140 mm wide. A small slit was cut into the dishes at one end to allow the rachis to fit through and the bunch to be completely covered by the two halves. The insides of the dishes were then coated by two coats of black acrylic paint. In the field the narrower dish was placed inside the wider dish and fitted in such a fashion that a 10 mm gap occurred on both sides of the narrower dish from the wider dish so as to provide sufficient air

movement. The two dishes were then set in place with heavy duty plastic adhesive tape (Plate 5-2).

**Plate 5-1, Light exclusion box placed over a bunch in the field.**

Treatments 2, 3 and 4 were applied using bags made from 90%, 70% and 50% exclusion, commercial shade cloth (Plate 5-3). Bags were made by cutting shade cloth to 200 mm by 500 mm strips. The shade cloth was then folded in half to measure 200 mm by 250 mm. The two longer sides were sewn together to form a bag. The bags were then placed over individual bunches and 4 staples placed around the opening and top of the bag to secure it in place.

Treatment 5 (Plate 5-4) was designated the untreated control. Eight basal bunches were harvested at random from a single pre-designated vine.

Treatment 6 (Green house) was designed to increase ambient temperature around the bunch. A 1 litre slightly opaque cylindrical bucket was used to completely surround bunches. Four 20 mm evenly spaced holes, were drilled 1 cm from the bottom of the bucket, in the side of the cylinder to allow air movement. In the base of the bucket a large cross was cut using a razor blade. This allowed passage of the bucket over the

bunch so as the bunch was able to fit neatly in place. Care was taken not remove berries from bunches (Plate 5-5).

All treated samples were taken from vines and processed as per the general methods.

**Plate 5-2, Treatment 1, 100% light exclusion**

**Plate 5-3, Treatments 2-4 light exclusion shade cloth**

**Plate 5-4, Treatment 5, untreated control**

**Plate 5-5, Treatment 6, green house, slightly opaque plastic bucket**

### *5.2.3 Monitoring and description of bunch microclimate*

In two replicates of this experiment, monitoring equipment was deployed to describe changes to temperature and light as a result of the imposed treatments. Four bunches were selected in each treatment to be monitored, with a sensor for both temperature and light. Four sensors, two for temperature and two for light, were also placed within a monitoring station suspended 1m above the ground and under canopy nets (Plate 5-6).

A Datataker DT80 and channel expansion model (data taker CEM) were used to record temperature and light readings every 10 minutes for a three week period between the 9$^{th}$ March 2007 and 29$^{th}$ of March 2007.

Light was monitored using calibrated handmade sensors from light sensitive photodiodes peak intensity at 525 nM. (Model EPD-525-0-1.4. senetivity range 480-560 nM, sensitive area 1.79 mm$^2$, Roithner Lasertechnik) soldered to commercial 2

mm dual core speaker wire. The light sensor was placed at a point immediately above the highest berries directly adjacent to the rachis and facing north and directly in line with the row. Temperature was measured using handmade thermocouples made from 1 mm Type K, wire with a 10 mm polystyrene ball attached using an epoxy resin to buffer temperature fluctuations. Temperature sensors were calibrated and standardised using a mercury thermometer at 10 °C and 20 °C in the laboratory. Temperature sensors were placed in close proximity to the outer edge of the bunch on the northern side.

The six different treatments were monitored in two replicates, three treatments in each replicate, due to the logistics of running sensors from a data logger to randomly allocated treatment and the number of channels available for monitoring. For each treatment, three temperature and three light sensors were placed in different bunches, within a single replicate of that treatment. Each bunch was assessed for fruit composition, length, width, bunch weight and berry number as per the general methods. Individual points of this subset were used for correlation analysis.

Measurements were averaged and converted to a percentage of the ambient light to estimate the degree to which each treatment shades the bunch.

**Plate 5-6 Monitoring equipment deployed in the field.**

### *5.2.4 Statistical Analysis*

All results were normally distributed and untransformed data were analysed using a ANOVA in SPSS version 19 using a type III sum of squares analysis. Means were compared using Fischers Least Significant Difference (LSD) calculated at P=0.05 after the method of Steele and Torrie (Steel & Torrie, 1980)

Correlation analysis was carried out using the univariate linear regression models package in SPSS version 19 to assess independent variables.

## 5.3 Results

Total anthocyanin concentration (mg/g) was found to be significantly increased only by the greenhouse treatment (Table 5-1). No significant differences were observed for any other recorded variables (Table 5-1). TA was lowest in the Greenhouse treatment. A significant correlation was observed between exposure (total accumulated light percentage of ambient) and total anthocyanin development when monitored bunches were processed individually (Figure 5.1). No relationship was recorded between any characteristic and average temperature.

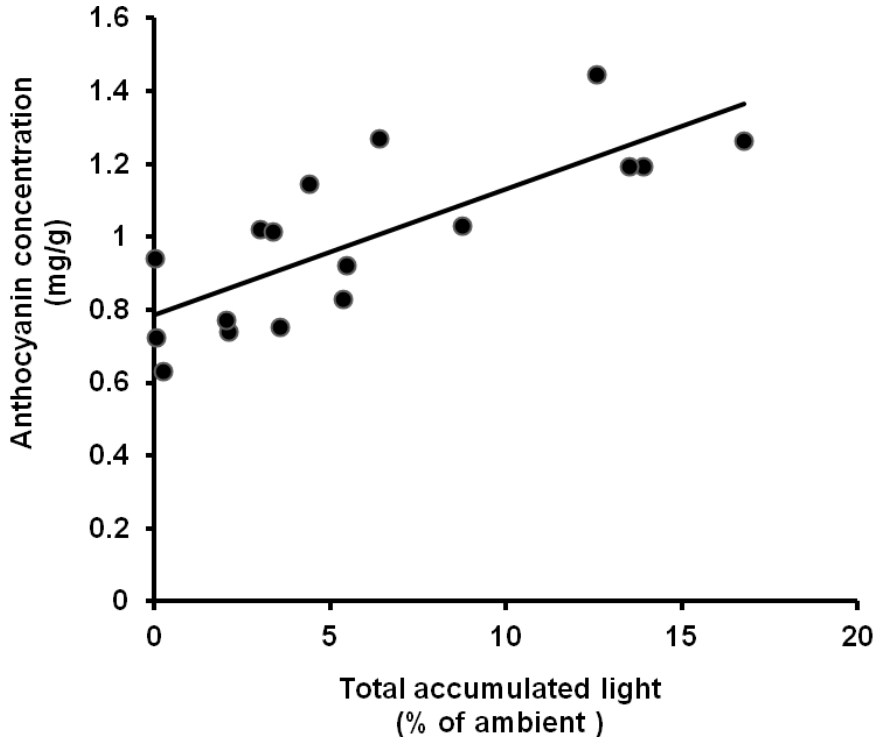


**Figure 5-1, Relationship between total accumulated light and accumulated anthocyanin in Pinot Noir**

**$y = 0.0345x + 0.7864$ and $R^2 = 0.61$.**

A significant correlation was also found between accumulated light and anthocyanin mg/g ($P<0.05$, $R^2 = 0.61$)

**Table 5-1 The response of fruit compositional and yield components to shading treatments in *Vitis vinifera* cv. Pinot Noir**

| | pH | Titratable acidity (g/L) | Total Anthocyanin (mg/g) | Total Anthocyanin per berry | Soluble solids (°Brix) | Mean bunch weight (g) | Berry number | Primary branch number | Mean berry weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| **Dark** | 4.13 | 6.06 | 0.70a | 0.32 | 25.9 | 59.0 | 129.3 | 14.4 | 0.47 |
| **90% shade** | 3.82 | 6.13 | 0.90a | 0.40 | 24.9 | 59.3 | 133.9 | 14.4 | 0.42 |
| **70% shade** | 3.66 | 6.48 | 0.92a | 0.39 | 24.6 | 51.4 | 119.4 | 13.5 | 0.44 |
| **50% shade** | 3.90 | 6.01 | 0.94a | 0.44 | 25.6 | 57.1 | 122.0 | 14.0 | 0.49 |
| **Untreated** | 4.12 | 6.55 | 0.87a | 0.41 | 24.3 | 51.5 | 108.6 | 12.0 | 0.48 |
| **Green house** | 3.95 | 5.27 | 1.15b | 0.50 | 26.3 | 44.2 | 102.1 | 12.9 | 0.44 |
| **$P_{value} \leq 0.05$** | ns | ns | 0.045 | ns | ns | ns | ns | ns | ns |

**Table 5-2, Total mean accumulated light for shaded bunches expressed as percentage of total ambient light**

| Treatment | Mean light interception ( % ambient) | Standard error |
|---|---|---|
| **Dark** | 0.03% | 0.02 |
| **90% shade** | 2.08% | 0.69 |
| **70% shade** | 2.73% | 0.63 |
| **50% shade** | 4.76% | 0.40 |
| **Untreated** | 9.56% | 2.45 |
| **Green House** | 14.28% | 2.98 |

**Table 5-3, Maximum temperature, minimum temperature and mean temperature of sensors located within the bunch in response to imposed shade treatments (Standard error represents variation between sensors)**

| | Maximum temperature (°C) | Standard error | Minimum temperature (°C) | Standard error | Mean temperature (°C) | Standard error |
|---|---|---|---|---|---|---|
| **Dark** | **37.16** | 0.39 | **3.41** | 2.80 | **20.33** | 0.19 |
| **90% shade** | **36.34** | 1.03 | **7.80** | 2.91 | **20.45** | 0.10 |
| **70% shade** | **40.20** | 0.89 | **4.00** | 2.60 | **20.58** | 0.12 |
| **50% shade** | **40.43** | 0.24 | **4.13** | 1.30 | **20.73** | 0.06 |
| **Untreated** | **41.85** | 0.14 | **4.85** | 0.58 | **21.02** | 0.04 |
| **Green House** | **39.92** | 0.81 | **0.03** | 3.18 | **20.30** | 0.23 |
| **Ambient** | **41.67** | 0.07 | **5.93** | 0.00 | **21.35** | 0.02 |

