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Effect of Irrigation, Glycine Betaine and Kaolin Particle Film on the Performance of *Vitis vinifera* L. 'Shiraz'

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DEDICATION

To the loving memory of

Ms Pauline Venn

(26th March 1964 – 8th January 2017)

A beautiful lady dedicated to living life to the full for the benefit of her family, her friends and the community.

Abstract

Environmental stresses, such as heat and water stress, are a current and emerging challenge to wine grape production in Australia. In Southern Australia, where wine grape production is predominately located, Global Circulation Models (GCMs) predict further increases in temperatures, increased frequency and severity of heatwaves and reduced rainfall. Wine grapes are particularly susceptible to heat and water stress due to the lengthy grapevine establishment times and the perennial nature of this crop. Therefore, there is a strong demand for management strategies to protect established vineyards from environmental stresses, such as heat and water stress.

Glycine betaine (GB) and kaolin particle film (KPF) are two products that can be exogenously applied to grapevines to potentially ameliorate the effects of heat and water stress. The objective of both foliar sprays is the same, but their mode of action is quite different. GB seeks to induce greater stress tolerance, while KPF seeks to physically protect the fruit and leaves. Management strategies, such as application of GB and KPF, implemented to ameliorate the effects of one stress may however have unintentional impacts when coupled with common management practices, such as deficit irrigation.

This research investigated the protective capacity of GB and KPF, individually and combined, on grapevine performance under varying levels of imposed water stress, and their associated interactions on the productive performance and physiology of *Vitis vinifera* L. 'Shiraz'. A field experiment was conducted on Shiraz (clone SA16) vines at The University of Melbourne's Dookie Campus Vineyard in North East Victoria, Australia (-36.395 °S, 145.703 °E) during the 2011-12 (Y1) and 2012-13 (Y2) growing seasons. The vines were planted in 1994, on Richter 99 rootstock on a cordon-trained Scott Henry trellis system, with two bilateral cordons. A randomised complete block design (RCBD) factorial experiment was established in 2011 comprising three irrigation treatments (I_{25}, I_{50} and I_{100}), two GB treatments (GB^- and GB^+) and two KPF treatments (KPF^- and KPF^+), with six replicates per treatment. Analysis was conducted on grape yield and yield components, fruit composition, plant water status, canopy growth and canopy temperature.

The research was conducted over two contrasting growing seasons. Y1 was largely cooler with high rainfall between véraison and harvest and Y2 was hotter with lower rainfall. In Y2, observed values of leaf water potential (ψ_{LEAF}) and canopy temperatures indicated that all the treatments were showing signs of severe to very severe water stress. Consequently, water availability (rainfall and irrigation) dominated treatment responses in yield and composition,

with average berry weight across all treatments 52% smaller, resulting in a 25% lower average yield in Y2 compared with Y1. Therefore, seasonal variation played a key role in the vines' response to the irrigation treatments, GB and KPF.

Under non-stressed conditions in Y1, applying GB resulted in a 16% reduction in grape yield, which was attributed in part to an 8% reduction in berry weight. However, under severe water stressed conditions in Y2 the impacts of GB on vine performance became less evident. Applying KPF resulted in a reduction in some yield components under the water stressed conditions of Y2. It is possible that KPF resulted in changes to transpiration resulting in the vines becoming more water stressed. In addition, the interaction of GB and KPF may have caused a response similar to the effect of KPF under water stressed conditions. This is evidenced by the combined effect of GB^+ and KPF^+ resulting in lower average cluster weight in Y1 and more negative values of midday ψ_{LEAF} (ψ_{MID}) post-irrigation in Y2. However, interpretation of this interaction is complicated by the impacts of the irrigation treatments and seasonality.

In conclusion, this research has shown how Shiraz vines respond to GB and KPF, when grown in commercial settings, under both high and low water availability conditions in North East Victoria, Australia. These findings are important as they indicate that the use of these compounds to ameliorate the impacts of environmental stresses, such as heat stress, needs to be carefully assessed alongside water availability. Importantly, these research findings have demonstrated that GB and KPF have different impacts across different environmental conditions; and there is a potential for interactions that could compromise production of grapes to meet commercial specifications.

Declaration

This is to certify that

i. the thesis comprises only my original work towards the towards the Doctor of Philosophy Degree;

ii. due acknowledgement has been made in the text to all other material used;

iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Sally Gwen Foletta

May 2017

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Table of Common Abbreviations

Abbreviations used in this thesis are detailed in the table below (Table 0.1).

Table 0.1. Abbreviations commonly used in this thesis, their meaning and units (if applicable).

Abbreviation	Meaning	Units
ABA	abscisic acid	
ABS	Australian Bureau of Statistics	
A_{CO2}	photosynthesis	
ANOVA	analysis of variance	
avg	average	
BADH	betaine aldehyde dehydrogenase	
BOM	Bureau of Meteorology	
$B_{\%DW}$	Berry percent dry weight	
C_b	colour per berry (anthocyanins per berry)	$mg \cdot berry^{-1}$
C_g	Colour concentration (anthocyanins per gram)	$mg \cdot g^{-1}$
CSIRO	Commonwealth Scientific and Industrial Research Organisation	
CMO	choline monooxygenase	
C_{min}	minimum canopy temperature (in infrared images)	°C
C_{max}	maximum canopy temperature (in infrared images)	°C
DOY	day of year	
ENSO	El Niño-Southern Oscillation	
ET_o	reference evapotranspiration	mm
ET_C	crop evapotranspiration	mm
gANOVA	general linear analysis of variance	
GB	glycine betaine	
GB^-	glycine betaine treatment (not applied)	
GB^+	glycine betaine treatment (applied)	
GDD_{10}	growing degree days	°C
GCMs	global circulation models	
GWRDC	Grape and Wine Research and Development Corporation	
g_l	leaf conductance	$mmol \cdot m^{-2} \cdot s^{-1}$
IR	infrared	
I_{25}	irrigation treatment (25% vineyard practice)	
I_{50}	irrigation treatment (50% vineyard practice)	
I_{100}	irrigation treatment (100% vineyard practice)	
IPCC	Intergovernmental Panel on Climate Change	
IRTI	Infrared thermal image	
KPF	kaolin particle film	
KPF^-	kaolin particle film treatment (not applied)	
KPF^+	kaolin particle film treatment (applied)	
L_{DW}	leaf dry weight	G
L_{FW}	leaf fresh weight	G
L_S	leaf size	cm^2
$L_{\%DW}$	leaf percent dry weight	
$LSD_{p = 0.05}$	Fisher's least significant difference at p = 0.05	
MDH	Malate dehydrogenase	
max	maximum	
min	minimum	
MJT	mean January temperature	

Table 0.1. Abbreviations commonly used in this thesis, their meaning and units (if applicable).

Abbreviation	Meaning	Units
ns	not significant	
PAR	Photosynthetically active radiation	
P_b	phenolics per berry (absorbance units per berry)	$au \cdot berry^{-1}$
P_g	phenolics concentration (absorbance units per gram)	$au \cdot g^{-1}$
PSI	photosystem I	
PSII	photosystem II	
PRD	partial rootzone drying	
rANOVA	repeated measures analysis of variance	
RCBD	random complete block design	
RDI	reduced deficit irrigation	
ROI	reactive oxygen intermediates	
ROS	reactive oxygen species	
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase	
SLA	specific leaf area	$cm^2 \cdot g^{-1}$
SIMRs	stress-induced morphogenic responses	
SSB	soluble solids per berry	$g \cdot berry^{-1}$
SSE	sum of squared errors	
SSV	soluble solids per vine	$kg \cdot vine^{-1}$
t	time (sample date)	
TA	titratable acidity	$g \cdot L^{-1}$
T_A	ambient air temperature	°C
T_C	average canopy temperature	°C
$T_C^{(BOT)}$	average canopy temperature (check p112)	°C
$T_C^{(MID)}$	average canopy temperature	°C
$T_C^{(TOP)}$	average canopy temperature	°C
T_C*	difference between average canopy temperature and ambient temperature	°C
T_{SD}	standard deviation of canopy temperature	°C
TSS	total soluble solids	°Brix
T_{MIN}	maximum temperature	°C
T_{MAX}	minimum temperature	°C
TA	titratable acidity	$g \cdot L^{-1}$
TCA	trunk cross-sectional area	cm^2
UV	ultraviolet	
VPD	vapour pressure deficit	
wt	weight	
Y1	year 1 of the experiment (2011-12 growing season)	
Y2	year 2 of the experiment (2012-13 growing season)	
$\%ET_O$	percentage of evapotranspiration	
ΔTCA	change in trunk cross-sectional area	cm^2
ψ_{LEAF}	leaf water potential	MPa
ψ_{PD}	pre-dawn leaf water potential	MPa
ψ_{MID}	midday leaf water potential	MPa

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Chapter 1. Introduction

1.1. The Australian Wine Industry

The wine industry is a major agricultural exporter in Australia, with wine exports valued at $1.96 billion in 2015 (Winetitles Media Pty Ltd, 2016). According to the Australian Bureau of Statistics (ABS) wine grape production was 94% of the total grape production in 2015, with 844,000 t of red wine grapes produced and red wine grapevines accounting for 64% of all grapevines planted (ABS, 2015). Shiraz has long been the most popular variety grown in Australia, accounting for almost half of red wine grape production in 2015, at 395,000 t (ABS, 2015). Wine grape production in Australia grew significantly from 1995 to 2005, with wine grape production reaching 1.92 million t for the 2005 crush (ABS, 2006), an increase of 205% compared with the 1995 crush of 0.63 million t (ABS, 1995). In the 2005-06 growing season, the Grape and Wine Research and Development Corporation (GWRDC) reported large quantities of good quality grapes being left on the grapevine or dropped onto ground (GWRDC, 2005). This period of oversupply and higher wine stocks has been referred to as the global 'wine glut'. Australian production has decreased since 2005, with a total grape production (wine and table) of 1.72 million t in 2015 from an area of 144,000 ha (ABS, 2016). Excess supply and global competition continue to provide impetus for improvements to grapevine performance through more efficient production processes with less resources and higher quality fruit.

Consistent wine quality has been paramount to the success of the Australian wine industry. However, significant seasonal fluctuations to water allocations for irrigation and more frequent heatwaves pose a serious threat to wine grape quality and the industry. For example, devastating impacts on viticulture were seen in the 2008-09 growing season, where two periods of extremely high temperatures (Figure 1.1) resulted in wide spread incidences of yield loss, decreased quality, "*stalled development, leaf burn, leaf drop, berry sunburn, berry 'bagging' and berry shrivel*" (Webb et al., 2010).

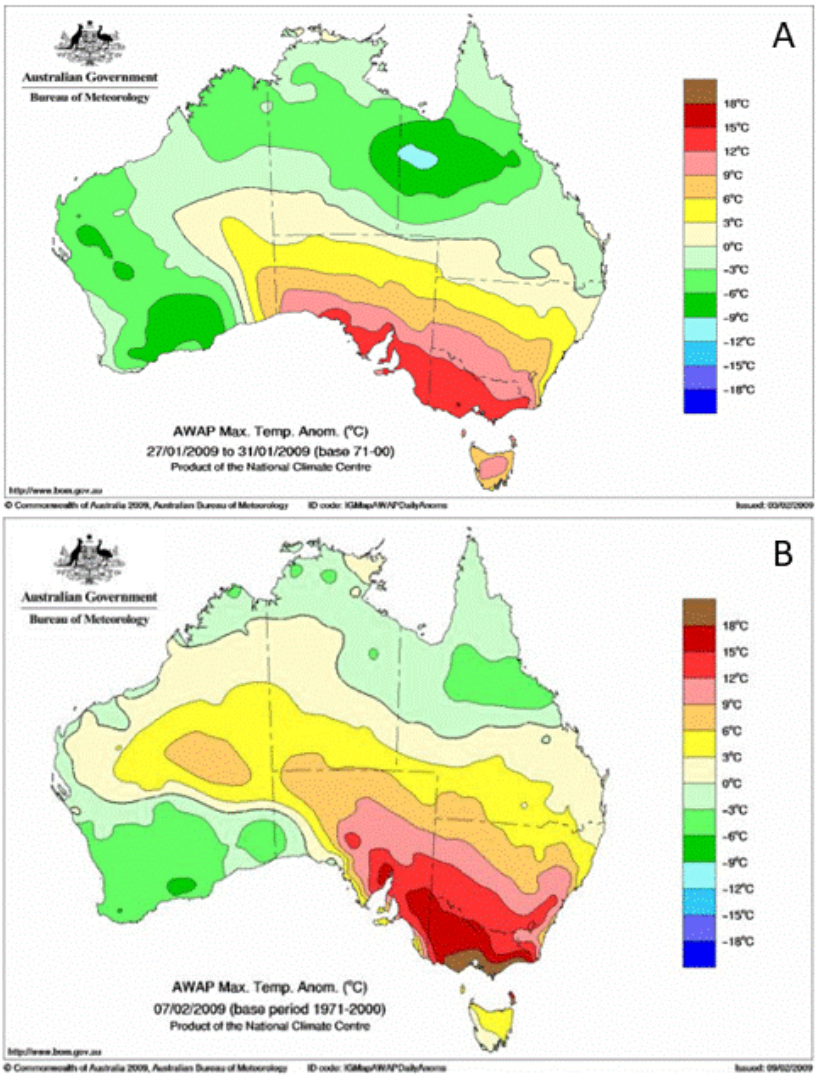

Figure 1.1. (A) Maximum temperature anomalies (base 1971-2000 average) for the period of 27th January to 31st January 2009; and (B) Maximum temperature anomalies (base 1971-2000 average) for the 7th February 2009 (Source: National Climate Centre, 2009).

This combination of heatwaves and reduced irrigation water supplies in some regions was blamed for the 7% (119,000 t) fall in production in the 2008-09 growing season, from 1.8 million to 1.7 million t in the 2007-08 and 2008-09 growing seasons, respectively (Webb et al., 2010). Similarly, an early season (November 2009) heatwave in south-eastern Australia and reduced irrigation water supplies in some regions adversely affected production in the 2009-10 growing season (Gunning-Trant & Shafron, 2010). A survey reported by Webb et al. (2010) shortly after the heatwaves, revealed that the variation in the heat-related impacts were "*not always related to the amount of heat above a certain threshold but to the management practices employed in the lead-up and through the event*". Given these environmental impacts on wine grape production, this thesis aims to evaluate adaptation options for heat and water stress.

1.2. The Australian Climate

Australia's climate is affected by many different weather systems, including El Niño-Southern Oscillation (ENSO) and the Indian Ocean Dipole [Commonwealth Scientific and Industrial Research Organisation (CSIRO) & Bureau of Meteorology (BOM), 2016a] . A statistically significant trend in climate over many decades, super-imposed on the natural climate variability, indicates that temperatures are increasing (CSIRO & BOM, 2015c). In Australia, temperatures have warmed approximately 0.7 °C·decade^{-1} from 1958 to 2011 (Jovanovic et al., 2017), with minimum temperatures increasing more than maximum temperatures at 0.06 and 0.12 °C·decade^{-1} for maximum and minimum temperatures, respectively (1910 to 2004; Nicholls & Collins, 2006). In general, the frequency of warm temperature events (> 35 °C day time temperature) has increased across Australia (Collins et al., 2000). Observed large-scale circulation changes have been linked to lower rainfall in southern Australia (CSIRO & BOM, 2015b), with an approximately 20 mm·decade^{-1} decrease in total annual rainfall for south eastern Australia (1950 to 2005; Gallant et al., 2007).

In Southern Australia, where wine grape production is predominately located, Global Circulation Models (GCMs) predict further increases in temperatures, increased frequency and severity of heatwaves and changes in rainfall patterns (CSIRO & BOM, 2015b; Timbal & Drosdowsky, 2013). The climate modelling uses four Representative Concentration Pathways (RCPs) to explore potential future land use changes and concentrations of greenhouse gases and aerosols (CSIRO & BOM, 2015b). There is little difference between the RCP projections for the period 2020 to 2039, with temperatures by 2030 projected to be 0.6 to 1.3 °C higher than the base of 1986 to 2005 (CSIRO & BOM, 2015b). However, for the period 2080 to 2099 the models begin to diverge as there is more uncertainty in the predicted future concentrations of greenhouse gases and aerosols, with predictions ranging from 0.6 to 1.7 °C (RCP2.6) to 2.8 to 5.1 °C (RCP8.5) higher than the base of 1986 to 2005 (CSIRO & BOM, 2015b). Furthermore, continued large-scale circulation changes are expected to further reduce rainfall in southern Australia (CSIRO & BOM, 2015b) and increasing temperatures are expected to accentuate the severity of droughts (Nicholls, 2004). These projected changes are likely to have an even greater impact on future wine grape production in Australia.

1.3. Australian Wine Grape Production and Climatic Factors

Environmental stresses can cause direct and indirect effects on grapevine physiology, yield, fruit integrity and fruit composition (Crippen & Morrison, 1986). Exposure to suboptimal environmental conditions may cause physical and/or chemical changes in the plant strain

(strain; Levitt, 1980); this strain may be reversible (elastic strain) or irreversible (plastic strain; Levitt, 1980). Environmental conditions (e.g. high temperature; Figure 1.2) capable of causing plastic strain (injury) may be classified as an abiotic (non-living) stress (Levitt, 1980). The strain caused by abiotic stress may cause direct plastic strain (primary direct injury), indirect plastic strain (primary indirect injury) or give rise to a secondary stress (secondary stress injury; Levitt, 1980).

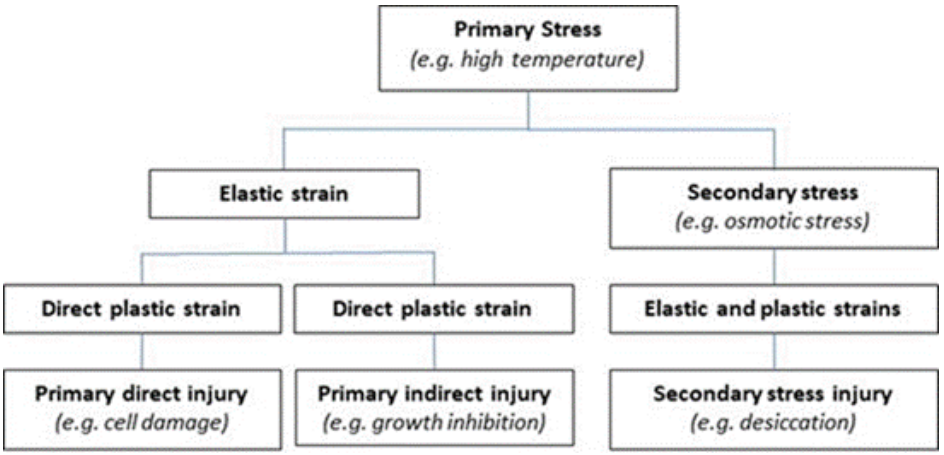

Figure 1.2. The different kinds of stress injury modified from Levitt (1980) to include examples.

The environmental conditions *per se* do not necessarily constitute an abiotic stress, rather it is the "*ability of plant mechanisms to function*" that determines if the conditions are stressful (Keller, 2015). Grapevines, like other plants, are plastic in response to their growing conditions (Dal Santo et al., 2013; Jurik, 1986; Martorell et al., 2015; Sadras et al., 2012a; Sadras et al., 2009; Young et al., 2016); responding to differences in water (Martorell et al., 2015; Sadras et al., 2009; Winkel & Rambal, 1993), light (Dokoozlian & Kliewer, 1995a, 1995b; Young et al., 2016), temperature (Atkin et al., 2005; Sadras et al., 2012a) and wind (De Langre, 2008; Gokbayrak et al., 2008) among other things. This phenotypic plasticity is what enables plants, such as grapevines, to cope with environmental factor variability (Gratani, 2014) and survive a range of "*environmental insults*" (stresses; Potters et al., 2009).

Phenotypic plasticity may be attributed to a combination of generic stress responses and stress specific responses by the grapevines (Potters et al., 2009). Changes in the metabolism of reactive oxygen species (ROS) and stress-induced morphogenic response (SIMRs) are generic stress responses made by plants in response to a range of biotic and abiotic stresses (Potters et al., 2009). An imbalance between pro-oxidants (ROS and its reaction products) generation and their antioxidants-mediated metabolism and scavenging leads to a physiological condition called oxidative stress (Anjum et al., 2014). The increase in ROS induced by stress is paralleled by the up-regulation of the ROS scavenging system (Potters et al., 2007). Morphogenic

responses (SIMRs) are characterised by localised inhibition of cell growth, localised stimulation of cell division and changes in cell differentiation (Potters et al., 2007). This response is largely driven by plant hormones (Keller, 2015). However, it has also been hypothesised that ROS may also play a role in SIMRs (Potters et al., 2007).

Exposure to suboptimal climatic conditions may have both direct and indirect impacts on grapevine physiology, fruit integrity and fruit composition (Scarlett et al., 2011). The severity, duration and timing of the environmental stress will ultimately determine the effect on photosynthetic efficiency, grapevine productivity (growth, yield and fruit composition) and long-term plant survival (Keller, 2015). Accordingly, not only can heat and water stress affect current season productivity, they may also affect productivity in the future seasons (sustained productivity), hence the importance of this research.

1.3.1. Heat Stress and Related Factors

When considering the effects of heat stress on grapevines, it is important to distinguish between "*radiative heat coupled to sun exposure and convective heat that results from mass air heating"* (Greer & Weston, 2010) and convective heating, which occurs when air temperatures exceed canopy temperatures (Greer & Weston, 2010). In simple terms, convective heating is the transfer of heat from one spot to another as a result of thermal movement of individual molecules, for example "*air movement within the intercellular spaces of plant leaves*" (Jones, 1992). The transfer of heat depends on temperature gradient and air-movement (wind and localised movement of small air pockets) at the interface between the canopy and the environment (boundary layer; Jones, 1992).

Radiation heating of the canopy occurs due to direct and indirect sun exposure (Greer & Weston, 2010). The sun emits a spectrum of electromagnetic radiation ranging from ultraviolet (UV; 100 to 400 nm) to infrared (IR; 700 nm to 1 mm), with peak radiation in the visible spectrum (380 to 780nm). Absorption, reflection and transmission of electromagnetic radiation by an object is dependent on the wavelength and dielectric properties of the object. Plants, such as grapevines, absorb and emit electromagnetic radiation resulting in a change in the potential energy of the plant organs (i.e. grapevine leaf; Jones, 1992), with the wavelength dependent on the magnitude of the energy change (Jones, 1992). Within the plant organ, atoms transition between different electron orbital states, and molecules transition between different vibrational and rotational states (Jones, 1992). Friction as a results of the transition between vibrational and rotational states creates heat as a bi-product, increasing the temperature of the plant tissue (radiative heating; Volokitin & Persson, 2007). This can

influence the rate of metabolic processes and the heat production can drive other radiation exchanges, such as transpiration (Jones, 1992).

The processes of transmission and absorption of electromagnetic radiation involve the attenuation and delay of the electromagnetic wave relative to the wave in free space (Brodie, 2008). Attenuation (loss of amplitude) and delay (shortening of the wavelength) are confined by the length of the wave (Lai et al., 2002). Consequently, shorter wavelengths (i.e. ultraviolet; UV) have a shallower depth of penetration (skin depth; Lai et al., 2002) resulting in more surface heating compared with longer wavelengths. Increased surface heating, due to shallower skin depth, may cause primary stress injury in the form of cell damage (i.e. leaf burn or berry sunburn; Levitt, 1980). UV and other very short wavelengths (i.e. X- and y-radiation) can also affect the structure of genetic material resulting in mutations (mutagenesis; Jones, 1992). Cyclobutane pyrimidine dimers (CPDs) are one of the major genetic mutations induced by UV radiation from the sun (Teranishi et al., 2008), with "*substantial levels of CPDs in samples irradiated with UVB wavelengths borderlined with UVA"* (Besaratinia et al., 2011). It is, therefore, important when considering the effects of heat stress in grapevines to consider radiation effects – including UV radiation – as well as canopy temperatures.

Ultraviolet Radiation

UV radiation has important implications on "*morphological, physiological and biochemical processes of many plant species*" (Kataria et al., 2014) and this is also the case with grapevines (Martínez-Lüscher et al., 2013). UV radiation is divided into three categories UV-A (315 – 400 nm), UV-B (280 – 315 nm) and UV-C (195 to 280 nm), with UV radiation accounting for approximately 7% of electromagnetic radiation emitted by the sun (Frohnmeyer & Staiger, 2003). Shorter wavelengths of UV radiation (i.e. UV-C) are completely attenuated (absorbed) by atmospheric gases, whereas longer wavelengths of UV radiation (i.e. UV-A) are hardly attenuated by atmospheric gases (Frohnmeyer & Staiger, 2003). UV-B, on the other hand, is partially attenuated by stratospheric ozone (O_3), with "*very small proportion transmitted to earth's surface*" (Frohnmeyer & Staiger, 2003). Depletion of the ozone layer may, therefore, increase transmitted UV-B radiation on the Earth's surface (Frohnmeyer & Staiger, 2003). However, attenuation of UV-B radiation by cloud-cover and anthropogenic aerosols coupled with topographic influences have contributed to observed temporal and spatial variability of transmitted UV-B radiation levels on the Earth's surface (Frohnmeyer & Staiger, 2003). Transmitted radiation reaching the Earth's surface rolls off sharply at around 300 nm (Besaratinia et al., 2011); therefore, UV-B radiation is the highest energy (shortest wavelength) portion of the sunlight spectrum that reaches the earth's surface (Kataria et al., 2014).

Cooley et al. (2001) found some ecotypes of *Arabidopsis thaliana* L. were sensitive to supplementary UV-A alone but not UV-A and UV-B together, whilst others showed the opposite sensitivity. Plants react to UV-B radiation by increasing leaf thickness, by thickening the cuticle and synthesising secondary metabolites capable of absorbing UV-B radiation (Girona et al., 2005). These secondary metabolites (i.e. phenolic compounds and flavonoids) accumulate in the vacuoles of epidermal cells in response to UV-B radiation and reduce the penetration of UV-B radiation (Frohnmeyer & Staiger, 2003). Some flavonoids are upregulated in the presence of UV radiation, whilst colourless and odourless, they play an important role in the colour stability by acting as a co-pigment for anthocyanins (Downey et al., 2006). Low levels of UV-B radiation have also been associated with *"transcription of genes involved in UV-protective responses"*(Brown et al., 2005), with filtering out UV-B radiation using polyester films significantly reducing UV-B absorbing flavonols, quercetins and kaempferol in one-year-old field grown *Vitis vinifera* (Berli et al., 2010). UV-A can enhance morphological and physiological changes (Cooley et al., 2001; Cooley et al., 2000a; Cooley et al., 2000b). The effects of UV-A alone are mostly inhibitory in *Arabidopsis thaliana* (Cooley et al., 2001)*, Bellis perennis* (daisy; Cooley et al., 2000a; Cooley et al., 2000b)*, Cynosurus cristatus* (crested dog's-tail; Cooley et al., 2000a; Cooley et al., 2000b), *Cardamine pratensis* (lessor smock; Cooley et al., 2000a) and *Ranunculus ficaria* (lesser celandine; Cooley et al., 2000a). Evidently, plants are not only sensitive to the intensity of UV radiation but also the balance of various wavelengths.

Canopy Temperature

Canopy temperature influences leaf function, berry function and the associated source-sink relationships (Smart, 1985), as well as the rates of metabolic processes, such as photosynthesis. Temperature induced changes to photosynthetic rate are reversible (elastic) over a "*considerable range (commonly 10° to 35°C)"* in most plants (Berry & Bjorkman, 1980), with the potential for irreversible (plastic) injury to the photosynthetic system at temperatures above or below this range (Berry & Bjorkman, 1980). Some plants, such as oleander (*Nerium oleander*), have shown the ability to acclimate to prevailing conditions, evidenced by a change in the optimum temperature for photosynthesis in response to the prevailing temperature (Raison et al., 1982), indicating the complexities in the plant responses to temperature.

Elastic and plastic changes to the photosynthetic system may be a result of factors, such as the loss of membrane integrity (Raison et al., 1982), photoinhibition (Aro et al., 1993; Barber & Andersson, 1992; Kato & Sakamoto, 2009; Sakamoto & Murata, 2002) and contributing to an inability to retain as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in an active form (Feller et al., 1998; Salvucci & Crafts-Brandner, 2004a, 2004b).

- **Membrane Integrity**

  High temperatures may modify the membrane composition, which can lead to leakage of ions and inhibition of metabolic processes, such as photosynthesis and respiration, which depend on membrane associated electron carriers and enzymes (Raison et al., 1982; Yang et al., 2005). Ion leakage, indicated by a change in electroconductivity, has been observed for *Zea mays* (Yang et al., 2005). In the research conducted by Raison et al (1982), *Nerium oleander* plants were grown in controlled environment chambers at 45/32 °C and 20/15 °C (day/night) were then extracted and assessed for changes in the molecular ordering of lipids in response to temperature. Changes in the molecular ordering of lipids occurred at 33 °C and 29 °C for the plants grown at 45/32 °C and 20/15 °C, respectively (Raison et al., 1982). Evidently, there is some degree of acclimation that occurs *in vivo*; however, there remains the potential for high temperatures to induce changes to the membrane.

- **Photoinhibition**

  The D1 protein in photosystem II (PSII) has a high turnover rate, the photodamaged D1 protein is degraded by protease and then recombined by a *de novo* synthesis, in what is referred to as the PSII repair cycle (Kato & Sakamoto, 2009). The extent of photoinhibition is determined by the balance of photo-induced damage, and light dependent repair of the D1 protein in the PSII complex (Aro et al., 1993). Photoinhibition is not only affected by light intensity but also "*superimposition of other environmental factors such as temperature, metabolic state, nutrient, water and carbon dioxide supply"* (Barber & Andersson, 1992). Melis (1999) observed a linear relationship between light intensity and the rate of photodamage in pumpkins and green algae, suggesting each time light reaches the PSII reaction centre there is a simple probability of photodamage. Additionally, the probability of photodamage was higher when the primary quinone acceptor was in a reduced state, which occurs more frequently under suboptimal conditions (Melis, 1999). More recent research has shown that damage to PSII by light occurs in two temporally separated steps: the first deactivation of the oxygen-evolving complex and the second impairment of the photochemical reaction centre and, the repair system involves several steps (Mohanty et al., 2007). Each of these steps are individually affected by temperature, light intensity and wavelength (Mohanty et al., 2007). For example, UV radiation is highly effective at inactivation of the first step in photodamage with increased rates above 34 °C; however, this step does not occur below 10 °C (Mohanty et al., 2007).

- **RuBisCO**

  RuBisCO is involved in the first major step of carbon fixation (Jones, 1992). High temperatures have been associated with the sequestration of RuBisCO activase from the soluble stroma fraction to the thylakoid membrane (Yang et al., 2005) reducing the plant's ability to retain RuBisCO in an active form (Feller et al., 1998; Salvucci & Crafts-Brandner, 2004a, 2004b). High temperatures are reported to have a direct effect on RuBisCO activase, inhibiting the activation of RuBisCO thus reducing CO_2 fixation. (Yang et al., 2005). Inhibition of carbon dioxide (CO_2) fixation is reported to have a greater impact on PSII in many plant species, with PSII only inhibited by heat stress when temperatures exceed 45 °C (Yang et al., 2005).

Ultimately, canopy temperature is influenced by the amount and the distribution of leaf area and its associated interaction with the above-ground climate (Smart, 1985). Subsequently, canopy temperatures vary temporally and spatially (Jones, 1999) due to environmental factors including wind, leaf angle, solar radiation and ambient temperature conditions (Sirvydas et al., 2011; Smart & Sinclair, 1976; Wahid et al., 2007; Wang et al., 2010) as well as stomatal conductance and transpiration (Jones, 1999; Smart & Sinclair, 1976). Transpiration lowers canopy temperatures via transpirational cooling (Smart & Sinclair, 1976), with transpiration rates being controlled via stomatal conductance (Smart & Sinclair, 1976). During the daytime, the temperature at which stomata close is determined by the availability of water, evaporative demand and the vines' isohydric behaviour; hence irrigation may play a role in reducing the negative impacts of heat stress.

1.3.2. Water Stress

A grapevine is water stressed when water availability is insufficient to meet evaporative demand. Water balance of grapevines is determined as the amount of water lost to the atmosphere via evapotranspiration and amount of water gained from the soil (Keller, 2015). A grapevine may become water stressed when the amount of water lost is greater than the amount of water gained (Keller, 2015). This could be due to a decrease in soil moisture availability (Cooley et al., 2017; Eagleson, 1978; Keller, 2015; Lebon et al., 2003; Pellegrino et al., 2006) or an increase in evapotranspiration (Eagleson, 1978; Fila et al., 1998; Keller, 2015; Lebon et al., 2003; Pellegrino et al., 2006). Plant physiologist use water potentials to measure how easily water moves from the soil through the plant to the atmosphere– the so-called soil plant atmosphere continuum. The relationship between soil water potential (ψ_{SOIL}), plant

water potential (ψ_{PLANT}), resistance to water flow in the grapevine (r_h) and the rate of transpiration (E) is described in Equation 1.1 (Keller, 2015).

$$\psi_{PLANT} = \psi_{SOIL} - Er_h \quad \text{(Equation 1.1)}$$

Soil moisture availability is affected by factors such as soil type (Ritchie, 1981; Saxton et al., 1986; Tramontini et al., 2013; Wear & Patterson, 1962); and rainfall and irrigation (Cooley et al., 2017; Grimes & Williams, 1990; Pellegrino et al., 2006; Pereira et al., 2002; Soar & Loveys, 2007). Most wine regions rely on irrigation to provide the shortfall between rainfall and crop water requirement, due to high evaporative demand (Shellie, 2006). Following each recharge, soil moisture is depleted via evaporation from the soil (Keller, 2015), transpiration by plants (Keller, 2015), use by plants (i.e. photosynthesis), run-off and drainage (Zotarelli et al., 2010). The amount of water in the soil available for plant uptake ("plant available water") is the difference between the soil's upper limit of water holding ("field capacity") and the lower limit ("permanent wilting point"; Zotarelli et al., 2010). Water is held in the soil pores, the space between soil components and roots, with the size of the pore affecting how water can move (Watt et al., 2006). Water drains quickly from pores greater than 30 µm, with only pores smaller than 30 µm containing water one to two days after a rainfall or irrigation event, with a water suction of about 10 kPa (Watt et al., 2006). Increased surface tension in smaller pores increases the water suction (Keller, 2015), with a water suction of about 1.5 MPa for pores smaller than 0.2 µm, which is "*approaching the limit for extraction of water by crop plants*" (Zotarelli et al., 2010). Grapevines respond to changes in soil-moisture availability, by changing patterns of root growth and distribution (Soar & Loveys, 2007). Additionally, soil texture and structure can have large impacts on plant available water; for example, sandy soils drain faster than clay soils thereby reducing holding capacity (Zotarelli et al., 2010) and increased organic matter content can increase water holding capacity (Hudson, 1994).

Evapotranspiration refers to the water lost from the land surface, via transpiration from plants (including grapevines) and evaporation from the soil surface (Keller, 2015). The rate of transpiration is determined by the difference in water vapour concentration (water potential) between the leaf air spaces and the surrounding air (Keller, 2015). As temperature rises the amount of moisture the air can hold increases, with the difference between the amount of moisture in the air and the amount of moisture in saturated air (at a given temperature) referred to as vapour pressure deficit (VPD; Keller, 2015). Transpiration rates increase with increasing VPD (Keller, 2015), and the plant controls transpiration rates via stomatal conductance (Smart & Sinclair, 1976). Various management practices may be used by

vineyards to reduce evaporation (i.e. from soil surface) and conserve water, such as mulching and weed control (Hoare, 2016).

Long-term adaptations to water stress include changes in leaf area and root extension, whilst shorter term changes include changes in leaf angle, stomatal conductance and hydraulic properties of the transport system (Jones, 2004). Most plants have some degree of autonomous control over the shoot or leaf water status in response to increases in evaporative demand and decreases in soil moisture, tending to minimise changes in shoot and leaf water status (Jones, 2004). Plants, which are highly effective in maintaining a stable leaf water status, are termed 'isohydric'; in contrast plants that are less effective are termed 'anisohydric' (Jones, 2004; Schultz, 2003). Shiraz, the grape variety used in this experiment, is considered to be an anisohydric (Schultz, 2003) or near-anisohyridic variety (Hochberg et al., 2013). It has less stomatal regulation, with stomata tending to remain open and daytime ψ_{LEAF} declining in response to water deficit and associated stress (Schultz, 2003). The anisohydric behaviour of Shiraz may be an innate adaptive response in terms of heat stress, with irrigated Shiraz up-regulating gas exchange in response to short periods of high maximum temperatures, at the expense of short-term transpiration efficiency (Soar et al., 2006). Alternatively, in water scarce situations it may be a response to competition for water, in other words "*use it or lose it*" (Kjelgren, 2009).

Low water availability (water stress) leads to osmotic stress, reducing the chemical activity of water in plant cells lowering cell turgor (Ashraf & Foolad, 2007). Osmotic stress (water deficit) is defined as "*any water content of a plant cell or tissue below its water content when fully hydrated"* (Taiz & Zeiger, 2002). A decrease in the relative water content of plant tissues, such as leaves, induces changes in the metabolism of ROS (Ashraf & Foolad, 2007) and SIMRs (as discussed in Section 1.3). Osmotic stress may also occur as a result of other primary stresses such as high temperatures (discussed above) and salinity (Munns, 2002). For example, salinity reduces the plants ability to uptake water causing a number of metabolic changes identical to those caused by water stress (for a review of the comparative effects of salinity and salt stress refer to Munns, 2002).

The severity, duration and timing of water stress influences how grapevines respond to the water scarcity (Keller, 2015), for a review refer to Deloire et al. (2004). The plant responses are complex, involving both adaptive changes (phenotypic plasticity; Potters et al., 2009) and potentially deleterious changes, with the effects of water stress further complicated by superimposition of other stresses (Chaves et al., 2002). Plants respond rapidly to changes in cell water relations, reducing leaf and growth, within hours plants exhibit a steady but lower

rate of leaf and root growth (Munns, 2002). As the duration of the water stress increases leaf growth becomes more affected than root growth, with reduced leaf emergence and leaf size (Munns, 2002), potentially influencing timing of various phenological stages (Deloire et al., 2004). Water stress can reduce photosynthesis, by reducing the diffusion of CO_2 into the leaf as a result of stomatal closure (Quick et al., 1992) and/or by inhibiting RuBisCO synthesis resulting in a loss of ATP synthase lowering ATP content (Tezara et al., 1999). Water stress can also have important implications on grapevine nutrition, reducing the uptake of nutrients from the soil (Deloire et al., 2004; Gonzalez-Dugo et al., 2010; Keller, 2005), coupled with the other effects of water stress the effects of grapevine nutrition can have important implications for berry growth and biochemistry (Deloire et al., 2004). In grapevines, timing of the water stress can have important implications; for example, water stress during the first stage of berry development (refer to Section 3.1 of Chapter 3) may restrict cell division influencing final berry size (Coombe & McCarthy, 2000; Kennedy, 2002), as a result water stress applied prior to véraison may have a larger effect on berry size than water stress applied after véraison (Matthews & Anderson, 1988; Matthews et al., 1987).

Frequency of water supply in addition to total water supplied may impact on plant growth (Novoplansky & Goldberg, 2001). In research conducted by Novoplansky and Goldberg (2001), higher total water and more frequent irrigation events improved the growth of three perennial grass species (*Sporobolus airoides, Hilaria jamesii* and *Scleropogon brevifolius*). Furthermore, the ability of the grass species from the least productive area (*Scleropogon brevilfolius*) to compete with neighbouring plants (tolerance and suppression) was improved under the infrequent irrigation (Novoplansky & Goldberg, 2001).

Whilst water stress has the potential to have negative impacts on grapevine performance, some stress is commonly associated with improvements in grape composition (Collins, 2006). Deficit irrigation strategies (e.g. partial rootzone drying (PRD; Loveys et al., 2000a) or regulation deficit irrigation (RDI; Kriedemann & Goodwin, 2003) use autonomous plant responses to manage water stress – i.e. water deficits are a commonly used management tool to reduce excessive vegetative growth (Collins, 2006). This is because excessive vegetative growth is often associated with poor fruit exposure (Dokoozlian & Kliewer, 1995a) which may have negative implications, such as lower grape quality (Dokoozlian & Kliewer, 1995a; Kriedemann & Goodwin, 2003; Smart & Robinson, 1991). Improvements in composition, such as high anthocyanin content, have been associated with reductions in vegetative growth with little effect on yield (Loveys et al., 2000b). Whilst in other cases, composition improvements have been associated with reductions in berry size (McCarthy et al., 2002) and in extreme

cases failure of fruit to mature (Hardie & Considine, 1976). The beneficial and unfavourable effects of water stress relative to severity and timing are reviewed in detail by Deloire et al. (2004).

Recent research, aiming to create a "*robust closed-loop decision support*" tool for soil moisture dynamics to predict the crop water requirement of a potato crop demonstrate the complexities of plant and soil water relations, due to non-linear relationships between parameters (Adeyemi et al., 2018). The research conducted by Adeyemi et al. (2018) used a combination of established soil and crop models and neural-networks with embedded memory mechanisms, which "*have a strong learning ability and are able to represent nonlinear relationships between inputs and outputs of a system",* coupled with real-time feedback from soil moisture and climatic sensors. These models are currently able to generate a one-day-ahead prediction of volumetric soil moisture content for potato crop production (R^2 = 0.94; Adeyemi et al., 2018). However, this tool does not currently include factors such as variety and changes in management practices (i.e. mulching). This demonstrates the need for further field-based experiments assessing the effects of various management practices in conjunction with varied water supply, across a range of crops including grapevines.

Furthermore, fluctuations in water allocations and common management strategies, such as deficit irrigation, may impact on sustained productivity and potentially increase grapevine susceptibility to heat stress, driving demand for alternative management strategies. Given these environmental impacts on wine grape production, this research aims to evaluate adaptation options for heat and water stress for viticulture, and associated interactions with water deficits. Further details and discussion on water stress and wine grape production are included in each of the analytical chapters of this thesis.

1.4. Management Strategies for Heat and Water Stress

A range of management strategies may be adopted to reduce the impacts of heat and water stress on wine grape production in an establish vineyard. However, under extreme cases this could even involve re-establishing the vineyard (e.g. changing location or variety) The lengthy establishment times and expensive setup costs of grapevines make changing varieties or establishing new varieties costly. Furthermore, there can also be many obstacles associated with gaining consumer acceptance of new varieties (Rose, 2007). Therefore, there is a need to develop and refine tools for protecting grapevines from seasonal weather events, such as heatwaves.

Decreasing Canopy Temperature via Evaporative Cooling

The process of evaporation of water from the grapevine (transition from water to gas) absorbs a large amount of energy (latent heat) reducing canopy temperature (Greer & Weedon, 2014). The reduction in canopy temperature due to this process, is referred to as transpirational cooling (Smart & Sinclair, 1976). Management practices, such as irrigation, that improve plant water status may potentially reduce the effects of heat stress through increased transpiration. This has been observed in irrigated Shiraz vines, which have been shown to up-regulate gas exchange and maintain berry growth, in response to short periods of high maximum temperature (Soar et al., 2006). Well-watered Cabernet Sauvignon vines showed less negative effects on the grapevine physiology when subjected to a high-temperature (heatwave) events compared with water stressed grapevines (Edwards et al., 2011).

Grape berries have low transpiration and therefore the evaporative cooling mechanisms that reduce leaf temperatures are not effective in the case of berry cooling (Keller, 2010). The beneficial effects of evaporative cooling may also be achieved through application of water to the canopy during the heat event, in the a process referred to as hydrocooling (Greer & Weedon, 2014). Hydrocooling may be achieved through application of water to the canopy via overhead sprinklers (Keever & Cobb, 1985). However, care should be taken as water droplets on the canopy can focus the sunlight (phyto-optical phenomenon) resulting in localised area of intensified light causing serious leaf burn (Egri et al., 2010). In research conducted by Greer and Weedon (2014), the beneficial effects of hydrocooling were observed in irrigated Semillon vines, that had additional water applied to the canopy via micro-sprinklers, controlled by automated irrigation systems which tracked canopy temperature using an external sensor (Greer & Weedon, 2014). The system was designed to apply water to the canopy for 30 sec when canopy temperature reached 35 °C, the vines were then allowed to dry for 5 min before the sensor checked the canopy temperature, reapplying if canopy temperatures exceeded 35 °C (Greer & Weedon, 2014). However, heatwaves may also be coupled with lower rainfall, prolonged droughts and fluctuations in water allocations, which may result in shortfalls in the supply of irrigation water.

Reducing Radiative Heating

The effects of radiative heating may be reduced by decreasing the amount of incident radiation that is absorbed by the canopy. This may be achieved by decreasing the amount of radiation that reaches the canopy (Greer et al., 2010). The grapevines' canopy may be altered to increase the amount of shade in the canopy; for example, additional foliage wires may be installed, such as a 'lazy-lift' foliage wire on the west face of north-south rows reducing the exposure of the western face (Webb et al., 2009b). Alternatively, artificial shading in the form

of netting (or shade cloth), may be used to reduce light intensity (Shahak et al., 2008) or photo-selective nets may be used to alter the light profile and intensity by selectively absorbing certain wavelengths (Shahak et al., 2004a; Shahak et al., 2004b). However, shade netting is expensive to implement, and inconsistent results have been reported for netting. Yellow netting increased berry weight, whereas grey netting reduced berry weigh of 'Superior' table grapes in grown in Ptahya, Israel (Shahak et al., 2008). Hail netting (20% shading) adversely affected budding percentage and decreased berry set of table grapes grown in a high rainfall region of South Africa (Avenant, 1994, 1995). Yield and berry quality decreased with increasing intensity of black polypropylene netting in Sangiovese grapes grown in central Italy (Cartechini & Palliotti, 1995). In the research conducted by Greer et al. (2011), the 70% shade cloth resulted in a 40% reduction in net photosynthesis and decreased mid-season biomass accumulations to the bunches and leaves, with an overall reduction in carbon balance with increased reliance on carbon reserves (Greer et al., 2011). The 70% shade cloth installed prior to budburst reduced photo flux density to < 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and lowered canopy temperature by 3 to 5 °C compared with ambient air temperatures of 'Semillon' grapevines (Greer et al., 2011).

For all these reasons, the management of heat and water stress in grapevines is complex and issues such as low supplies of water can often mean that the full range of management options is frequently unavailable. Therefore, increasing the range of tools available for managing heat and water stress is important. Foliar sprays are one commonly available option that are growing in popularity. In this thesis, the two foliar sprays investigated were glycine betaine (GB) and kaolin particle film (KPF).

1.5. Glycine Betaine

A plant's ability to adapt to a constantly changing environment is aided by the accumulation of low molecular mass organic compounds, known collectively as compatible solutes (Sakamoto & Murata, 2002). GB (N, N, N-trimethyl glycine) is one of these compounds, which is produced in varying levels in some plants (Sakamoto & Murata, 2002). In higher plants, there are two enzymes [choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH)] that are involved in the metabolic pathway for the synthesis of GB, in which choline is oxidised to produce GB (Figure 1.3). CMO, the catalyst for the first step in the production of GB, is a ferredoxin-dependent enzyme (Rathinasabapathi et al., 1997). In barley (*Hordeum vulgare* L.), GB is primarily accumulated in the mature leaves moving to the younger leaves after watering (Ladyman et al., 1980).

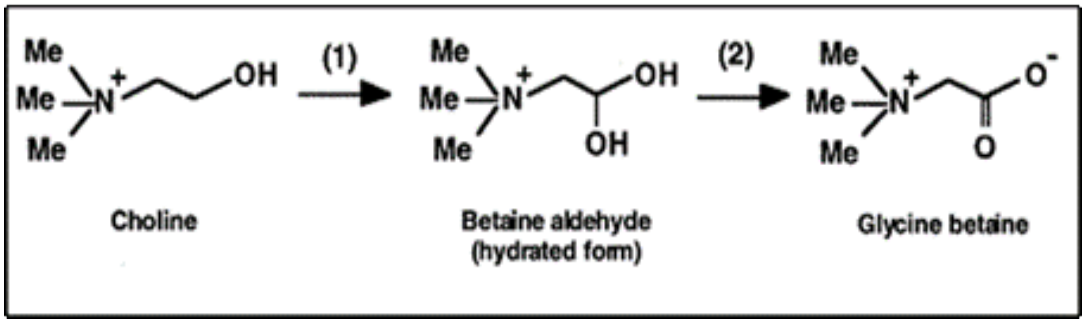

Figure 1.3. Metabolic pathway for the synthesis of glycine betaine (GB) from choline. Choline is oxidised to GB in two steps as indicated by (1) and (2). In higher plants, two enzymes are involved in this process; reaction (1) is catalysed by choline monooxygenase (CMO) and reaction (2) is catalysed by betaine aldehyde dehydrogenase (BADH; Sakamoto & Murata, 2002).

GB is involved in the protection of many macro-components in plant cells, including proteins, enzymes and membranes, under stress conditions (Sakamoto & Murata, 2002; Yang et al., 2005). GB plays an important role in several environmental stresses, including salinity, heat stress and low temperature stress, with stress inducing its biosynthesis (Sakamoto & Murata, 2002).

In research on the seeds of peanuts (*Arachis hypogaea* L. 'JL-24'), there were increased concentrations of GB and proline in response to sodium chloride (NaCl) salinity stress treatment during germination (Girija et al., 2002). The addition of calcium chloride (CaCl) to the NaCl-stressed seedlings further increased endogenous GB, whilst reducing proline levels (Girija et al., 2002). *In vitro* analysis of fragments of thylakoid membrane containing PSII complexes extracted from spinach indicated that GB prevented dissociation of two extrinsic proteins (18 kDa and 23 kDa) in PSII, in the presence NaCl (Murata et al., 1992; Papageorgiou & Murata, 1995).

Loquat (*Eriobotrya japonica* L. 'Jiefangzhong') fruit harvested at commercial maturity that were subjected to low temperature conditioning (10 °C for 6 days) prior to refrigeration (1 °C for up to 5 weeks) had higher endogenous GB content, higher BADH activity, lower levels of ion leakage and lower values for the chilling injury index (Jin et al., 2015).

1.5.1. Glycine Betaine Modes of Action

Certain areas of GB's modes of action are still unclear (Scarlett et al., 2011); however, the following modes have been identified.

- **Protection of Membrane Stability**

  GB has demonstrated the ability to protect membrane stability under environmental stress (Jolivet et al., 1982; Sakamoto & Murata, 2000, 2002).

- **Stabilisation of the Photosystem II and I Complexes**

  GB has demonstrated a profound effect on the stabilisation of the oxygen-evolving photosystem II (PSII) complex (Sakamoto & Murata, 2002). GB has been shown to stimulate the repair of the PSII complex whilst not effecting rate photodamage (Sakamoto & Murata, 2002). In other research, glycine betaine has been shown to reduce stress-induced inactivation of the PSII complex (Kurepin et al., 2015). For example, Allakhverdiev et al. (1996) observed evidence of sucrose and glycine betaine acting as co-solutes PSII against thermal inactivation. Additionally, reduced ferredoxin (required for the first step in production of GB) is produced by photosystem I (PSI) directly linking the production of glycine betaine to photosynthesis (Kurepin et al., 2015). Therefore, GB biosynthesis may act as an additional sink for electrons generated during photosynthesis (Kurepin et al., 2015). Inactivation of the oxygen evolving complex (OEC) as a result of 10 min exposure to 45 °C, was alleviated by exogenous application of GB to Barley leaves, as evidenced by a K-step in the OJIP transient (~200 to 400 µs) in the control plants but not the plants with GB applied (Oukarroum et al., 2012). This was measured using the JIP test (Bussotti et al., 2010).

- **Protection of Enzymes**

  GB has been shown to protect the activities of enzymes, such as RuBisCO and malate dehydrogenase (MDH), from environmental stresses, such as salinity (Sakamoto & Murata, 2002) and heat stress (Yang et al., 2005). GB has been shown to reduce the movement of RuBisCO activase from the soluble stroma fraction to the thylakoid membrane under high temperature conditions (Yang et al., 2005). Therefore, playing an important role in retaining RuBisCO in the active form (Feller et al., 1998; Salvucci & Crafts-Brandner, 2004a, 2004b).

- **Up-regulation of Reactive Oxygen Species Scavengers**

  GB may play a role in reducing oxidative stress. Generation of reactive oxygen species (ROS) is a common plant response to a range of environmental stresses. Oxidative stress occurs when there is an imbalance between pro-oxidants (ROS and their reaction products) generation and their antioxidants-mediated metabolism and scavenging (Anjum et al., 2014). One mode of action proposed for GB, is that it activates the expression of genes for the production of ROS scavengers (Chen & Murata, 2011; Kurepin et al., 2015), therefore potentially playing a role in stopping or reducing oxidative stress. In research conducted by Park et al. (2006), exogenous application of GB to Tomato plants (*Lycopersicon esculentum* Mill. cv. Money-maker) caused immediate increased hydrogen

peroxide (H_2O_2) levels, catalase activity and expression of the catalase gene (CAT1). In this research, H_2O_2 levels remained high for one day after chilling treatment, decreasing to below the control two days after chilling treatment (Park et al., 2006). H_2O_2 is a strong oxidiser (Halliwell, 1991), which is broken down by the enzyme catalase (Nishikawa et al., 2009). This research indicates that up-regulation of ROS scavengers may be H_2O_2 mediated (Park et al., 2006).

- **Osmoregulation**

  It is proposed that GB may play a role in osmoregulation (Dragolovich & Pierce, 1994; Jolivet et al., 1982; Robinson & Jones, 1986). In research conducted by Robinson and Jones (1986), GB accumulated in the chloroplast of salt (NaCl) stressed spinach leaves (*Spinacia oleracea*) resulting in an equivalent water potential of approximately -0.75 MPa. This helps the plant maintain chloroplast volume and photosynthetic capacity as Na^+ and Cl^- are largely excluded from the chloroplast (Robinson & Jones, 1986).

1.5.2. Use of Glycine Betaine in Agriculture

Most of the research into the use of GB in agriculture has been focused around genetically engineering the plant to enable it to produce GB (for reviews refer to: Ahmad et al., 2013; Chen & Murata, 2008; Chen & Murata, 2011; Sakamoto & Murata, 2000, 2002). It has been proposed that exogenous application of GB to plants that are unable to produce GB or produce GB at low levels, may alleviate some of the negative effects of environmental stresses (Ashraf & Foolad, 2007).

Exogenous GB has been found to enhance grain yield of maize, sorghum and wheat (Agboma et al., 1997). In that research, GB was applied at 0, 2, 4 and 6 $kg \cdot ha^{-1}$ under optimal and suboptimal supplementary irrigation regimes; GB marginally increased above ground biomass in all three crops. However, it is important to note that reduction in biomass due to suboptimal irrigation was only observed in maize (Agboma et al., 1997). The beneficial effects of exogenous GB for amelioration of salt stress have been observed in lettuce (Shams et al., 2016; Yildirim et al., 2015). Yildirim et al. (2015) found that salt stress reduced gibberellic acid (GA) and salicylic acid (SA) content in salt stressed plants, with application of 10 and 25 $mmol \cdot L^{-1}$ of GB enhancing GA and SA content in the salt stressed plants. In research conducted by Shams et al. (2016), GB reduced the content of compounds involved in the partitioning of ROS, such as superoxide dismutase, and increased dry matter content of salt stressed lettuce. In tomatoes, GB has been shown to increase stomatal conductance under well-watered, water-

deficient and saline conditions (Mäkelä et al., 1998b) and, increase fruit number and yield under high temperatures (Mäkelä et al., 1998a).

Uptake and translocation of exogenously applied GB with and without surfactants has been studied in summer turnip rape (*Brasicca rapa* L. ssp. *oleifera*), soybean [*Glycine max* (L.) Merr.], pea (*Pisum sativum*) tomato (*Lycopersicon esculentum* Mill.) and spring wheat (*Triticum aestivum* L.) in greenhouse experiments (Mäkelä et al., 1996). The differing modes of action for the surfactants and the differing canopy surface properties likely influenced differences in the uptake of GB between species, with many species benefiting from the use of a surfactant (Mäkelä et al., 1996). Within 2 h of applying [^{14}C] glycine betaine to summer turnip rape it was translocated to the roots and thereafter to younger parts of the shoots, with trace amounts of labelled GB detected in almost all plant parts after 6 h under optimum conditions (Mäkelä et al., 1996). Water stress, low light and low air humidity all reduced the rate of uptake and translocation of GB in summer turnip rape (Mäkelä et al., 1996). It is possible that the rapid translocation of GB may also have influenced the ability to accurately estimate and compare the uptake of GB in the other species measured. In the research conducted by Mäkelä et al. (1996), GB remained unmetabolised in turnip rape for up to 17 days after application. However, at present, it appears that there is no unifying hypothesis describing the duration of GB effects in grapevines.

1.5.3. Use of Glycine Betaine in Viticulture

Grapevines have been shown to exhibit lower endogenous levels of GB compared to other plants (Mickelbart et al., 2006). Endogenous GB content increased with increasing levels of imposed salinity (Na^+ and Cl^-) in a nine *Vitis vinifera* L. genotypes (Mohammadkhani et al., 2013). However, accumulation of GB in grapevines under other environmental stresses has not been confirmed. Endogenous levels of GB in the lamina differed among genotypes in response to various levels of salinity (Mohammadkhani et al., 2013). For example, *Vitis vinifera* L. 'Gharashani' had higher levels of GB than other genotypes for all treatments (control and imposed salinity treatments). Furthermore, the rate of accumulation of GB in response to increasing levels of imposed salinity was lower in *Vitis vinifera* L. 'GhezelUzum' compared to other genotypes (Mohammadkhani et al., 2013). Differences in the endogenous levels and the magnitude of the response to increasing levels of imposed salinity have also been observed between *Vitis* L. species (Mohammadkhani et al., 2014); for example, 'H6' (*Vitis vinifera* x *Vitis riparia*) had lower endogenous level of GB and a lower rate of accumulation of GB in response to imposed salinity, compared with 'Shirazi' (Mohammadkhani et al., 2014). Following an initial increase in GB content in response to increasing imposed salinity some genotypes, such as

'Anghootkeh' (*Vitis labrusca*), showed a drop in GB content at higher levels of salinity (Mohammadkhani et al., 2014). It is possible that the species and genotype of the rootstock and scion may both influence endogenous levels and accumulation of GB in response to various environmental stresses. In summary, there has been limited research into the effects of exogenous GB on grapevines or perennial plants; hence the importance of the research. Additional literature on the effects of GB on yield and composition can be found in Chapters 3 and 4 of this thesis.

1.6. Kaolin Particle Film

The weathering of naturally occurring primary minerals, feldspar and quartz, gives rise to secondary minerals, such as aluminosilicate clays (Glenn et al., 2005). Historically, aluminosilicates are one of many "*soil dusts*" (Glenn et al., 2005) developed and used for their insecticide benefits (Glenn et al., 2005; Glenn et al., 1999). However, it is the reflective properties of aluminosilicate clays that are of interest for moderating heat stress in plants. Research conducted by Abou-Khaled et al. (1970) reported increased reflection of visible light and reduced transpiration in orange, lemon, rubber and bean plants applied with aluminosilicate clay, kaolinite [$Al_2Si_2O_5(OH)_4$]. This research "*stimulated considerable research"* (Glenn & Puterka, 2005) into the engineering and development of 'particle films' that can protect plants from insects and/or environmental stresses (Glenn & Puterka, 2004). Since the commercialisation of the first particle film (Surround®, Engelhard Corporation, Iselin, New Jersey, USA) several products ("*Raynox®, Cacoon™, Purshade™, Parasol®, Screen®, Eclipse™, etc*") have been developed (Sharma et al., 2015).

The particle film used in this research, Surround®WP (herein referred to as KPF) is based on the naturally occurring kaolin (Glenn et al. 2010), a *"white, non-porous, non-swelling, low-abrasive, fine-grained, plate-shaped, aluminosilicate mineral ($Al_4Si_4O_{10}(OH)_8$) that easily disperses in water and is chemically inert over a wide pH range*" (Glenn & Puterka, 2004). Surround®WP comprises 95% the active constituent (kaolin) and "*5% adjuvants to aid spreading and adhesion of the particles*" (Glenn et al., 2010), with a portion of the kaolin that has been made hydrophobic by coating it with silicone (Glenn & Puterka, 2005). KPF has been proposed as a potential management tool to save water (Boari et al., 2015; Sharma et al., 2015) and reduce radiation stress (Glenn, 2012; Sharma et al., 2015).

1.6.1. Kaolin Particle Film Modes of Action

KPF alters the absorption, reflection and transmission of solar radiation, changing the quantity and quality of light within a plant's canopy (Abou-Khaled et al., 1970; Rosati et al., 2007; Shellie & King, 2013a).

- **Increased Reflection of Ultraviolet Radiation**

  There have been observed increases in the reflectance of harmful ultraviolet (UV) radiation from the surface of apples (Glenn et al., 2002). In research conducted by Glenn et al. (2002), KPF in the form of Surround®WP (Engelhard Corporation, New Jersey, USA) increased the reflectance of UV-A (320 to 400 nm) for all KPF treatments (two and four weekly applications of 45 to 56 $kg \cdot ha^{-1}$) compared with the control. However, increased reflectance of UV-B (280 to 320 nm) and UV-C (200 to 280 nm) was not observed for 'Fuji' apples after two applications of KPF compared with the control (no KPF applied). Glenn et al. (2002) concluded that it is likely that the amount of reflectance is affected by the amount of particle film residue on the fruit surface. Similar results have been reported for *Vitis vinifera* L. 'Malbec' with three applications of 57 $kg \cdot ha^{-1}$ of KPF in the form of Surround®WP (Engelhard Corporation, New Jersey, USA), with the reflectance of UV-A (315 to 400 nm) increasing with each of the KPF applications (Shellie & King, 2013a); however, the authors did not report any changes in UV-B or UV-C.

- **Increased Reflection of Visible Light**

  The spectral range of solar radiation, which photosynthetic organisms can use in the process of photosynthesis, is referred to as photosynthetically active radiation (PAR; McCree, 1972). In the literature, it is defined as either the wavebands 400 to 700 nm or 380 to 710 nm, which corresponds to the portion of the electromagnetic spectrum that is visible to the human eye (McCree, 1972). Increased reflection of visible light has been observed for several plant species with KPF applied, including soybean (380 - 750 nm; Doraiswamy & Rosenberg, 1974), almond (PAR; Rosati et al., 2007), walnut (PAR; Rosati et al., 2007), black pecan (350 - 850 nm; Cottrell et al., 2002), grapefruit (PAR; Jifon & Syvertsen, 2003), pistachio (Vatandoost et al., 2017) and grapevines (400 - 700 nm; Shellie & King, 2013a). On a leaf scale, KPF reduced absorption of PAR (estimated from measurements of reflection and transmission for almond (Rosati et al., 2007), walnut (Rosati et al., 2007) and pistachio trees (Vatandoost et al., 2017). KPF increased the distribution of light to the lower canopy of soybeans (Doraiswamy & Rosenberg, 1974) and interior canopy of almond (Rosati et al., 2007), walnut (Rosati et al., 2007) and pistachio trees (Vatandoost et al., 2017). Modelled whole canopy absorption was reduced to a lesser

extent than for a single-leaf for almond (Rosati et al., 2007) and walnut (Rosati et al., 2007). In contrast, KPF increased radiation use efficiency (based on dry weight production) for pistachios, which the authors attributed to an increase in whole canopy absorption of PAR (Vatandoost et al., 2017).

It is proposed, that increased reflection of radiation results in a reduction in canopy (Cooley et al., 2008) and fruit (Glenn et al., 2002) temperatures. Reductions in canopy temperature from the application of KPF have been observed in olives (Denaxa et al., 2012), apples (Glenn et al., 2002), grapefruit (Jifon & Syvertsen, 2003) and grapevines (Cooley et al., 2008; Glenn et al., 2010; Shellie & Glenn, 2006; Shellie & King, 2013a, 2013b). For example, Glenn et al. (2002) showed that KPF coated fruit had cooler temperatures than the non-coated fruit, and there was less evidence of a zone of heating on the upper half of the fruits, which rotated from the east to the west side of the fruit throughout the day on non-coated fruit. Furthermore, increased reflection of PAR within the canopy is proposed to increase photosynthesis under some circumstances (Glenn et al., 1999; Puterka et al., 2000), potentially benefiting production. Additionally, it is proposed that KPF reduces susceptibility to photoinhibition, evidenced by a higher quantum yield of PSII photochemistry under warmer conditions (Dinis et al., 2016b).

1.6.2. Use of Kaolin Particle Film in Viticulture

The interactive effects of KPF and water deficits have been studied on the white grape variety Viognier (Glenn et al., 2010; Shellie & Glenn, 2008) and on red grape varieties: Merlot (Glenn et al., 2010; Ou et al., 2010; Shellie, 2015; Song et al., 2012), Malbec (Shellie & King, 2013a, 2013b), and Cabernet Sauvignon (Shellie & King, 2013b) grown in Idaho, which has a semi-arid climate. More recently, the effects of KPF have been studied on Cabernet Sauvignon in Southern Italy (Brillante et al., 2016) and Touriga Nacional grown in Northern Portugal (Dinis et al., 2016a; Dinis et al., 2016b). In Australia, the effects of KPF and water deficits have been studied on the red grape varieties Cabernet Sauvignon (Cooley et al., 2008; Glenn et al., 2010) and Shiraz (Foletta, 2006).

The impact of KPF on yield and composition has differed among cultivars (Glenn et al., 2010). KPF has been linked with higher water use as a result of an increase in crop load, fruit weight and leaf conductance (g_l), prompting the conclusion that irrigation scheduling may need to be altered to ensure adequate supply of water (Glenn et al., 2001) as it changes ET_C demand. Shiraz, the variety used in this research, is considered to be an anisohydric (Schultz, 2003) or near-anisohydric variety (Hochberg et al., 2013), with less control over stomatal conductance

under diminishing water supply. Shellie and King (2013a) have proposed that differences in the mechanisms of stomatal control among *Vitis vinifera* cultivars may influence the grapevine response to KPF. Therefore, this research will assist in gaining better insights into the effects of KPF on Shiraz vines, grown in Australia. Additional literature on the effects of KPF on grapevines under deficit irrigation can be found in Chapters 3 and 4 of this thesis.

1.7. Glycine Betaine and Kaolin Particle Film

Comparison of the combined effects of GB and KPF under deficit irrigation has been conducted on olives (Denaxa et al., 2012; Roussos et al., 2010). In grapevines, comparison of a physical protectant and metabolic protectant is limited to the comparison of KPF to an anti-transpirant (pinolene). This research was conducted on *Vitis vinifera* L. 'Cabernet Sauvignon' grown under field conditions in Italy over three growing seasons (Brillante et al., 2016), with inconsistent effects on yield observed. However, there is no research to the author's knowledge comparing the effects of GB and KPF under deficit irrigation on grapevines; or assessing the potential for interactions of GB and KPF under deficit irrigation on grapevines or other plants. Furthermore, as these management tools may potentially be used individually or combined under different levels of soil moisture availability, it is important to establish if any interactive relationships exist. Further details and discussion on the effects of water stress, GB and KPF on wine grape production is included in each of the analytical Chapters of this thesis.

1.8. Research Objectives and Research Approach

The principal objective of this research was to investigate the protective capacity of GB and KPF, individually and combined, on grapevine performance under varying levels of imposed water stress, and their associated interactions on the productive performance and physiology of *Vitis vinifera* L. 'Shiraz'.

More specifically, the objectives of this research were to:

(1) Investigate the effectiveness of a metabolic protectant GB on the performance of grapevines under water stress conditions;
(2) Investigate the effectiveness of a physical protectant KPF on the performance of grapevines under water stress conditions;
(3) Determine potential interactions between these protectants on grapevine performance under water stress conditions; and
(4) Discuss the potential value of these protectants under future hotter and drier climatic conditions.

A single large experiment was conducted during the 2011-12 (Y1) and 2012-13 (Y2) growing seasons on *Vitis vinifera* L. 'Shiraz' grown at The University of Melbourne's Dookie Campus vineyard located in North East Victoria, Australia (36.395° S, 145.703 °E, elevation 189 m) to assess the effects or irrigation treatments, GB and KPF on grapevine performance. Chapter 2 describes the experimental site, planting material, experimental design, implementation of treatments, sampling procedures and statistical analysis.

Analysis of data collected from the experiment is divided into three analytical chapters.

- **Chapter 3. Yield and Yield Components**

  To maximise profits, growers aim to produce as much crop per hectare as the wineries are willing to purchase. Therefore, for growers, yield per hectare is a constant trade-off between grower revenue, costs of production and the constraints imposed by the wineries that purchase the grapes (i.e. grape composition). Therefore, Chapter 3 assesses the effects of GB (objective 1), KPF (objective 2) and the interaction of GB and KPF (objective 3) on yield and yield components.

- **Chapter 4. Grape Composition**

  Grape composition, like yield, is an essential measure of grapevine performance. Primary metabolites (carbohydrates and organic acids) and secondary metabolites (phenolics) are assessed by wineries to inform grape payment. Therefore, Chapter 4 assesses the effects of GB (objective 1), KPF (objective 2) and the interaction of GB and KPF (objective 3) on carbohydrates, organic acids and phenolics.

- **Chapter 5. Canopy Growth, Plant Water Status and Canopy Temperature**

  Chapter 5 provides measurements of canopy growth, plant water status and canopy temperature conducted in Y2 of the experiment, to provide insights into the mechanisms behind the observed changes in yield and grape composition reported in Chapter 3 and 4.

Chapter 6 provides a discussion of key findings of the research, the implications of the key findings and areas for further research. This chapter includes a discussion of the potential value of KPF and GB under future hotter and drier climatic conditions (Objective 4).

Chapter 2. Field Experiment Materials and Methods

2.1. Introduction

A field experiment was conducted on *Vitis vinifera* L. 'Shiraz' vines during the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March) to investigate the impacts of irrigation treatments and two commercially available foliar sprays, glycine betaine (GB) and kaolin particle film (KPF), on grapevine performance. This chapter describes the experimental site (Section 2.2), planting material (Section 2.3), experimental design (Section 2.4), implementation of treatments (Section 2.5), sampling procedures (Section 2.6) and statistical analysis (Section 0). Analysis was conducted on grape yield and yield components (Chapter 3), grape composition (Chapter 4) and, canopy growth, plant water status and canopy temperature (Chapter 5).

2.2. Experimental Site

The field experiment was established in a commercial vineyard located at The University of Melbourne's Dookie Campus Vineyard located in North East Victoria, Australia (36.395° S, 145.703° E, elevation 189 m), on the lower southern slope of Mount Major (Figure 2.1). The soil at the site is referred to locally as a Dookie clay loam (Downes, 1949), a red ferrosol soil (Isbell & the National Committee on Soil and Terrain, 2016).

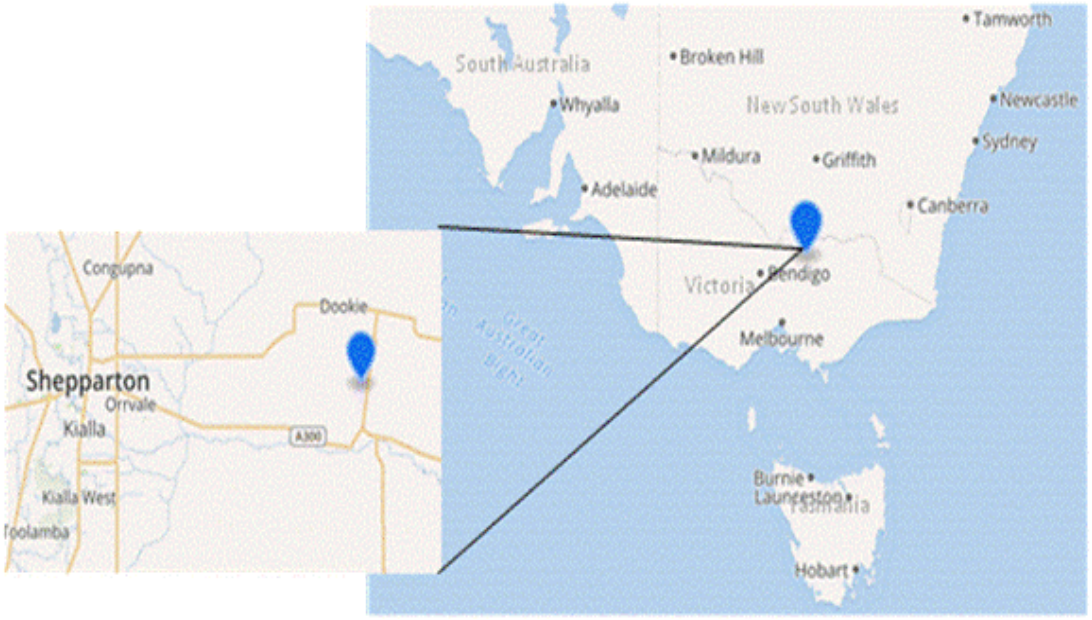

Figure 2.1. Location of the field experiment conducted at The University of Melbourne's Dookie Campus commercial vineyard in North East Victoria, Australia (36.395° S, 145.703° E, elevation 189 m).

2.2.1. *Long Term Climate Data for the Dookie Region*

The Dookie region is a warm or temperate climate region, with warm to hot summers and milder winters (Figure 2.2). The average annual rainfall for the region is 575 mm (1986 to 2012), with slightly higher rainfall in the winter months than summer months (Figure 2.3). Historical climate averages for the Dookie region were calculated using extrapolated data obtained from the SILO website (www.longpaddock.qld.gov.au/silo). SILO data sets are constructed using continuous daily time step records from approximately 4600 sites across Australia of rainfall, maximum and minimum temperature, Class A pan evaporation, reference crop evapotranspiration (ET_o), solar radiation and vapour pressure (Jeffrey et al., 2001). According to the SILO website, ET_o was calculated using the FAO Penman-Monteith formula (Allen et al., 1998) with a default wind value of 2 $m \cdot s^{-1}$ and radiation values derived from cloud oktas and hours of sunshine duration [Department of Science (Queensland Government), 2016] . Monthly long-term rainfall, temperature, reference evapotranspiration (ET_o) and humidity data is tabulated in the appendices (refer to Section 8.1 of Chapter 8).