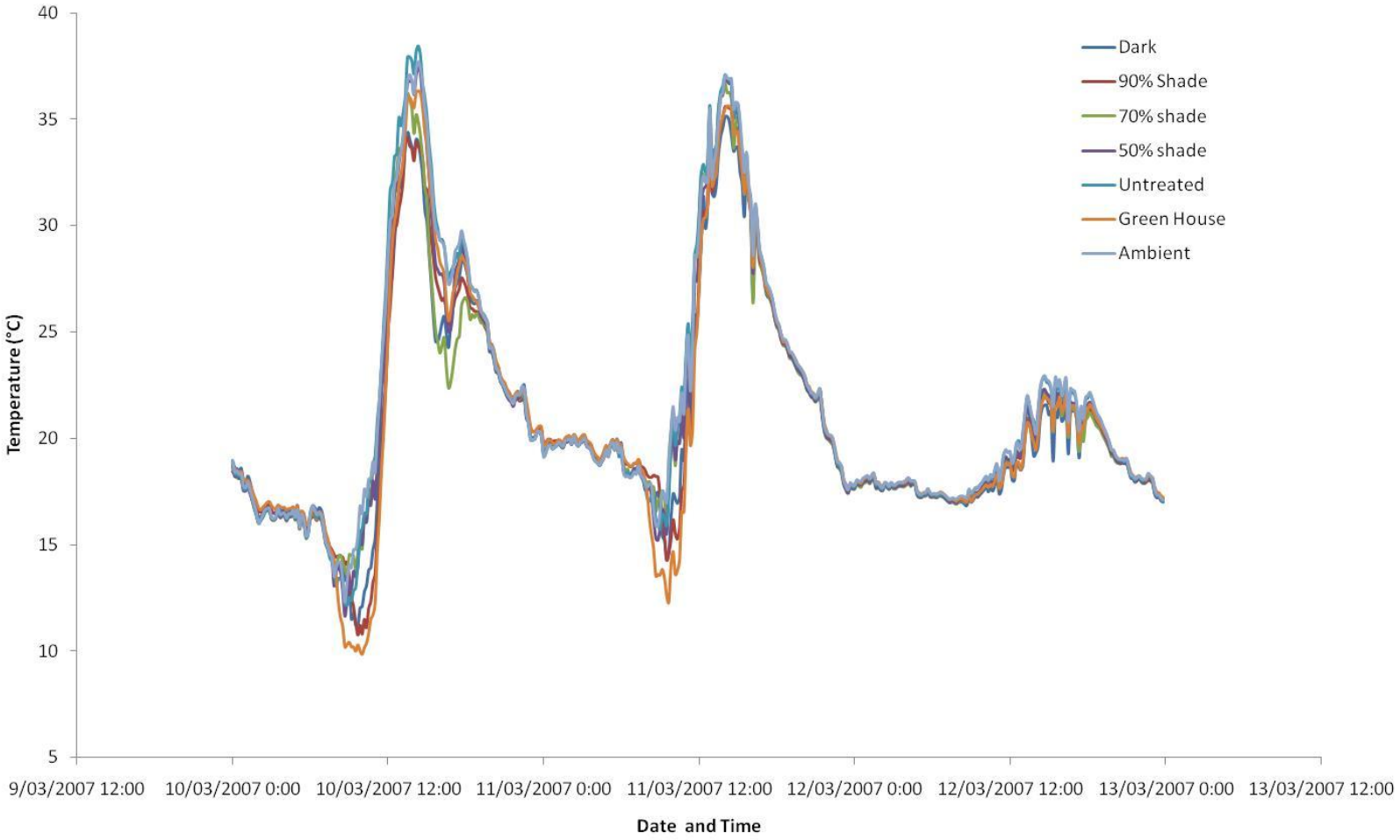


**Figure 5-2, 24 hour mean temperature of sensors located within shaded bunches of Pinot Noir and ambient between 10/03/2007 – 12/03/2007**

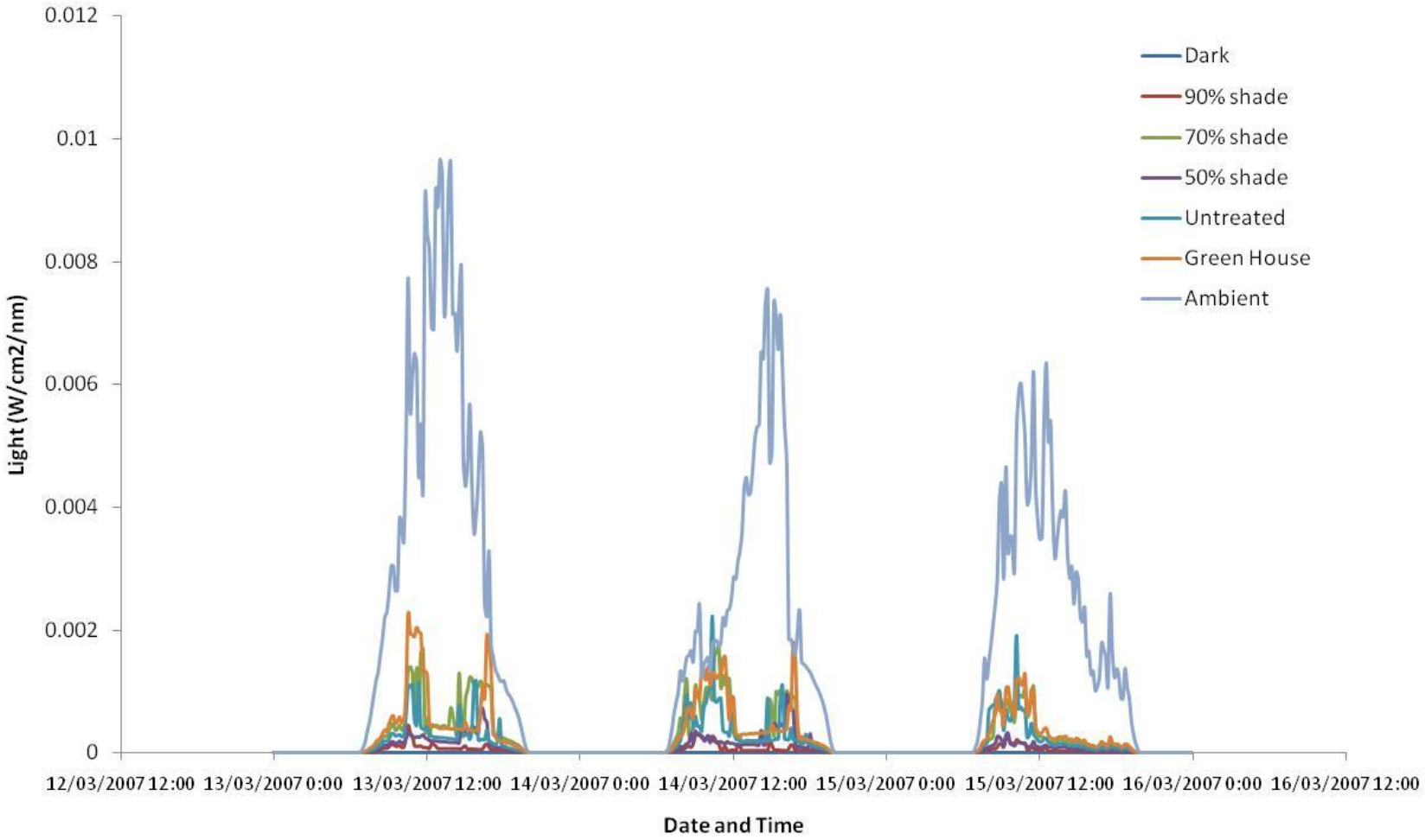


**Figure 5-3, 24 hour mean light curves located within shaded bunches of Pinot Noir and ambient between 10/03/2007 – 12/03/2007**

## 5.4 Discussion

Shading did not significantly influence anthocyanin composition of shaded treatments (Table 5-1). This result is similar to that of Cortell and Kennedy, (2006) and Downey et al., (2004) in Pinot Noir and Shiraz respectively. Both studies reported a significant shift in the proportion of individual anthocyanin glucosides, but no impact on total anthocyanin concentration. The correlation between increasing exposure and total anthocyanin development (Figure 5.1) on the other hand, contradicts the analysis of variance results for in this experiment and also the results of Cortell and Kennedy, (2006) and Downey et al., (2004). Though not significantly different from the control or shaded treatments the lowest average anthocyanin concentration was recorded in the dark treatment.

UV radiation has been implicated in increasing the anthocyanin concentration in fruits such as peach (Kataoka & Beppu, 2004). Anthocyanins have been implicated as scavengers of free radicals in berries (Kahkonen et al., 2003). Cortell and Kennedy, (2006) present an argument that differences in UV exposure shift the ratio of anthocyanin biosynthesis in the skin tissues as a response to stress. Though not significantly different, the lowest amount of anthocyanin was recorded in the shaded treatment. In the present experiment berry size was consistently small. High total anthocyanins were recorded across all treatments and were twice that observed by Cortell and Kennedy, (2006). Greater replication in this experiment may have lead to significant differences between treatments being observed by accounting for more natural variation. It is not known if high absolute values recorded in the present study are an artefact of differing method, differing levels of plant "stress" associated with environmental influence (Cortell et al., 2007), differing genotype (Mori et al., 2005), or possibly increases in UV B radiation as a result of a cyclic pattern in stratospheric ozone depletion associated with proximity to Antarctica (Solomon et al., 2005).

It has been observed that gene expression of key enzymes of the two biosynthetic pathways for anthocyanin in grapevine may be significantly altered in response as a result of temperature (Mori et al., 2005), a similar influence may have occurred here.

The present experiment should be repeated with specific attention to shifts in individual polyphenols and monitoring of radiation in the UV spectrum.

Total anthocyanin concentration of fruit in this experiment was significantly affected by only the "green house" treatment, in which the highest level of accumulated anthocyanin was observed (Table 5-1). This treatment was designed to increase the temperature of fruit by trapping solar radiation inside the cylinder structure, in a greenhouse like effect. Anthocyanin concentration of fruit has been shown to increase as a result of higher temperature (Dokoozlian & Kliewer, 1996). Observations of minimum temperatures and maximum temperatures (Table 5-3) recorded for the green house treatments did not appear to vary markedly from other treatments. An unexpected observation recorded for this treatment was a reduction in overnight temperatures, as displayed by mean minimum temperature (Table 5-2) and when viewing pre-dawn temperature curves (Figure 5-3). This observation was only recorded in only the green house treatment. There are a number of possible explanations for this observation. Equipment failure can't be ruled out as factor. The equipment was calibrated in laboratory conditions between 10 °C and 20 °C. Examining the curves in Figure 5-3 much of the variability in temperature between treatments occurs over two periods, during pre-dawn measurement or during light exposure in the fruiting zone during the middle of the day before and directly after the canopy is shaded as a result of the mid day sun being directly shaded by the canopy (Figure 5.4). Given the absence of intense light on the sensor or within the bunch zone, little variation between sensors occurs. A consistent curve is observed particularly for large parts of the night leading up to the predawn period, the evening and during cloudy cooler days for example the 12$^{th}$ of January 2007 (Figure 5.3). This would suggest that equipment was operating normally for the majority of the day.

High daily maximum and minimum temperatures recorded in Figure 5.2 are well above those recorded at the nearby Hobart Airport for similar days. For example, maximum temperature recorded at the Hobart airport from the 10$^{th}$-12$^{th}$ of March 2007 were 23.5, 26.3 and 16.1. In some cases there was over 10 °C difference between the recorded maximum in the experiment and the recorded daily maximum

at the Hobart airport. The two sites have different microclimates as a result of differing aspect and the surrounding geography. This may explain some of the variability in maximum temperatures between the Hobart Airport and the Experimental site, but it is unlikely that it explains a 10 °C shift in temperature. The equipment used to measure differences in voltage produced by thermocouples, utilises an internal standard, on which it bases temperature values. For this reason polystyrene protection was added around the recording device. Insufficient insulation around the device, which was located in a large metal box (Plate 5.6), may be responsible for some of the differences in daily maximum temperatures recorded. Although it is reasonable to question the accuracy of temperatures reported in this study, the differences between treatments are expected to have been affected similarly, given the utilisation of a standard internal measure from which all measurements are compared at any given time. The standard error calculated for temperature measurements does not suggest that a particular treatment exhibits more variability than other treatments (Table 5-3). The low standard error term for the ambient sensors may also suggest that the variability in measurements may be either in response to treatments or natural variability in the bunch microclimate. This explanation may take into account high maximum temperatures, but does not explain low temperatures, particularly those recorded in the green house treatment.

A plausible explanation for this observation may be that the lack of a buffering leaf layer immediately surrounding the bunch caused lower pre-dawn fruit temperatures. Increasing diurnal fluctuation in temperature has been observed to increase anthocyanin accumulation in grape berries (Mori et al., 2005). Differences in gene expression of key enzymes in the anthocyanin biosynthesis pathway were discussed as the likely cause for this increase (Mori et al., 2005), and that absisic acid may control the expression of anthocyanin biosynthetic enzyme genes (Yamane et al., 2006). Speculating, it may also be the case that increased capture of radiation within the cylinder, created by the bucket, did indeed result in intensified levels of radiation of a particular wave length. Without further experimentation the mechanism of this observation remains unknown.

Other fruit compositional parameters were unaffected as a result of changes to in exposure to light. While soluble solids have been shown to increase with fruit exposure (Petrie et al., 2003) the lack of a result is in agreement with other authors (Cortell & Kennedy, 2006; Hunter et al., 1995). Though lowest in the greenhouse treatment, TA was unaffected by treatment. TA is directly influenced by the respiration rate of the berry which is a direct function of temperature (Kliewer & Lider, 1968). It would have been expected that if exposure significantly impacted on fruit temperature TA would have been lower in less shaded treatments (Jackson, DI & Lombard, 1993). Diurnal fluctuations in temperature have also been discussed as being important in maintaining acid and lowering pH for longer during maturation, with high day temperatures and low night temperatures being the most favourable (Kliewer & Torres, 1972). The lack of an observable difference in pH due to the complicated nature of conditions imposed by this experiment and the complicated nature of changes to pH in the vineyard (Bisson, 2001) is therefore not surprising.

# Chapter 6 - The effect of bunch architecture and internal shading on fruit composition in Pinot Noir

## 6.1 Introduction

In some *Vitis vinifera* varieties, particularly Pinot Noir and Chardonnay, berries may grow and expand so that the bunches of some varieties form almost solid bodies (May, 2000). This observation has created speculation (Heazlewood, 2005) that fruit composition may be influenced by the degree to which individual berries are shaded within the greater structure of the bunch (cluster). Visual examination of clusters with a "tight" structure often reveals berries which appear to be of reduced colour intensity. The degree of "compactness" of a bunch is directly influenced by berry number and size, in comparison to the volume occupied by the extremity of the bunch. The final volume occupied is largely determined by the growth of the inflorescence and in particular the cluster rachis which provides the framework to which berries attach (Shavrukov et al., 2004). It has also been observed that a relationship between anthocyanin (absorbance at 520 nm of skin discs taken from Pinot Noir) and the number of berries per bunch, may indicate that bunch size or compactness could be related to the fruit composition of Pinot Noir. A review by May (2000) has suggested that rachis branch number, inflorescence and berry number and the overall morphology of the bunch, may all be important in determining the final compactness. Inflorescence primordia growth begins in the previous vegetative season in latent buds (Mullins et al., 1992). Following bud burst, a sigmoidal pattern of inflorescence growth is observed. The most rapid growth immediately precedes capfall, after which the growth rate slows then plateaus until the point of harvest (Shavrukov et al., 2004).

Environmental factors such as temperature and day length influence inflorescence initiation and development (Buttrose & Hale, 1973; Sugiura et al., 1975). Rachis internode length has also been shown to be a major factor in bunch compactness and is likely to be under the control of a single major gene in *Vitis vinifera* (Dry, IB & Thomas, 2003). Studies reveal that changes to inflorescence architecture may be a

function of cell division, cell expansion, or both within the rachis of the inflorescence (Goosey & Sharrock, 2001). In *Vitis* extension of the rachis has been shown to be almost solely the function of internode cellular expansion prior to anthesis (Shavrukov et al., 2004).

Application of Gibberelic Acid ($GA_3$) has been used experimentally in *Vitis vinifera* since the mid 1950's. It has widely been discussed as a tool for reducing the compactness of the bunch and as a tool for yield control in various species including *Vitis vinfera.* Early studies indicated that the response of *Vitis vinfera* to application of $GA_3$ were numerous and diverse. Spraying and dipping treatments at various developmental stages in *Vitis vinfera* resulted in an increase in shoot and internode length, berry elongation, hastened onset of flowering, increase in cluster and rachis length, an increase in the number of shot (seedless) berries and that the colouration of the red wine grape variety Zinfandel was advanced following application (Weaver & McCune, 1959b) and further that treatment at bloom resulted in many shot berries, reduced frequency of bunch rot, crop reduction and reduced berry number (Weaver & McCune, 1959a). The translocation of $GA_3$ within the vine was also observed to be readily translocated from leaves into developing clusters and also from clusters and lower leaves to the shoot apex, but not from one shoot to another. (Weaver & McCune, 1959a).

Studies have indicated that the ability of $GA_3$ to manipulate bunch architecture and compactness fall into two groups, treatments which affect fruit set (Dokoozlian et al., 2001; Teszlak et al., 2005; Weaver & McCune, 1959b) and those that affect bunch length (Ferree et al., 2003; Shavrukov et al., 2004). Treatments applied later in the development of the cluster, for example those applied at bloom or fruit set, reduce berry number and lead to an increase in berry size. This response may or may not lead to significant reduction in yield but do not significantly increase cluster length (Weaver & Pool, 1971). Pre-bloom treatment tends to lengthen clusters and hence decrease the cluster compactness (Ferree et al., 2003). $GA_3$ application has been shown to affect a number of growth and developmental processes in plants including the stimulation of cell division and elongation in rapidly growing tissues (Davies, 1995). As a factor of increased cell division and enlargement it is hypothesised that

application of $GA_3$ two weeks prior to the beginning of capfall (Ferree et al., 2003) and coinciding with the most rapid period of cluster elongation (Shavrukov et al., 2004) will induce elongation of the rachis.

The aim of the experiment is to examine the effect of $GA_3$ on bunch architecture in *Vitis Vinifera* cv Pinot Noir through application at EL15 approximately two weeks before the beginning of capfall. The expectation was that early application would increase cluster length with limited effects on berry number or size, thereby reducing the compactness of the bunch.

## 6.2 Materials and methods

### *6.2.1 Site Characteristics*

The experiment was carried out in a commercial vineyard located in southern Tasmania over three consecutive vintages between 2006 and 2008. The canopy was trained to a modified lyre trellis with shoots positioned vertically. Vines were Pinot Noir clone D5V12 (2051) greater than 25 years in age. They were pruned to two, two bud spurs and two, ten bud canes. Rows were orientated in an east west direction. BCV 42°49'12"S 147°50'26"E, elevation 65 m 27 km from the Hobart Airport Bureau of Meteorology weather station (BOM station 094008).

### *6.2.2 Treatment Design*

Pro Gibb (Sumitomo Chem, $GA_3$) was applied as a single application at EL 15 approximately two weeks preceding the initial stages of capfall using 2.5 ml / L of Horti Oil (Synertrol) as a wetter. Previously treated vines were not used in subsequent seasons.

No reference to application of $GA_3$ on Pinot Noir could be found in the literature, studies have reported effective concentrations between 5 and 1000 ppm (Weaver & McCune, 1959b) with most in the range of 0 – 50 ppm (Ferree et al., 2003). In vintage 2006 concentrations of 0, 5, 10, 25 and 50 ppm $GA_3$ and were applied to individual vines both by dipping bunches in a beaker of solution and spraying to the point of runoff in the fruiting zone in a 5 treatment, 2 application method, 4 replicate random block design. Vines were separated by untreated buffer vines on both sides. No significant differences were observed in relation to the bunch structure and the concentrations were concluded to be too low to significantly impact rachis length and width. Results are not presented.

In vintage 2007 concentrations of 0, 150, 300 and 600 ppm $GA_3$ were applied to two vines on opposite (north and south) sides of the modified lyre. Bunches were either dipped in a beaker of solution of containing the four different $GA_3$ concentrations or

the fruiting zone was sprayed by hand to the point of runoff with the four different concentrations. Vines immediately neighbouring treated vines were left untreated and designated as buffer vines. The experiment was set up as an 8 treatment by 4 replicate random block design.

In the 2008 vintage concentrations of 0, 150, 300 and 600 ppm GA3 were applied to two vines on opposite (north and south) sides of the modified lyre. Bunches were sprayed with the 4 different concentrations in the fruiting zone, by hand to the point of runoff. Vines immediately neighbouring treated vines were left untreated and designated as buffer vines. The experiment was set up as a 4 treatment by 8 replicate random block design.

### *6.2.3 Measurement and observation*

Treatment application took place on the 23rd of November 2006 and the 25th of November 2007 for harvest on the 8th April 2007 and 15th of April 2008 respectively.

Observations of yield and fruit composition were carried out as per the general methods presented in chapter 3.

Theoretical bunch volume was estimated using rachis width and length.

$$\text{Theoretical volume} = 1/3\ \pi r^2\ l, \text{ where } r = \text{width}/2 \text{ and } l = \text{length}$$

Shavrukov et al., (2004) used displacement volume to measure the degree of bunch "compactness". Extremely ripe fruit cracked and disintegrated when plunged into water and the likely influence on fruit composition deemed the method inappropriate. Other authors such as Ferree *et al.,* (2003) used bunch length divided by bunch weight as a measure of compactness. The approach taken in the present study is therefore a combination of these two methods. 'Bunch density' was calculated to estimate the compactness of the bunch. This measure is calculated by dividing the weight of the bunch by its theoretical volume.

Density = Bunch weight / Theoretical Volume

### *6.2.4 Statistical Analysis*

All results were normally distributed and untransformed data were analysed using a type III ANOVA in SPSS 17. Means were compared using Least Significant Difference (LSD) calculated at P=0.05 after the method of Steele and Torrie (1980). For the 2007 vintage application method was found not to be significantly different and the experiment was therefore analysed as an 8 replicate by 4 treatment random block design.

## 6.3 Results

Application of $GA_3$ significantly increased the length and width of clusters in the 2007 vintage by a mean of up to 20 and 27 mm respectively (Table 6-1). In the 2008 vintage $GA_3$ had no significant effect on length but significantly increased width by a mean of 22 mm (Table 6-1). Volume of the bunch was significantly increased in both the 2007 and 2008 vintages and density was significantly reduced in both vintages, density and volume were impacted by treatment with $GA_3$ to a greater extent in the 2007 than the 2008 vintage (Table 6-1).

Berry weight was significantly increased by the 600 ppm treatment in the 2007 vintage (Table 6-2) Mean berry weight for all but the 600 ppm treatment was similar for both the 2007 and 2008 vintages. Bunch weight and berry number were not significantly affected in either vintage. Berry number and bunch weights were approximately 25 -30% larger in the 2008 vintage.

Significant differences in fruit composition were observed in the 2007 vintage but not the 2008 vintage in the $GA_3$ application treatments (Table 6-3). Total anthocyanin per unit weight and per berry, were both significantly increased while TA and pH were both reduced. Soluble solids concentrations were not significantly different between treatments.