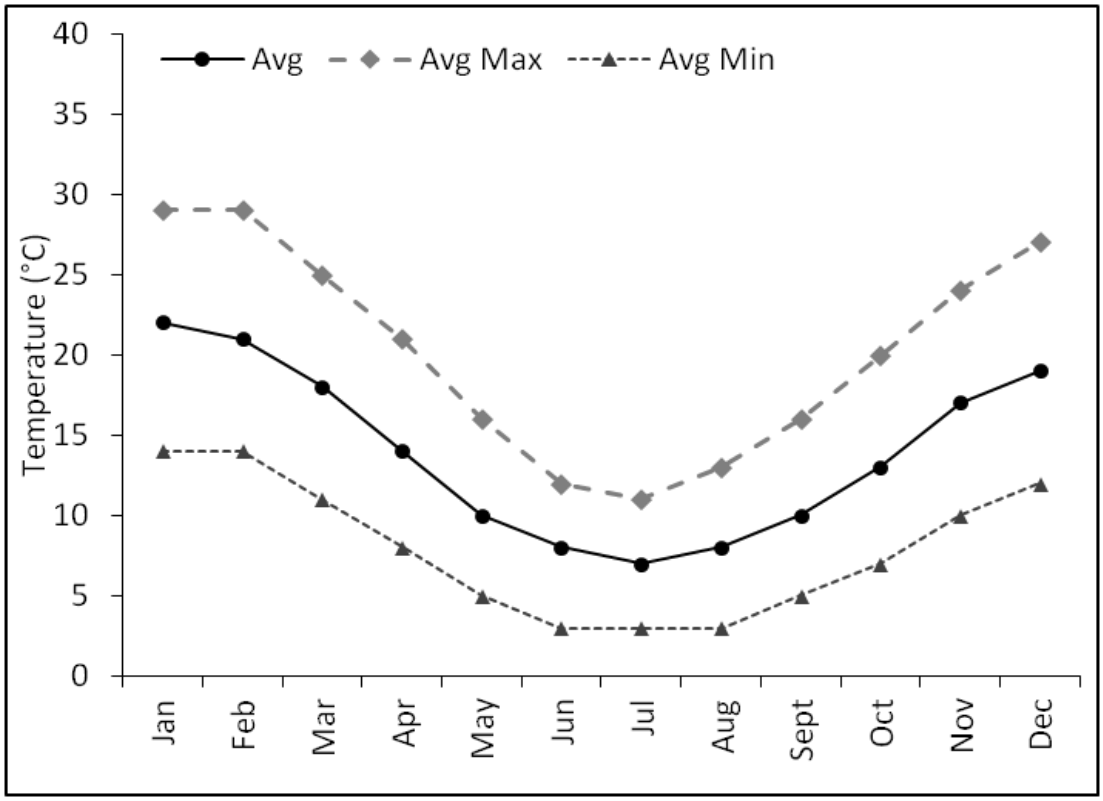

Figure 2.2. Historical monthly average temperature (Avg; °C), average maximum temperature (Avg Max; °C) and average minimum temperature (Avg Min; °C) for the Dookie Region, calculated from extrapolated climate data (Jeffrey et al., 2001) from 1986 to 2012 obtained from SILO website (www.longpaddock.gov.au/silo).

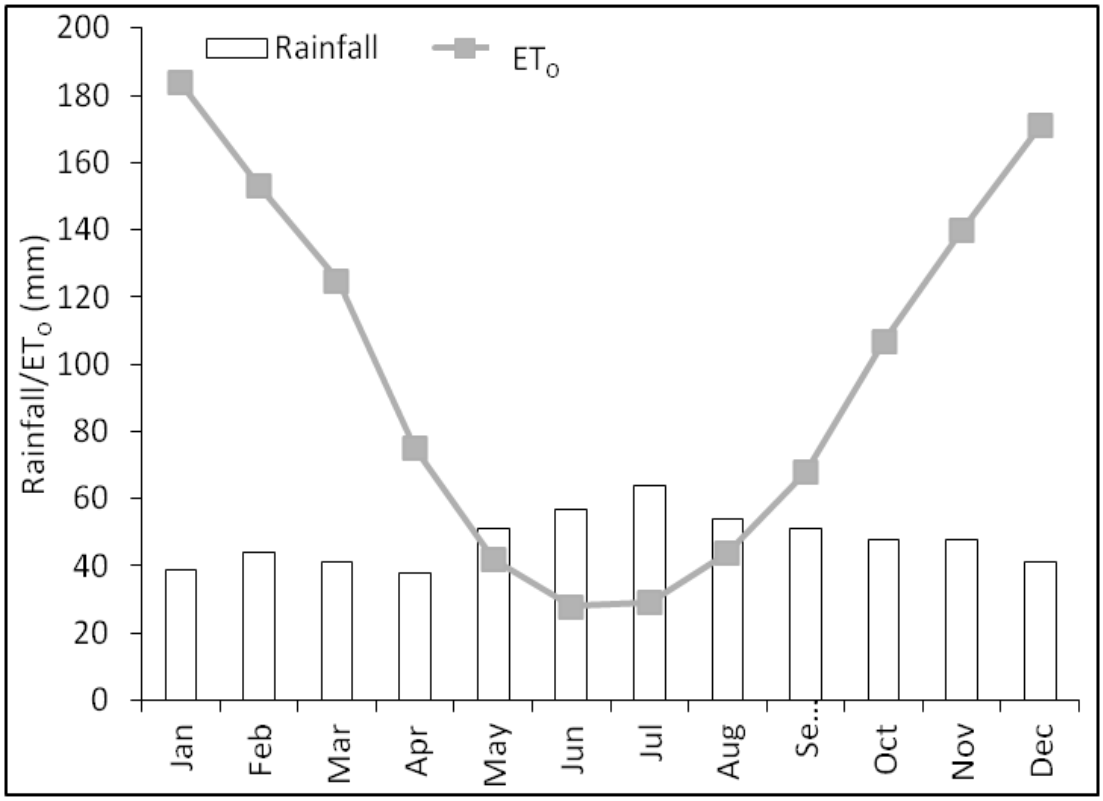

Figure 2.3. Historical monthly rainfall (mm) and reference evapotranspiration (ET_o; mm) averages for the Dookie region, calculated from extrapolated climate data (Jeffrey et al., 2001) from 1986 to 2012 obtained from the SILO website (www.longpaddock.qld.gov.au/silo).

Growing Degree Days

GDD_{10} was calculated as the sum of the mean monthly temperatures above 10 °C to represent the biologically effective heat accumulated throughout the growing season (1st October to 31st of March) using Equation 3.1 (Winkler et al., 1974). The summation expressed as degree-days – i.e. if the mean temperature for January was 20 °C, the summation is 310 degree-days (10 degrees times 31 days; Winkler et al., 1974)

$$GDD_{10} = \sum_{m=1}^{m=3} \max\left[\left(\frac{Tmin_m + Tmax_m}{2} - 10\right) \times days_m, 0\right] \quad \text{(Equation 3.1)}$$

Where m = month number

Tmin = average minimum monthly temperature (°C)

Tmax = average maximum monthly temperature (°C)

days = number of days in month m

The long-term average GDD_{10} for the growing season from 1986 to 2012 is 1,539.5 C° units. Inclusion of the long-term average degree days for April in the calculation of GDD_{10} (135 C°), results in this region being classified as a Region III (out of 5) on the Winkler index or a "*moderately warm climate*" (Winkler et al., 1974).

Mean January Temperature

MJT, the equivalent to the mean July temperature in the Northern Hemisphere, was calculated using average daily temperatures (T_{mean}) for January using Equation 3.2 (Jarvis et al., 2017).

$$MJT = \frac{\left(\sum_{1\ \mathrm{Jan}}^{31\ \mathrm{Jan}} T_{mean}\right)}{31} \quad \text{(Equation 3.2)}$$

The long-term average MJT from 1986 to 2012 is 21.6 °C.

2.2.2. Environmental Conditions during the Experiment

The environmental conditions during the experiment were obtained from a weather station (WXT510; Vaisala Group; Helsinki, Finland) located within approximately 100 m of the experiment site, outside of the vineyard rows. Ambient air temperature, humidity, rainfall, radiation, wind speed and wind direction were recorded at a ten min frequency over the experimental period. Reference evaporation (ET_o) was obtained from the SILO website (www.longpaddock.qld.gov.au/silo). Monthly rainfall, temperature, ET_o and relative humidity data during the experiment is tabulated in the appendices (refer to Section 8.2 of Chapter 8).

Ambient Temperature

The growing season (1st October – 31st March), mean temperatures were hotter than average in Y1 and Y2, with similar but higher than average GDD_{10} and MJT for Y1 and Y2 (Table 2.1).

Table 2.1. Growing degree day (GDD_{10}), mean January temperature (MJT) and average temperatures for Dookie, Victoria, Australia for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March). GDD_{10} was calculated using a base of 10 °C for the 2011-12 (Y1) and 2012-13 (Y2) growing season (1st October – 31st March); MJT was calculated as the average of the mean daily maximum temperature and mean daily minimum temperature. Long-term climate averages were calculated using extrapolated daily minimum and maximum temperature data (Jeffrey et al., 2001) from 1986 to 2012 obtained from the SILO website (www.longpaddock.qld.gov.au/silo).

	Y1	Y2	Long-term Average
	(°C)	(°C)	(°C)
GDD_{10}	1793.3	1838.8	1539.5
MJT	23.3	23.0	21.6
October	15.5	14.5	13.0
November	19.6	19.7	16.8
December	20.3	21.5	19.0
January	23.5	23.0	21.6
February	22.0	23.1	21.2
March	17.8	19.2	18.3

In Y1 and Y2, average maximum temperature was higher than the long-term average (Table 2.2). However, there were only two days above 40 °C during the experimental period – 5th January 2013 (43 °C) and 7th January 2013 (41 °C).

Table 2.2. Average maximum temperature (Avg Max) and absolute maximum temperature (Max*) for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March). Long-term climate average maximums monthly temperature was calculated as the average of extrapolated temperature data from 1986 to 2012 (Jeffrey et al., 2001) obtained from the SILO website (www.longpaddock.qld.gov.au/silo).

	Y1		Y2		Long-Term Average Maximum
	Avg Max (°C)	Max* (°C)	Avg Max (°C)	Max* (°C)	(°C)
October	21.3	30.8	21.0	32.4	19.5
November	25.9	35.4	27.2	39.9	23.9
December	27.0	34.3	28.7	38.2	26.7
January	30.4	39.1	32.0	43.0	29.3
February	28.8	35.5	30.8	37.5	28.7
March	23.2	29.1	26.1	33.5	25.4

In Y2, lower minimum temperatures were observed during the growing season compared with Y1; however, both years had higher than average minimum temperatures (Table 2.3).

Table 2.3. Average minimum temperature (Avg Min) and absolute minimum temperature (Min*) for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March). Long-term climate average minimums monthly temperature was calculated using extrapolated temperature data from 1986 to 2012 (Jeffrey et al., 2001) obtained from the SILO website (www.longpaddock.qld.gov.au/silo).

	Y1		Y2		Long-Term Average Minimum
	Avg Min (°C)	Min* (°C)	Avg Min (°C)	Min* (°C)	(°C)
October	9.9	4.8	8.1	4.1	6.5
November	13.4	8.4	12.4	6.8	9.7
December	13.4	6.5	13.9	8.7	11.6
January	16.2	8.3	14.0	8.0	13.9
February	15.7	10.3	15.4	8.5	13.8
March	12.7	7.8	12.2	4.5	11.2

Rainfall, Irrigation and Evapotranspiration

Rainfall in the first half of the growing season (1st October – 31st December) was 155.1 compared with 95.7 mm for Y1 and Y2, respectively (refer to Section 8.2 of Chapter 8). There was higher rainfall in January of Y1, with 48.4 compared with 3.6 mm for Y1 and Y2, respectively (refer to Section 8.2 of Chapter 8), with véraison occurring during the final week of January years in both years. In Y1, there was very high rainfall in the lead up to harvest (5th of March 2013; DOY = 65, Y2) compared with low rainfall in the lead up to harvest in Y2 (25th February 2013; DOY = 56, Y2). Growing season rainfall, irrigation and temperatures are summarised in Figure 2.4.

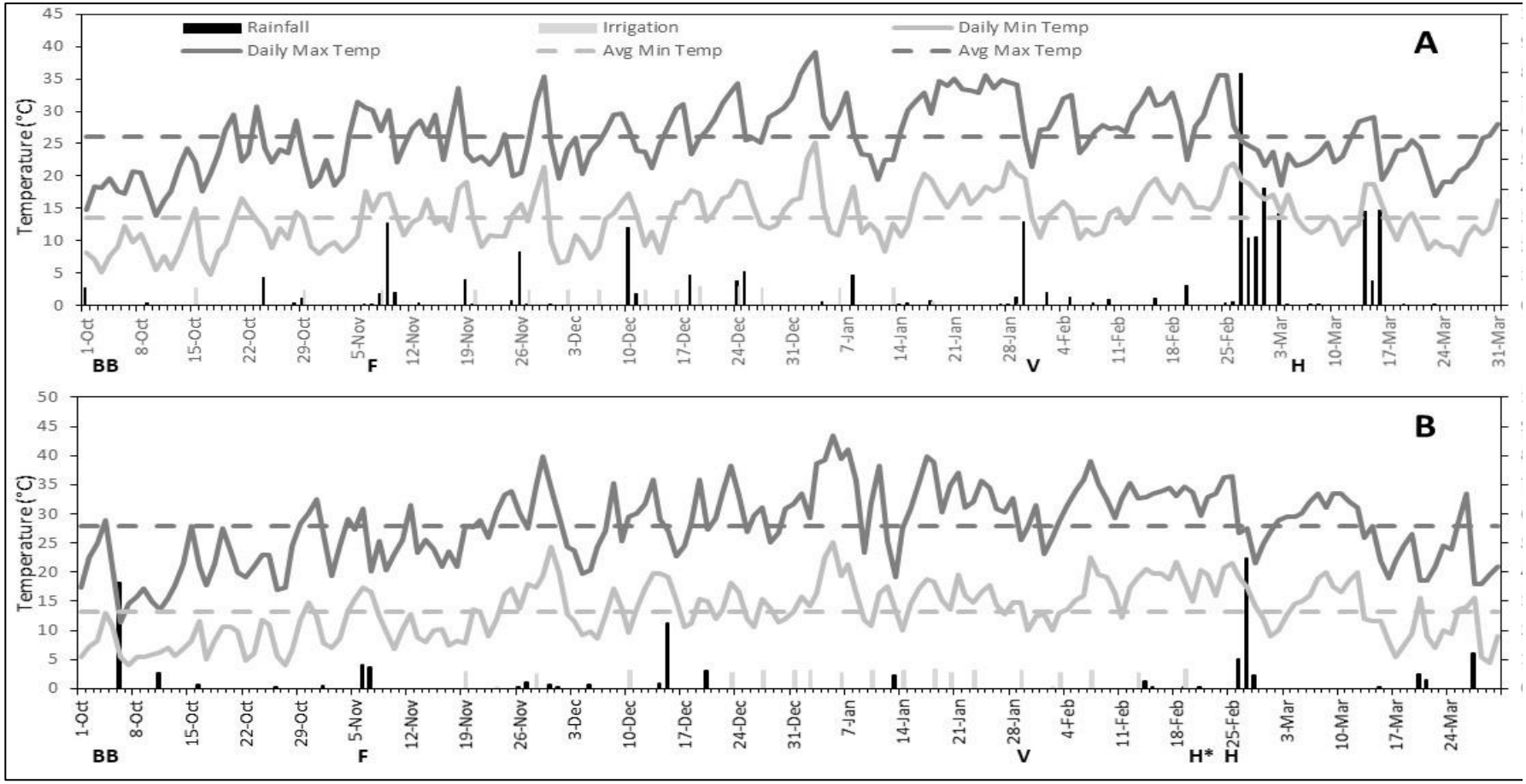

Figure 2.4. Daily minimum and maximum temperatures, rainfall and irrigation received (vineyard practice; I_{100}) at the experimental site for the (A) 2011-12 (Y1); and (B) 2012-13 (Y2) growing seasons (1st October to 31st March). Budburst (BB) and flowering (F) dates were estimated from the dates of a neighbouring vineyard and, véraison (V) and harvest (H) dates estimated from vineyard observations. The dashed lines represent the average minimum and maximum temperature for each of the growing seasons.

NB: Sub-sample collection for analysis of grape composition (H*) was conducted prior to the full harvest for analysis of yield (H).

The site was chosen as it was a proposed pilot site for the IrriSat SMS technology, which uses satellite images to estimate site specific crop coefficients and thus estimate crop water use (Montgomery & Hornbuckle, 2010). This information is then used to provide customised irrigation scheduling information to growers via SMS messaging or the internet (Montgomery & Hornbuckle, 2010; Montgomery et al., 2015). Whilst the site did not proceed as a pilot site, "vineyard practice" resulted in similar amounts of irrigation being in both Y1 and Y2. This allowed for the treatments to be assessed under contrasting seasonal conditions, with high rainfall received between véraison and harvest in Y1 and, low rainfall received véraison and harvest in Y2. However, it is important to acknowledge given that vineyard practice did not consider water demand, vine water severity could not be repeated between seasons. There were three soil moisture probes located in the block in which this experiment was based. However, scrutiny of the data after the experiment revealed that the probes were damaged (data omitted from the thesis).

Analysis of flow meter data revealed that the vineyards irrigation scheduling strategy ("vineyard practice") used in Y1 and Y2 of the experiment approximated a 'time-date on-off' strategy with the aim of irrigating for 4 h every 10 to 14 days from mid- to late-December and every three to six days from late-December until just after harvest. Irrigation events were scheduled to occur overnight, starting between 2000 and 2100 h AEDT each time. Total irrigation received each growing season (1st October – 31st March) for each of the irrigation treatments (Section 2.5.1) is shown in Table 2.4.

Table 2.4. Irrigation applied to vines for each of the irrigation treatments (I_{25}, I_{50} and I_{100}) for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March) expressed as litres per vine ($L \cdot vine^{-1}$), mega litres per hectare ($ML \cdot ha^{-1}$) and percentage of reference evapotranspiration ($\%ET_o$).

Treatment	Y1			Y2		
	$L \cdot vine^{-1}$	$ML \cdot ha^{-1}$	$\%ET_o$	$L \cdot vine^{-1}$	$ML \cdot ha^{-1}$	$\%ET_o$
I_{25}	125	0.20	2.4%	162	0.26	2.8%
I_{50}	250	0.41	4.9%	324	0.53	5.7%
I_{100}	500	0.82	9.8%	648	1.05	11.3%

Cumulative reference evapotranspiration (ET_o, mm), rainfall and irrigation received for each of the irrigation treatments was calculated daily for each of the growing seasons (1st October – 31st March). Growing season ET_o was lower than the long-term average in Y1 at 833 mm (Figure 2.5) compared with the long-term average of 880 mm from 1986 to 2012 (refer to Table 8.1 in Chapter 8). In Y1, growing season rainfall was approximately double the long-term average at 500.8 mm (Figure 2.5) compared with the long-term average of 261.2 for 1986 to 2012 (refer to Table 8.1 in Chapter 8). In contrast in Y2, ET_o was higher than the long-term

average at 933 mm and growing season rainfall was less than the long-term average at 176.9 mm (Figure 2.5). There was a similar amount of irrigation water applied in the Y1 and Y2 growing seasons (Figure 2.5).

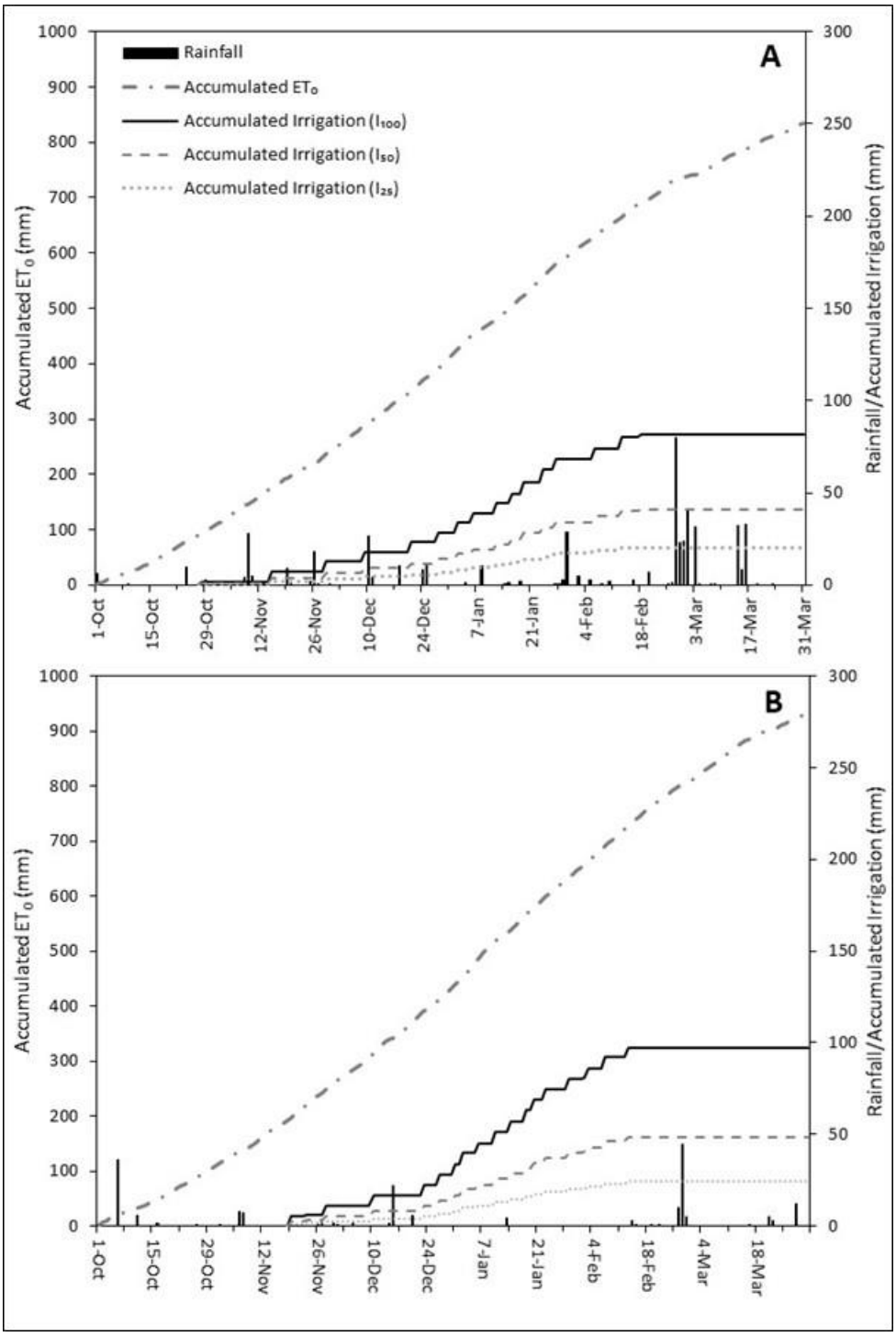

Figure 2.5. Accumulated reference evapotranspiration (ET_o), rainfall and irrigation received for the irrigation treatments (I_{25}, I_{50} and I_{100}) for the (A) 2011-12 (Y1); and (B) 2012-13 (Y2) growing seasons (1st October – 31st March).

2.3. Planting Material

The Shiraz vines (clone SA16) were planted in 1994, on Richter 99 rootstock spaced 1.8 m apart on magnetic north-south orientated rows spaced 3.4 m apart. The vines were spur-pruned to two bud spurs with approximately 20 spurs per m (ten per cordon m) on a cordon-trained Scott Henry trellis system, with two bilateral cordons (Figure 2.6). The trellis system allowed the western side of the canopy to sprawl like a fan, with the aim of protecting the canopy from the afternoon sun; whilst the eastern side of the canopy was positioned vertically using shoot positioning wires (fruiting wires; Figure 2.6) to maximise cluster exposure in the morning. Vines were considered to have medium to high vigour, with an average trunk circumference measured prior to the experiment (refer to Section 2.6.1) of 18.3 cm.

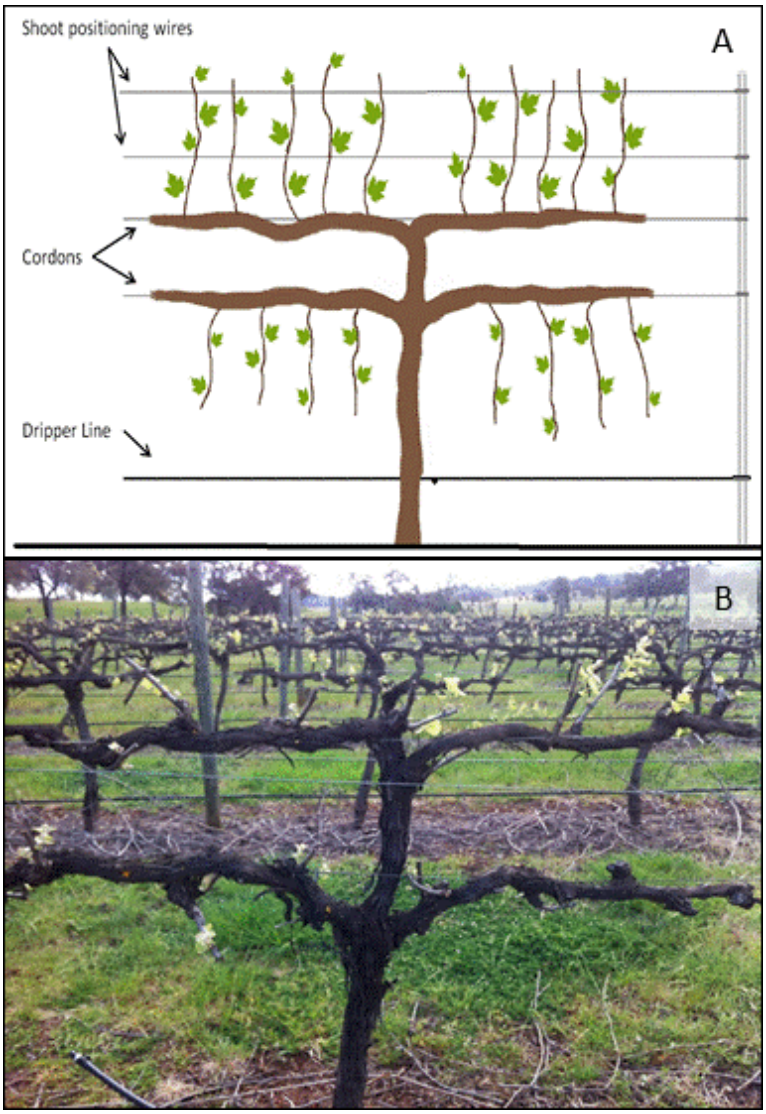

Figure 2.6. (A) Diagram of the cordon-trained Scott Henry trellis system, with two bilateral cordons, used in the vineyard (adapted from Christensen et al., 2003); (B) Photo of the trellis system used in the vineyard.

2.4. Experimental Design

The statistical design for this research project was a 3 x 2 x 2 factorial with six replicates (Table 2.5). There were three treatment levels for irrigation amount and the GB and KPF treatments each had two levels, which consisted of a single rate of application and a control that had no treatment application. The field design was a randomised complete block design (RCBD) with some row-column balance – i.e. all 12 treatment combinations were randomised within each block (Table 2.7). The experimental design and layout were optimised by a biometrician (Dr Leigh Callinan) using trunk cross-sectional area data (Section 2.6.1).

Table 2.5. Summary of the 12 combinations of the three factorial treatments: irrigation (I_{25}, I_{50} and I_{100}), glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+)

	GB^- (not applied)		**GB^+ (applied)**	
	KPF^- (not applied)	**KPF^+ (applied)**	**KPF^- (not applied)**	**KPF^+ (applied)**
I_{25} (2 L·h^{-1}) (25% vineyard practice)	$I_{25}GB^-KPF^-$	$I_{25}GB^-KPF^+$	$I_{25}GB^+KPF^-$	$I_{25}GB^+KPF^+$
I_{50} (4 L·h^{-1}) (50% vineyard practice)	I_{50} GB^-KPF^-	I_{50} GB^-KPF^+	I_{50} GB^+KPF^-	I_{50} GB^+KPF^+
I_{100} (8 L·h^{-1}) (100% vineyard practice)	$I_{100}GB^-KPF^-$	$I_{100}GB^-KPF^+$	$I_{100}GB^+KPF^-$	$I_{100}GB^+KPF^+$

The experimental area (0.88 ha) comprised 12 rows, with 120 vines in each row. There were 6 untreated buffer vines located on the northern (vines 1 – 6) and southern (vines 115 – 120) ends of each row. Each treatment plot (Table 2.6) comprised 18 vines located in three adjacent rows with six vines per row; treatments were applied to the six vines in the interior row with six untreated buffer vines located in the two adjacent rows. Measurements and sampling were conducted on the central two vines.

Table 2.6. Layout of each of treatment plots, comprising 18 vines located in three adjacent rows.

	Row		
Vine	**1**	**2**	**3**
1	Buffer Vine (Untreated)	Buffer Vine (Treated)	Buffer Vine (Untreated)
2	Buffer Vine (Untreated)	Buffer Vine (Treated)	Buffer Vine (Untreated)
3	Buffer Vine (Untreated)	***Sample Vine (Treated)***	Buffer Vine (Untreated)
4	Buffer Vine (Untreated)	***Sample Vine (Treated)***	Buffer Vine (Untreated)
5	Buffer Vine (Untreated)	Buffer Vine (Treated)	Buffer Vine (Untreated)
6	Buffer Vine (Untreated)	Buffer Vine (Treated)	Buffer Vine (Untreated)

There was a total of 72 treatment plots, which were split into six blocks running from north to south. Each block comprised 12 treatment plots, with each treatment combination appearing once (Table 2.7).

Table 2.7. The randomised complete block design (RCBD) arranged over 12 rows, comprising three factorial treatments: three irrigation treatments (I_{25}, I_{50} and I_{100}), two glycine betaine treatments (GB^- and GB^+) and two kaolin particle film treatments (KPF^- and KPF^+), with six replicates per treatment.

		Row			
	Vine	**1 – 3**	**4 – 6**	**7 – 9**	**10 – 12**
	7 – 12	$I_{25}GB^-KPF^-$	I_{50} GB^+KPF^-	$I_{100}GB^+KPF^-$	$I_{100}GB^-KPF^-$
Block 1	**13 – 18**	$I_{25}GB^+KPF^-$	I_{50} GB^-KPF^-	$I_{100}GB^+KPF^+$	I_{50} GB^-KPF^+
	19 – 24	I_{50} GB^+KPF^+	$I_{25}GB^-KPF^+$	$I_{25}GB^+KPF^+$	$I_{100}GB^-KPF^+$
	25 – 30	$I_{25}GB^-KPF^-$	$I_{100}GB^-KPF^-$	$I_{25}GB^+KPF^+$	I_{50} GB^- KPF^+
Block 2	**31 – 36**	$I_{100}GB^+KPF^-$	I_{50} GB^+KPF^-	$I_{100}GB^+KPF^+$	$I_{100}GB^-KPF^+$
	37 – 42	I_{50} GB^+KPF^+	I_{50} GB^-KPF^-	$I_{25}GB^-KPF^+$	$I_{25}GB^+KPF^-$
	43 – 48	I_{50} GB^-KPF^-	I_{50} GB^-KPF^+	$I_{100}GB^+KPF^-$	$I_{100}GB^-KPF^+$
Block 3	**49 – 54**	$I_{25}GB^+KPF^-$	$I_{25}GB^+KPF^+$	$I_{100}GB^+KPF^+$	$I_{100}GB^-KPF^-$
	55 – 60	I_{50} GB^+KPF^-	$I_{25}GB^-KPF^+$	$I_{25}GB^-KPF^-$	I_{50} GB^+KPF^+
	61 – 66	$I_{25}GB^+KPF^+$	I_{50} GB^+KPF^+	$I_{100}GB^-KPF^-$	$I_{100}GB^+KPF^-$
Block 4	**67 – 72**	$I_{100}GB^-KPF^-$	$I_{100}GB^+KPF^+$	$I_{25}GB^-KPF^-$	$I_{25}GB^+KPF^-$
	73 – 78	I_{50} GB^+KPF^-	I_{50} GB^+ KPF^-	$I_{25}GB^-KPF^+$	$I_{25}GB^-KPF^+$
	79 – 84	$I_{25}GB^-KPF^+$	$I_{100}GB^-KPF^-$	$I_{25}GB^+KPF^-$	$I_{100}GB^+KPF^-$
Block 5	**85 – 90**	I_{50} GB^+KPF^+	I_{50} GB^+KPF^-	$I_{100}GB^+KPF$	$I_{100}GB^-KPF^+$
	91 – 96	I_{50} GB^-KPF^-	I_{50} GB^-KPF^+	$I_{25}GB^-KPF^-$	$I_{25}GB^+KPF^+$
	97 – 102	$I_{25}GB^+KPF^+$	I_{50} GB^+KPF^-	$I_{25}GB^-KPF^+$	$I_{100}GB^-KPF^-$
Block 6	**103 – 108**	$I_{25}GB^-KPF^-$	I_{50} GB^+KPF^+	$I_{100}GB^-KPF^+$	$I_{100}GB^+KPF^-$
	109 – 114	$I_{100}GB^+KPF^+$	I_{50} GB^-KPF^+	I_{50} GB^-KPF^-	$I_{25}GB^+KPF^-$

2.5. Implementation of Treatments

2.5.1. Irrigation

The irrigation schedule was controlled by central management. Therefore, it was the same for all plots. Different amounts of irrigation were achieved by installing pressure compensating emitters that had different emitter delivery rates. Vineyard practice (I_{100}) was compared to two reduced rates of irrigation: 50% of vineyard practice (I_{50}) and 25% of vineyard practice (I_{25}). Irrigation treatments were implemented using pressure compensating emitters spaced at one dripper per vine. Irrigation outputs were dependent on the emitter types used for each treatment. Irrigation outputs were as follows: I_{100} at 8 $L \cdot h^{-1}$ per vine, I_{50} at 4 $L \cdot h^{-1}$ per vine and I_{25} at 2 $L \cdot h^{-1}$ per vine, such that I_{50} and I_{25} treatments applied 50 and 25% of the volume of water applied by I_{100} each irrigation event, respectively. Irrigation events were scheduled to occur for 4 h, equating to approximately 1.97, 3.94 and 5.88 mm each irrigation event for the I_{25}, I_{50} and I_{100} treatments, respectively. Electronic flow meters tracked the duration and frequency of irrigation events during the experiment with the output data logged at ten min intervals. Total irrigation received each growing season (1st October – 31st March) for each treatment is shown in Table 2.5.

2.5.2. ***Glycine Betaine***

GB in the form GreenStim™ (Agrimm Pty Ltd; New Zealand) was applied every three to four weeks from flowering. Each application was at a rate of 4 kg·ha^{-1}, which equated to approximately 245 mL of a 0.01 kg·L^{-1} solution per vine (refer to Table 8.3 in Chapter 8 for calculations). GreenStim™ comprises 97% active constituent (glycine betaine; $C_5H_{11}NO_2$), thus each application applied approximately 20 mmol·vine^{-1} of GB (refer to Table 8.3 in Chapter 8 for calculations). At the time of this research there was limited research available on exogenous application of GB to grapevines, with no clear consensus on application rates, timing or frequency (Table 2.8). Therefore, the application rate was based on the upper limit recommended on the label. The frequency and timing of the applications was based on advice provided by the agronomist who supplied the product. The vines were sprayed using an 8 L hand-held garden sprayer (FH220919; Hills Holding Limited; Pennant Hills, Australia). Manual calibration of the sprayer output rate was conducted before each use.

Table 2.8. Application rates and timing of glycine betaine (GB) used in the literature for various crops.

Crop Details	Application Details[¥]	Reference
Pinot Noir (*Vitis vinifera*)	Solutions ranging from 0 to 200 mM of GB to the point of dripping every seven to ten days before leaf collection (three to four applications).	(Mickelbart et al., 2006)
Maize (*Zea mays*) Wheat (*Triticum aestivum*) Sorghum (*Sorghum bicolor*)	0, 2, 4 and 6 kg·ha^{-1}	(Agboma et al., 1997)
Summer turnip rape (*Brassica rapa)* Soybean (*Glycine max*) Pea (*Pisum sativum*) Tomato (*Lycopersicon esculentum*) Spring wheat (*Triticum aestivum*)	One application of a 100 mM solution at approximately four-leaf stage.	(Mäkelä et al., 1996)
Tomato (*Lycopersicon esculentum*)	Solutions ranging from 0 to 140 mM of GB applied at three different timings (early flowering, mid flowering, and late flowering).	(Mäkelä et al., 1998a)
Tomato (*Lycopersicon esculentum*)	Solutions ranging from 0 to 10 mM.	(Park et al., 2006)
Lettuce (*Lactuca sativa*)	Solutions ranging from 0 to 25 mM of GB following transplanting and 10 and 20 days later.	(Shams et al., 2016)
Potted Olive (*Olea europaea*)	152 mL per tree prior to the onset of drought stress at a rate of 500 g per 100 L.	(Denaxa et al., 2012)

[¥] Application rate, timing and frequency based on information provided by the author(s).