**Table 6-1, The effect of pre-bloom gibberellic acid ($GA_3$) application at varying concentrations on bunch length, width, theoretical volume and estimated density of *Vitis vinifera* cv. Pinot Noir over two vintages. (Significant differences are represented by letter groupings in superscript)**

| | 2007 | | | | 2008 | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration GA3 (ppm)** | **Bunch Length (mm)** | **Bunch Width (mm)** | **Theoretical Bunch Volume ($cm^3$)** | **Bunch Density ($g/cm^3$)** | **Bunch Length (mm)** | **Bunch Width (mm)** | **Theoretical Bunch Volume ($cm^3$)** | **Bunch Density ($g/cm^3$)** |
| **0** | $109.1^{a}$ | $81.6^{a}$ | $286.6^{a}$ | $0.294^{a}$ | 128.6 | $86.4^{a}$ | $384.5^{a}$ | $0.313^{a}$ |
| **150** | $111.7^{ab}$ | $82.7^{a}$ | $316.9^{a}$ | $0.266^{a}$ | 130.5 | $96.0^{b}$ | $481.1^{ab}$ | $0.249^{b}$ |
| **300** | $121.0^{ab}$ | $97.2^{b}$ | $464.3^{b}$ | $0.179^{b}$ | 137.0 | $97.7^{b}$ | $518.6^{ab}$ | $0.244^{b}$ |
| **600** | $132.2^{b}$ | $107.1^{b}$ | $619.9^{c}$ | $0.154^{b}$ | 134.0 | $108.2^{c}$ | $621.8^{b}$ | $0.220^{b}$ |
| **$P_{value}$ ≤0.05** | 0.028 | 0.001 | 0.003 | 0.001 | ns | 0.001 | 0.001 | 0.002 |

**Table 6-2, The effect of pre-bloom gibberellic acid ($GA_3$) application at varying concentrations on yield components of *Vitis vinifera* cv. Pinot Noir over two vintages. (Significant differences are represented by letter groupings in superscript)**

| | 2007 | | | 2008 | | |
|---|---|---|---|---|---|---|
| **Concentration GA3 (ppm)** | **Mean Bunch Weight (g)** | **Mean Berry Number** | **Mean Berry Weight (g)** | **Mean Bunch Weight (g)** | **Mean Berry Number** | **Mean Berry Weight (g)** |
| **0** | 84.3 | 119.4 | $0.71^{a}$ | 116.6 | 130.6 | 0.67 |
| **150** | 74.6 | 115.4 | $0.66^{a}$ | 116.3 | 140.2 | 0.69 |
| **300** | 78.0 | 113.9 | $0.72^{a}$ | 125.1 | 142.9 | 0.69 |
| **600** | 86.6 | 105.3 | $0.84^{b}$ | 133.5 | 153.3 | 0.71 |
| **$P_{value}$ ≤0.05** | ns | ns | 0.016 | ns | ns | ns |

**Table 6-3. The effect of pre-bloom gibberellic acid ($GA_3$) application at varying concentrations on fruit composition of Vitis vinifera cv. Pinot Noir over two vintages. (Significant differences are represented by letter groupings in superscript)**

| | 2007 | | | | | 2008 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration GA3 (ppm)** | **pH** | **Titratable Acidity (g/L)** | **Anthocyanin (mg/g)** | **Soluble solids (°Brix)** | **Anthocyanin (mg /berry)** | **pH** | **Titratable Acidity (g/L)** | **Anthocyanin (mg/g)** | **Soluble solids (°Brix)** | **Anthocyanin (mg /berry)** |
| **0** | $3.26^{a}$ | $6.45^{a}$ | $0.99^{a}$ | 23.8 | $0.70^{a}$ | 3.78 | 5.39 | 0.86 | 25.8 | 0.76 |
| **150** | $3.19^{b}$ | $5.89^{b}$ | $1.22^{c}$ | 23.6 | $0.79^{a}$ | 3.78 | 5.71 | 1.05 | 26.3 | 0.87 |
| **300** | $3.21^{ab}$ | $5.67^{b}$ | $1.03^{ab}$ | 23.5 | $0.74^{a}$ | 3.65 | 5.52 | 0.94 | 25.9 | 0.82 |
| **600** | $3.13^{b}$ | $5.99^{ab}$ | $1.14^{bc}$ | 23.1 | $0.93^{b}$ | 3.89 | 5.49 | 0.94 | 25.5 | 0.80 |
| **$P_{value}$ ≤0.05** | 0.006 | 0.034 | 0.008 | ns | 0.002 | ns | ns | ns | ns | ns |

a)


b)

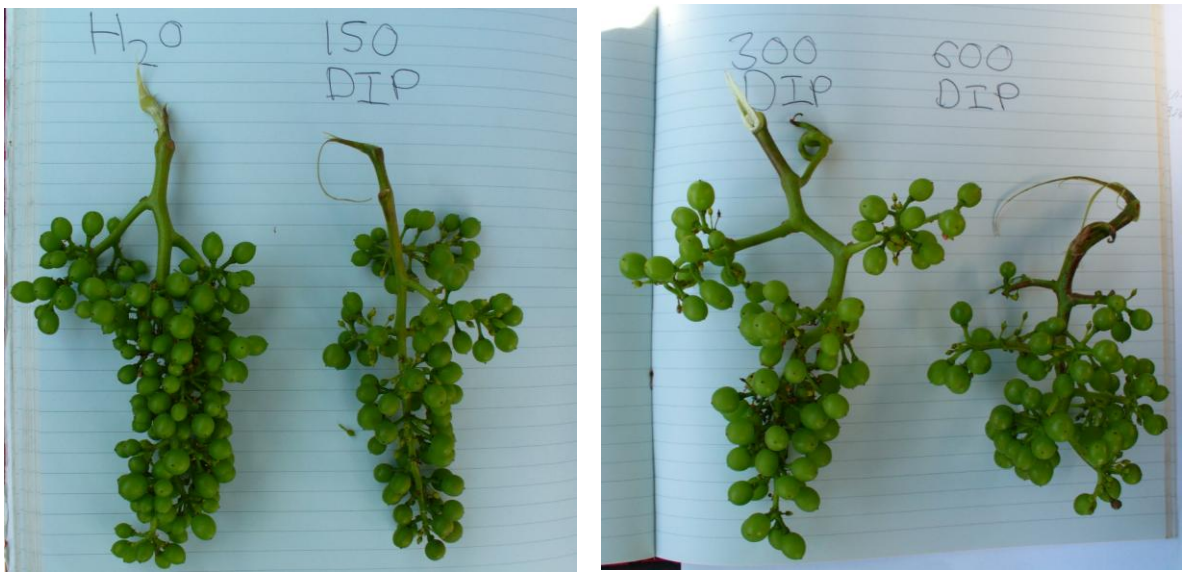


**Plate 6-1, Comparison of bunches taken from *Vitis vinifera* cv Pinot Noir treated with $GA_3$ at three concentrations (ppm) and a water control using two application methods spray (a) and dip (b).**

a)


b)


**Plate 6-2, Comparison of bunches taken from *Vitis vinifera* cv Pinot Noir treated with a water control (a) and 600ppm $GA_3$ (b) applied at EL15 following harvest.**

By combining the results of chapter 3 and chapter 5 into a bulk data set and comparing total anthocyanin and mean berry weight via a linear regression a significant negative correlation is observed between mean berry weight and total anthocyanin concentration (Figure 6-1).

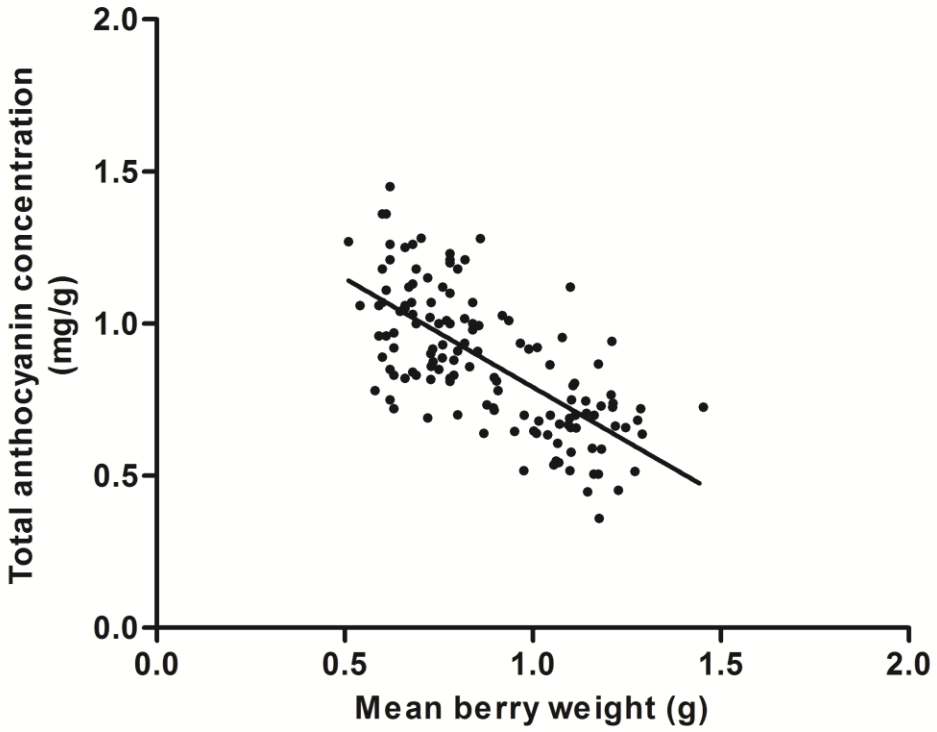


**Figure 6-1, The relationship between mean berry weight and total anthocyanin concentration in Pinot Noir over 4 vintages and from two commercial vineyards in southern Tasmania ($R^2$ = 0.47, P<0.0001, y = -0.741x + 1.505).**

## 6.4 Discussion

A single application of $GA_3$ to Pinot Noir grapevines significantly affected bunch length in the 2007 vintage and width in both the 2007 and 2008 vintages. For the 2007 vintage both width and length were increased incrementally and at the highest concentration by 23% and 18% respectively. In the 2008 vintage width increased by up to 20% in the 600 ppm $GA_3$ Treatment (Table 6-1, Plate 5-1 & 5-2). These observations indicate that pre-flowering application of $GA_3$ may increase the length of clusters by extending the rachis (Ferree et al., 2003). However the observation that width, not length was extended during the 2008 vintage may indicate a number of things. Studies suggest that the response of *Vitis vinifera* to $GA_3$ is affected by timing (Weaver & McCune, 1959b). It is possible that treatment of clusters in this study may have occurred following the period of rapid growth in bunch length and was only active during the period when bunch width increased. The timing of extension of secondary branches is a research question that arises from this study. It may also be argued that a reduction in berry number may also have been observed due to a pollenicide effect of $GA_3$ application timings closer to anthesis (Weaver & McCune, 1960). However in cool climates such as Tasmania, flowering and other developmental processes may occur over an extended period (Heazlewood, 2005) which may have reduced the ability of $GA_3$ application to act in this manner. The effect of $GA_3$ on plants is also known to be affected by environmental influence, such as temperature (Olszewski et al., 2002), which may have also moderated the influence of $GA_3$ application in this instance. If the results of this study were to be considered for commercial use in Pinot Noir, careful consideration would need to be given to the timing and rate of the spray. Unpublished data suggests that applications of $GA_3$ at concentrations lower than 50 ppm at EL15 were not sufficient to induce changes to bunch structure in Pinot Noir. Variation in the sensitivity of different varieties of *Vitis vinifera* to the exogenous application of $GA_3$ rates between 10 ppm to 1000 ppm have been described (Ferree et al., 2003; Harell & Williams, 1987; Weaver & McCune, 1959a, 1959b; Weaver & Pool, 1971) .

Berry weight was significantly larger in the 600 ppm $GA_3$ treatment in 2007 but not in the 2008 vintage (Table 6-2). Changes to berry weight and shape as a result of $GA_3$ have been observed in many previous studies (Ferree et al., 2003; Harell & Williams, 1987; Weaver & McCune, 1959a, 1959b; Weaver & Pool, 1971), though these results were due to reduced berry number and increases in shot berries which may not have significantly impacted yield. No treatment effect on berry number was observed for either the 2007 or 2008 vintage suggesting that fruit set was not impacted in this study. The 600 ppm $GA_3$ treatment though not significant ($p=0.61$) had the least number of berries. This may in part explain the observation of larger berries and no significant treatment effect on bunch weight and hence yield (not measured).

It was observed, but not measured, that hen and chickens (millerendage) seemed to be more prevalent at lower concentrations, whereas at higher concentrations of $GA_3$, a higher number of shot berries (collure) were observed. Further investigation would be required to confirm this observation in Pinot Noir. Previous studies have highlighted the theory that the number of berries in a tight bunch may be reduced as a result of competition for space and that berries may effectively be pushed off the pedicel (May, 2000). The results of this experiment do not support this theory. It is worth noting that bunches at maturity in the control treatments were not so compact as to see major abnormality to the shape of the berry.

There was no treatment effect on bunch weight in either vintage though bunch weights were lower in the 2007 vintage which also corresponded to shorter bunches in the control treatment. It is not known if the larger size of bunches could have impacted the influence of $GA_3$ on extension growth in 2008.

One objective of the experiment was to modify bunch width and length and thereby increase the volume occupied by the extremity of the bunch and decrease the density or ‘compactness’ of the bunch. In 2007 and 2008 bunch volume was significantly increased by application of $GA_3$ by as much as 100% in 2007 and 50% in 2008. In the 600ppm treatment bunch density was reduced by almost half in 2007 and by approximately a quarter in the 2008 vintage. This result confirms the observations of

previous authors who reported an increase in cluster length and decrease in compactness with pre-bloom application (Ferree et al., 2003).

Reduced bunch density significantly altered the visual appearance of bunches which may have had significant implications for the microclimate of the bunch. Bunches were no longer a single solid mass at harvest with all berries being visible from the exterior in the 600ppm (Plate 6-2).

Application of $GA_3$ significantly influenced fruit composition for the 2007 vintage but not the 2008 vintage. This observation would seem to both support and reject the hypothesis that changes to the architecture and therefore the density of the bunch can directly influence fruit composition. The results of previous chapters suggest that large changes to the microclimate of the fruiting zone have a limited influence on fruit composition of Pinot Noir grown in the cool maritime climate of Tasmania (Chapter 4). The observation that $GA_3$ application altered volume and density far less in the 2008 vintage may also further assist to serve as explanation.

In 2007 the application of $GA_3$ significantly increased total anthocyanin concentration per mg and per berry. This observation would seem to support the hypothesis that increasing the exposure of individual berries in grape varieties, which form dense bunches, may lead to a significant increase in total anthocyanin biosynthesis. Notably the highest concentration of anthocyanin mg/g in both seasons was in the 150 ppm treatment which, though not significant, in 2007 had the smallest mean berry size and was also observed (though not measured) to have a greater proportion of ‘chickens”.

One of the justifications of the present study was the observation that berry number per bunch was correlated ($R^2 = 0.61$) with anthocyanin concentration (Heazlewood, 2005). In this study a correlation was observed between increasing berry number and decreasing anthocyanin concentration. No correlation between berry number and anthocyanin mg per g ($R^2 = 0.05$) or between berry number and anthocyanin mg per berry ($R^2 = 0.02$) was observed in this study.

It has been suggested that berry size may influence the total anthocyanin composition of red grape varieties and therefore the resultant wines as a function of

the skin to juice ratio (surface area to volume) (Dunn et al., 2004; Roby et al., 2004). Not only does it assist to explain the increased levels of anthocyanin in the 150ppm treatment, but the relationship may also have implications for assessing fruit quality, site selection, canopy management strategies and selection of clones for new plantings.

Small berries may result in improved spectral properties and higher wine sensory scores (Johnstone et al., 1996). Skins are the primary site of important phenolics associated with Pinot Noir quality (Cortell & Kennedy, 2006; Cortell et al., 2008). Increases in wine quality are thought to occur because of the ratio for skin surface area to the volume of the flesh, in small berries, relative to large berries (Matthews and Anderson 1988). Cultural practices which influence berry size, such as partial rootzone drying, also influences berry skin thickness which determines the amount of tissue available for anthocyanin and tannin accumulation (Mathews & Kriedemann, 2006). Small berries have therefore been reported to have a similar skin to fruit ratio, and a similar juice yield when compared to large berries (Roby et al., 2004; Walker et al., 2005). Further work should identify weather the berry size influence reported in the present study is associated with an increase in skin thickness.

In the context of the present experiment it is notable that despite the relationship between berry size and anthocyanin concentration, in the 2007 season the 600 ppm treatment was the only treatment to have significantly increased concentrations of total anthocyanin concentration in both mg/g and mg per berry. This observation would seem to further support the hypothesis that lower bunch density in some seasons could lead to an increase in total anthocyanin concentration.

Coombe (1987) suggested that light exposure effects on fruit may be more dependent on temperature than exposure to light per se. The results of chapter 4 of this thesis and other authors (Cortell et al., 2007; Cortell & Kennedy, 2006; Downey et al., 2004) suggest that light exposure may have some influence on total anthocyanin composition, though the degree to which fruit may be impacted is limited. May (2000) highlights that in warm climates canopy manipulation to limit over exposure may be important, whereas in cooler climates manipulation, that reduce bunch compactness

or techniques that encourage a greater number of smaller bunches, may be an important for increasing fruit quality.

Application of $GA_3$ significantly reduced both pH and TA in 2007, though to a limited extent as displayed by a lack of separation between a number of treatments and the control. Maintenance of low pH with sufficient acid degradation has been linked to the quality and capacity of red wines to age (Somers & Evans, 1977). In cool climates such as that of Tasmania, achieving suitable acid degradation in association with low pH and optimal sugar concentrations can be a challenge, particularly in less favourable vintages. Acid metabolism is largely a function of temperature (Jackson, DI & Lombard, 1993). It may be expected that if exposure was increased to the extent whereby it could induce temperature up-regulated anthocyanin biosynthesis that TA may also have decreased in response to temperature induced heating and or cooling. The pattern of acid metabolism observed in this study seems to support this conclusion.

Changes to pH are complex and not necessarily a function of berry age (Bisson, 2001). It is therefore not surprising that pH remained low while TA also decreased. A combination of reduced TA in association with maintained low pH suggest that management techniques which promote a less dense bunch structure may lead to an improvement in fruit composition and in turn wine quality in cool climates.

The observation that there was no treatment effect on soluble solids in either vintage, suggests earlier reports of hastened ripening following treatment at fruit set or bloom (Harell & Williams, 1987; Weaver & McCune, 1959b) are likely to be the result of either crop reduction and hence carbohydrate availability, or the influence of greater cluster or berry exposure. Dreier et al., (2000) suggests that evapotranspiration is an important driver of carbohydrate loading of berries during ripening. A less compact cluster is likely to have a similar effect through increased airflow and solar heating of fruits, and therefore increase the evapotranspiration of individual berries within clusters. The bulk of the experiments utilising $GA_3$ describe warm climate situations where exposure has been shown to be very important for sugar accumulation (Coombe, B. G., 1987; Jackson, DI & Lombard, 1993). It is plausible that different

results would be observed in cool climates where berries heat to temperatures above ambient for only a very small period of the day as a direct result of exposure to solar radiation. A combination of carbohydrate source limitation combined with limited exposure effects on fruit evapotranspiration may explain the lack significant differences in the onset of ripening or sugar accumulation in this study.

# Chapter 7 - Conclusions industry recommendations and further research

This study has shown that leaf removal in the fruiting zone of Pinot Noir had no positive impact on fruit composition over a three year trial period. Instead leaf removal was shown to delay ripening and significantly affect vine balance. Ongoing sustained defoliation at high levels is likely to significantly reduce the quality of wines through a delay in ripening. The results of the study therefore neither confirm nor reject the hypothesis that fruiting zone defoliation lead to an improvement in fruit composition of Pinot Noir. It instead highlights the challenges and variability associated with field research.

Seasonal influences were shown to account for greater variability in fruit composition than for applied defoliation treatments. In this instance increasing severity of fruiting zone defoliation did not lead to a reduction in yield components and primary branching was not shown to be a better predictor in bunch size than other commonly used measures. Measurement of bunch morphology, shows little promise to be used in yield prediction models due to the strong over arching problem of variability in fruit set as a result of poor weather during flowering (Heazlewood, 2005; May, 2000). The decision to use this tool in vineyard management should consider overwintering carbohydrate reserves of the plant, vine balance as defined by Y:P and vigour of the vine canopy. Though not examined in this thesis leaf removal should be considered for the management of both powdery mildew and bunch rots.

Since the commencement of this thesis, much progress has been made in relation to our understanding of the development of phenolics in grapes and wine. The report here of a relationship between exposure and total anthocyanin per berry and potentially with diurnal temperature fluctuation, requires further research to examine the underlying mechanisms. The results of this study suggest that increased shading of bunches did lead to a negative influences on basic fruit composition in particular anthocyanin development. There is a growing body of evidence to suggest that ratios of particular phenolics may be influenced by exposure to UV light (Cortell &

Kennedy, 2006; Downey et al., 2004) or vine (Cortell et al., 2007; Cortell et al., 2008). The lack of an observed difference in total anthocyanin composition between treatments for the 2008 vintage in chapter six suggests that the relationship between fruit exposure and anthocyanin development is not a simple temperature driven metabolic process, which is in agreement with the conclusions of other studies (Downey et al., 2004). It is suggested instead that various other factors, such as primary metabolism, may also influence anthocyanin biosynthesis in any particular vintage. Despite the range of evidence presented for the relative effect of bunch exposure on total anthocyanin accumulation in Vitis *vinifera*, the relationship presented in Figure 5.1 suggests that fruit exposure should not be dismissed in relation to achieving maximum levels of total anthocyanin in fruit and hence the potential to achieve maximum levels in must and wine. It should be stressed that measures of fruit composition did not take into account many flavour and aroma compounds which have been shown to be effected by shading, for example methoxypyrazines which cause green or bell pepper aromas (Allen et al., 1995).