2.5.3. ***Kaolin Particle Film***

KPF in the form Surround®WP (Engelhard Corporation, New Jersey, USA) was applied at véraison and one week after véraison. Each application was at a rate of 50 kg·ha^{-1}, which equated to approximately 612 mL of a 0.05 kg·L^{-1} solution per (refer to Table 8.4 in the Chapter 8). Surround®WP comprises 95% active constituent (kaolin), thus each application was applied approximately 29.1 g·vine^{-1} of kaolin (refer to Table 8.4 in the Chapter 8). At the time of application there was limited published research on KPF application to grapevines (Table 2.9). Therefore, application rate was based on the upper limit recommended on the label. The frequency and timing of the applications was based on advice provided by the agronomist who supplied the product. Consequently, the grapevines received an intermediary rate of application, with less product applied than research conducted in Idaho, USA and more product applied than research conducted in Sunraysia, Australia (Table 2.9). The vines were sprayed using a 100 L sprayer (Silvan Selecta 12-Volt Spotpak Spray; Silvan Australia Pty Ltd; Dandenong South, Australia), with a 6.8 L·min^{-1} pump at 4.14 bar pressure rate. To keep the product in solution it was regularly manually agitated. Manual calibration of the sprayer output rate was conducted before each use. Figure 2.7 shows an example of a canopy with and without KPF applied.

Figure 2.7. Digital image of canopy (A) no kaolin particle film application; and (B) kaolin particle film applied.

Table 2.9. Application rates and timing of kaolin particle film (KPF) used in the literature, including grape variety, row by vine spacing, location and vintages.

Variety	Row x Vine Spacing	Location	Vintage(s)	Application Details[¥]	Reference
Viognier	2.7 x 2.1 m	Idaho, USA	2005	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Glenn et al., 2010)
	2.7 x 2.1 m	Idaho, USA	2005 & 2006	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Shellie & Glenn, 2008)
Merlot	2.7 x 2.1	Idaho, USA	2005	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Glenn et al., 2010)
	£	Idaho, USA	2006 - 2008	60 $g \cdot L^{-1}$ weekly for three weeks	(Ou et al., 2010)
	2.4 x 1.8 m	Idaho, USA	2006-2010	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Shellie, 2015)
	2.7 x 2.1 m	Idaho, USA	2005 & 2006	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Shellie & Glenn, 2008)
	2.4 x 1.8 m	Idaho, USA	2007 & 2008	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Song et al., 2012)
Cabernet Sauvignon	2.2 x 0.9 m	Southern Italy	2012 - 2014	One application at 6 $L \cdot hL^{-1}$ at bunch closure	(Brillante et al., 2016)
	3 x 2.4 m	Sunraysia, Australia	2003-04	Two applications 12 days apart at 30 $g \cdot L^{-1}$ just after fruit set	(Glenn et al., 2010)
	2 x 2.7 m	Idaho, USA	2007-2009	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Shellie & King, 2013b)
Malbec	2 x 2.7 m	Idaho, USA	2009-2011	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Shellie & King, 2013a)
	2 x 2.7 m	Idaho, USA	2007-2009	60 $g \cdot L^{-1}$ in 950 $L \cdot ha^{-1}$ weekly for 3 weeks from fruit set	(Shellie & King, 2013b)
Touriga Nacional	£	Northern Portugal	2012 & 2013	Two applications at 50 $g \cdot L^{-1}$ on the same day soon after véraison	(Dinis et al., 2016a)
	£	Northern Portugal	2012 & 2013	One application at 50 $g \cdot L^{-1}$ soon after véraison	(Dinis et al., 2016b)

[¥] Application rates, timing and frequency used to establish the initial coat of KPF based on information provided by the author(s).

[£] Information not provided by the author(s).

2.6. Sampling Procedures

Analysis was conducted on grape yield and yield components (Chapter 3), grape composition (Chapter 4) and, plant water status, canopy temperature and canopy growth (Chapter 4). Sampling and measurements utilised for results presented in more than one chapter are presented here, whereas specific procedures used in one chapter only, are reported in the materials and methods section of that chapter.

2.6.1. Trunk Cross-Sectional Area

Trunk circumference (C, cm) was measured to the nearest 0.1 cm using a flexible tape, 30 cm from the base of both sample vines during dormancy. Measurements were conducted prior to Y1 (8th, 12th and 13th September 2011) and after harvest in Y2 (24th July 2013). Trunk cross-sectional area (TCA, cm^2) was calculated using the circumference measurements, on the assumption that the trunks were circular, using Equation 2.1.

$$TCA = \frac{C^2}{4\pi} \qquad \text{(Equation 2.1)}$$

Analysis of the trunk cross-sectional area was used to select the experiment location and was used by the biometrician to assist with optimisation of the experimental design.

2.6.2. Harvest Sampling

Harvest sampling comprised cluster sub-sampling and whole vine harvest for analysis of harvest yield and composition (Table 2.10). In Y1, cluster sub-sampling and whole harvest was based on the commercial harvest date of the experimental plot to allow for the buffer vines to be commercially harvested, with harvest occurring on the 5th March 2012 (DOY = 65, Y1). In Y2, cluster sampling was conducted on the 20th of February 2013 (DOY = 51, Y2) and whole vine harvest was conducted on the 25th of February 2013 (DOY = 56, Y2) as access to the vineyard was limited by commercial management during commercial harvest.

Table 2.10. Details of harvest sampling timing for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons with reference to the day of year (DOY) and analysis conducted on the sample.

Sample	Date in Year 1	Date in Y2	Analysis
Cluster sub-sample	5 March 2012 (DOY = 65, Y1)	20 February 2013 (DOY = 51, Y2)	Average berry weight (Chapter 3) Grape composition (Chapter 4)
Whole vine harvest	5 March 2012 (DOY = 65, Y2)	25 February 2013 (DOY = 56, Y2)	Cluster weight (Chapter 3) Yield per vine (Chapter 3)

Prior to harvest, a sample of six clusters per sample vine (12 clusters per plot) was collected and placed into bags and placed on ice in the field. Sampling was split evenly between each side of the canopy (Shellie, 2006) and each sample vine. The cluster samples were weighed

using a two decimal point balance (Sartorius ED2202S-CW Extend; Sartorius AG; Goettingen, Germany). A subsample of 100 berries, randomly selected from the 12 clusters and weighed using the same two decimal point balance. The cluster samples were stored in the -20 °C freezer for analysis of soluble solids and acidity and 50 of 100-berry samples were stored in the -20°C freezer for analysis of colour, phenolics and berry percent dry weight (Chapter 4). The number of clusters per vine were counted *in situ* prior to whole vine harvest. The remaining clusters, on the two sample vines in each plot, were then harvested by hand and weighed using a two decimal point field balance (FG-30KBM Digital Scale; A & D Co Ltd; Tokyo, Japan) for analysis of yield per vine (Chapter 3).

2.6.3. Maturity Sampling

Maturity sampling was conducted four times between véraison and harvest in Y1 and Y2. Berries were collected from randomly selected clusters located on both sides of the canopy of the two sample vines in each plot. Sampling was split evenly between each side of the canopy (Shellie, 2006) and each sample vine. Five berries per random cluster were collected *in situ* and placed into zip lock plastic bags: two berries from the top, two berries from the middle and one berry from the bottom of each of the clusters (Iland et al., 2000). The zip lock plastic bags were placed onto ice in the field until they could be weighed on a two decimal point balance (Sartorius ED2202S-CW Extend; Sartorius AG; Goettingen, Germany) to assess average berry weight (Chapter 3). The berry samples were then stored in zip lock bags in a -20 °C freezer for analysis of soluble solids and acidity (Chapter 4). The total number of clusters sampled each time was adjusted upwards in Y1 and Y2 after it was found more berries were required to complete desired analysis (Table 2.11).

Table 2.11. Details of sampling timing for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March) with reference to the day of year (DOY) and sample size.

	Sample Date	Day of Year (DOY), Year	# Cluster Sampled	Total # Berries
Sample Date 1, Y1	2nd February 2012	DOY = 33, Y1	6	30
Sample Date 2, Y1	7th February 2012	DOY = 38, Y1	12	60
Sample Date 3, Y1	14th February 2012	DOY = 45, Y1	12	60
Sample Date 4, Y1	25th February 2012	DOY = 56, Y1	12	60
Sample Date 1, Y2	6th February 2013	DOY = 37, Y2	12	60
Sample Date 2, Y2	9th February 2013	DOY = 40, Y2	16	80
Sample Date 3, Y2	12th February 2013	DOY = 43, Y2	16	80
Sample Date 4, Y2	16th February 2013	DOY = 47, Y2	16	80

2.7. Statistical Analysis

The field experiment was a RCBD with some row-column balance (refer to Section 2.4). The design could have been analysed as a RCBD, a row-column design or a spatial design. Statistical advice provided by the biometrician (Dr Leigh Callinan) recommended the use of RCBD, as it was the model that fit best.

Treatment effects on individual response variables were undertaken using a statistical software package (GenStat 16th Edition; VSN International Limited; Oxford, UK), using methodology described here. Methodology for assessing the association between two response variables [i.e. pre-dawn (ψ_{PD}) and midday (ψ_{MID}) leaf water potential] and any treatment effects on the association are described within the relevant chapters. Repeated response measurements were measured on the same experimental unit, both within each season and between the two seasons. As a result, repeated measurements are non-independent and underestimate the experimental error (Fernandez, 2007). Therefore, repeated measures analysis of variance (rANOVA) should be used "*to test the significance of the repeated-measures factor and any interaction effects associated with the repeated-measures factor*" (Fernandez, 2007). Repeated measures analysis (rANOVA) requires a minimum of three time points (Lund Research Ltd, 2018). Therefore, in the absence of a third year of data, analysis of the effects of season (Y1 versus Y2) was unable to be assessed. The lack of repeatability in vine water stress severity between seasons (refer to Section 2.2.2), further supported the independent analysis of Y1 and Y2.

The effects of the treatments (irrigation, GB and KPF) and their associated interactions on the response variables with three or more sample dates in one year (i.e. berry weight), and response variables with less than three sample dates were assessed with general linear model analysis of variance (gANOVA). The assumptions of normality of residuals and homogeneity of variance were assessed by visual inspection of the histogram of residuals, residual versus fitted values, normal probability and the half-normal probability plots generated by GenStat using methodology described by Payne (2014).

2.7.1. General Linear Model Analysis of Variance

Each of the response variables, that were measured on two or less occasions in Y1 or Y2, were assessed using general linear model analysis of variance (gANOVA). Irrigation, GB and KPF were used as the fixed model (treatment) terms and block was used as the random (error) model term; and each measurement was input as the response variable (Table 2.12). Additional analysis for violations of the ANOVA assumptions was conducted using the *"automatic testing*

of the assumption" option for available for gANOVA, which assesses the assumptions of the Levene's and Shapiro-Wilk tests using methodology described in Payne (2014).

Table 2.12. Degrees of freedom for the analysis of response variables using general linear model analysis of variance (gANOVA) with irrigation (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

Source of Variation	Degrees of Freedom	
Block stratum	5	
Block x Treatment Stratum		
I	2	
GB	1	
KPF	1	
I x GB	2	
I x KPF	2	
GB x KPF	1	
I x GB x KPF	2	
Error (Block x Treatment)	55	
Total	**71**	(3 x 2 x 2 x 6) - 1

The ANOVA determined the significant effect of each of the treatments (irrigation, GB and KPF) on the response variable individually as well as the effect of any interactions between each of the treatment. Fisher's protected least significant difference at p = 0.05 ($LSD_{p = 0.05}$) was chosen *a priori* to conduct pairwise comparison of means when a treatment or interaction effect was found to be significant.

2.7.2. Repeated Measures Analysis of Variance

Each of the response variables, that were measured on three or more occasions in Y1 or Y2, were analysed using repeated measures analysis of variance (rANOVA). Irrigation, GB and KPF were used as the fixed model (treatment) terms and block\plot was used as the random (error) model terms; and each measurement was input as the response variable (Table 2.13). In addition to the visual check of the assumptions for normality of residuals and homogeneity of variance (described above). The assumption of compound symmetry (sphericity) was assessed using the Box test (Box, 1950) and a Greenhouse-Geiser epsilon adjustment (Greenhouse & Geisser, 1959) was applied to the degrees of freedom to adjust for compound symmetry (VSN International, 2015).

Table 2.13. Degrees of freedom for the analysis of response variables measured on five sample dates (time, t) using repeated measures linear model analysis of variance (ANOVA) with irrigation (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

Source of Variance	Degrees of Freedom	
Block stratum	5	
Block x Treatment Stratum		
I	2	
GB	1	
KPF	1	
I x GB	2	
I x KPF	2	
GB x KPF	1	
I x GB x KPF	2	
Residual	55	
Block x Treatment x Time Stratum		
t	4	
t x I	8	
t x GB	4	
t x KPF	4	
t x I x GB	8	
t x I x KPF	8	
t x GB x KPF	4	
t x I x GB x KPF	8	
Residual	240	
Total	**359**	(3 x 2 x2 x 6 x 5) -1

The rANOVA determined the overall significance of treatments (irrigation, GB and KPF) and any their interactions on the response variable; whilst the interaction between time and the treatments determined if there was a change in the effect over time. Fisher's least significant difference a p = 0.05 ($LSD_{p = 0.05}$) was chosen *a priori* to conduct pairwise comparison of means when a treatment or interaction was found to be significant.

Chapter 3. Impact of Irrigation, Glycine Betaine and Kaolin Particle Film on Yield and Yield Components

3.1. Literature Review of Yield and Yield Components

Yield is a measure of the how much a vineyard produces. On a commercial scale it is commonly reported as fresh weight of fruit per unit area (i.e. $t \cdot ha^{-1}$), whilst on a smaller scale it may be reported on a per vine basis (e.g. $kg \cdot vine^{-1}$). Ultimately, yield per unit area depends on the planting density (i.e. $vines \cdot ha^{-1}$), buds per vine, shoots per bud, clusters per vine, berries per cluster and berry weight. The number of inflorescences per plant accounts for most inter-annual fluctuations in yield (Clingeleffer et al., 2001; Vasconcelos et al., 2009). It is estimated that berries per bunch explains 20 to 30% of annual variation in grape yield, whereas bunch number per grapevine typically accounts for 60 to 70% of year-to-year variation in yield (Krstic et al., 2005).

The primary aim and challenge of wine grape production is "*to consistently produce a quantity of grapes sufficient to cover all costs of production and return a profit"* (Howell, 2001). To maximise profits, growers aim to produce as much crop per hectare as the wineries are willing to purchase. Therefore, for growers, yield per hectare is a constant trade-off between grower revenue, costs of production and the constraints imposed by the wineries that purchase the grapes (i.e. grape composition). Optimal yield and grape composition depend on the purpose for which the grapes are produced. In some cases, a premium may be paid for fruit that meets certain compositional criteria. However, this may come as a trade-off, as the industry has long associated an inverse correlation between yield and quality (Robinson & Smart, 1999). As a perennial plant, a grapevine requires enough vegetative growth to produce carbohydrates to ripen fruit during the current season and replenish carbohydrates stored in the woody parts of the grapevine required for bud burst and initial shoot growth in the following season (Lebon et al., 2005; Lebon et al., 2008). Too much vegetative growth, on the other hand, may result in a dense canopy with poor light penetration (Dokoozlian & Kliewer, 1995a), which may have negative implications, such as lower grape quality (Dokoozlian & Kliewer, 1995a) and higher pest and disease risk (Smart & Robinson, 1991).

The grapevine reproductive cycle, referred to as the flowering and fruiting cycle (Figure 3.1), is approximately 18-months long. It begins with development of leaf and inflorescence primordia for the following seasons fruit (induction) in the current season buds and ends with the grape harvest the following year (Watt et al., 2008), with the induction, initiation and differentiation

of inflorescence primordia for the following seasons fruit coinciding with flower/cluster development of the current season fruit (Figure 3.1). Environmental conditions and management practices may affect yield at any time induction from through to harvest (Vasconcelos et al., 2009; Watt et al., 2008). Yield components may be affected directly or indirectly – i.e. influences on the source-sink balance and associated carbon partitioning (Fischer et al., 2012; Gifford & Evans, 1981; Howell, 2001; Vivin et al., 2001). For example, interruption of carbohydrate reserve replenishment may impact on yield for the following year, for example by reducing inflorescences per plant and/or the number of flowers per inflorescence (Lebon et al., 2008; Watt et al., 2008).

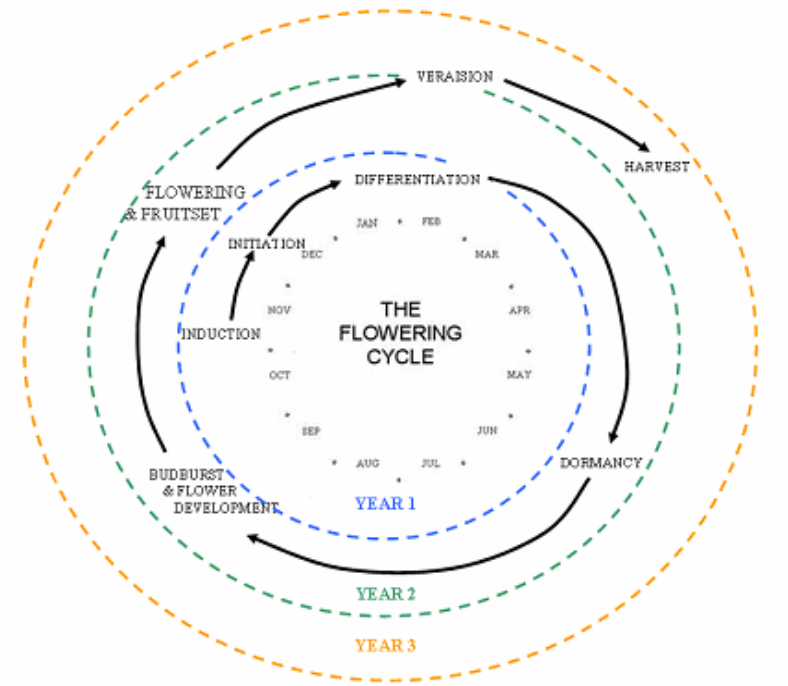

Figure 3.1. Approximate timing of the phenology of flowering and fruiting of *Vitis vinifera* L. 'Shiraz' grown in North East Victoria, Australia. The cycle begins in the middle and spirals clockwise outwards [Source: Foletta (2006) adapted from a diagram provided by A.M. Watt, personal communication, 2006].

Environmental conditions during initiation and differentiation of inflorescence primordia may have a significant influence on the following seasons yield (May, 2004; Watt et al., 2008), with bud fruitfulness (maximum number of inflorescence per bud) determined approximately 3 months after budbreak (Keller, 2015). Factors, such as temperature and apical dominance, can influence initiation and differentiation affecting the number and position of inflorescence primordia formed (May, 2004). For example, Watt et al. (2008) found that the extent of inflorescence primordia differentiation in *Vitis vinifera* L. 'Chardonnay' buds measured just prior to véraison [stage 33, modified Eichorn and Lorenz (E-L) scale] varied with climate. Hot climate buds were found to be more advanced, larger and have more widespread secondary

branching compared with cool climate buds (MJT 18.1 °C). For further discussion on factors (prior to budburst) affecting the initiation and position of flowers refer to May (2004).

Following dormancy, "*the conversion of inflorescence primordium to inflorescence starts as soon as spring growth commences*" (May, 2004). When the buds are reactivated, it is commonly accepted that flower initiation and branching commence (Keller, 2015). Subsequent flower differentiation (development of individual organs of each flower) occurs approximately 5 week after budbreak (Keller, 2015). Nearly all varieties of *Vitis vinifera* have hermaphroditic flowers (functional stamen and pistils on the one flower; Iland et al., 2011). A range of factors can result in the loss of inflorescence and flowers before anthesis (flowering), with many cases attributed to "*lack of vigour of the shoot carrying the inflorescence" (May, 2004)*, as a result of factors such as inadequate nutrition. Water stress was considered "*unlikely to impact on inflorescence development in most Australian vineyards because of sufficient levels of soil moisture during spring, either through natural rainfall or through supplementary irrigation"* (May, 2004). However, according to May (2004), vintage reports from 1978 for South Australia have cited lack of soil moisture as a contributing factor for poor fruit set. Projected increases in temperatures and changes in rainfall patterns (discussed in Section 1.2 of Chapter 1), may see this becoming an issue in the future. Furthermore, fruit-set may also be affected by factors, such as cap fall, stigma viability, pollen viability and fertilisation (May, 2004). Each of these factors may be affected by environmental condition as discussed in more detail in May (2004).

After flowering and fruit set, berry development is split up into two stages with a transition phase – "*two successive sigmoidal growth periods separated by a lag phase*" at véraison (Coombe & McCarthy, 2000; Kennedy, 2002; Winkler et al., 1974). Alternatively, other literature refers to this as a double sigmoid growth pattern (Shellie, 2006). The first stage (berry formation; Coombe & McCarthy, 2000) lasts for approximately 60 day from flowering (Kennedy, 2002). During this time the berry is formed (fruit set) and seed embryos are produced (Kennedy, 2002; Pratt, 1971), with seed number and fruit set established during fertilisation (Ruan et al., 2012). This is minimal dry matter accumulation (Coombe & McCarthy, 2000) and water accumulation is primarily coming from xylem flow (Pratt, 1971). The sap in the xylem is a dilute aqueous solution of inorganic ions and some root-derived organic metabolites (Coombe & McCarthy, 2000). Pericarp growth during this stage is attributed to cell division and cell growth (Harris et al., 1968; Kennedy, 2002), with total number of cells within the berry being established by the end of this stage (Kennedy, 2002). Thus the extent of cell division during this stage can influence on final berry size (Coombe & McCarthy, 2000;

Kennedy, 2002) and the "*direction*" of cell division can influence the shape (Coombe & McCarthy, 2000).

The transition between the two stages is termed véraison, during which time the berries soften and change colour from green to red, as a result of the rapid synthesis and accumulation of anthocyanins (Kennedy, 2002; Pérez et al., 2000). In some of the literature véraison is referred to as a separate growth stage (Krstic et al., 2005; Pratt, 1971; Shellie, 2006).

The second stage, berry ripening or berry maturation, is where berry size increases due to cell expansion and growth, sugars accumulate, acidity reduces, tannins and aromas are developed, and skin colour darkens (Pratt, 1971). During this stage the primary source of the growth for Shiraz berries is from the influx of phloem sap as the movement of xylem sap into the berry becomes impeded (Coombe & McCarthy, 2000) or has a reduced function (Kennedy, 2002). As a result, some degree of berry weight loss in Shiraz is normal prior to berries being ready for harvest, even under adequate irrigation (McCarthy & Coombe, 1999; Rogiers et al., 2004). Impendence of the xylem in *Vitis vinifera* L. 'Muscat Gordo Blanco' has been attributed to "*stretched tracheids in the network of dorsal bundles",* evidenced by irregularities in cell wall thickness, breaks in cell membranes and the formation of aerenchyma (air pockets; Findlay et al., 1987). Based on analysis of cell vitality using fluorescein diacetate, Tilbrook and Tyerman (2008) proposed that some varieties (i.e. Shiraz) exhibit programmed cell death in the pericarp resulting in a reduction in hydraulic conductance by the xylem. Others maintain continued cell vitality that can allow higher hydraulic conductance (i.e. Thompson Seedless; Tilbrook & Tyerman, 2008). More recent research, has challenged this view, proposing that the "*decrease in xylem inflow at the onset of ripening may be a consequence of sink-driven increase in phloem flow"* (Keller et al., 2015). In research conducted by Keller et al. (2015), interrupting (girdling) phloem flow through the peduncle stopped accelerated berry growth following application of water, whereas interruption of xylem did not, in pot-grown Merlot and Concord grapevines. Keller et al. (2015) proposed that excess phloem water partly evaporated from the berry surface and partly flows back to the plant via the xylem.

3.1.1. Impact of Deficit Irrigation, Glycine Betaine and Kaolin Particle Film on Yield and Yield Components

Climate variability and climate change present a major challenge to management. Management to reduce the effects of one risk, such as heat stress, may have unintentional impacts when coupled with common management practices, such as deficit irrigation. Glycine betaine (GB) and kaolin particle film (KPF), as discussed in Chapter 1, are two products that can

be exogenously applied to grapevines to ameliorate the effects of environmental stresses, such as heat and water stress. Whilst they may be used as a valuable management tool, the impacts on yield and yield components are important to determine, and an interaction with the irrigation treatments needs to be considered. GB is a compatible solute that is involved in the protection of many macro-components in plant cells under stress conditions, including proteins, enzymes and membranes (Sakamoto & Murata, 2000; Yang et al., 2005). KPF is a kaolin mineral particle that alters the quantity and quality of light within a plant's canopy (Abou-Khaled et al., 1970; Rosati et al., 2007; Shellie & King, 2013a).

The timing of deficit irrigation has important implications on yield and yield components. Water deficits pre-véraison, during the first stage of berry growth, may result in a reduction in cell division and growth resulting in reduced berry weight (Freeman et al., 1979, 1980). Changes in cell division are commonly associated with the inability of berries to recover in size after early season water stress (Ojeda et al., 2001). After véraison, the effects of water stress on berry weight is due to differences in cell growth (Ojeda et al., 2001), with evidence that the effects of water stress during this stage may be partially or fully alleviated if water becomes available (McCarthy, 1997; Ojeda et al., 2001). In recent research conducted by Keller et al. (2016), applying 25% of crop evapotranspiration (ET_C) to Cabernet Sauvignon (*Vitis vinifera* L.) vines grown in south eastern Washington strongly limited gas exchange and reduced vine capacity and productivity compared with vines irrigated at 70 and 100% of ET_C. In the same research, irrigating the vines at 25% of ET_C from berry set until véraison and 100% of ET_C from véraison to harvest, resulted in open canopies and smaller berries without a reduction in vine capacity and yield (Keller et al., 2016). Similarly in research conducted by Shellie (2006), Merlot vines irrigated with 35% of ET_C prior to véraison and 70% after véraison used 30% less water and produced similar yield and quality compared with vines that were maintained at 70% ET_C. Furthermore, the impacts of water stress in one growing season, can have flow on effects for future growing seasons. In research conducted by Petrie et al. (2004) on Shiraz vines, deficit irrigation after véraison negatively impacted on yield in the current as well as the following season, even when irrigation was returned to standard practice in the following season. In the current season lower yield was driven by reduced berry and cluster weight. In the following season lower yield was attributed to fewer shoots per vine reducing cluster number per vine. Evidently, changes in yield and yield components due to water stress are intrinsically linked with associated changes in canopy growth (Keller et al., 2016) and complicated by the perennial nature of the grapevine (Petrie et al., 2004).

In some grapevine cultivars, such as Shiraz, a small degree of berry weight loss is normal prior to berries being ready for harvest, even under adequate irrigation (McCarthy & Coombe, 1999; Rogiers et al., 2004). Additional berry weight loss may also occur in response to environmental stresses, such as heat stress (Webb et al., 2009; Webb et al., 2010). For example, sunburn can crack the skin of the berry or result in damage to the rachis dehydrating the berry (Krasnow et al., 2010). GB has shown positive results in reducing berry shrivel of Shiraz grapes grown in Western Victoria and the Yarra Valley, Victoria (Scarlett, 2009; Scarlett et al., 2011), thus potentially reducing the negative impacts of berry shrivel on yield. The research conducted by Mickelbart et al. (2006), assessing the endogenous levels of GB and exogenous application of GB did not asses the effects of GB on yield or composition. Minimal research has been conducted on grapes to determine the effects of GB on yield.

Conflicting observations have been reported in the literature, for the effects of KPF on yield and yield components of grapevines, with no clear links to variety, location, season or irrigation method. KPF had no effect on yield of warm climate Cabernet Sauvignon which were irrigated to maintain them close to full point and at 50% of full point (Sunraysia, Victoria, Australia). Furthermore, the authors reported no interactions between irrigation and KPF (Cooley et al., 2008). Similarly, KPF had no significant effect on yield of Cabernet Sauvignon vines grown in Idaho, USA during the 2007, 2008 and 2009 growing seasons, but KPF increased berry weight of Malbec vines during the 2007 and 2009 growing seasons in the same field experiment under standard industry practice for the region (~70% ET_C) and reduced irrigation (~23% ET_C; Shellie & King, 2013a). Inconsistent results are also reported for Merlot vines, with KPF increasing berry weight of Merlot vines grown under four irrigation strategies ranging from 35 to 100% of crop evapotranspiration (ET_C) in Idaho, USA during the 2005 and 2006 growing seasons (Shellie & Glenn, 2008). In contrast, there was no significant effect of KPF or interaction of the irrigation treatments and KPF on berry weight reported for Merlot vines during 2007 and 2008 growing seasons (Song et al., 2012). There was no significant effect of KPF on yield or yield components of Shiraz vines, the variety used in this research, grown in North East Victoria, Australia under two different crop loads during the 2005-06 growing season (Foletta, 2006). Therefore, at this stage there is no unifying hypothesis correlating KPF with yield or yield components in grapevines. This would suggest complexities in the underlying mechanisms of KPF application methodologies.

To date, comparison of the effects of GB and KPF under deficit and non-deficit irrigation has only been assessed on olives (Denaxa et al., 2012; Roussos et al., 2010). In this research, GB resulted in higher yield whilst KPF resulted in lower yield under deficit and non-deficit

conditions (Roussos et al., 2010). Therefore, this chapter examines the effects of irrigation, GB and KPF on yield and yield components of field grown *Vitis vinifera* L. 'Shiraz' in North East Victoria, Australia.

3.2. Yield and Yield Components Materials and Methods

The experiment site, planting material, experimental design, implementation of treatments, sampling procedures and statistical analysis are described in Chapter 2.

3.2.1. Berry Maturation

In Y1 and Y2, berries were sampled from randomly selected clusters four times between véraison and at harvest (Section 2.6.3 of Chapter 2) and at harvest (Section 2.6.2 of Chapter 2). The samples were placed on ice in the field until they could be weighed on a two decimal point balance (Sartorius ED22024-CW Extend; Sartorius AG; Goettingen, Germany) later in the day. Average berry fresh weight (berry wt, g) was calculated from the total weight of the berries sampled for each plot divided by the number of berries sampled at each time point. Change in berry weight (Δ berry wt, g) was calculated as the difference in berry weight between two consecutive sample dates with in each of the growing seasons.

3.2.2. Harvest Measurements

In Y1 and Y2, yield per vine was measured by collecting and weighing all the clusters, from the two sample vines from each plot, using a two decimal point field balance (FG-30KBM Digital Scale; A & D Co Ltd; Tokyo, Japan). Yield per vine was calculated by dividing the yield per plot by the number of sample vines in each plot. Yield per unit area ($t \cdot ha^{-1}$) was calculated from yield per vine (kg) and planting density (1.8 x 3.4 m). The number of clusters per plot was counted and average number of clusters per vine was calculated by dividing the number of clusters per plot by the number of vines in each plot. Average cluster weight was estimated using yield per plot divided by clusters per plot. Average number of berries per cluster was calculated by dividing average cluster weight by average berry weight for each plot.

3.3. Yield and Yield Components Results

A summary, of the significant effects of irrigation, GB, KPF and their associated interactions on yield and yield components is provided in Table 3.1.

Table 3.1. Significance of the p values for yield and yield components measurements for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons from the repeated measures analysis of variance (rANOVA) and general analysis of variance (gANOVA), with irrigation treatments (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

	Y1						**Y2**					
	Berry Wt (g)	**Δ Berry Wt (g)**	**Cluster #**	**Berries per Cluster**	**Cluster Wt (g)**	**Yield (kg·vine^{-1})**	**Berry Wt$^{£}$ (g)**	**Δ Berry Wt$^{£}$ (g)**	**Cluster #**	**Berries per Cluster**	**Cluster Wt (g)**	**Yield (kg·vine^{-1})**
I	< 0.001 ***	ns	ns	ns	0.049 *	0.049 *	< 0.001 ***	ns	ns	ns	< 0.001 ***	< 0.001 ***
GB	<0.001 ***	ns	ns	< 0.001 ***	< 0.001 ***	< 0.001 ***	0.042 *	ns	ns	ns	ns	ns
KPF	ns	ns	ns	ns	ns	ns	0.019 *	ns	ns	ns	ns	ns
I x GB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
I x KPF	0.017 **	ns	ns	ns	0.038 *	ns	ns	ns	ns	ns	0.05 *	0.019 *
GB x KPF	ns	ns	ns	ns	0.033 *	ns	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Epsilon adjustment	***0.8659***						***0.7233***					
Time (t)	< 0.001 ***						< 0.001 ***					
t x I	0.036 *						ns					
t x GB	ns						ns					
t x KPF	ns						ns					
t x I x GB	ns						ns					
t x I x KPF	0.038 *						ns					
t x GB x KPF	ns						ns					
t x I x GB x KPF	ns						ns					

Average yield per hectare (calculated from average yield per vine and planting density) across all treatments was 12.0 and 9.6 $t \cdot ha^{-1}$ for Y1 and Y2, respectively. At harvest, average berry weight across all treatments was 52% smaller in Y2 compared with Y1, with average berry weight in Y2 being smaller than the minimum berry weight in Y1 (Table 3.2).

Table 3.2. Average, minimum and maximum berry weight (g), cluster weight (g), clusters per vine and yield per vine (kg) measured at harvest in the 2011-12 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March). n = 72.

		Average	Minimum	Maximum
Average Berry Wt (g)	**Y1**	1.56	1.14	2.08
	Y2	0.82	0.41	1.31
Average Cluster Wt (g)	**Y1**	129.1	68.1	210.3
	Y2¥	89.7	27.5	181.7
Average Cluster #	**Y1**	57	32	86
	Y2¥	65	40	91
Average Yield per Vine (kg)	**Y1**	7.33	4.10	11.31
	Y2¥	5.87	1.10	11.50

¥ due to missing values n = 67

In Y1 and Y2, there was no significant effect of any of the treatments (irrigation, GB and KPF) or their associated interactions on cluster number per vine or the change in berry weight between sample dates ($p > 0.05$; data not presented).

3.3.1. Irrigation Treatments

In Y1, there was a significant effect of the irrigation treatments on yield per vine, with I_{100} resulting in higher yield per vine than I_{25} ($p = 0.049$; Figure 3.2). In Y2, there was a significant effect of the irrigation treatments on yield per vine, with I_{100} resulting in higher yield per vine than I_{25} and I_{50} ($p < 0.001$; Figure 3.2).

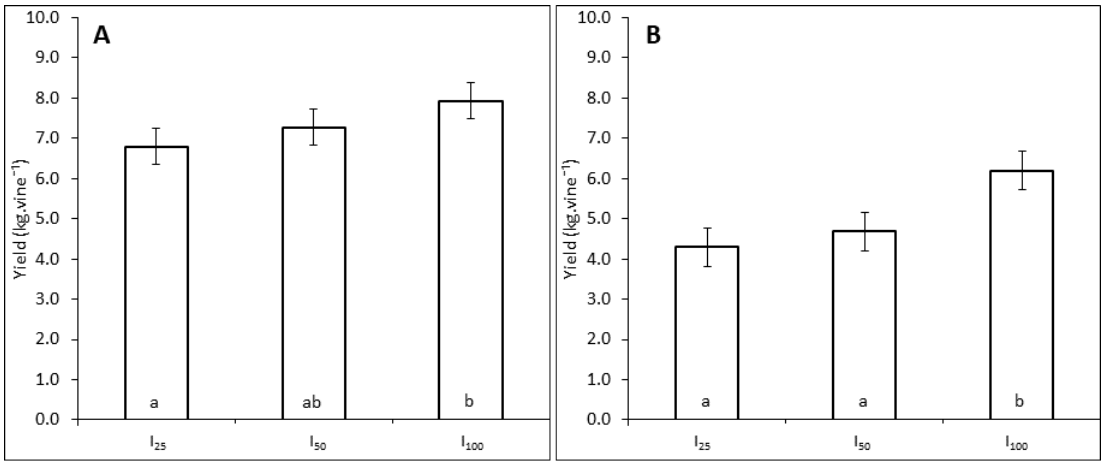

Figure 3.2. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on yield per vine for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).
NB: when the p value (I) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I) > 0.05 no means was conducted.

In Y1, there was a significant effect of the irrigation treatments on cluster weight, with I_{100} resulting in higher cluster weight than I_{25} ($p = 0.049$; Figure 3.3). In Y2, there was a significant effect of the irrigation treatments on cluster weight, with I_{100} resulting in higher cluster weight than I_{25} and I_{50} ($p < 0.001$; Figure 3.3).