Pre-bloom $GA_3$ application shows promise to improve the quality of fruit and reduce the compactness of varieties such as Pinot Noir and it can be neither confirmed nor rejected that shading within the bunch, leads to negative influences on fruit composition. While it was not the aim of the experiment to influence bunch architecture for disease control, many authors (Dry, IB & Thomas, 2003; Ferree et al., 2003; Shavrukov et al., 2004; Vail & Marois, 1991) have highlighted the influence a reduction in bunch compactness may have on disease control particularly for bunch rots. Bunch rot by *Botrytis cinerea* has been identified as a major issue by the Tasmanian wine industry. Pre bloom application of $GA_3$ shows significant promise to reduce the influence of bunch rot, though further work would be required to optimise timing and rates and likely to differ for different varieties. Though not measured in the present experiment, return bunch number in the following season should be a consideration in further work, as a reduction in number has been observed as the result of application of $GA_3$ (Harell & Williams, 1987). Further work should also focus on the relationship presented between anthocyanin and berry size to examine

juice to skin ratios and if this may be used practically to improve the quality of wines made from Pinot Noir.

# Chapter 8 - References


ABS 2005, *1384.6 - Statistics - Tasmania*.

ACE-CRC 2010, *Climate Futures for Tasmania general climate impacts: the summary*, Antarctic Climate and Ecosystems Cooperative Research Centre, Hobart, Tasmania.

Allen, MS 1997, 'Phenolics Demystified', in *ASVO Oenology Seminar, Phenolics and Extraction*, Adelaide pp. 4-11.

Allen, MS, Lacey, MJ & Boyd, SJ 1995, 'Methoxypyrazines in Red Wines - Occurrence of 2-Methoxy-3-(1-Methylethyl)Pyrazine', *Journal of Agricultural and Food Chemistry*, vol. 43, no. 3, pp. 769-772.

AWBC 2010, *Annual Report*, National Wine Center, Adelaide

Bergqvist, J, Dokoozlian, N & Ebisuda, N 2001, 'Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California', *American Journal of Enology and Viticulture*, vol. 52, no. 1, pp. 1-7.

Bisson, LF 2001, 'In search of optimal grape maturity', *Practical Winery and Vineyard Journal*, vol. July/August, pp. 32-43.

Bledsoe, AM, Kliewer, WM & Marois, JJ 1988, 'Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines', *American Journal of Enology and Viticulture*, vol. 39, no. 1, pp. 49-54.

Boss, PK, Davies, C & Robinson, SP 1996a, 'Analysis of the expression of anthocyanin pathway genes in developing Vitis vinifera L cv Shiraz grape berries and the implications for pathway regulation', *Plant Physiology*, vol. 111, no. 4, pp. 1059-1066.

Boss, PK, Davies, C & Robinson, SP 1996b, 'Expression of anthocyanin biosynthesis pathway genes in red and white grapes', *Plant Molecular Biology*, vol. 32, no. 3, pp. 565-569.

Boulton, R 2001, 'The copigmentation of anthocyanins and its role in the color of red wine: A critical review', *American Journal of Enology and Viticulture*, vol. 52, no. 2, pp. 67-87.

Bravdo, B, Hepner, Y, Loinger, C, Cohen, C & Tabacman, H 1985, 'Effect of crop level and crop load on growth yield, and must and wine composition, and quality of

Cabernet Sauvignon', *American Journal of Enology and Viticulture*, vol. 36, pp. 125-131.

Buttrose, MS & Hale, CR 1973, 'Effect of temperature on development of grapevine inflorescence after bud burst', *American Journal of Enology and Viticulture*, vol. 24, no. 1, pp. 14-16.

Candolfivasconcelos, MC, Koblet, W, Howell, GS & Zweifel, W 1994, 'Influence of Defoliation, Rootstock, Training System, and Leaf Position on Gas-Exchange of Pinot-Noir Grapevines', *American Journal of Enology and Viticulture*, vol. 45, no. 2, pp. 173-180.

Caspari, HW, Lang, A & Alspach, P 1998, 'Effects of girdling and leaf removal on fruit set and vegetative growth in grape', *American Journal of Enology and Viticulture*, vol. 49, no. 4, pp. 359-366.

Chapman, DM, Matthews, MA & Guinard, JX 2004, 'Sensory attributes of cabernet sauvignon wines made from vines with different crop yields', *American Journal of Enology and Viticulture*, vol. 55, no. 4, pp. 325-334.

Chellemi, DO & Marois, JJ 1992, 'Influence of leaf removal, fungicide applications, and fruit maturity on incidence and severity of grape powdery mildew', *American Journal of Enology and Viticulture*, vol. 43, no. 1, pp. 53-57.

Coombe, BG 1959, 'Fruit set and development in seeded grape varieties as affected by defoliation, topping, girdling, and other treatments', *American Journal of Enology and Viticulture*, vol. 10, no. 2, pp. 85-100.

Coombe, BG 1987, 'Influence of temperature on composition and quality of grapes', *Acta Horticulturae*, vol. 206, pp. 23–35.

Coombe, BG 1995, 'Adoption of a system for identifying grapevine growth stages', *Australian Journal of Grape and Wine Research*, vol. 1, no. 100-110.

Coombe, BG & McCarthy, MG 1997, ' Identification and naming of aroma development in ripening grape berries', *Australian Journal of Grape and Wine Research*, vol. 3, no. 18-20.

Coombe, BG & McCarthy, MG 2000, 'Dynamics of grape berry growth and physiology of ripening', *Australian Journal of Grape and Wine Research*, vol. 6, no. 2, pp. 131-135.

Cortell, JM, Halbleib, M, Gallagher, AV, Righetti, TL & Kennedy, JA 2007, 'Influence of vine vigor on grape (Vitis vinifera L. cv. Pinot Noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit', *Journal of Agricultural and Food Chemistry*, vol. 55, no. 16, pp. 6575-6584.

Cortell, JM & Kennedy, JA 2006, 'Effect of shading on accumulation of flavonoid compounds in (Vitis vinifera L.) Pinot noir fruit and extraction in a model system', *Journal of Agricultural and Food Chemistry*, vol. 54, no. 22, pp. 8510-8520.

Cortell, JM, Sivertsen, HK, Kennedy, JA & Heymann, H 2008, 'Influence of vine vigor on Pinot noir fruit composition, wine chemical analysis, and wine sensory attributes', *American Journal of Enology and Viticulture*, vol. 59, no. 1, pp. 1-10.

CRCV 2006, *AWRI Industry Standard Methods - Determination of total anthocyanins (colour) in red grape berries*, Cooperative Research Centre for Viticulture, www.crcv.com.au.

Creasy, GL, Price, SF & Lombard, PB 1993, 'Evidence for Xylem Discontinuity in Pinot-Noir and Merlot Grapes - Dye Uptake and Mineral-Composition During Berry Maturation', *American Journal of Enology and Viticulture*, vol. 44, no. 2, pp. 187-192.

Davies, PJ (ed.) 1995, *Plant Hormones: Physiology, Biochemistry and Molecular Biology*., Kluwer Academic Publishers, Dordrecht.

Deloire, A, Carbonneau, A, Wang, ZP & Ojeda, H 2004, 'Vine and water a short review', *Journal International Des Sciences De La Vigne Et Du Vin*, vol. 38, no. 1, pp. 1-13.

Dokoozlian, NK, Ebisuda, NC & Hashim, JM 2001, 'Gibberellic acid bloom sprays reduce fruit set and improve packable yield of 'Autumn Royal' table grapes', *Journal American Pomological Society*, vol. 55, no. 1, pp. 52-57.

Dokoozlian, NK & Kliewer, WM 1996, 'Influence of light on grape berry growth and composition varies during fruit development', *Journal of the American Society for Horticultural Science*, vol. 121, no. 5, pp. 869-874.

Downey, MO, Dokoozlian, NK & Krstic, MP 2006, 'Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research', *American Journal of Enology and Viticulture*, vol. 57, no. 3, pp. 257-268.

Downey, MO, Harvey, JS & Robinson, SP 2004, 'The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes', *Australian Journal of Grape and Wine Research*, vol. 10, no. 1, pp. 55-73.

Doyle, R & Farquhar, D 2011, *Tasmanian viticultural soils and geology*, http://www.dpipwe.tas.gov.au/inter.nsf/WebPages/CART-6F56YE?open viewed 24/09/2011.

Dreier, LP, Stoll, GS & Ruffner, HP 2000, 'Berry ripening and evapotranspiration in Vitis vinifera L', *American Journal of Enology and Viticulture*, vol. 51, no. 4, pp. 340-346.

Dry, IB & Thomas, MR 2003, *Report CRV 99/14d: Modification of grape cluster architecture for the control of fungal diseases*, Cooperavtive research center for viticulture.

Dry, PR, Iland, P & Ristic, R 2004, 'What is vine balance?', in R Blair, Williams, P. and Pretorius, S. (ed.), *Proceedings of the 12th Australian Wine Industry Technical Conference*, Melbourne, Australia, pp. 68–74.

Dry, PR & Smart, RE 1988, 'The grape growing regions of Australia', in BG Coombe & PR dry (eds), *Viticulture: Volume 1, Resources*.

Duncan, RA, Stapleton, JJ & Leavitt, GM 1995, 'Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as influenced by leaf removal', *Plant Pathology*, vol. 44, no. 6, pp. 956–965.

Dunn, GM, Martin, DJ & Petrie, PR 2004, 'Managing yield variation in vineyards', in *Proceedings of the 12th Australian Wine Industry Technical Conference*, Melbourne, pp. 51-56.

Dunn, GM & Martin, SR 2000, 'Do temperature conditions at budburst affect flower number in Vitis vinifera L. cv. Cabernet Sauvignon?', *Australian Journal of Grape and Wine Research*, vol. 6, no. 2, pp. 116-124.

English, JT, Kaps, ML, Moore, JF, Hill, J & Nakova, M 1993, 'Leaf removal for control of Botrytis bunch rot of wine grapes in the midwestern United States', *Plant Disease*, vol. 77, no. 12, pp. 1224-1227.

Ezzahouani, A & Williams, LE 2003, 'Trellising, fruit thinning and defoliation have only small effects on the performance of 'Ruby Seedless' grape in Morocco', *Journal of Horticultural Science and Biotechnology*, vol. 78, no. 1, p. 79—83.

Fang, Y & Qian, M 2005, 'Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA)', *Flavour and Fragrance Journal*, vol. 20, no. 1, pp. 22-29.

Ferree, DC, Ellis, MA, McArtney, SJ, Brown, MV & Scurlock, DM 2003, 'Comparison of fungicide, leaf removal and gibberellic acid on development of grape clusters and botrytis bunch rot of 'Vignoles' and 'Pinot Gris'', *Small Fruits Review*, vol. 2, no. 4, pp. 3-18.

Gal, Y, Naor, A & Bravdo, B 1996, 'Effect of shoot density, crop level and crop load on fruit and wine quality of 'Sauvignon Blanc' grapes', in S Poni, E Peterlunger, F

Iacono & C Intrieri (eds), *First ISHS Workshop on strategies to optimize wine grape quality.*, ISHS, Act Horticulturae., Conegliano, Italy, vol. 427, pp. 151-160.

Goosey, L & Sharrock, R 2001, ' The Arabidopsis compact inflorescence genes: phase-specific growth regulation and the determination of inflorescence architecture', *Plant Journal*, vol. 2, pp. 549-559.

Gubler, WD, Bettiga, LJ & Heil, D 1991, 'Comparisons of hand and machine leaf removal for the control of Botrytis bunch rot', *American Journal of Enology and Viticulture*, vol. 42, no. 3, pp. 233-236.