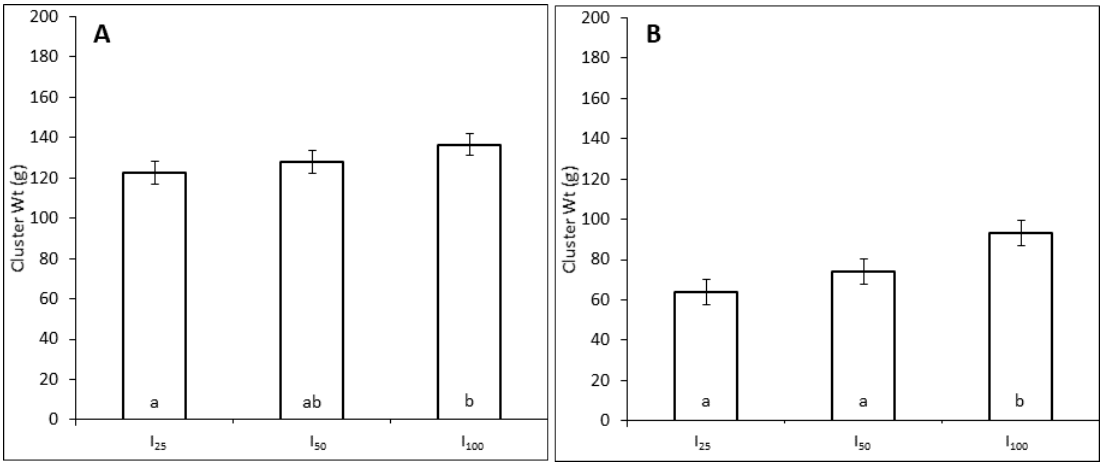

Figure 3.3. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on cluster weight for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).
NB: when the p value (I) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I) > 0.05 no means was conducted.

In Y1 and Y2, there was no significant effect of the irrigation treatments on the number of berries per cluster ($p > 0.05$; Figure 3.4).

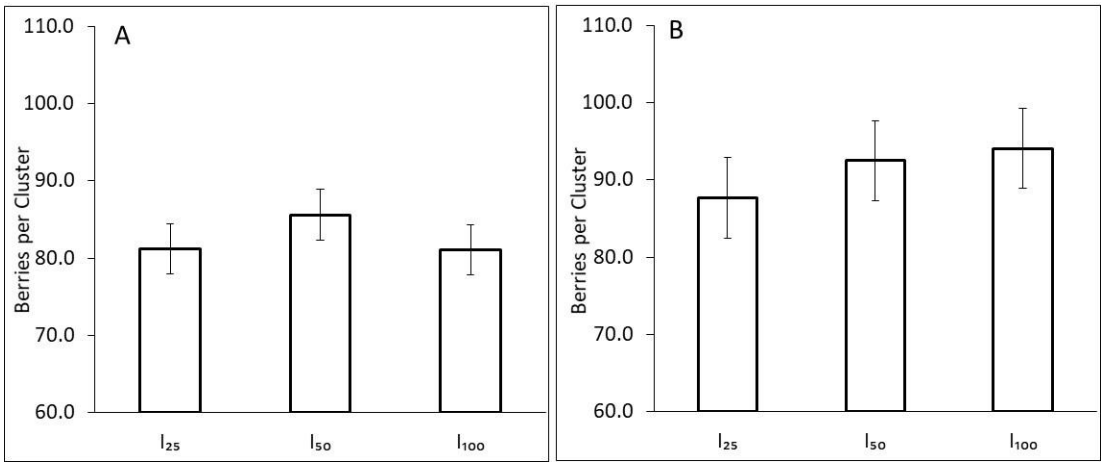

Figure 3.4. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on the number of berries per cluster for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).
NB: when the p value (I) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I) > 0.05 no means was conducted.

In Y1, there was a significant effect of the irrigation treatments on berry weight, with I_{100} resulting in a higher berry weight than I_{25} and I_{50} ($p < 0.001$; Figure 3.5). There was a significant interaction of the irrigation treatments and sample date (time), with I_{50} and I_{100} converging for the fourth sample date in Y1 (day 56; $p = 0.036$; Figure 3.5). In Y2, there was a highly significant effect of the irrigation treatments on berry weight, with I_{100} resulting in higher berry

weight than I_{50} and I_{25} ($p < 0.001$; Figure 3.5). In Y2, there was no significant interaction of the irrigation treatments and sample date (time), indicating this response was consistent for all sample dates in Y2 ($p > 0.05$; Figure 3.5).

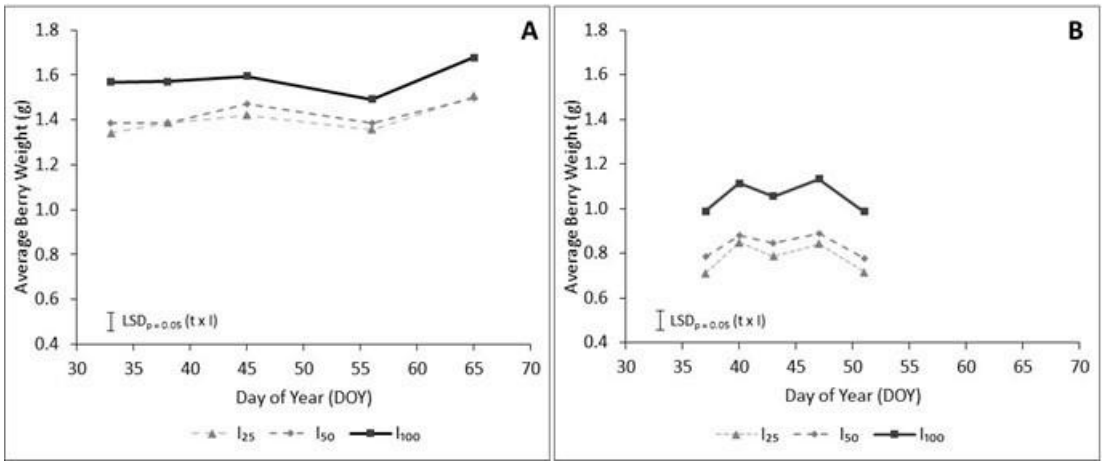

Figure 3.5. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on berry weight for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p=0.05}$) for the interaction of sample date (time, t) and irrigation treatments (I).

3.3.2. Glycine Betaine

In Y1, GB reduced yield per vine at harvest, with means of 8.0 and 6.7 kg·vine^{-1} for GB^- and GB^+, respectively ($LSD_{p=0.05} = 0.74$; $p < 0.001$). In Y2, there was no significant effect of GB on yield per vine at harvest, with means of 5.2 and 4.9 kg for GB^- and GB^+, respectively ($LSD_{p=0.05} = 0.91$; $p > 0.05$). In Y1 and Y2, there was no significant interaction of the irrigation treatments and GB on yield per vine ($p > 0.05$; Figure 3.6).

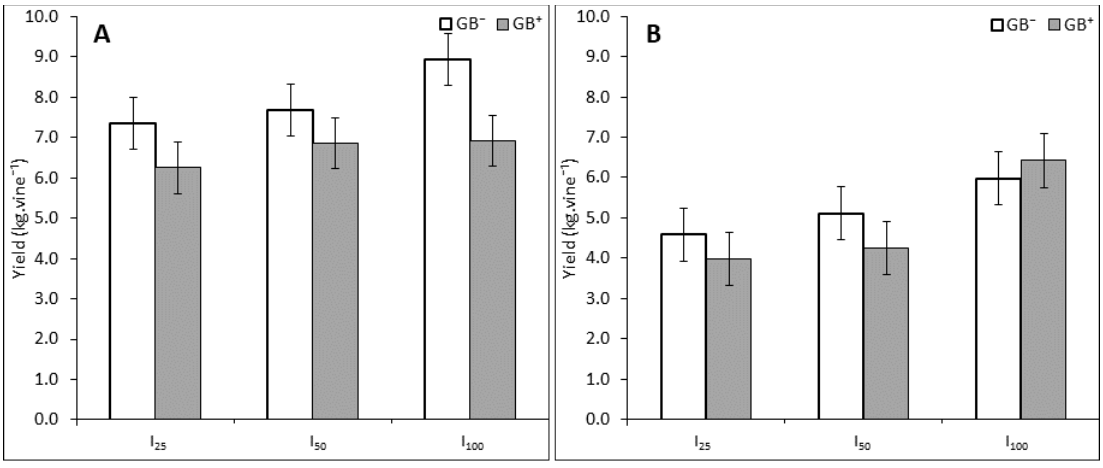

Figure 3.6. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on yield per vine for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and glycine betaine (GB).
NB: when the p value (I x GB) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I x GB) > 0.05 no means was conducted.

In Y1, GB reduced cluster weight, with means of 142 and 116 g for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 9.3; $p < 0.001$). In Y2, there was no significant effect of GB on cluster weight, with means of 80.0 and 74.0 g for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 11.7; $p > 0.05$). In Y1 and Y2, there was no significant interaction of the irrigation treatments and GB on cluster weight ($p > 0.05$; Figure 3.7).

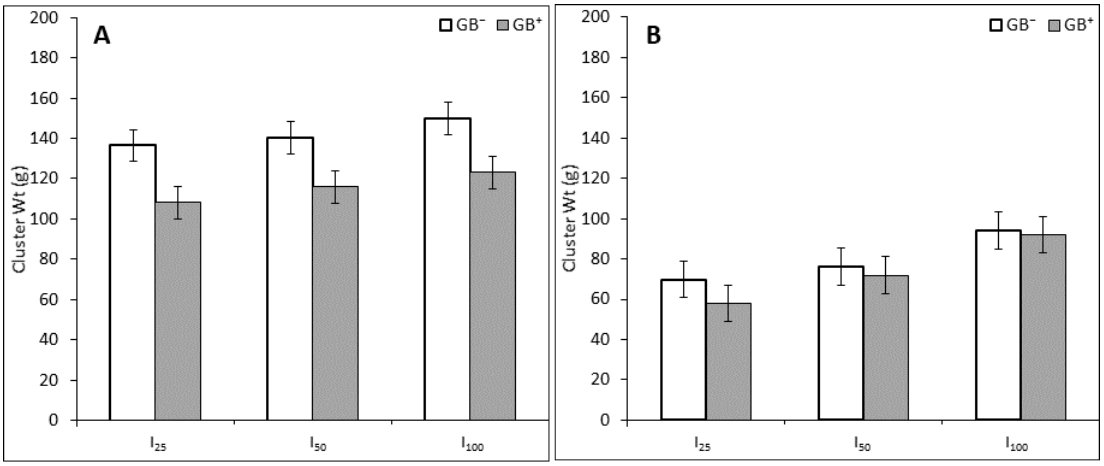

Figure 3.7. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on cluster weight for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of irrigation treatments (I) and glycine betaine (GB).
NB: when the p value (I x GB) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I x GB) > 0.05 no means was conducted.

In Y1, GB reduced number of berries per cluster, with means of 87.2 and 76.7 for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 5.34; $p < 0.001$). In Y2, there was no significant effect of GB on the number of berries per cluster ($p > 0.05$). In Y1 and Y2, there was no significant interaction of irrigation treatments and GB on the number of berries per cluster ($p > 0.05$; Figure 3.8).

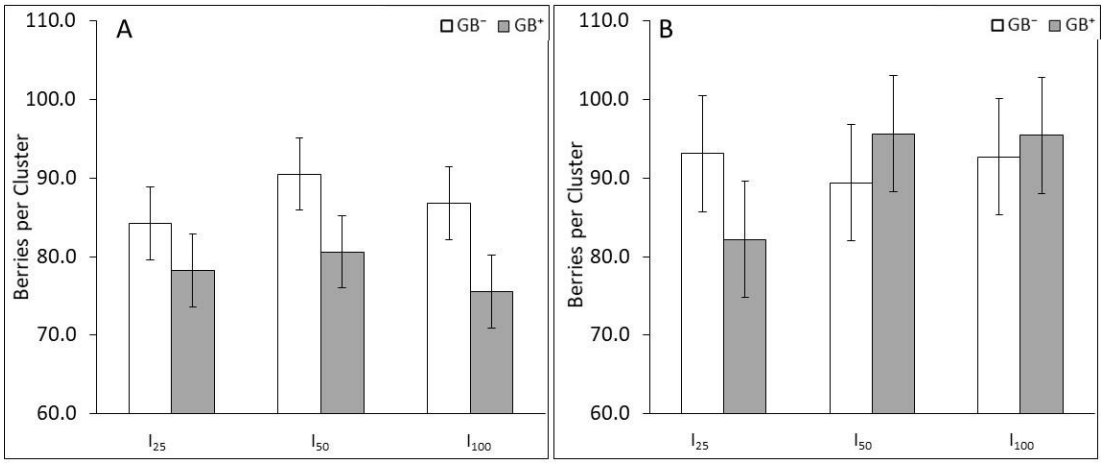

Figure 3.8. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on the number of berries per cluster for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of irrigation treatments (I) and glycine betaine (GB).
NB: when the p value (I x GB) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I x GB) > 0.05 no means was conducted.

In Y1 ($p < 0.001$) and Y2 ($p = 0.042$), GB reduced berry weight ($p < 0.001$). This response was consistent for all sample dates, with no interaction of GB and time ($p > 0.05$; Figure 3.9).

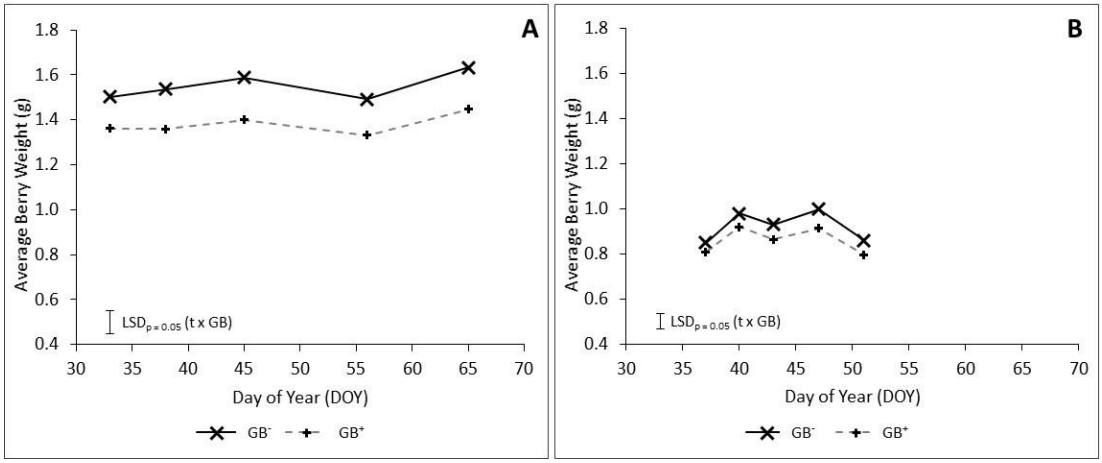

Figure 3.9. Impact of glycine betaine (GB^- and GB^+) on berry weight measured in the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of sample date (time, t) and glycine betaine (GB).

3.3.3. Kaolin Particle Film

In Y1 and Y2, there was no significant effect of KPF on yield per vine measured at harvest ($p > 0.05$). In Y1, there was no significant interaction of irrigation treatments and KPF on yield per vine measured at harvest ($p > 0.05$; Figure 3.10). In Y2, there was a significant interaction of irrigation treatments and KPF on yield per vine measured at harvest, with the combination of I_{25} and KPF^+ resulting in the lowest yield per vine and the combination of I_{100} and KPF^- resulting in the highest yield per vine ($p = 0.019$; Figure 3.10).

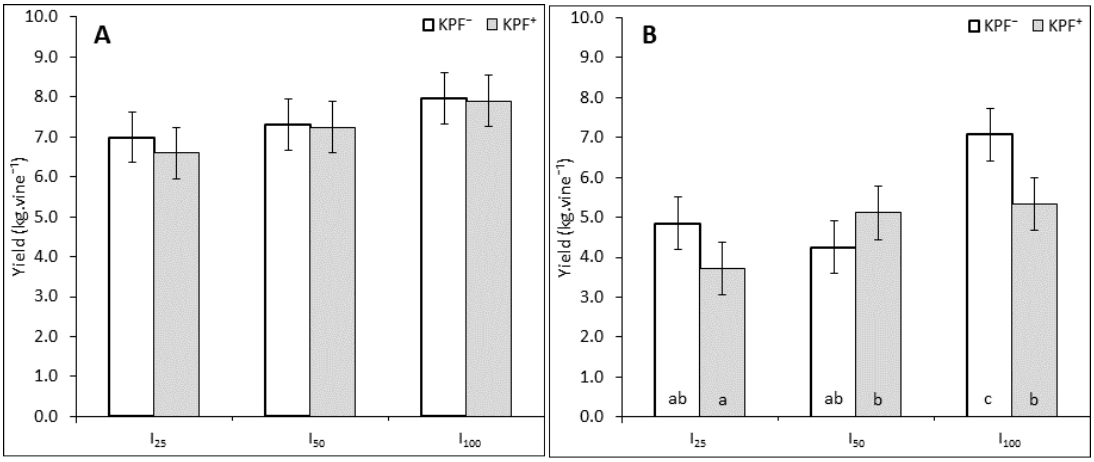

Figure 3.10. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on yield per vine for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I x KPF) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I x KPF) > 0.05 no means was conducted.

In Y1 and Y2, there was no significant effect of KPF on cluster weight measured at harvest ($p > 0.05$; data not presented). In Y1 ($p = 0.038$) and Y2 ($p = 0.049$), there was a significant

interaction of irrigation treatments and KPF on average cluster weight measured at harvest, with the combination of I_{25} and KPF^+ resulting in the lowest average cluster weight and the combination of I_{100} and KPF^- resulting in the highest average cluster weight (Figure 3.11).

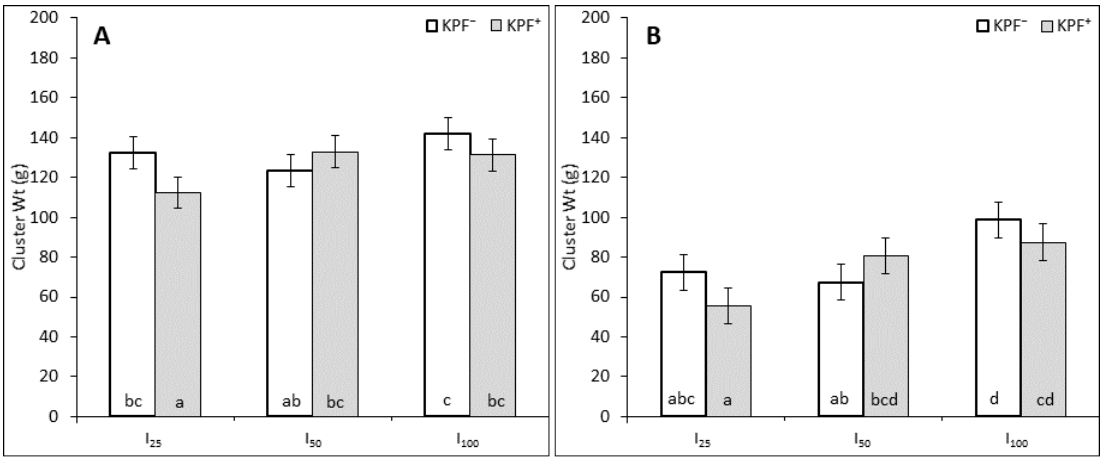

Figure 3.11. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on cluster weight for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I x KPF) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I x KPF) > 0.05 no means was conducted.

In Y1 and Y2, there was no significant effect of KPF the number of berries per cluster (p > 0.05).

In Y1 and Y2, there was no significant interaction of irrigation treatments and KPF on the number of berries per cluster (p > 0.05; Figure 3.12).

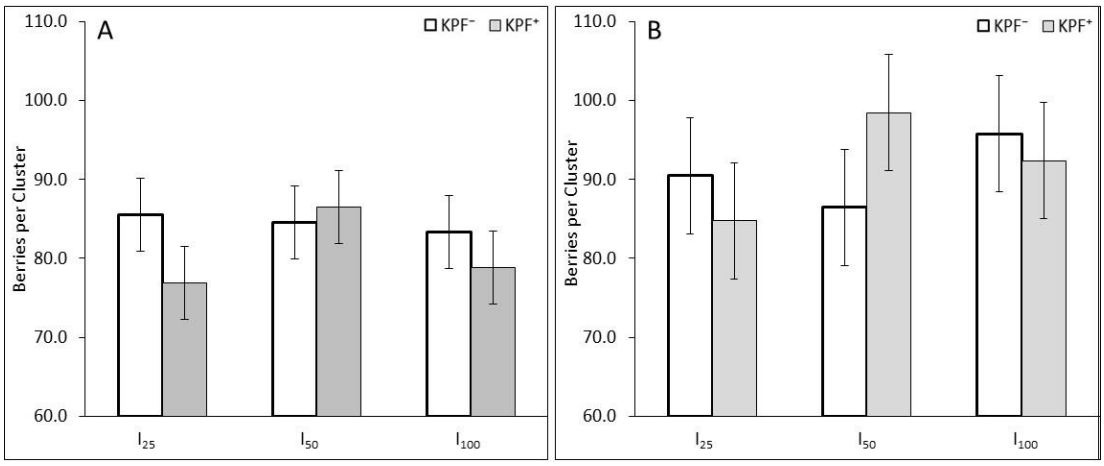

Figure 3.12. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on the number of berries per cluster for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I x KPF) < 0.05 dissimilar letters indicate significant difference between means and when the p value (I x KPF) > 0.05 no means was conducted.

In Y1, there was no overall significant effect of KPF on berry weight (p > 0.05; Figure 3.13). In Y2, KPF reduced berry weight (p = 0.019; Figure 3.13).

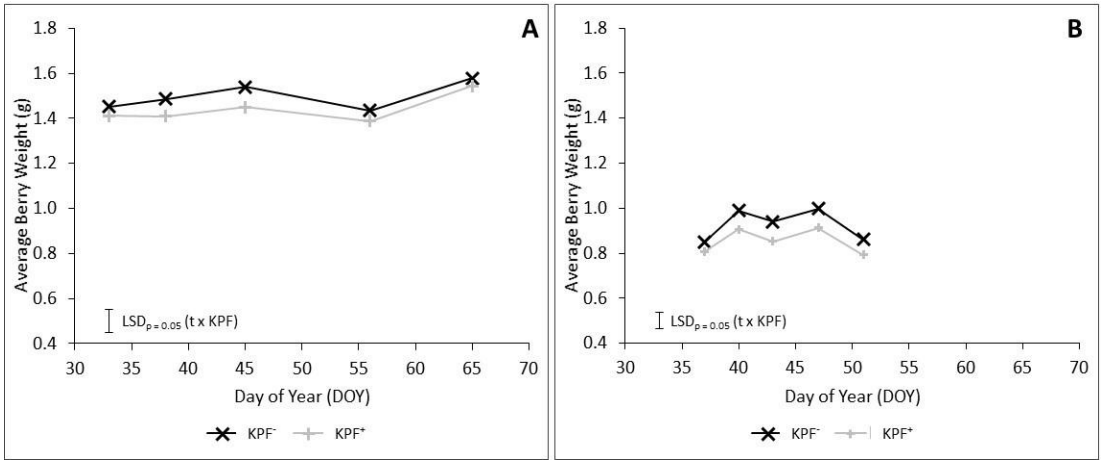

Figure 3.13. Impact of kaolin particle film (KPF^- and KPF^+) on berry weight measured in the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of sample date (time, t) and kaolin particle film (KPF).

In Y1, there was a significant interaction of irrigation treatments and KPF on berry weight (p = 0.017; Figure 3.14). However, the interaction effect was not consistent for all sample dates, indicated by a significant interaction of irrigation treatments, KPF and sample date (p = 0.038; Figure 3.14).

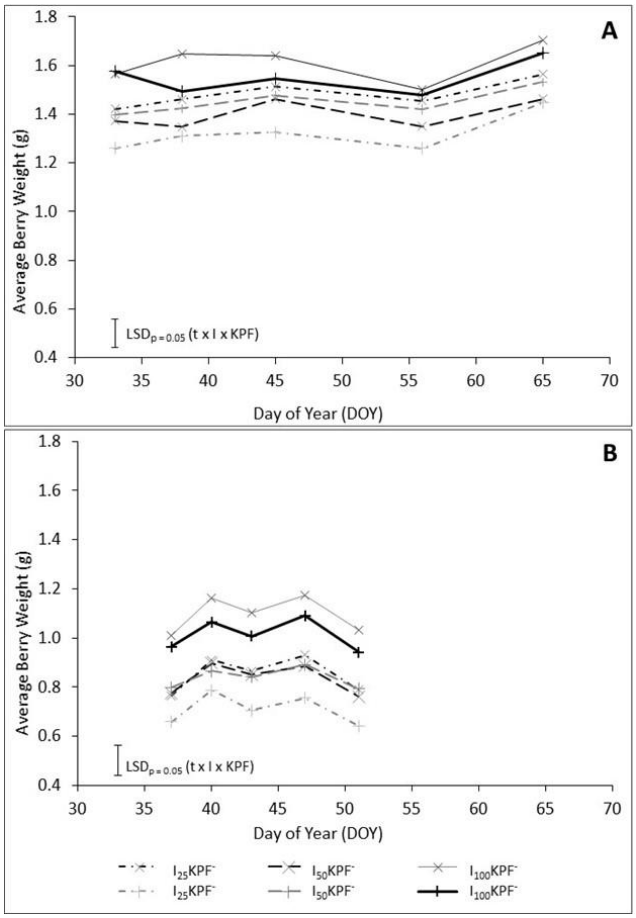

Figure 3.14. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on berry weight in the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of sample date (time, t), irrigation treatments (I) and kaolin particle film (KPF).

3.3.4. Glycine Betaine and Kaolin Particle Film

In Y1 and Y2, there was no significant interaction of GB and KPF on yield per vine ($p > 0.05$; Figure 3.15).

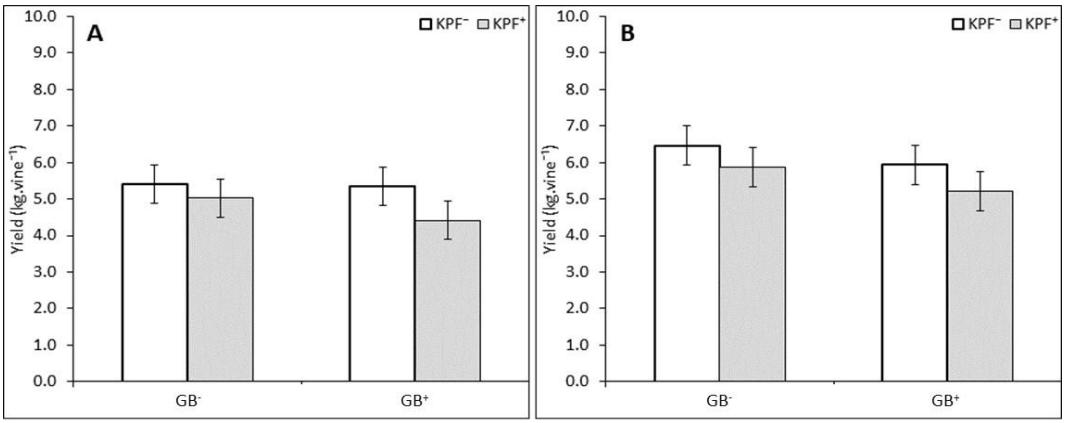

Figure 3.15. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on yield per vine for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).
NB: when the p value (GB x KPF) < 0.05 dissimilar letters indicate significant difference between means and when the p value (GB x KPF) > 0.05 no means was conducted.

In Y1, there was a significant interaction of GB and KPF on cluster weight, with the combination of KPF^+ and GB^+ resulting in the lowest cluster weight ($p = 0.033$; Figure 3.16). In Y2, there was no significant interaction of GB and KPF on cluster weight ($p > 0.05$; Figure 3.16).

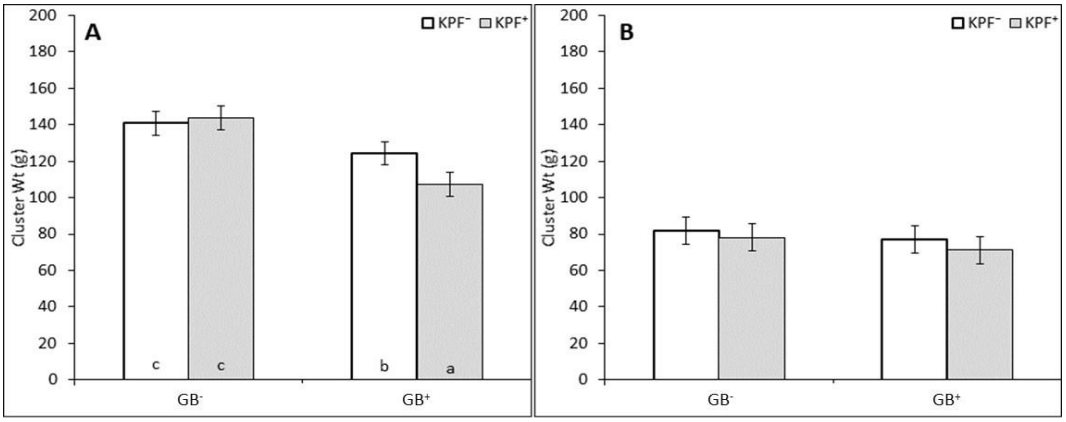

Figure 3.16. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on cluster weight for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (growing season). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).
NB: when the p value (GB x KPF) < 0.05 dissimilar letters indicate significant difference between means and when the p value (GB x KPF) > 0.05 no means was conducted.

In Y1 and Y2, there was no significant interaction of GB and KPF on the number of berries per cluster ($p > 0.05$; Figure 3.17).

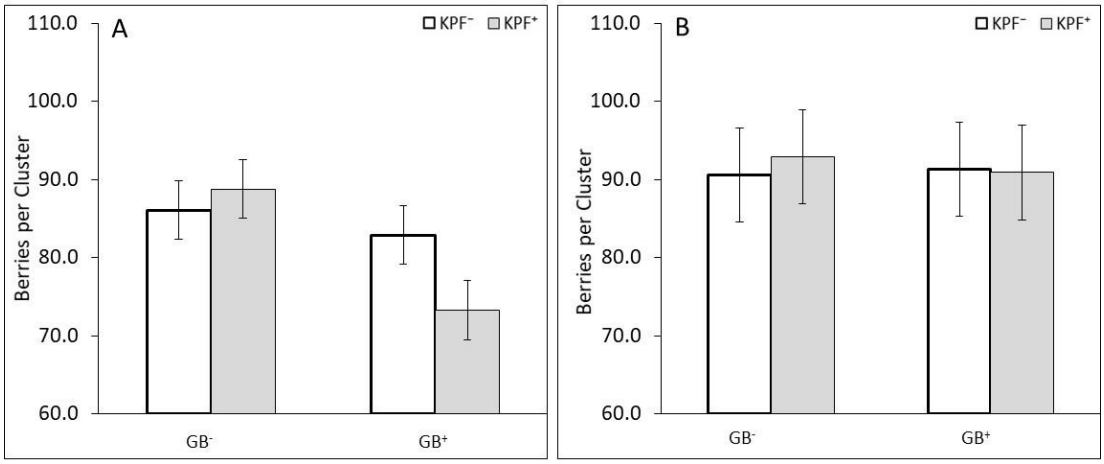

Figure 3.17. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on the number of berries per cluster for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).
NB: when the p value (GB x KPF) < 0.05 dissimilar letters indicate significant difference between means and when the p value (GB x KPF) > 0.05 no means was conducted.

In Y1 and Y2, there was no significant interaction of GB and KPF on berry weight ($p > 0.05$; Figure 3.18).

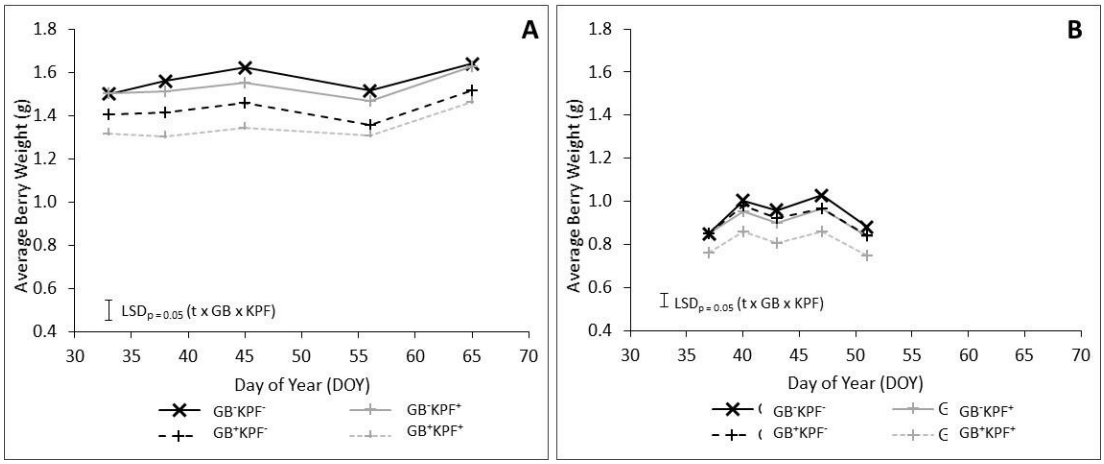

Figure 3.18. Impact of the glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on berry weight in the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of sample date (time, t), glycine betaine (GB) and kaolin particle film (KPF).

3.4. Yield and Yield Components Discussion

Weather data were recorded at the vineyard as they are both seasonally variable and influence berry yield and composition. For the experimental period, Y1 and Y2, it was hotter than average (Section 2.2.2 of Chapter 2). In Y1, growing season (1st October – 31st March), reference evapotranspiration (ET_o) was lower than average with approximately double average growing season rainfall. In contrast in Y2, growing season ET_o was higher than average with lower than average growing season rainfall (refer to Section 2.2.2 of Chapter 2). Irrigation treatments applied a similar proportion of ET_o in each year (refer to Table 2.4 in Chapter 2 and Table 8.2 in Chapter 8. However, total water received (cumulative irrigation and rainfall) as a proportion of ET_o for the growing season was higher in Y1 than Y2 (refer to Figure 2.5 in Chapter 2 and Table 8.2 in Chapter 8).

Yield is known to be subject to seasonal effects (Caprio & Quamme, 2002; Clingeleffer et al., 2001; Krstic et al., 2005; Lobell et al., 2007; Vasconcelos et al., 2009) and the observations recorded here indicate large seasonal effects with a 20% lower average yield per vine in Y2 compared with Y1. Whilst, statistical comparison of yield and yield components between the two seasons would have required a third season of data (refer to Section 2.7). Average berry weight in Y2 was lower than the minimum berry weight in Y1 (Table 3.2) indicates it is likely the differences in yield components between seasons are significant. The causes of seasonal variation are complicated and are driven by the interaction of a number of factors including weather, soil properties and vineyard management (Bramley et al., 2011; Dai et al., 2011; Howell, 2001). In this research, the irrigation strategy, time-date on-off (Section 2.5.1 of Chapter 2), was not altered despite the contrasting rainfall received in each of the growing seasons. As a result, a similar amount of irrigation water was applied in Y1 and Y2 for each of the irrigation treatments. Consequently, it is likely that most of the seasonal variation can be attributed to differences in water availability, which has driven a large difference in berry weight and subsequently yield between the two seasons.

Comparison of total water received (cumulative rainfall and irrigation) to estimated crop evapotranspiration (ET_C) derived from commonly used crop coefficients (K_C) for a medium vigour vineyard (Pudney et al., 2004) indicate that all treatments received more water than required by harvest in Y1 (Table 3.3). In contrast in Y2, it is estimated that the vines received approximately 30, 40 and 60% of crop water requirement by harvest for I_{25}, I_{50} and I_{100}, respectively (Table 3.3). Consequently, in Y2, comparatively I_{100} received less total water (rainfall and irrigation) relative to ET_C than I_{25} in Y1. As a result, higher yields were observed

across all treatments in Y1 compared with Y2, indicating the vines were under a greater water stress in Y2.

Table 3.3. Total water received (irrigation and rainfall) for each of the irrigation treatments (I_{25}, I_{50} and I_{100}) as a proportion of estimated crop evapotranspiration, based on commonly used crop coefficients (K_C[£]) for a medium vigour vineyard (Pudney et al., 2004) for the 2011-2 (Y1) and 2012-13 (Y2) growing seasons (1st October – 31st March).

	Y1 (2011-12)				Y2 (2012-13)			
	Date	**I_{25}**	**I_{50}**	**I_{100}**	**Date**	**I_{25}**	**I_{50}**	**I_{100}**
Sample Date 1	2nd Feb	1.01	1.10	1.26	6th Feb	0.33	0.43	0.62
Sample Date 2	7th Feb	0.98	1.07	1.23	9th Feb	0.33	0.43	0.63
Sample Date 3	14th Feb	0.94	1.02	1.20	12th Feb	0.32	0.42	0.62
Sample Date 4	25th Feb	0.90	0.97	1.13	16th Feb	0.32	0.42	0.61
Harvest	5th Mar	1.64	1.71	1.86	25th Feb	0.31	0.40	0.59

[£] Crop coefficients used were October (0.28), November (0.35), December (0.42), January (0.42), February (0.43) and March (0.43).

3.4.1. ***Irrigation Treatments***

In Y1, substantial rainfall fell between véraison and harvest (Figure 2.4). Therefore, by harvest there were minimal differences in water received between the different irrigation treatments, with total water received by harvest being 1.64, 1.71 and 1.86 times more than estimated ET_C for I_{25}, I_{50} and I_{100}, respectively (Table 3.3). In Y1 and Y2, differences between the irrigation treatments were observed for yield, with higher amount of irrigation water applied resulting in higher yield (Figure 3.2), despite the contrasting rainfall between seasons. It is likely that while all irrigation treatments provided well in excess of the crop water requirements by harvest in Y1, changes cell division and growth during the first stage of berry growth resulted in the reduction in average berry weight (Freeman et al., 1979, 1980). Therefore, small differences in water applied between flowering and véraison have had an irreversible effect on berry size (Ojeda et al., 2001). In Y1, there was no observed change in berry number per cluster (Figure 3.4). Therefore, increases in berry weight for I_{100} has driven differences in cluster weight (Figure 3.3) and yield per vine (Figure 3.2).

In Y2, I_{25} and I_{50} both resulted in significant reductions in yield (Figure 3.2), cluster weight (Figure 3.3) and berry weight (Figure 3.5). The effect of irrigation treatments on berry weight was also evident at véraison in Y2, with no significant differences in the change in berry weight between sample dates from véraison to harvest. Hence, it appears that the difference in berry weight may again have been driven by differences in cell division during the early stages of berry development rather than cell growth. In contrast to Y1, the rainfall levels in Y2 were at the other extreme where all treatments were under water stress and consequently cell growth

during stage two of berry development was lower across all treatments. This highlighted by the difference the overall higher yields and berry weights observed in Y1 compared with Y2.

Significant effects of irrigation treatments on berry weight, cluster weight and yield in Y1 and Y2 support the finding of Petrie et al. (2004), with respect to the effects of current season water deficits on yield and yield components. However, in contrast to research conducted by Petrie et al. (2004) in Y2, there was no observed reduction in the number of clusters per vine in response differences in the amount of irrigation applied in Y1. High summer rainfall, in Y1, coincided with induction and initiation of the inflorescence primordia in the latent buds, two major drivers of yield potential for Y2. Consequently, the irrigation treatments effects on differentiation of inflorescence primordia and yield potential for Y2 was likely to be dominated by the high rainfall (Figure 2.4) resulting in no significant difference in number of clusters per vine between the irrigation treatments in Y2.

3.4.2. Glycine Betaine

In Y1, when water was not considered to be a limiting factor, GB resulted in a reduction in berry weight (Figure 3.9), number of berries per cluster, cluster weight and yield per vine (Section 3.3.2). In Y2, there was an overall effect of GB on berry weight; however, pair-wise comparison shows that the change in berry weight was not significant at harvest. In Y1, yield per vine was reduced by 16% compared with an 8% reduction in berry weight (as seen from the maturity samples). The difference in yield was attributable to a reduction in fruit set (lower number of berries per cluster) as well as a reduction in berry weight. Berries are more prone to "*abortion*" (abscission) during the first stage of berry growth, during this phase they are highly sensitive to internal and external stresses (Ruan et al., 2012). It has been proposed that abscisic acid (ABA) induces the biosynthesis of ethylene, which is involved in ovary abscission, up until berries reach 5 mm in diameter (May, 2004). Exogenous application of ABA increased GB content of strawberry (*Fragaria X ananassa* Duch.) plants (Rajashekar et al., 1999) and *Arabidopsis thaliana* (Xing & Rajashekar, 2001). Therefore, the reduction in berry set observed in Y1 indicates that GB may be involved in some of the stress signalling processes induced by ABA.

In some plants, GB is synthesised in response to a range of stresses including water stress, heat stress and salinity (Kurepin et al., 2015). Endogenous GB content increased with increasing levels of salinity (Na^+ and Cl^-) in nine Iranian table grape (*Vitis vinifera* L.) genotypes (Mohammadkhani et al., 2014). Although, accumulations under other stresses has not yet been assessed. Grapevines have been shown to exhibit relatively low concentrations of

naturally occurring GB compared to other plants that accumulate this solute (Mickelbart et al., 2006). A slight but significant correlation was found between relative water content and GB in one of the three years it was measured, indicating that further research is required to determine if GB is synthesised in response to stress in grapevines. Additionally, Mickelbart et al. (2006) found that high concentrations of GB can potentially have negative impacts including decreased or ceased leaf growth and severe phytotoxic symptoms in grapevines. The lower yields observed in Y1, in the absence of stress, may be due to the accumulation of GB over the growing season given that GB was applied every three to four weeks from flowering. Whilst there were no visible signs of phytotoxic symptoms. It is possible that GB concentrations reached levels sufficient to reduce leaf growth and leaf area, thus reducing the total pool of assimilate for yield. In Y2, it is possible that the vines without exogeneous application of GB synthesised sufficient endogenous GB due to water stress such that there was no observed difference in yield in Y2. It is evident that the grapevines' response to GB is complex. Therefore, this response will be explored in more detail in the upcoming chapters.

3.4.3. Kaolin Particle Film

The impacts of KPF may be influenced by the application technique (Glenn, 2016), formulation (Glenn & Puterka, 2004) frequency and timing of application. In this experiment, two applications of KPF were made, one at véraison and a week later after véraison, to establish the desired coat. Therefore, the effects of KPF on berry weight were limited to the final stage of berry development due to the timing of KPF application. The second application of KPF coincided with the first berry sample date in Y1 and Y2.

In Y1, there was no effect of KPF on berry weight (Figure 3.13); however, there was a complex interaction found between irrigation and KPF, which changed over time (Figure 3.14). By the sample date 3 (DOY = 45, Y2), the combination of I_{100} and KPF^- had significantly larger berries and, I_{25} and KPF^+ had significantly smaller berries. Inconsistencies, prior to the third sample may have been a combination of the differences in first coat of KPF (two coats were used to get full coverage) and the smaller sample size used to assess berry weight for the first sample date (30 berries; Table 2.11 of Chapter 2). In Y1, estimated crop water requirements exceeded total water received for the I_{25} treatments in the lead up to the high rainfall between the fourth sample date and harvest (Figure 3.14). The combination of I_{25} and KPF^+ resulted in lower berry weight for the second, third and fourth sample dates (Figure 3.14). In Y2, when less water (rainfall and irrigation) was received following véraison, KPF reduced berry weight (Figure 3.13). There was no observed effect of KPF on cluster weight or yield per vine (Section 3.4.3) indicating the inherent complexities of KPF effects.

In Y2, there was an overall reduction in berry size (Figure 3.13); however, the short duration (one week) and/or thickness of film (one coat) may explain why pair-wise comparison revealed no difference for the first sample date. This supports the recommendation by Glenn et al. (2001) that irrigation scheduling may need to be altered to ensure adequate supply of water, when KPF is applied. In contrast Shellie (2015), observed no negative impact of KPF on yield or yield components for Merlot vines under sustained deficit irrigation (35% ET_C). Furthermore, Shellie (2015) found that whilst KPF did not alleviate the effects of the sustained deficit irrigation (35% ET_C compared with 90% ET_C), KPF did increase the ratio of soluble solids to anthocyanins. This highlights the potential for KPF to have a positive effect on grape composition. This will be explored in Chapter 4.

Changes in carbon assimilation may also have contributed to changes in berry weight. The canopy structure (refer to Figure 2.6 in Chapter 2) may be described as a wall of canopy and pre-véraison water stress may have resulted in a smaller canopy. Therefore, canopy structure, trellis and/or canopy size may have limited the benefits of reflected photosynthetically active radiation (PAR) within the canopy when KPF is applied under water stress conditions. Alternatively, others have proposed that KPF may block stomata (Moreshet et al., 1979) decreasing carbon assimilation. However, this does not appear to be the case in this research. In research conducted by Shellie and King (2013a), KPF increased reflection of visible light (400 to 700 nm) and decreased photosynthesis (A_{CO2}) for three of the measurement dates, with no relationship found between the magnitude of reflection of PAR and the decrease in assimilation. Whilst there was an observed decrease assimilation due to KFP for three of the sample dates, there was no change in yield components. This indicated that "*net primary productivity was sufficient to ripen fruit to maturity"* (Shellie & King, 2013a). In addition to the reflection of PAR, KPF decreases leaf and berry temperature under well-watered and water deficits, reducing canopy microclimate vapour pressure deficit (Shellie & King, 2013a). On the occasions that KPF reduced A_{CO2}, measurements of A_{CO2} were conducted between 1000 and 1200 with average ambient temperatures of 30.4, 25.2 and 32.9 °C (Shellie & King, 2013a). Therefore, it is possible that higher assimilation rates were observed under higher temperature later in the day. Evidently, grapevines response to KPF is complex; therefore, this response will be explored further in the next two chapters.

3.4.4. Glycine Betaine and Kaolin Particle Film

Novel findings presented in Section 3.3.4, indicate there is the potential for an interactive effect of applying both products. The combined effect of GB^+ and KPF^+ resulted in the lowest cluster weight at harvest in Y1. GB may have influenced the vines' response to KPF in a similar

manner to how KPF responded when less irrigation water was applied (i.e. I_{25}). In Y2, when there was less water provided between véraison and harvest, water stress and the effects of GB on yield become less evident whilst KPF tended to reduce berry weight. Therefore, these results indicate that canopy systems and water management need to be considered carefully before both KPF and GB are applied. This response will be explored in more detail in the next two chapters.

3.5. Yield and Yield Components Conclusion

Water availability has dominated responses in yield and yield components compared to the effects of application of GB or KPF. In the absence of stress, GB has been found to reduce yield, which may be attributable to the amount, timing and/or frequency of GB applications. The inhibitory effects may pose a commercial risk, and a greater understanding is required to inform more appropriate application methodology. KPF was not shown to influence yield or yield components under high available water. In contrast, KPF reduced berry weight under lower available water. Therefore, it is evident that complicated interactions of yield characteristics seem to exist, and adaptation strategies need to consider this. Applying a different application methodology of KPF may change this outcome. This is important to be considered as interactions in GB and KPF can occur. To further understand the role of water on the grapevines' response to GB and KPF, the following chapter will assess the impact on grape composition.

Chapter 4. Impact of Grape Irrigation, Glycine Betaine and Kaolin Particle Film on Grape Composition

4.1. Literature Review of Grape Composition

Consistent quality has been paramount to the success of Australian wines domestically and internationally (Marsh & Shaw, 2000). The "*concept of quality is not something that is constant in time and space but, on the contrary, something dynamic, whose changes depend on a number of factors*" (Gargiulo, 1991). Thus grape quality is not easily defined, and it has long been established that even the most sophisticated chemical analysis cannot reliably determine grape quality (Jackson & Lombard, 1993). Ultimately, quality is still and will most likely remain judged by the consumer and therefore is highly subjective (Jackson & Lombard, 1993). Nonetheless, presently, there are several components of a grape that can be measured and used as an indication of grape quality. Primary metabolites (carbohydrate and organic acids) and secondary metabolites (phenolics) are three major compositional components, which may be correlated to consumer interpretation of grape quality (Rusjan et al., 2008). Therefore, grape composition, like yield, is an essential measurement of grapevine performance as it may influence the vineyard payment for wine grapes.

Second to water, carbohydrates make up the largest proportion of compounds in a grape ranging from 150 to 250 $g \cdot L^{-1}$ at harvest (Winkler et al., 1974). The composition of carbohydrates varies depending on the stage of ripening and at harvest consists of reducing sugars (glucose and fructose). Reducing sugars comprise the majority of soluble solids (99%; Jackson & Lombard, 1993). Total soluble solids (TSS, °Brix), a measure of the collective concentration of all solutes, therefore, provides an indication of the amount of sugar present in grape juice. Sugars are fermented to produce alcohol, so TSS provides an indication of alcohol level and the potential likelihood of residual sugar in the final product (Jackson & Lombard, 1993). Alcohol and residual sugar levels are particularly important in wine as they can mask other quality parameters and influence spoilage risk (Jackson & Lombard, 1993). Therefore, TSS is commonly used to track maturity within a growing season, and fruit is generally picked at a predetermined TSS based on the target market for the fruit (Winkler et al., 1974).

During the first stage of berry development following flowering and fruit set (refer to Section 3.1) there is minimal dry matter accumulation (Coombe & McCarthy, 2000), with this stage being characterised by rapid cell division (Spencer & Peynaud, 1984). Following véraison,

during the second stage of berry development, there is a rapid accumulation of sugar (Pérez et al., 2000), most of which is manufactured in the leaves (Winkler et al., 1974). Sugars synthesised in the leaves migrate to the berries in the form of sucrose (Swanson & El-Shishiny, 1958; Winkler et al., 1974). Sucrose is then hydrolysed into glucose and fructose by an invertase enzyme in the plasma membrane (Hardy, 1968). A series of metabolic reactions provide energy to reassemble glucose and fructose into sucrose which passes through the tonoplast into the vacuole, where it is cleaves back into glucose and fructose (Ribéreau-Gayon et al., 2006). As a result, the rate of sugar accumulation is influenced by the of production and distribution of sucrose in the plant as well as the metabolic reactions inside the berry cell. It is important to note that this is a simplistic view, as *in vivo* there is more involved in the accumulation of reducing sugars than translocation and hydrolysis of sucrose (Hawker, 1969). For example, grapes are capable of synthesising carbohydrates from the transformation of organic acids; but it is believed the total amount formed in this manner is "*undoubtedly quite small*" (Winkler et al., 1974).

Organic acids influence the organoleptic properties of wines, they are responsible for the sharp or sour taste (Fowles, 1992), which is perceived as refreshing in an acid balanced wine (Volschenk et al., 2006). Additionally, organic acids and their salts act as buffers, keep the pH low which helps in keeping the wine microbiologically stable (Fowles, 1992). The principal organic acids contained in grapes are d-tartaric acid and l-malic acid, constituting 90% or more of total acidity (Winkler et al., 1974). L-malic acid is formed via glycolysis and the citric acid cycle, whereas L-tartaric acid is primarily formed from ascorbic acid (Volschenk et al., 2006). Traditionally in many red wines, excess acid (L-malic acid) is removed during malolactic fermentation (Volschenk et al., 2006).

Organic acids are commonly measured as either pH or titratable acidity expressed in tartaric acid equivalents (TA, $g \cdot L^{-1}$). TA measures acidity, which is both fixed and volatile, it is determined by titration with sodium hydroxide (NaOH) to an end point of pH 8.2 (Iland et al., 2000). Berry juice pH has a well-established link with final wine quality (Boulton, 1980), as higher pH is generally combined with higher potassium (K) concentrations, lower acidity and a diminished wine quality. Wines with higher pH tend to taste flat or flabby, have reduced colour and are more susceptible to bacterial attack (Fowles, 1992). Wine pH can also influence the degree of co-pigmentation of anthocyanins; thus impacting on the colour (red) intensity (Volschenk et al., 2006).

During stage one of berry development, L-malic and L-tartaric accumulate in the berries causing the vacuole to increase in size with berry acidity reaching a maximum during véraison

(Volschenk et al., 2006). Following véraison, organic acids in the berry break down, reducing total acidity and increasing pH (Spencer & Peynaud, 1984). At véraison, degradation of chlorophyll reduces the availability of sucrose for respiration resulting in the breakdown of organic acids (Spencer & Peynaud, 1984). In warm climate regions grapes tend to develop with higher levels of sugar and low total acid, compared with less sunny regions (Schultz & Lider, 1964). Therefore, in warm climate regions of Australia, like Dookie where this research was conducted, sugar accumulation is not considered to be a limiting factor, hence harvest date and balance of flavour may be more reliable measures of quality (Webb et al., 2011). However, it is common for wine ferments in hotter regions to be 'acid adjusted'.

Phenolics (sometimes referred to as polyphenolics or polyphenols) are secondary metabolites, which are derived from the phenylpropanoid pathway (Iriti et al., 2005; Sparvoli et al., 1994). They are a very large and highly reactive group of chemical compounds of which phenol (C_6H_5OH) is the basic building block (Waterhouse, 2002). Wine phenolics include non-flavonoids and flavonoids [refer to Waterhouse (2002) for a review of wine phenolics]. Phenolics in grapes of primary interest here include tannins, colour pigments, such as anthocyanins, and many other flavour compounds.

Tannins, which include the flavan-3-ols monomers and proanthocyanidins, are a compound capable of interacting with proteins to increase their molecular size (Waterhouse, 2002). They may be present in the skin, pulp and seeds of grapes (Winkler et al., 1974). Tannins have two sensory characteristics, true taste bitterness and tactile astringency (Lesschaeve & Noble, 2005; McRae & Kennedy, 2011). The astringency (drying and a puckering sensation in the mouth) in wine can provide 'structure' and 'body' to a red wine influencing its wine quality (McRae & Kennedy, 2011).

Anthocyanins are primarily located in the skin of red varieties, such as Shiraz. They are responsible for the red, blue, purple and black colour in grapes (Iland et al., 2000; Mori et al., 2007; Winkler et al., 1974) and contribute to the sensory attributes of wine (Mori et al., 2007). Measurement of the concentration of anthocyanins gives an indication of the potential colour of wine made from the grapes, with a strong association observed between skin disk colour and eventual wine score (Holgate, 2000). Hence, many wineries will pay a premium for grapes with higher levels of anthocyanins.

Biosynthesis of proanthocyanidins (tannins) and anthocyanins occur at different stages of development (Ollé et al., 2011). Proanthocyanidins are primarily synthesised in the lead up to véraison, with proanthocyanidin levels in seed and skin of Shiraz decreasing from véraison to harvest. Anthocyanin accumulation starts at véraison (Ollé et al., 2011), resulting in the change

of colour from green to red. This is accompanied by berry softening and influx of sugar (Pérez et al., 2000). Berry anthocyanins content has been shown to be higher in cooler climates (Kliewer & Torres, 1972).

In addition to carbohydrates, organic acids and phenolics grapes comprise a range of volatile compounds including pyrazines, terpenes, shikimic acid derivatives, lactones, esters and sulphur containing compounds (González-Barreiro et al., 2015). Shiraz, the variety used in this experiment, has in excess of 800 volatile compounds which contribute to the flavour and aroma of wine and hence its quality (Mayr et al., 2014). Recent research has involved the characterisation of many of these volatile flavour compounds (Mayr et al., 2014). For example, rotundone has been identified as the primary compound responsible for the spicy, peppercorn aroma (Wood et al., 2008), which is considered to be important to some Australian red wine styles (Siebert et al., 2008). Measurement methods of volatile compounds currently often involve sophisticated and expensive instrumentation (Olarte Mantilla et al., 2012). Therefore, research will focus on carbohydrates, organic acids and phenolics as they are commonly assessed by wineries to inform grape prices.

4.1.1. Impact of Deficit Irrigation, Glycine Betaine and Kaolin Particle Film on Grape Composition

Management decisions can influence yield at any time from induction through to harvest (Vasconcelos et al., 2009; Watt et al., 2008); whereas fruit composition is primarily determined during grape maturation from fruit set through to harvest (Spencer & Peynaud, 1984). Water deficits are a management tool used to reduce vigour, leading to indirect changes in grape composition (Kriedemann & Goodwin, 2003). The implementation of deficit irrigation post-flowering primarily reduces cell division and growth (Freeman et al., 1979, 1980) and it can result in changes to the biochemistry (i.e. shikimic acid pathway; Martinelli et al., 2012). By-products of the shikimic acid pathway have been linked to grape quality (Figueiredo et al., 2008). For example, phenylpropanoid compounds are precursors of several phenolics, including flavonoids and isoflavonoids (Figueiredo et al., 2008).

Improvements in composition, such as higher anthocyanin content, have been associated with reductions in vegetative growth with little effect on yield (Loveys et al., 2000b). Whilst in other cases, improvements have been associated with reductions in berry size (Matthews & Anderson, 1988; Matthews et al., 1987; McCarthy et al., 2002). Changes in composition have been linked to changes in berry size, with smaller berries being associated with more skin surface per unit of berry weight (Singleton, 1972). Results presented by Roby et al. (2004) indicate there are effects of grapevine water deficit that arise independently of changes in

berry size. Furthermore, others have reported a reduction in yield and composition due to grapevine water deficits and in extreme cases failure of fruit to mature (Hardie & Considine, 1976).

Glycine betaine (GB) and kaolin particle film (KPF), as discussed in Chapter 1, are two products that can be exogenously applied to grapevines to potentially ameliorate the effects of environmental stresses. The effects of the irrigation treatments, GB and KPF and their associated interactions on yield and yield components of Shiraz vines grown over two growing seasons were reported and interpreted in Chapter 3. The vines' response to GB and KPF was influenced by water availability (cumulative irrigation and rainfall), with yield reductions observed for both GB (under high water availability) and KPF (under low water availability). Given that commercial wine grape production is not only characterised by yield and its drivers but importantly by composition, further research is required to assess the impacts of the irrigation treatments, GB and KPF on grape composition.

GB is a compatible solute involved in the protection of many macro-components in plant cells, including proteins, enzymes and membranes, under stress conditions (Sakamoto & Murata, 2000; Yang et al., 2005). Exogenous application of GB has been attributed to a reduction in berry raisining (Scarlett, 2009). Therefore, it may help maintain berry integrity in response to stresses, such as heat stress (Scarlett et al., 2011). Furthermore, reducing berry raisining may potentially have positive impacts on grape composition, with anecdotal evidence suggesting berry raisining may impart a 'dead fruit' characteristic (Scarlett, 2009). However, to the best of the author's knowledge there has been no research conducted on the effects of GB on grape composition.

KPF increased the ratio of anthocyanins to total soluble solids (between 18 and 24%) of Merlot (*Vitis vinifera* L.) grapes irrigated to provided 90 and 35% of estimated water demand (Shellie, 2015). It was unclear why the effectiveness of the film decreased soluble solids higher than 24%. Shellie (2015) proposed that it may have been due to a decrease in the films thickness or the onset of cooler air temperatures and lower radiation intensities. Similar results were observed by Shellie and King (2013a), with KPF increasing the total content of monomeric anthocyanins of Malbec (*Vitis vinifera* L.) grapes irrigated at 23 and 70% of ET_C during the 2009, 2010 and 2010 growing seasons. During the 2007, 2008 and 2009 growing seasons the increase in total anthocyanin concentration of Malbec (*Vitis vinifera* L.) and Cabernet Sauvignon (*Vitis vinifera* L.) was accompanied by a decrease in berry weight (Shellie & King, 2013b). In apples, KPF has been shown to have positive impacts on fruit colour in some cases (Glenn & Puterka, 2007; Glenn et al., 2001; Scarlett, 2009; Wand et al., 2006), whilst no effect

(Glenn & Puterka, 2007) or reductions (Gindaba & Wand, 2005) on colour are reported in other cases.

In research conducted by Shellie and Glenn (2008) on viognier (*Vitis vinifera* L.), the correlation between TSS and titratable acidity (TA) was stronger in vines with KPF applied than without KPF applied grown in southwestern Idaho. The same research showed TA declining more rapidly with increasing TSS for vines with KPF applied. This response was not observed for Merlot (*Vitis vinifera* L.) grown the same region and growing seasons (Shellie & Glenn, 2008). Cooley et al. (2008) observed an increase in organic acid (tartaric, malic and citric) along with sugar concentrations (glucose and fructose) for Cabernet Sauvignon vines grown in Victoria, Australia. Additionally, KPF had minimal impact on free and bound volatile compounds of Merlot vines, with no interactive effect of KPF and deficit irrigation (Song et al., 2012).

Comparison of the effects of GB and KPF under deficit and non-deficit irrigation has only been assessed on olives (Denaxa et al., 2012; Roussos et al., 2010). Consequently, there is still the need to investigate the impacts of the irrigation treatments, GB and KPF and their associated interactions on grape composition. This research examines the effects of irrigation, GB and KPF on grape of field grown *Vitis vinifera* L. 'Shiraz' in North East Victoria, Australia.

4.2. Grape Composition Materials and Methods

The experiment site, planting material, experimental design, implementation of treatments, sampling procedures and statistical analysis are described in Chapter 2.

4.2.1. Berry Maturation

In Y1 and Y2, berry sampling was conducted four times between véraison and harvest using methodology described in Section 2.6.3 of Chapter 2. Sampled berries were stored in zip lock bags in a -20 °C freezer until compositional analysis could be conducted. The berries were defrosted at room temperature before being crushed by hand in the zip lock bags to release free run juice. The free run juice was centrifuged for 6 min at 3000 rpm prior to analysis of soluble solids and organic acids (Iland et al., 2000).

Soluble Solids

TSS was measured using a temperature compensating refractometer (WM-7; ATAGO; Tokyo, Japan). The refractometer was cleaned using distilled water and a tissue prior to use and between each sample. Distilled water was used to zero the refractometer every three to four samples. For each sample, approximately 0.2 to 0.5 mL was placed onto the prism of the refractometer using a disposable pipette for analysis.

Soluble solids per berry (SSB, g·berry^{-1}) was estimated from TSS and average berry fresh weight (BW, g) using Equation 4.1.

$$TSS = \frac{SSC}{100} \times BW \quad \text{(Equation 4.1)}$$

Soluble solids per vine (SSV, kg·vine^{-1}) was estimated from TSS and yield per vine (Y, kg·vine^{-1}) using Equation 4.2 (Considine, 2004).

$$TSS = \frac{SSC}{100} \times Y \quad \text{(Equation 4.2)}$$

Total soluble solids (TSS, °Brix) provides an indication the amount of sugar present in grape juice (g sucrose equivalent per 100 g of solution; Considine, 2004). Therefore, SSB provides an estimate of the amount of sugar per berry and SSV provides an estimate of yield of sugar per vine (Considine, 2004).

Organic Acids

Acidity was measured as pH and TA (g of tartaric acid equivalents per litre of grape juice; g·L^{-1}) using methodology described by Iland et al. (2000). The pH of the centrifuged juice was measured using an automatic titrator (Ω Metrohm Titrando; Metrohm; Herisau, Switzerland). The centrifuged juice was then diluted with distilled water (1:3) and TA was determined by titration with sodium hydroxide (0.1 M NaOH) to an end point of pH 8.2 using an automatic titrator (Ω Metrohm Titrando; Metrohm, Herisau, Switzerland). Calibration was performed using buffers of pH 7.0 and 4.0 at the start and end of each analysis set, and the probe will be cleaned prior to each sample using distilled water and a tissue.

4.2.2. Harvest Analysis

At harvest in Y1 and Y2, cluster sampling was conducted using methodology described in Section 2.6.2 of Chapter 2. Sampled clusters were stored in zip lock bags in a -20 °C freezer until compositional analysis could be conducted. The clusters were defrosted at room temperature before being crushed by hand in the zip lock bags to release free run juice. The free run juice was centrifuged for 6 min at 3000 rpm prior to analysis of soluble solids and organic acids (Iland et al., 2000) using methodology described above.

At harvest in Y1 and Y2, a subsample of 50 randomly selected berries per treatment plot was collected using methodology described in Section 2.6.2. The 50 berry subsamples were then stored in -20 °C freezer until analysis could be conducted on colour, phenolics and berry percent dry weight.

Colour and Phenolics

The 50 berry subsamples were defrosted at room temperature before being homogenised for analysis of colour (anthocyanins) and phenolics. The samples were homogenised for approximately 30 sec at 24,000 rpm using a 220V homogeniser dispenser (T25 Ultra-Turrax; Fabr-Nr 611554; Terra Universal, Inc; Fullerton, USA) until there were no visible pieces of skin or seeds larger than 1 mm^3 (Iland et al., 2000). Colour and phenolics were extracted using 50% $v{\cdot}v^{-1}$ aqueous ethanol adjusted to a pH of 2.0 and hydrochloric acid (1.0 M HCl) and analysed using an ultraviolet-visible (UV-Vis) ratio beam spectrophotometer (Halo RB-10; Dynamica Scientific Ltd; Newport Pagnell, United Kingdom) using the methodology described in (Iland et al., 2000). Colour concentration (C_g, mg anthocyanins per gram berry weight; $mg{\cdot}g^{-1}$) and colour per berry (C_b, mg anthocyanins per berry; $mg{\cdot}berry^{-1}$) were calculated based on absorbance at 520 nm (Iland et al., 2000). Phenolics concentration (P_g, absorbance units per gram berry weight; $au{\cdot}g^{-1}$) and phenolics per berry (P_b, absorbance units per berry; $au{\cdot}berry^{-1}$) were calculated based on absorbance at 280 nm (Iland et al., 2000).

Berry Percent Dry Weight

The homogenised berries used for the analysis of anthocyanins and phenolics were placed into a pre-weighed weighing boat to obtain fresh weight (H_{FW}, g) using a two decimal point balance (Sartorius ED2202S-CW Extend, Sartorius AG; Goettingen, Germany). They were then dried at 65 °C until consistent weight was achieved between hourly measurements to obtain dry weight (H_{DW}, g) using the two decimal point balance. $B_{\%DW}$ was then estimated using Equation 4.3.

$$\mathbf{B_{\%DW}} = \frac{\mathbf{H_{FW}}}{\mathbf{H_{DW}}} \times \mathbf{100} \qquad \text{(Equation 4.3)}$$

4.3. Grape Composition Results

Table 4.1 and Table 4.2 provided a summary of the significant effects of irrigation, GB, KPF and their associated interactions on soluble solids (Table 4.1), organic acids (Table 4.1), colour (Table 4.2), phenolics (Table 4.2) and berry percent dry weight (Table 4.2).

Table 4.1. Significance of the p values for soluble solids and organic acids measurements for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons from the repeated measures analysis of variance (rANOVA) with irrigation treatments (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

	Y1					Y2				
	TSS (°Brix)	**SSB (g·berry^{-1})**	**SSV (kg)**	**pH**	**TA (g·L^{-1})**	**TSS (°Brix)**	**SSB (g·berry^{-1})**	**SSV (kg)**	**pH**	**TA (g·L^{-1})**
I	ns	< 0.001 ***	ns	ns	ns	< 0.001 ***	< 0.001 ***	< 0.001 ***	ns	0.017 *
GB	< 0.001 ***	< 0.001 ***	0.001 ***	ns	ns	0.020 *	ns	ns	ns	ns
KPF	ns	ns	ns	ns	ns	0.004 **	ns	ns	ns	ns
I x GB	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.036 *
I x KPF	ns	0.014 *	ns	ns	ns	ns	ns	0.022 *	ns	ns
GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Epsilon adjustment	0.8657	0.8574		*0.8269*	0.9400	0.8932	0.7785		*0.8156*	0.4308
Time (t)	< 0.001 ***	< 0.001 ***		< 0.001 ***	< 0.001 ***	< 0.001 ***	<0.001 ***		< 0.001 ***	< 0.001 ***
t x I	ns	ns		ns	ns	ns	ns		ns	0.046 *
t x GB	ns	ns		ns	ns	0.015 *	ns		0.047 *	ns
t x KPF	ns	ns		ns	ns	ns	ns		ns	ns
t x I x GB	ns	ns		ns	ns	ns	ns		ns	0.026 *
t x I x KPF	ns	ns		ns	ns	ns	ns		ns	ns
t x GB x KPF	ns	ns		ns	ns	ns	ns		ns	ns
t x I x GB x KPF	ns	ns		ns	ns	ns	ns		ns	ns

Table 4.2. Significance of the p values for the colour, phenolics and berry percent dry weight measurements for the 2011-12 (Y1) and 2012-13 (Y2) growing seasons from the general analysis of variance (gANOVA) with irrigation treatments (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

	Y1					Y2				
	C_g (mg·g^{-1})	C_b (mg·berry^{-1})	P_g (au·g^{-1})	P_b (au·berry^{-1})	$B_{\%DW}$	C_g (mg·g^{-1})	C_b (mg·berry^{-1})	P_g (au·g^{-1})	P_b (au·berry^{-1})	$B_{\%DW}$
I	ns	ns	ns	ns	ns	0.003 **	0.024 *	0.002 **	ns	< 0.001 ***
GB	ns	0.031 *	ns	0.029 *	ns	ns	0.010 **	ns	ns	ns
KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.003 **
I x GB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
I x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

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At harvest, average TSS was lower and average SSB was higher in Y1 compared with Y2 (Table 4.3). In Y1, TSS decreased between the fourth sample date and harvest with TSS ranging from 22.0 to 27.0 °Brix, with an average across all treatments of 24.9 °Brix for the fourth sample date. In contrast in Y2, TSS increased between the fourth sample date and harvest, with TSS ranging from 20.9 to 27.8 °Brix, with an average across all treatments of 24.4 °Brix for the fourth sample date. Average berry juice pH across all treatments was similar in Y1 and Y2 (Table 4.3). At harvest in Y2, equipment failure mid-analysis resulted in the loss of the TA data. TA ranged from 2.75 to 4.01 $g \cdot L^{-1}$ for the fourth sample date in Y2, with an average across all treatments of 3.33 $g \cdot L^{-1}$. In contrast, TA ranged from 3.01 to 3.88 $g \cdot L^{-1}$ for the fourth sample date in Y1 with an average across all treatments of 3.49 $g \cdot L^{-1}$. Average C_g and P_g across all treatments were lower in Y1 compared with Y2; and average C_b and P_b across all treatments was higher in Y1 than Y2 (Table 4.3).

Table 4.3. Average, minimum and maximum across all treatments of total soluble solids (TSS, °Brix), soluble solids per berry (SSB, $g \cdot berry^{-1}$), soluble solids per vine (SSV, $kg \cdot vine^{-1}$), pH, titratable acidity (TA, $g \cdot L^{-1}$), colour concentration (C_g, $mg \cdot g^{-1}$), colour per berry (C_b, $mg \cdot berry^{-1}$), phenolics concentration (P_g, $au \cdot g^{-1}$) and phenolics per berry (P_b, $au \cdot berry^{-1}$) and berry percent dry weight ($B_{\%DW}$) measured at harvest in the 2011-12 (Y1) and 2012-13 (Y2) growing seasons. n = 72.

		Average	Minimum	Maximum
TSS (°Brix)	**Y1**	23.8	21.4	25.7
	Y2	26.7	22.8	29.4
SSB ($g \cdot berry^{-1}$)	**Y1**	0.370	0.282	0.490
	Y2	0.219	0.120	0.329
SSV ($kg \cdot vine^{-1}$)	**Y1**	1.732	0.996	2.546
	Y2	0.246	1.322	2.374
pH	**Y1**	4.24	3.92	4.54
	Y2	4.16	3.68	4.51
TA ($g \cdot L^{-1}$)	**Y1**	2.76	2.08	3.60
	Y2	‡	‡	‡
C_g ($mg \cdot g^{-1}$)	**Y1**	1.52	1.12	2.03
	Y2	1.63	0.95	2.25
C_b ($mg \cdot berry^{-1}$)	**Y1**	2.37	1.04	3.33
	Y2	1.31	0.72	1.81
P_g ($au \cdot g^{-1}$)	**Y1**	1.45	1.14	1.86
	Y2	1.76	0.96	2.38
P_b ($au \cdot berry^{-1}$)	**Y1**	2.25	1.07	3.47
	Y2	1.40	0.71	2.06
$B_{\%DW}$	**Y1**	28.2	18.2	46.0
	Y2	29.9	25.7	35.28

‡ Data not recorded

4.3.1. Irrigation Treatments

In Y1, there was no significant effect of irrigation treatments on TSS ($p > 0.05$; Figure 4.1). In Y2, there was a significant effect of the irrigation treatments on TSS, with I_{100} resulting in lower TSS than I_{25} and I_{50} ($p < 0.001$; Figure 4.1). In Y1 and Y2, there was a significant effect of the irrigation treatments on SSB, with I_{100} resulting in higher SSB than I_{25} and I_{50} ($p < 0.001$; Figure 4.1).