Hamilton, RP & Coombe, BG 2004, 'Harvesting wine grapes.', in BG Coombe & PR Dry (eds), *Viticulture, Volume 2 - Practices* Winetitles, Adelaide, Australia, pp. 302–327.

Harell, DC & Williams, LE 1987, 'The influence of gridling and gibberelic acid application at fruitset on ruby seedless and thompson seedless grapes', *American Journal of Enology and Viticulture*, vol. 38, no. 2, pp. 83-88.

Haselgrove, L, Botting, D, Heeswijck, Rv, Hoj, PB, Dry, PR, Ford, C & Iland, PG 2000, 'Canopy microclimate and berry composition: the effect of bunch exposure on the phenolic composition of Vitis vinifera L cv. Shiraz grape berries', *Australian Journal of Grape and Wine Research*, vol. 6, no. 2, pp. 141–149.

He, JJ, Liu, YX, Pan, QH, Cui, XY & Duan, CQ 2010, 'Different anthocyanin profiles of the skin and the pulp of Yan7 (Muscat Hamburg x Alicante Bouschet) grape berries', *Molecules*, vol. 15, no. 3, pp. 1141-1153.

Heazlewood, JE 2005, 'Aspects of Vitis vinifera L. cv Pinot Noir fruitfulness in Tasmania', Ph.D thesis, University of Tasmania

Heuvel, JEV, Robidoux, JL, Autio, WR & Neto, CC 2005, 'Timing of partial defoliation affects carbohydrate concentration of vegetative tissues and concentration of phenolics in berries of potted De Chaunac (Vitis sp.) grapevines', *Canadian Journal of Plant Science*, vol. 85, no. 3, pp. 703-708.

Howell, GS 2001, 'Sustainable grape productivity and the growth-yield relationship: A review', *American Journal of Enology and Viticulture*, vol. 52, pp. 165-174.

Howell, GS, Candolfi-Vasconcelos, MC & Koblet, W 1994, 'Response of Pinot Noir grapevine growth, yield, and fruit composition to defoliation the previous growing season', *American Journal of Enology and Viticulture*, vol. 45, no. 2, pp. 188-191.

Hummell, AK & Ferree, DC 1998, 'Interaction of crop level and fruit cluster exposure on 'Seyval blanc' fruit composition', *Journal of the American Society for Horticultural Science*, vol. 123, no. 5, pp. 755-761.

Hunter, JJ & Le Roux, DJ 1992, 'The effect of partial defoliation on development and distribution of roots of Vitis-Vinifera L Cv Cabernet-Sauvignon grafted onto rootstock 99 Richter', *American Journal of Enology and Viticulture*, vol. 43, no. 1, pp. 71-78.

Hunter, JJ, Ruffner, HP, Volschenk, CG & Roux, DJl 1995, 'Partial defoliation of Vitis vinifera L. cv. Cabernet Sauvignon/99 Richter: effect on root growth, canopy efficiency, grape composition, and wine quality', *American Journal of Enology and Viticulture*, vol. 46, no. 3, pp. 306-314.

Intrieri, C, Poni, S, Rebucci, B & Magnanini, E 1997, 'Effects of canopy manipulations on whole-vine photosynthesis: results from pot and field experiments', *Vitis*, vol. 36, no. 4, pp. 167-173.

Intrieri, C, Poni, S, Silvestroni, O & Filippetti, I 1992, 'Leaf age, leaf position and photosynthesis in potted grapevines', *Advances in Horticultural Science*, vol. 6, pp. 23-27.

Jackson, DI & Lombard, PB 1993, 'Environmental and management practices affecting grape composition and wine quality - a review', *American Journal of Enology and Viticulture*, vol. 44, no. 4, pp. 409-430.

Jackson, DI & Schuster, D (eds) 1987, *The production of grapes and wines in cool climates*, Gypsum Press, Wellington, New Zealand.

Jackson, RS 2000, *Wine Science. Principles, practice perception*, Academic Press, San Diego, California.

Johnstone, RS, Clingeleffer, PR & Lee, TH 1996, 'The composition of Shiraz grape berries – implications for wine. ', in CS Stockley, AN Sas, RS Johnstone & TH Lee (eds), *Proceedings of the Ninth Australian Wine Industry Technical Conference*, Adelaide, SA., pp. 105-108.

Joscelyne, VL, Downey, MO, Mazza, M & Bastian, SEP 2007, 'Partial shading of Cabernet Sauvignon and Shiraz vines altered wine color and mouthfeel attributes, but increased exposure had little impact', *Journal of Agricultural and Food Chemistry*, vol. 55, no. 26, pp. 10888-10896.

Kahkonen, MP, Heinamaki, J, Ollilainen, V & Heinonen, M 2003, 'Berry anthocyanins: isolation, identification and antioxidant activities', *Journal of the Science of Food and Agriculture*, vol. 83, no. 14, pp. 1403-1411.

Kataoka, I & Beppu, K 2004, 'UV irradiance increases development of red skin color and anthocyanins in 'Hakuho' peach', *Hortscience*, vol. 39, no. 6, pp. 1234-1237.

Keller, M, Smithyman, RP & Mills, LJ 2008, 'Interactive effects of defict irrigation and crop load on Cabernet Sauvignon in an arid climate. ', *American Journal of Enology and Viticulture*, vol. 59, pp. 221-234.

Kennedy, JA 2008, 'Grape and wine phenolics: Observations and recent findings', *Agraria*, vol. 35, pp. 107-120.

Kennedy, JA, Hayasaka, Y, Vidal, S, Waters, EJ & Jones, GP 2001, 'Composition of grape skin proanthocyanidins at different stages of berry development', *Journal of Agricultural and Food Chemistry*, vol. 49, no. 11, pp. 5348-5355.

Kennedy, JA, Saucier, C & Glories, Y 2006, 'Grape and wine phenolics: History and perspective.', *American Journal of Enology and Viticulture*, vol. 57, pp. 239-248.

Kerslake, F 2011, 'The effect of vineyard managment practices on Pinot noir fruit and wine composition', Ph.D thesis, University of Tasmania.

Kliewer, WM 1970, 'Effect of day temperature and light intensity on coloration of Vitis vinifera L. grapes', *Journal of the American Society for Horticultural Science*, vol. 95, pp. 693-697.

Kliewer, WM 1971, 'Effect of of day temperature and and light intensity on the concentration of malic and tartaric acids in *Vitis vinifera* L. grapes.', *Journal of the American Society for Horticultural Science*, vol. 96, pp. 327-377.

Kliewer, WM 1977, 'Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes', *American Journal of Enology and Viticulture*, vol. 28, no. 2, pp. 96-103.

Kliewer, WM & Antcliff, AJ 1970, 'Influence of defoliation, leaf darkening, and cluster shading on the growth and composition of Sultana grapes ', *American Journal of Enology and Viticulture*, vol. 21, no. 1, pp. 26-36.

Kliewer, WM & Dokoozlian, N 2005, 'Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality.', *American Journal of Enology and Viticulture*, vol. 56, pp. 170-181.

Kliewer, WM & Lider, LA 1968, 'Influence of cluster exposure to the sun on the composition of Thompson Seedless fruit', *American Journal of Enology and Viticulture*, vol. 19, no. 3, pp. 175-184.

Kliewer, WM & Torres, RE 1972, 'Effect of controlled day and night temperatures on grape coloration', *American Journal of Enology and Viticulture*, vol. 23, no. 2, pp. 71-77.

Kliewer, WM, Wolpert, JA & Benz, M 2000, 'Trellis and vine spacing effects on growth, canopy microclimate, yield and fruit composition of Cabernet Sauvignon', *Acta Horticulturae*, no. 526, pp. 21-32.

Koblet, W, Candolfivasconcelos, MC, Zweifel, W & Howell, GS 1994, 'Influence of leaf removal, rootstock, and training system on yield and fruit composition of Pinot-Noir grapevines', *American Journal of Enology and Viticulture*, vol. 45, no. 2, pp. 181-187.

Lakso, AN & Kliewer, WM 1975, ' Physical properties of phosphoenolpyruvate carboxylase and malic enzyme in grape berries', *American Journal of Enology and Viticulture*, vol. 26, pp. 75-78.

Lebon, G, Wojnarowiez, G, Holzapfel, BP, Fontaine, F, Vallant-Gaveau, N & Clement, C 2008, 'Sugars and flowering grapevine (Vitis vinifera L.)', *Journal of Experimental Botany*, vol. 59, no. 10, pp. 2565-2578.

Main, GL & Morris, JR 2004, 'Leaf-removal effects on Cynthiana yield, juice composition, and wine composition', *American Journal of Enology and Viticulture*, vol. 55, no. 2, pp. 147-152

Mathews, M & Kriedemann, PE 2006, 'Water deficit, yield and berry size as factors for composition and sensory attributes of red wine', paper presented to Finishing the Job - Optimal ripening of Cabernet Sauvignon and Shiraz, Mildura Arts Center, Mildura, Victoria, Australia.

May, P 2000, 'From bud to berry, with special reference to inflorescence and bunch morphology in Vitis viniferaL', *Australian Journal of Grape and Wine Research*, vol. 6, no. 2, pp. 82-98.

May, P, Shaulis, NJ & Antcliff, AJ 1969, 'The effect of controlled defoliation in Sultana vine', *American Journal of Enology and Viticulture*, vol. 20, pp. 237-250.

Mazza, G, Fukumoto, L, Delaquis, P, Girard, B & Ewert, B 1999, 'Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia', *Journal of Agricultural and Food Chemistry*, vol. 47, no. 10, pp. 4009-4017.

Mazza, G & Miniati, E (eds) 1993, *Anthocyanins in fruits, vegetables, and grains*, CRC Press Inc, USA.

McCarthy, MG, Cirami, RM & Furkaliev, DG 1987, 'The effect of crop load and vegetative growth control on wine quality', in *Proceedings of the 6th Australian Wine Industry Technical Conference*, pp. 75-77.

Mori, K, Sugaya, S & Gemma, H 2005, 'Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition', *Scientia Horticulturae*, vol. 105, no. 3, pp. 319-330.

Morrison, JC & Noble, AC 1990, 'The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties', *American Journal of Enology and Viticulture*, vol. 41, no. 3, pp. 193-200.

Mullins, MG, Bouqet, A & Williams, LE (eds) 1992, *Biology of the Grapevine*, Cambridge University Press, United Kingdom.

Ollat, N, Diakou-Verdin, P, Carde, JP, Barrieu, F, Gaudillere, JP & Moing, A 2002, 'Grape berry development: A review', *Journal International Des Sciences De La Vigne Et Du Vin*, vol. 36, no. 3, pp. 109-131.

Ollat, N & Gaudillere, JP 1998, 'The effect of limiting leaf area during stage I of berry growth on development and composition of berries of Vitis vinifera L. cv. Cabernet Sauvignon', *American Journal of Enology and Viticulture*, vol. 49, no. 3, pp. 251-258.

Olszewski, N, Sun, T, Gubler, F & Cabanne, C 2002, 'Gibberellin signaling: biosynthesis, catabolism, and response pathways', *The Plant Cell*, vol. 14 no. Suppl., pp. 61-80.