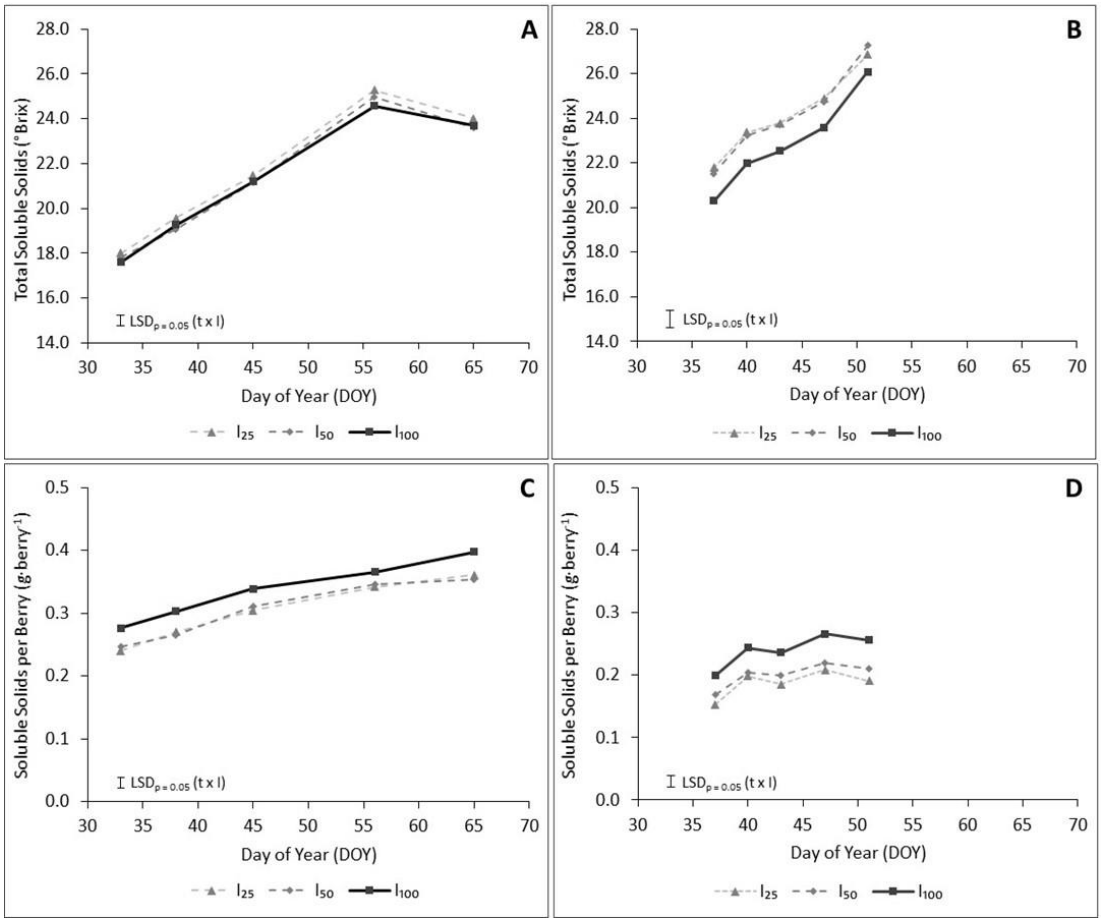


Figure 4.1. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on (A) total soluble solids (TSS, °Brix) in the 2011-12 growing season (Y1); and (B) TSS in 2012-13 growing season (Y2); (C) soluble solids per berry (SSB; g·berry^{-1}) in Y1; and (D) SSB in Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of sample date (time, t) and irrigation treatments (I).

In Y2, there was a significant effect of the irrigation treatments on SSV, with I_{100} resulting in higher SSV than I_{25} and I_{50} ($p < 0.001$; Figure 4.2). A similar trend approached conventional levels of significance in Y1 ($p = 0.053$; Figure 4.2).

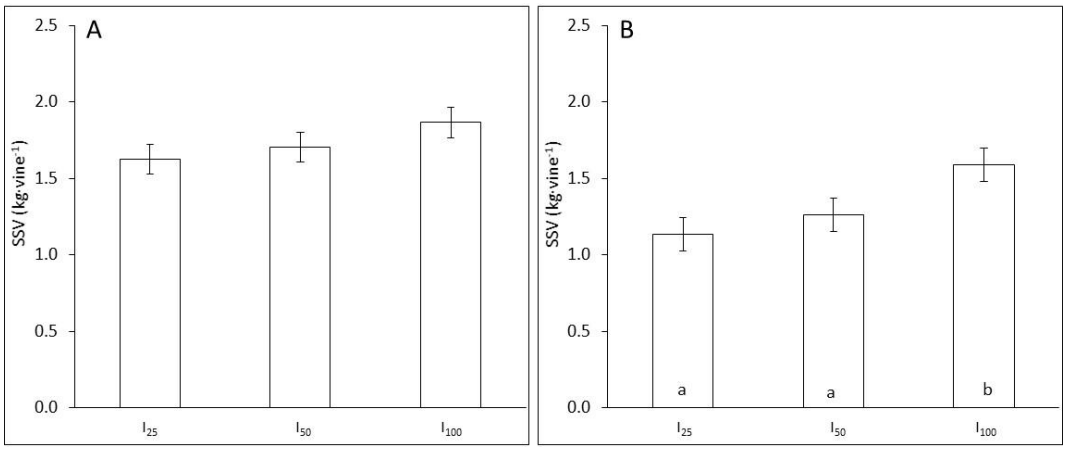


Figure 4.2. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on soluble solids per vine (SSV; kg·vine^{-1}) for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).
NB: when the p value (I) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I) > 0.05 means separation was not conducted.

In Y1, there was no significant effect of irrigation treatments on $B_{\%DW}$ ($p > 0.05$; Figure 4.3). In Y2, there was a significant effect of the irrigation treatments on $B_{\%DW}$, with I_{100} resulting in significantly lower $B_{\%DW}$ than I_{25} and I_{50} ($p < 0.001$; Figure 4.3).

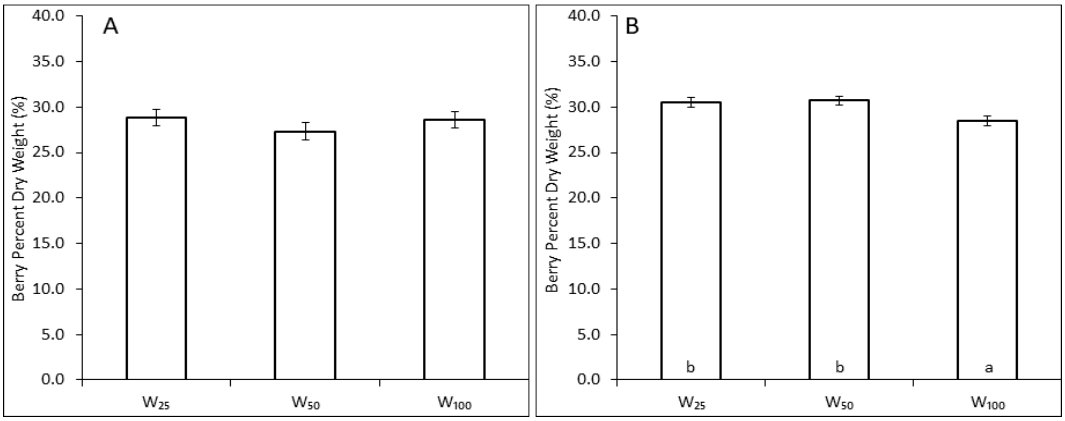


Figure 4.3. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on berry percent dry weight ($B_{\%DW}$) for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).
NB: when the p value (I) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant effect of the irrigation treatments on berry juice pH ($p > 0.05$; Figure 4.4). In Y2, there was no significant effect of the irrigation treatments on TA ($p > 0.05$; Figure 4.4). In Y2, there was an overall significant effect of the irrigation treatments on TA, with I_{100} resulting in lower TA than I_{25} ($p = 0.017$ Figure 4.4). However, the irrigation treatments converged with no significant difference observed for the final sample date, indicated by the significant interaction of sample date (time, t) and irrigation treatments ($p = 0.046$; Figure 4.4).

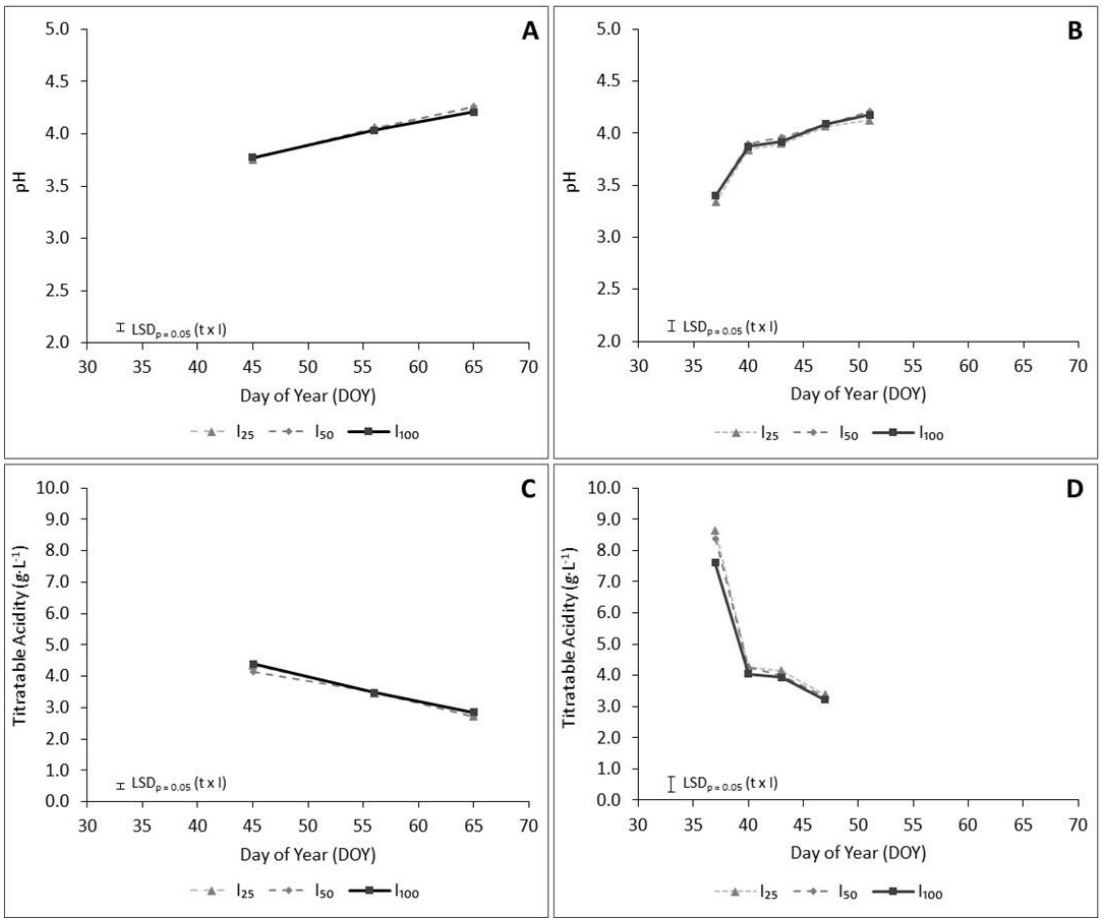


Figure 4.4. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on (A) pH for the 2011-12 growing season (Y1); (B) pH for the 2012-13 growing season (Y2); (C) titratable acidity (TA, $g \cdot L^{-1}$) for Y1; and (D) TA in Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of sample date (time, t) and irrigation (I).

In Y1, there was no significant effect of the irrigation treatments on C_b or C_g ($p > 0.05$; Figure 4.5). In Y2, there was a significant effect of the irrigation treatments on C_g, with I_{100} resulting in significantly lower C_g than I_{50} and I_{25} ($p = 0.003$; Figure 4.5) and C_b, with I_{100} resulting in significantly higher C_b than I_{25} ($p = 0.002$; Figure 4.5).

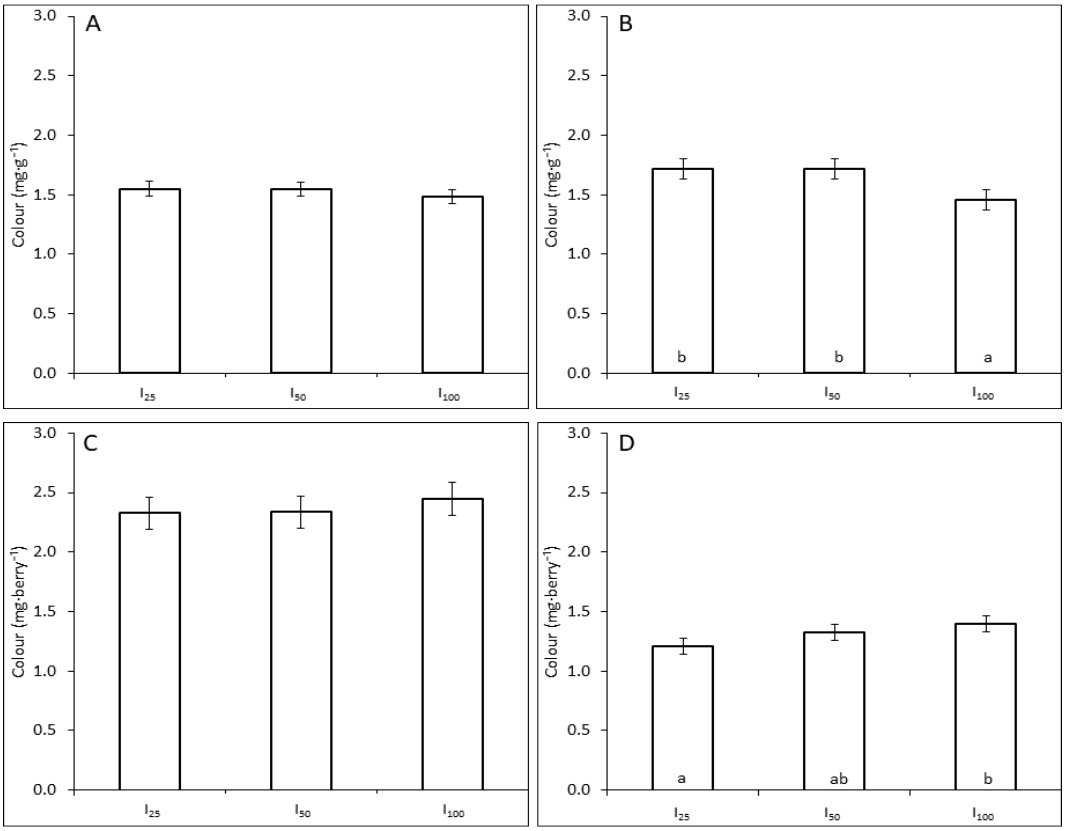


Figure 4.5. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on (A) colour concentration (C_g, $mg \cdot g^{-1}$) for 2011-12 growing season (Y1); (B) C_g for the 2012-13 growing season (Y2); (C) colour per berry (C_b, $mg \cdot berry^{-1}$) for Y1; and (D) C_b for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).

NB: when the p value (I) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I) > 0.05 means separation was not conducted.

In Y1, there was no significant effect of the irrigation treatments on P_b or P_g ($p > 0.05$; Figure 4.6). In Y2, there was a significant effect of the irrigation treatments on P_g, with I_{100} resulting in significantly lower P_g than I_{50} and I_{25} ($p = 0.002$; Figure 4.6). In Y2, there was no significant effect of the irrigation treatments P_b in Y2 ($p > 0.05$; Figure 4.6).

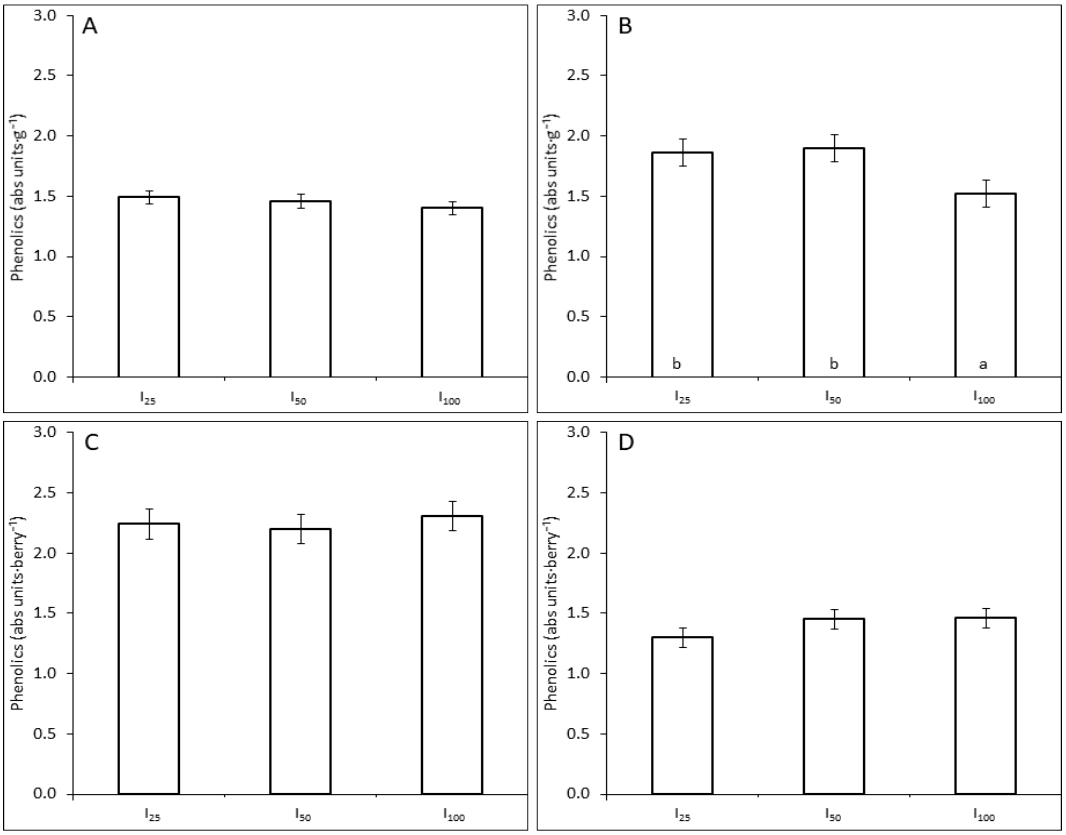


Figure 4.6. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on (A) phenolics concentration (P_g, au·g^{-1}) for the 2011-12 growing season (Y1); (B) P_g for the 2012-13 growing season (Y2); (C) phenolics per berry (P_b, au·berry^{-1}) for Y1; and (D) P_b for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for irrigation treatments (I).
NB: when the p value (I) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I) > 0.05 means separation was not conducted.

4.3.2. Glycine Betaine

In Y1, GB significantly increased TSS and reduced SSB ($p < 0.001$; Figure 4.7). In Y2, there was an overall significant effect of GB on TSS, with GB increasing TSS ($p = 0.020$; Figure 4.7). However, the GB treatments converged with no significant difference observed from the third sample date onwards, indicated by the significant interaction of sample date (time, t) and glycine betaine treatments ($p = 0.015$; Figure 4.7). In Y2, there was no significant effect of GB on SSB ($p > 0.05$; Figure 4.7). In Y1 and Y2, there was no significant interaction of GB and irrigation treatments on TSS or SSB ($p > 0.05$; data not presented).

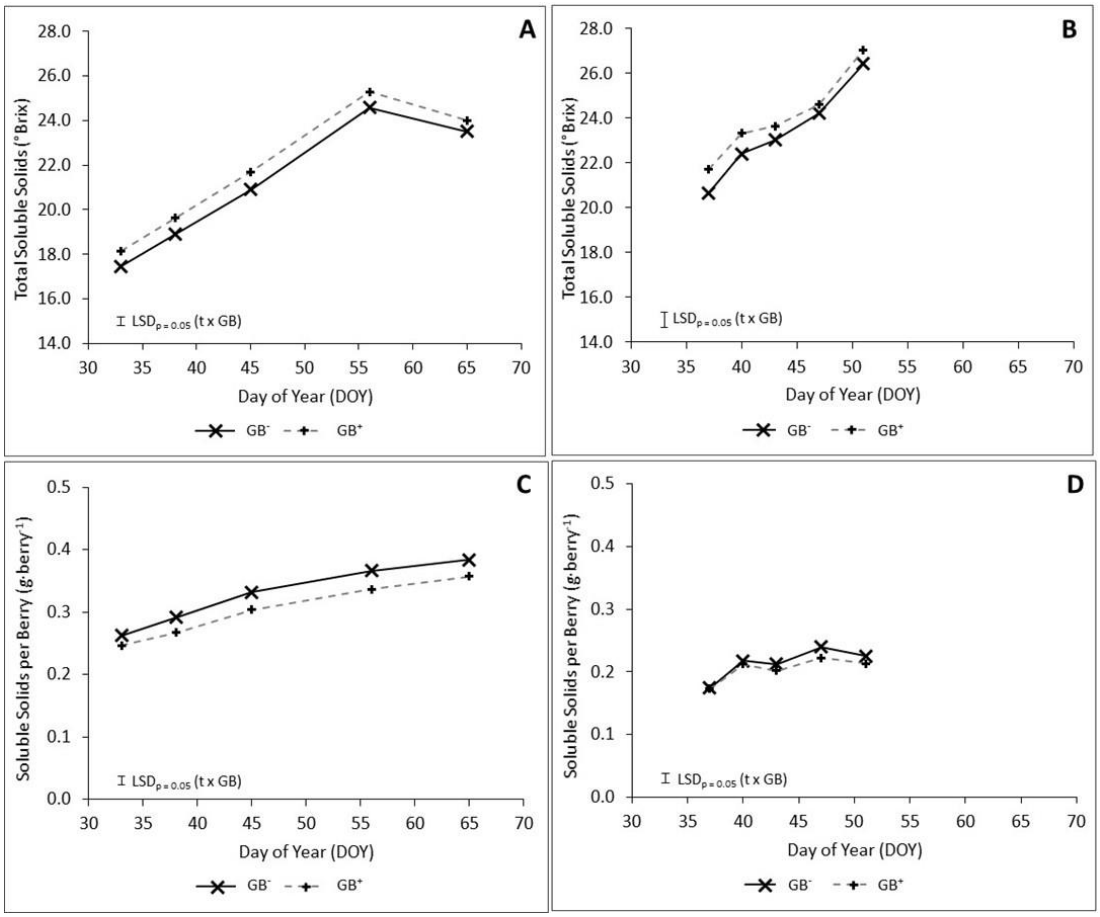


Figure 4.7. Impact of glycine betaine (GB^- and GB^+) on (A) total soluble solids (TSS, °Brix) for the 2011-12 growing season (Y1); (B) TSS for the 2012-13 growing season (Y2); (C) soluble solids per berry (SSB, $g \cdot berry^{-1}$) for Y1; and (D) SSB for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of sample date (time, t) and glycine betaine (GB).

In Y1, GB significantly reduced SSV with means of 1.87 and 1.60 kg·vine^{-1} for GB$^-$ and GB$^+$, respectively ($LSD_{p = 0.05}$ = 0.16; p = 0.001). In Y2, there was no significant effect of GB on SSV (p > 0.05). In Y1 and Y2, there was no significant interaction of irrigation treatments and GB on SSV (p > 0.05; Figure 4.8).

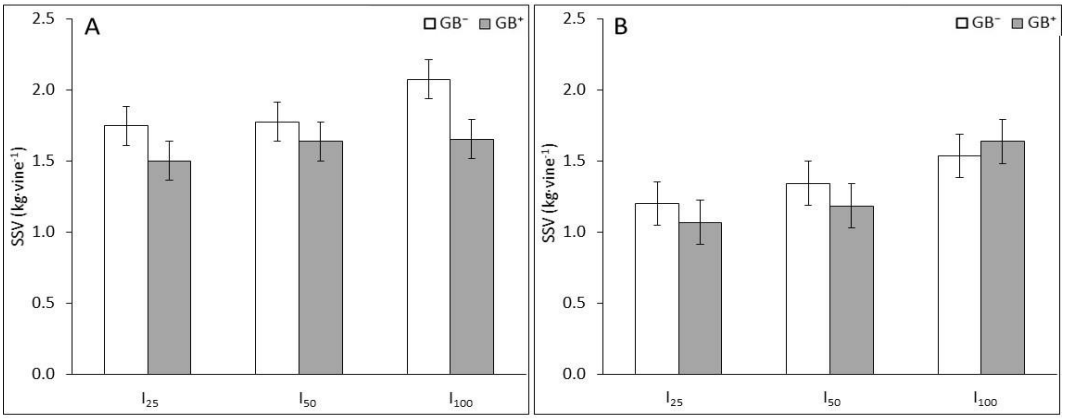


Figure 4.8. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB$^-$ and GB$^+$) on soluble solids per vine (SSV, kg·vine^{-1}) for the (A) 2011-12 growing season (Y1); and (B) 2011-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of irrigation (I) and glycine betaine (GB).
NB: when the p value (I x GB) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x GB) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant effect of GB or the interaction of irrigation treatments (p > 0.05; Figure 4.9) or GB on $B_{\%DW}$ (p > 0.05).

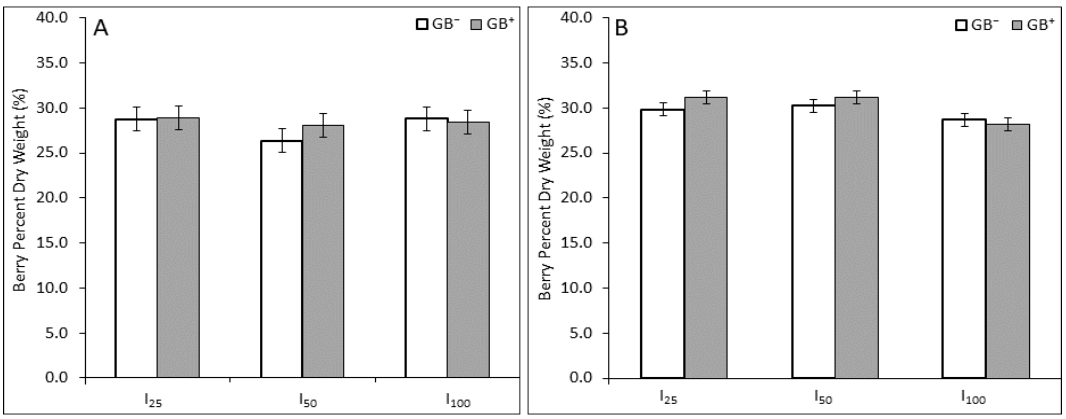


Figure 4.9. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB$^-$ and GB$^+$) on berry percent dry weight ($B_{\%DW}$) for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of irrigation treatments (I) and glycine betaine (GB).
NB: when the p value (I x GB) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x GB) > 0.05 means separation was not conducted.

In Y1, there was no significant effect of GB on berry juice pH or TA for any of the sample dates, or at harvest ($p > 0.05$; Figure 4.10). In Y2, overall there was no significant effect of GB on berry juice pH ($p > 0.05$). However, the GB treatments diverged at sample date 2, with GB increasing pH for this sample date indicated by the significant interaction of t and GB ($p = 0.047$; Figure 4.10). In Y2, there was no significant effect of GB on TA ($p > 0.05$; Figure 4.10).

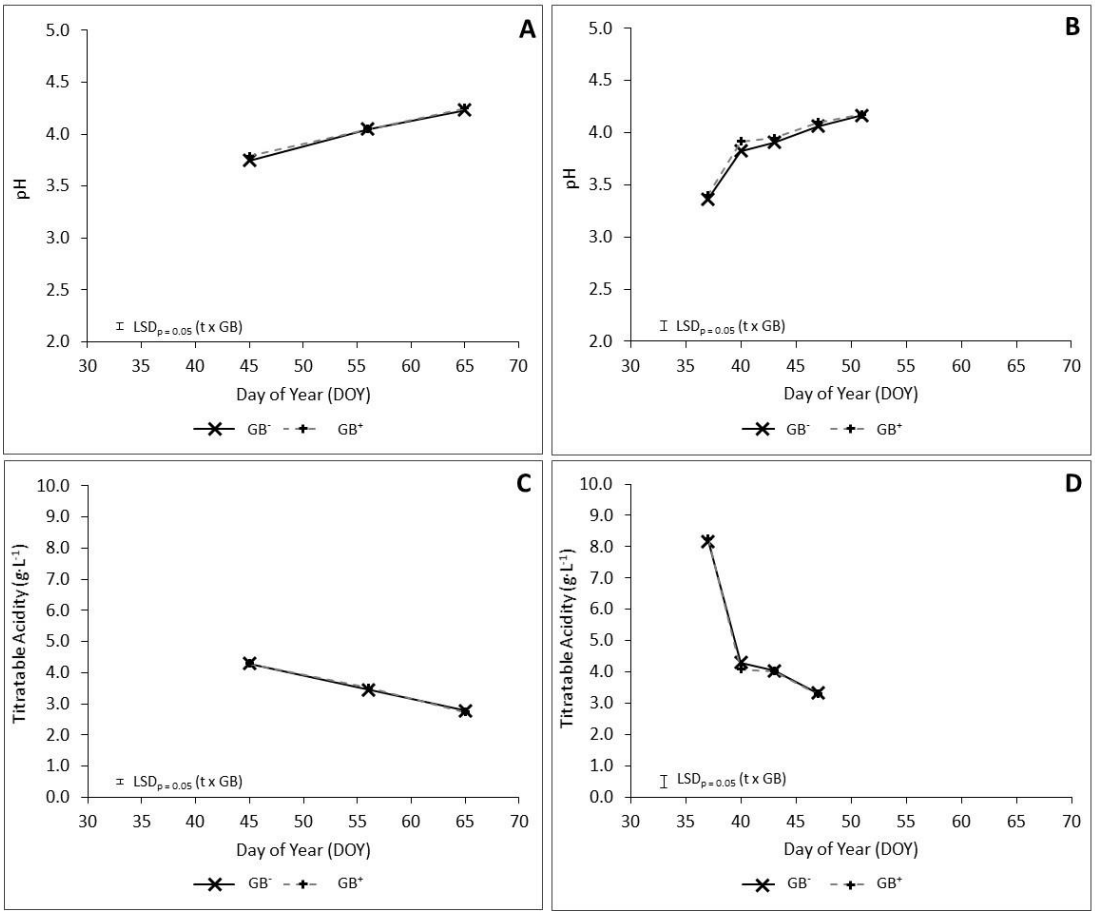


Figure 4.10. Impact of glycine betaine (GB^- and GB^+) on (A) pH for the 2011-12 growing season (Y1); (B) pH for the 2012-13 growing season (Y2); (C) titratable acidity (TA, $g \cdot L^{-1}$) for Y1; and (D) TA for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of sample date (time, t) and glycine betaine (GB).

In Y1 and Y2, there was no significant effect of GB on C_g ($p > 0.05$). In Y1, GB significantly reduced C_b, with means of 2.49 and 2.24 mg·berry^{-1} for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 0.22; p = 0.031). In Y2, GB significantly reduced C_b, with means of 1.39 and 1.24 mg·berry^{-1} for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 0.11; p = 0.010). In Y1 and Y2, there was no significant interaction of irrigation treatments and GB on C_g or C_b ($p > 0.05$; Figure 4.11).

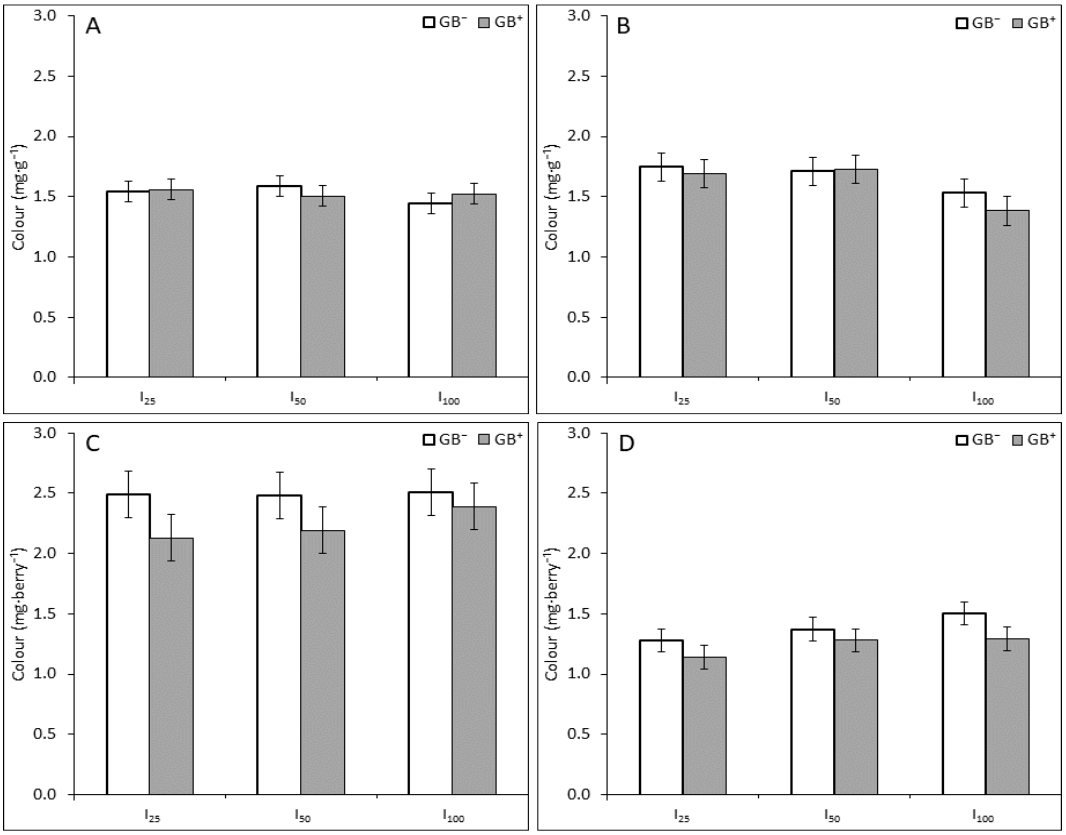


Figure 4.11. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on (A) colour concentration (C_g, mg·g^{-1}) for the 2011-12 growing season (Y1); (B) C_g for the 2011-13 growing season (Y2); (C) colour per berry (C_b, mg·berry^{-1}) for Y1; and (D) C_b for Y2. Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of irrigation (I) and glycine betaine (GB).

NB: when the p value (I x GB) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x GB) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant effect of GB on P_g ($p > 0.05$). In Y1, GB significantly reduced P_b, with means of 2.36 and 2.14 au·berry^{-1} for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 0.20; p = 0.029). In Y2, there was no significant effect of GB on P_b ($p > 0.05$). In Y1 and Y2, there was no significant interaction of irrigation treatments and GB on P_g or P_b ($p > 0.05$; Figure 4.12).

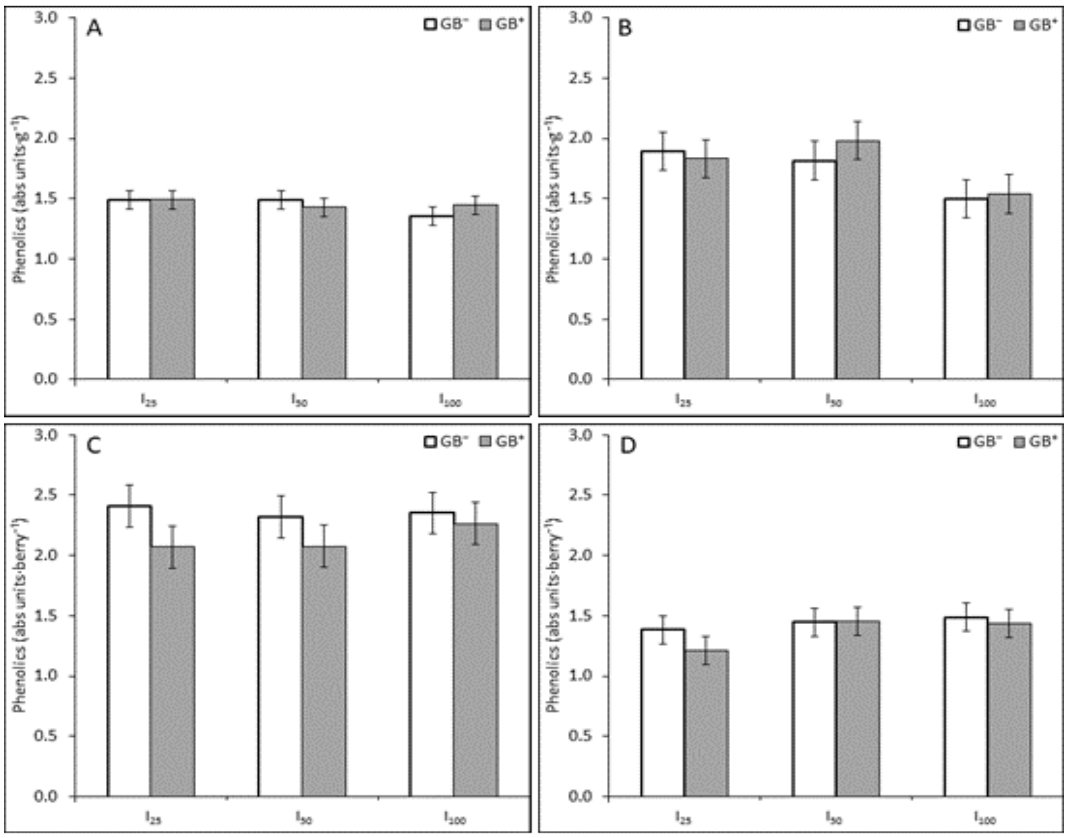


Figure 4.12. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on (A) phenolics concentration (P_g, au·g^{-1}) for the 2011-12 growing season (Y1); (B) P_g for the 2012-13 growing season (Y2); (C) phenolics per berry (P_b, au·berry^{-1}) for Y1; and (D) P_b for Y2. Error bars indicate least significant difference at p = 0.05 ($LSD_{p = 0.05}$) for the interaction of irrigation treatments (I) and glycine betaine (GB).

NB: when the p value (I x GB) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x GB) > 0.05 means separation was not conducted.

4.3.3. Kaolin Particle Film

In Y1, there was no significant effect of KPF on TSS or SSB ($p > 0.05$; Figure 4.13). In Y2, KPF increased TSS ($p = 0.004$; Figure 4.13) with no effect on SSB ($p > 0.05$; Figure 4.13).