Partridge, NL 1925, 'The use of the growth-yield relationship in field trials with grapes.', in *Proceedings of the American Society for Horticultural Science*, vol. 23, pp. 131-134.

Pearce, I & Coombe, BG 2004, 'Grape vine phenology. In: Viticulture, Volume 1 - Resources', in PR Dry & BG Coombe (eds), Wine Titles, Adelaide, vol. 1, pp. 150–166.

Percival, DC, Fisher, KH & Sullivan, JA 1994a, 'Use of fruit zone leaf removal with Vitis vinifera L. cv. Riesling grapevines. I. Effects on canopy structure, microclimate, bud survival, shoot density, and vine vigor', *American Journal of Enology and Viticulture*, vol. 45, no. 2, pp. 123-132.

Percival, DC, Fisher, KH & Sullivan, JA 1994b, 'Use of fruit zone leaf removal with Vitis vinifera L. cv. Riesling grapevines. II. Effect on fruit composition, yield, and occurrence of bunch rot (Botrytis cinerea Pers.:Fr.)', *American Journal of Enology and Viticulture*, vol. 45, no. 2, pp. 133-140.

Petrie, PR, Trought, MCT & Howell, GS 2000a, 'Fruit composition and ripening of Pinot Noir (Vitis vinifera L.) in relation to leaf area', *Australian Journal of Grape and Wine Research*, vol. 6, no. 1, pp. 46–51.

Petrie, PR, Trought, MCT & Howell, GS 2000b, 'Growth and dry matter partitioning of Pinot Noir (Vitis vinifera L.) in relation to leaf area and crop load', *Australian Journal of Grape and Wine Research*, vol. 6, no. 1, pp. 40-45.

Petrie, PR, Trought, MCT & Howell, GS 2000c, 'Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (Vitis vinifera L. cv. Pinot noir) leaves', *Vitis*, vol. 39, no. 1, pp. 31-36.

Petrie, PR, Trought, MCT, Howell, GS & Buchan, GD 2003, 'The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of Vitis vinifera L. Sauvignon Blanc', *Functional Plant Biology*, vol. 30, no. 6, pp. 711-717.

Price, SF, Breen, PJ, Valladao, M & Watson, BT 1995, 'Cluster sun exposure and quercetin in Pinot noir grapes and wine', *American Journal of Enology and Viticulture*, vol. 46, no. 2, pp. 187-194.

Ravaz, ML 1903, '"Sur la brunissure de la vigne."', *Comptes Rendus de l Academie des Sciences*, vol. 136, pp. 1276-1278.

Ravaz, ML 1906, 'Influence de la surproduction sur la vegetation de la vigne. ', in *Ann. De l'E.N.A*, Montpellier, vol. VI, pp. 1-41.

Reynolds, AG, Wardle, DA & Dever, M 1996, 'Vine performance, fruit composition, and wine sensory attributes of Gewurztraminer in response to vineyard location and canopy manipulation', *American Journal of Enology and Viticulture*, vol. 47, no. 1, pp. 77-92.

Robinson, JB (ed.) 2006, *The Oxford Companion to Wine*, Oxford University Press, Oxford.

Roby, G, Harbertson, JF, Adams, DA & Matthews, MA 2004, 'Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins', *Australian Journal of Grape and Wine Research*, vol. 10, no. 2, pp. 100-107.

Roby, G & Matthews, MA 2004, 'Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit', *Australian Journal of Grape and Wine Research*, vol. 10, no. 1, pp. 74-82.

Shabala, S & Wilson, S 2001, 'Fluctuations in light intensity modulate ion fluxes from grape berry mesocarp: direct evidence from microelectrode ion flux estimations', *Australian Journal of Grape and Wine Research*, vol. 7, no. 3, pp. 137-143.

Shaulis, NJ, Jordan, TD & Tomkins, JP 1966, ' Cultural practices for New York vineyards ', *Cornell Extension Bulletin*, vol. 805, pp. 33-34.

Shavrukov, YN, Dry, IB & Thomas, MR 2004, 'Inflorescence and bunch architecture development in Vitis vinifera L', *Australian Journal of Grape and Wine Research*, vol. 10, no. 2, pp. 116-124.

Smart, RE 1985, 'Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review', *American Journal of Enology and Viticulture*, vol. 36, no. 3, pp. 230-239.

Smart, RE, Dick, JK, Gravett, IM & Fisher, BM 1990, 'Canopy management to improve grape yield and wine quality - principles and practices', *South African Journal of Enology and Viticulture*, vol. 11, no. 1, pp. 3-17.

Smart, RE & Dry, PR 1980, 'A climatic classification for Australian viticultural regions', *Australian Grapegrower and Winemaker*, vol. 17, pp. 8-10.

Smart, RE, Robinson, JB, Due, GR & Brien, CJ 1985, 'Canopy microclimate modification for the cultivar Shirza. II. Effects on must and wine composition', *Vitis*, vol. 24, no. 2, pp. 119-128.

Solomon, S, Portmann, RW, Sasaki, S, Hofmann, DJ & Thompson, DWJ 2005, 'Four decades of ozonesonde measurements over Antarctica', *Journal of Geophysical Research*, vol. 21, pp. D21311-D21326.

Somers, TC & Evans, JC 1974, 'Wine quality: correlations with colour density and anthocyanin equilibria in a group of young red wines', *Journal of the Science of Food and Agriculture*, vol. 25, pp. 1369-1379.

Somers, TC & Evans, ME 1977, 'Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO2, “chemical age”', *Journal of the Science of Food and Agriculture*, vol. 28, no. 3, pp. 279-287.

Souquet, JM, Cheynier, V, Brossaud, F & Moutounet, M 1996, 'Polymeric proanthocyanidins from grape skins', *Phytochemistry*, vol. 43, pp. 509-512.

Spayd, SE, Tarara, JM, Mee, DL & Ferguson, JC 2002, 'Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries', *American Journal of Enology and Viticulture*, vol. 53, no. 3, pp. 171-182.

Staff, SL, Percival, DC, Sullivan, JA & Fisher, KH 1997, 'Fruit zone leaf removal influences vegetative, yield, disease, fruit composition, and wine sensory attributes of Vitis vinifera L 'Optima' and 'Cabernet franc'', *Canadian Journal of Plant Science*, vol. 77, no. 1, pp. 149-153.

Stapleton, JJ & Grant, RS 1992, 'Leaf removal for nonchemical control of the summer bunch rot complex of wine grapes in the San Joaquin Valley', *Plant Disease*, vol. 76, no. 2, pp. 205-208.

Stapleton, JJ, Leavitt, GM & Verdegaal, PS 1995, 'Leaf removal improves fungicide control of powdery mildew in SJV grapes', *California Agriculture*, vol. 49, no. 5, pp. 33-36.

Steel, RGD & Torrie, JH (eds) 1980, *Principles and Procedures of Statistics: a Biometrical Approach*, McGraw-Hill, New York.

Sugiura, A, Utsunomiya, N & Kobayashi, A 1975, ' Effects of daylength and temperature on growth and bunch differentiation of grapevines. ', *Journal of the Japanese Society for Horticultural Science*, vol. 43, pp. 387–392.

Teszlak, P, Gaal, K, Shahin, M & Nikfardjam, MSP 2005, 'Influence of grapevine flower treatment with gibberellic acid (GA(3)) on polyphenol content of Vitis vinifera L. wine', *Analytica Chimica Acta*, vol. 543, no. 1-2, pp. 275-281.

Vail, ME & Marois, JJ 1991, 'Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*', *Phytopathology*, vol. 81, no. 2, pp. 188-191.

van Leeuwen, C, Friant, P, Chone, X, Tregoat, O, Koundouras, S & Dubourdieu, D 2004, 'Influence of climate, soil, and cultivar on terroir', *American Journal of Enology and Viticulture*, vol. 55, no. 3, pp. 207-217.

Vasconcelos, MC & Castagnoli, S 2000, 'Leaf canopy structure and vine performance', *American Journal of Enology and Viticulture*, vol. 51, no. 4, pp. 390-396.

Vasconcelos, MC & Castagnoli, S 2001, 'Leaf canopy structure and vine performance', *Practical Winery and Vineyard Journal*, vol. Sep-Oct, pp. 42-45.

VAT 2002, *Tasmanian Wine Industry Strategic Plan 2002 -2007*, Vineyards Association of Tasmania, Launceston.

Walker, RR, Blackmore, DH, Clingeleffer, PR, Kerridge, GH, Ruhl, EH & Nicholas, PR 2005, 'Shiraz berry size in relation to seed number and implications for juice and wine composition', *Australian Journal of Grape and Wine Research*, vol. 11, no. 1, pp. 2-8.

Weaver, JW & McCune, SB 1959a, 'Effects of gibberelin on seeded Vitis vinifera and its translocation within the vine', *Hilgardia*, vol. 28, no. 20, pp. 625-645.

Weaver, JW & McCune, SB 1959b, 'Response of certain varieties of Vitis Vinefera to gibberelin', *Hilgardia*, vol. 28, no. 13, pp. 297-347.

Weaver, JW & McCune, SB 1960, 'Further studies with gibeberellin on *Vitis vinifera* grapes', *Botanical Gazette*, vol. 121, no. 3, pp. 155-162.

Weaver, JW & Pool, RM 1971, 'Thining 'Tokay' and 'Zinfandel' grapes by bloom sprays of gibberelin', *Journal of the American Society for Horticultural Science*, vol. 96, no. 6, pp. 820-822.

Williams, LE 1996, 'Grape', in E Zamski & AA Scaffer (eds), *Photoassimilate distribution in plants and crops: source sink relationships*, Marcel Dekker, Inc, New York pp. 851–881.

Wilson, S 2002, *Personal communication* Hobart.

Yamane, T, Jeong, ST, Goto-Yamamoto, N, Koshita, Y & Kobayashi, S 2006, 'Effects of temperature on anthocyanin biosynthesis in grape berry skins', *American Journal of Enology and Viticulture*, vol. 57, no. 1, pp. 54-59.

Zoecklein, BW, Wolf, TK, Duncan, NW, Judge, JM & Cook, MK 1992, 'Effects of fruit zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and White Riesling (Vitis-Vinifera L) grapes', *American Journal of Enology and Viticulture*, vol. 43, no. 2, pp. 139-148.

Zoecklein, BW, Wolf, TK, Duncan, SE, Marcy, JE & Jasinski, Y 1998, 'Effect of fruit zone leaf removal on total glycoconjugates and conjugate fraction concentration of Riesling and Chardonnay (Vitis vinifera L.) grapes', *American Journal of Enology and Viticulture*, vol. 49, no. 3, pp. 259-265.

Zoecklein, BW, Wolf, TK, Marcy, JE & Jasinski, Y 1998, 'Effect of fruit zone leaf thinning on total glycosides and selected aglycone concentrations of Riesling (Vitis vinifera L.) grapes', *American Journal of Enology and Viticulture*, vol. 49, no. 1, pp. 35-43.

Zoecklein, BW, Yoder, CC, Wolf, TK & Jasinski, Y 1997, 'Effect of fruit zone leaf removal and shoot thinning on Vitis vinifera L. grape glycosides', in T Henick-Kling, TE Wolf & EM Harkness (eds), *Proceedings of the fourth international symposium on cool climate viticulture & enology*, Rochester, New York, USA, pp. V-74-V-79.