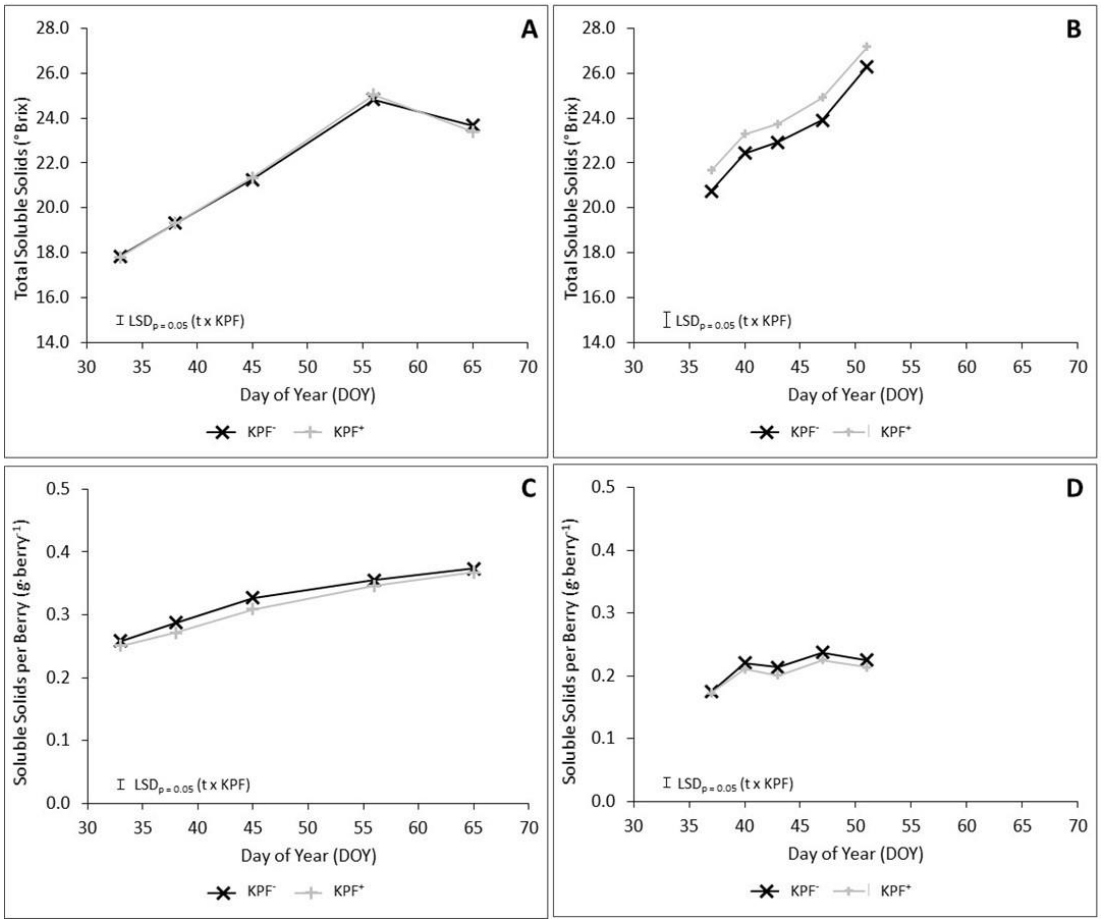


Figure 4.13. Impact of kaolin particle film (KPF^- and KPF^+) on (A) total soluble solids (TSS, °Brix) for 2011-12 growing season (Y1); (B) TSS for the 2012-13 growing season (Y2); (C) soluble solids per berry (SSB, $g \cdot berry^{-1}$) for Y1; and (D) SSB for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of kaolin particle film (KPF) and sample date (time, t).

In Y1 and Y2, there was no significant interaction of irrigation treatments and KPF on TSS ($p > 0.05$; data not presented). In Y1, there was an overall significant interaction of irrigation treatments and KPF on SSB ($p = 0.014$; Figure 4.14). Post hoc analysis of the overall means showed that with the combination of I_{25} and KPF^+ resulting in the lowest SSB and the combination of I_{100} and KPF^- resulting in the highest SSB. Additionally, the combination of KPF^+ and I_{25} had lower SSV than KPF^- and I_{25}. In Y2, there was no significant interaction of irrigation treatments and KPF on SSB ($p > 0.05$; Figure 4.14).

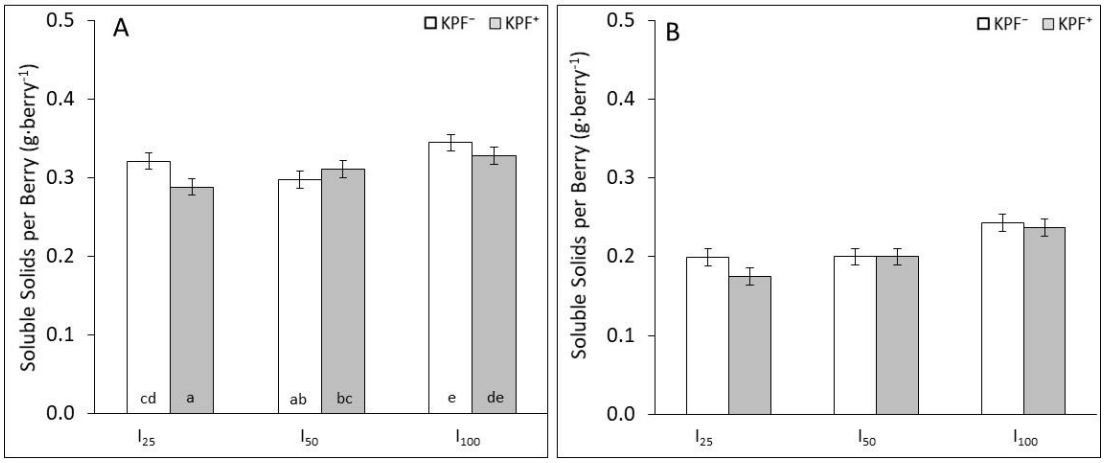


Figure 4.14. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on soluble solids per berry (SSB, $g \cdot berry^{-1}$) for the (A) 2011-12 growing season (Y1); and (B) 2012-13 (Y2) growing seasons. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x KPF) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant effect of KPF on soluble solids per vine ($p > 0.05$). In Y1, there was no significant interaction of irrigation treatments and KPF on SSV ($p > 0.05$; Figure 4.15). In Y2, there was a significant interaction irrigation treatments and KPF on SSV, I_{25} and KPF^+ resulting in the lowest SSV and the combination of I_{100} and KPF^- resulting in the highest SSV ($p = 0.022$; Figure 4.15).

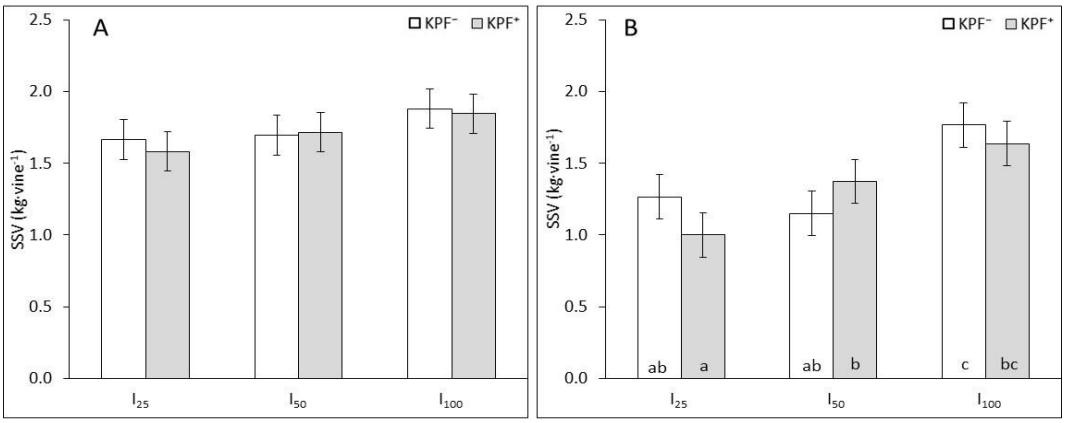


Figure 4.15. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on soluble solids per vine for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x KPF) > 0.05 means separation was not conducted.

In Y1, there was no significant effect of KPF on $B_{\%DW}$, ($p > 0.05$). In Y2, KPF increased $B_{\%DW}$, with means of 29.2 and 30.6% for KPF^- and KPF^+, respectively ($LSD_{p=0.05} = 0.84$; $p = 0.003$). In Y1 and Y2, there was no significant interaction of irrigation treatments and KPF on $B_{\%DW}$ ($p > 0.05$; Figure 4.16).

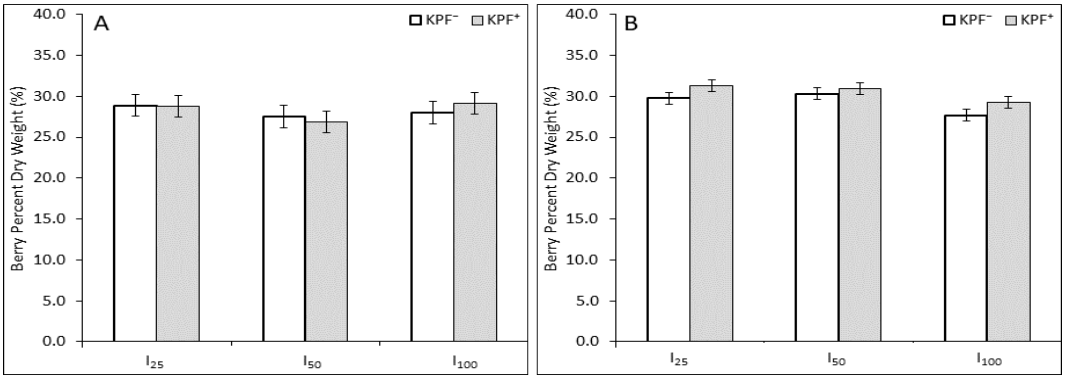


Figure 4.16. Impact of irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^-, KPF^+) on berry percent dry weight for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x KPF) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant effect of KPF on berry juice pH or TA for any of the sample dates ($p > 0.05$; Figure 4.17). In Y1 and Y2, there was no significant interaction of irrigation treatments and KPF on berry juice pH or TA ($p > 0.05$; data not presented).

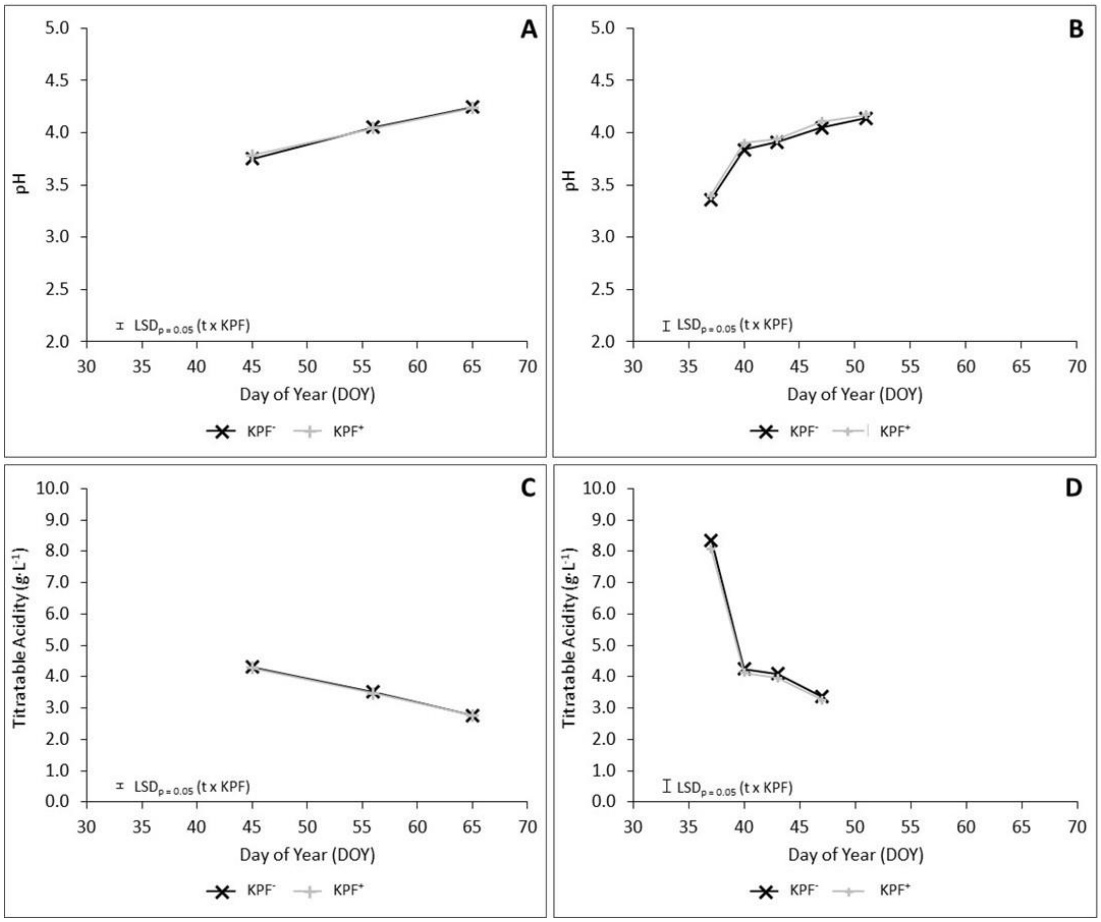


Figure 4.17. Impact of kaolin particle film (KPF^- and KPF^+) on (A) pH for the 2011-12 growing season (Y1); (B) pH for the 2012-13 growing season (Y2); (C) titratable acidity (TA, $g \cdot L^{-1}$) for Y1; and (D) TA for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of sample date (time, t) and kaolin particle film (KPF).

In Y1 and Y2, there was no significant effect of KPF or the interaction of irrigation and KPF on C_g or C_b ($p > 0.05$; Figure 4.18).

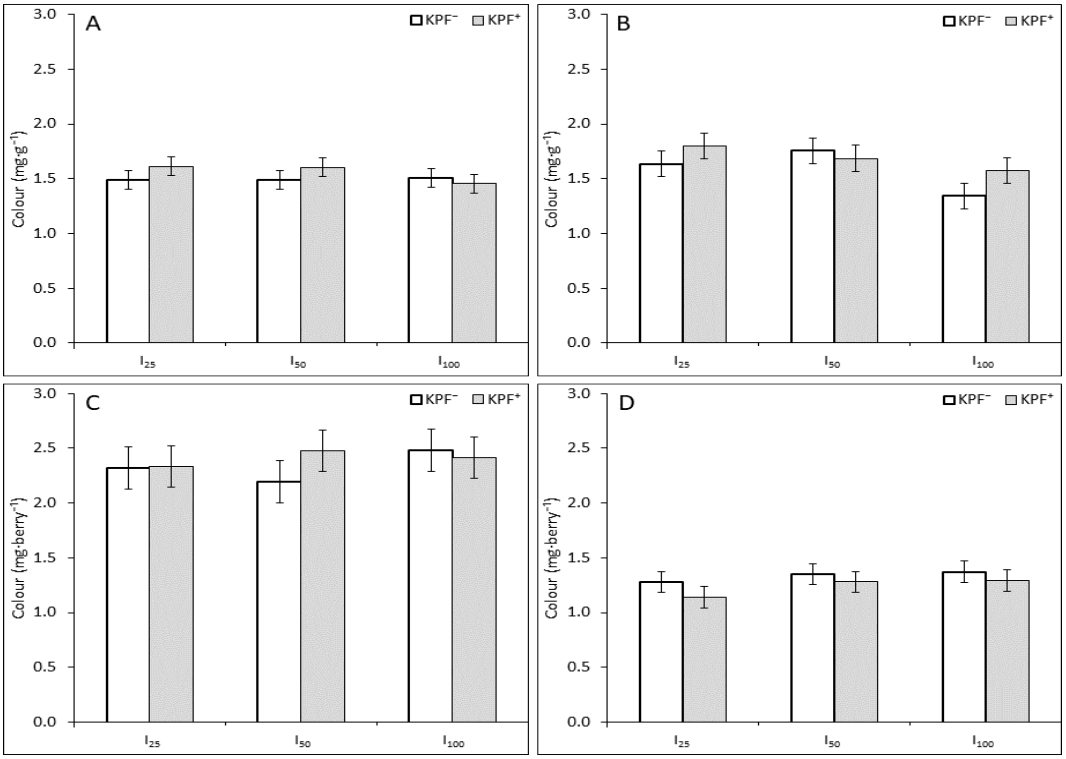


Figure 4.18. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on (A) colour concentration (C_g, $mg \cdot g^{-1}$) for the 2011-12 growing season (Y1); (B) C_g for the 2012-13 growing season (Y2); (C) colour per berry (C_b, $mg \cdot berry^{-1}$) for Y1; and (D) C_b for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).
NB: when the p value (I) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant effect of KPF or the interaction of irrigation and KPF on P_g or P_b ($p > 0.05$; Figure 4.19).

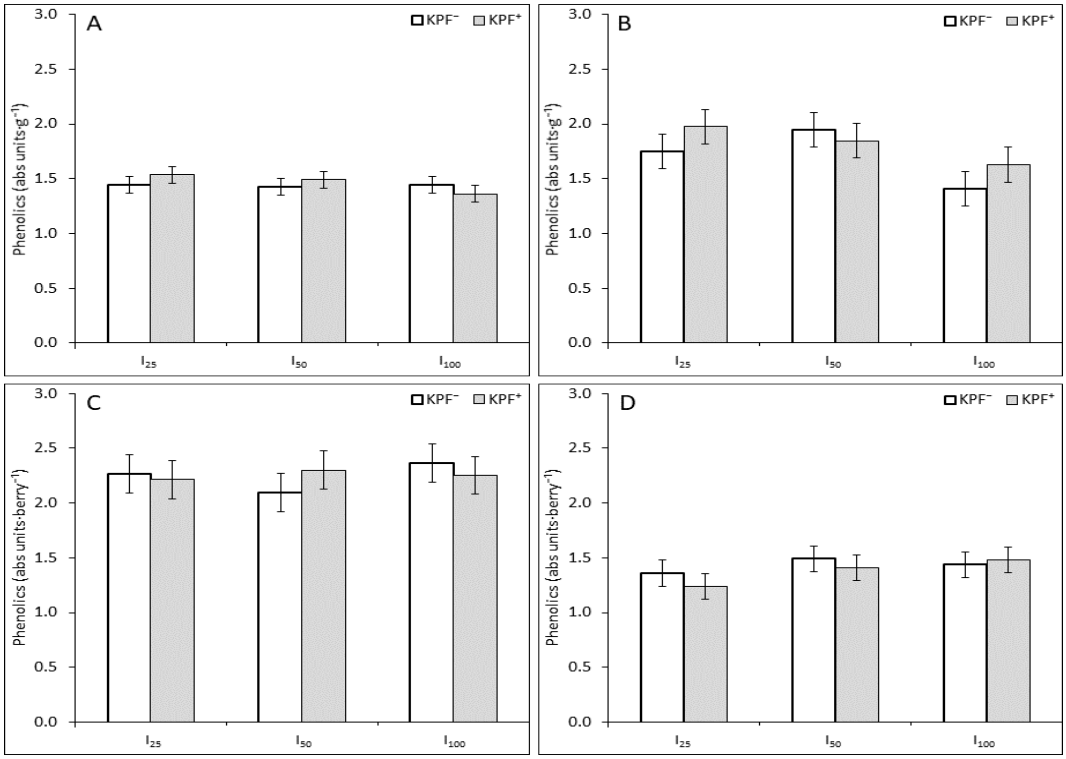


Figure 4.19. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on (A) phenolics concentration (P_g, au·g^{-1}) for the 2011-12 growing season (Y1); (B) P_g for the 2012-13 growing season (Y2); (C) phenolics per berry (P_b, au·berry^{-1}) for Y1; and (D) P_b for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of irrigation treatments (I) and kaolin particle film (KPF).

NB: when the p value (I x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (I x KPF) > 0.05 means separation was not conducted.

4.3.4. Glycine Betaine and Kaolin Particle Film

In Y1 and Y2, there was no significant interaction of GB and KPF on TSS or SSB ($p > 0.05$; Figure 4.20).

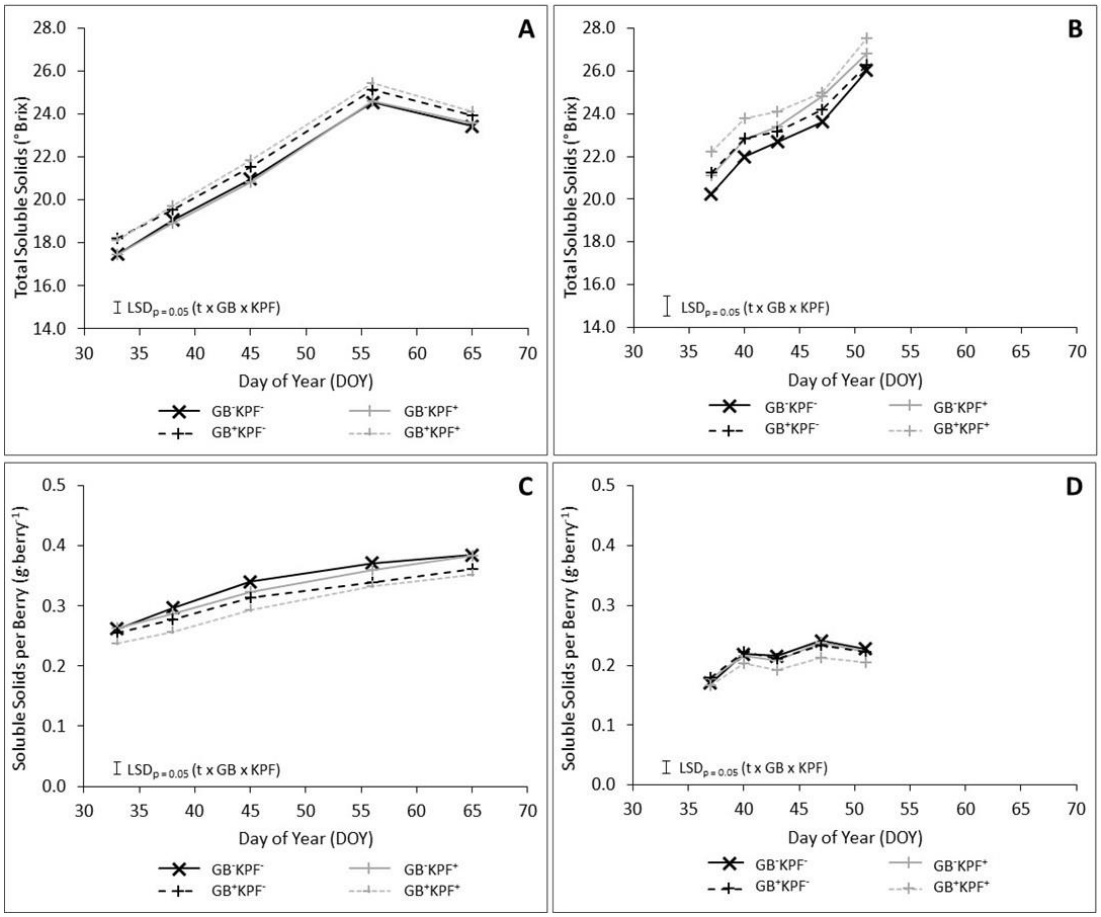


Figure 4.20. Impact of the glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on (A) total soluble solids (TSS, °Brix) for the 2011-12 growing season (Y1); (B) TSS for the 2012-13 growing season (Y2); (C) soluble solids per berry (SSB; $g \cdot berry^{-1}$) for Y2; and (D) SSB in Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).

In Y1 and Y2, there was no significant interaction of GB and KPF on SSV ($p > 0.05$; Figure 4.21).

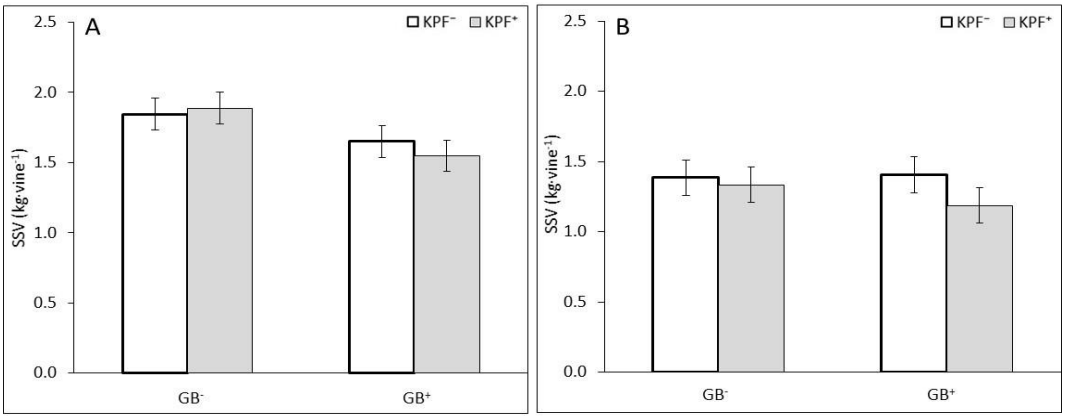


Figure 4.21. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on soluble solids per vine (SSV; $kg \cdot vine^{-1}$) for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).

NB: when the p value (GB x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (GB x KPF) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant interaction of GB and KPF on $B_{\%DW}$ ($p > 0.05$; Figure 4.22).

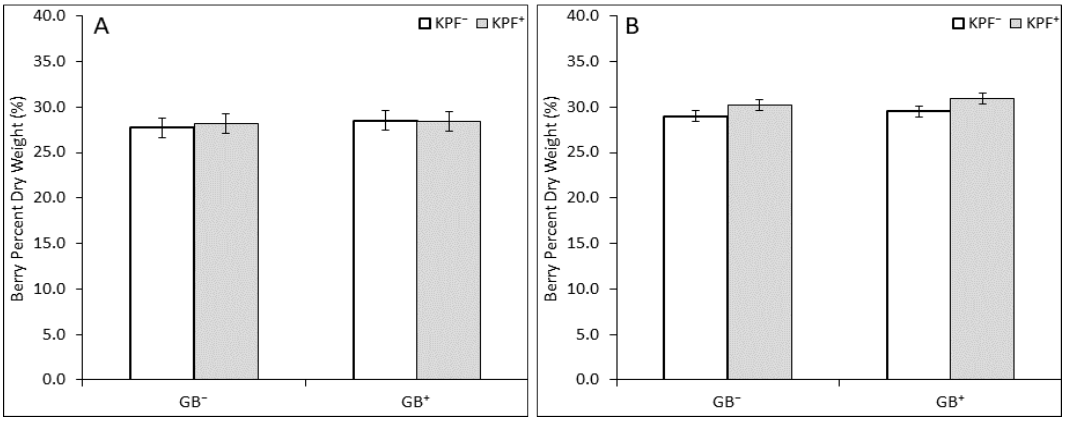


Figure 4.22. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on berry percent dry weight ($B_{\%DW}$) for the (A) 2011-12 growing season (Y1); and (B) 2012-13 growing season (Y2). Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).

NB: when p value (GB x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (GB x KPF) > 0.05 no means separation was conducted.

In Y1 and Y2, there was no significant interaction of GB and KPF on pH or TA ($p > 0.05$; Figure 4.23).

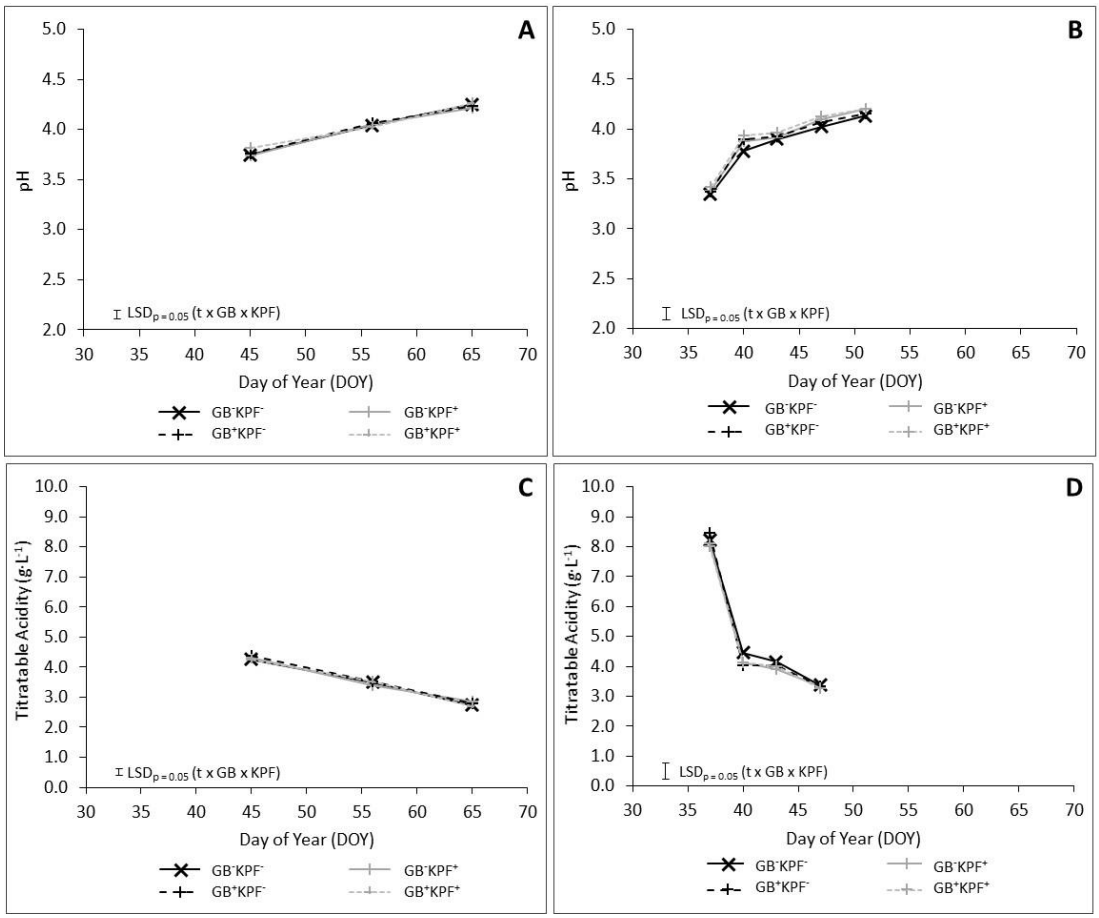


Figure 4.23. Impact of the glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on (A) pH for the 2011-12 growing season (Y1); (B) pH for the 2012-13 growing season (Y2); (C) titratable acidity (TA, $g \cdot L^{-1}$) for Y1; and (D) TA for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).

In Y1 and Y2, there was no significant interaction of GB and KPF on C_g or C_b ($p > 0.05$; Figure 4.24).

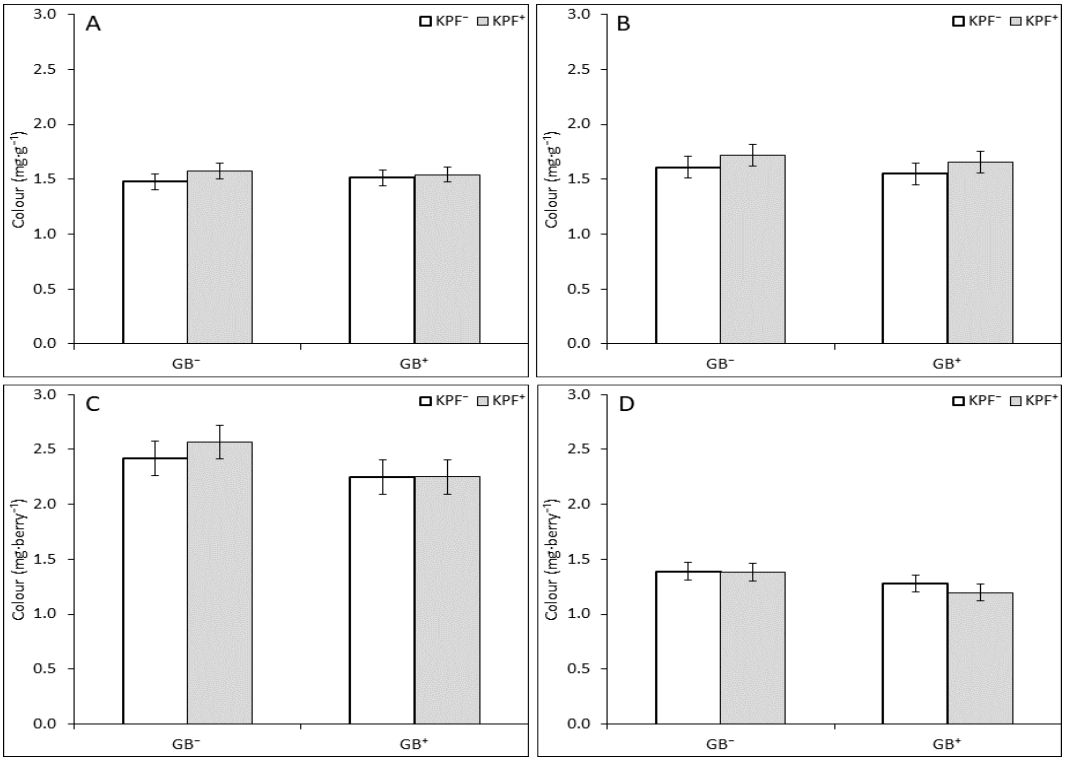


Figure 4.24. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on (A) colour concentration (C_g, $mg \cdot g^{-1}$) for the 2011-12 growing season (Y1); (B) C_g for the 2012-13 growing season (Y2); (C) colour per berry (C_b, $mg \cdot berry^{-1}$) for Y1; and (D) C_b for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).
NB: when the p value (GB x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (GB x KPF) > 0.05 means separation was not conducted.

In Y1 and Y2, there was no significant interaction of GB and KPF on P_g or P_b ($p > 0.05$; Figure 4.25).

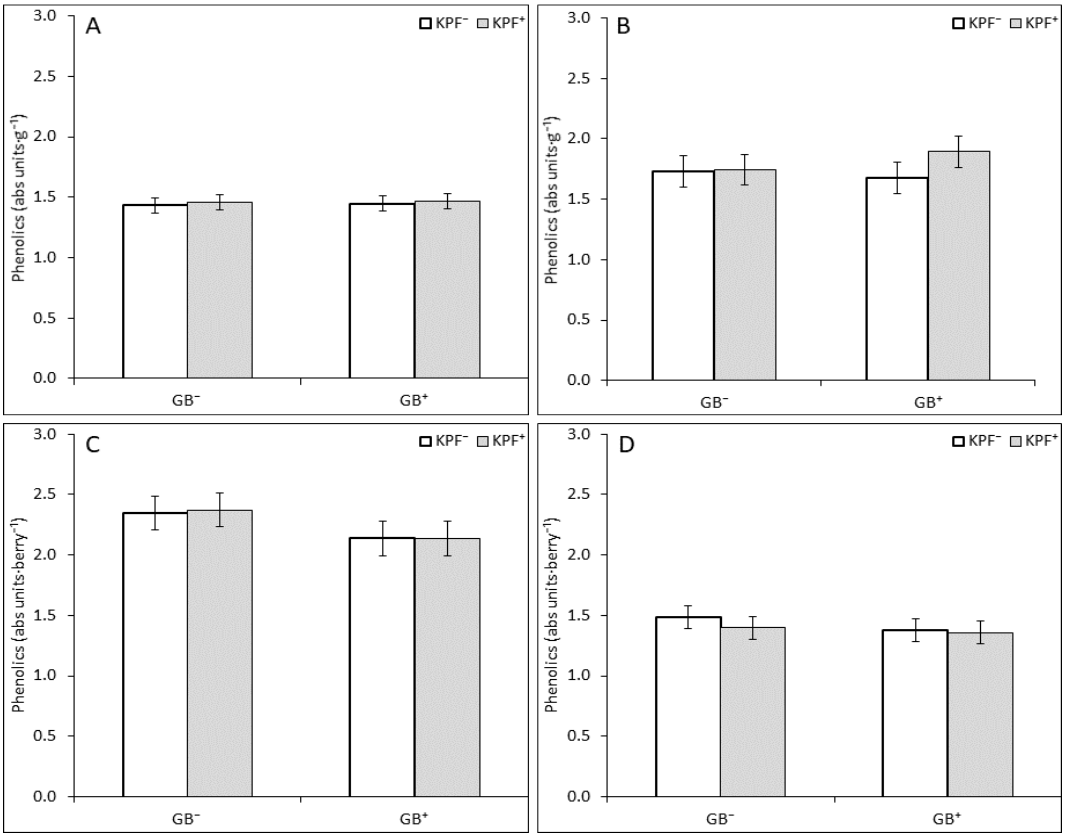


Figure 4.25. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on (A) phenolics concentration (P_g, au·g^{-1}) for the 2011-12 growing season (Y1); (B) P_g for the 2012-13 growing season (Y2); (C) phenolics per berry (P_b, au·$berry^{-1}$) for Y1; and (D) P_b for Y2. Error bars indicate least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) for the interaction of glycine betaine (GB) and kaolin particle film (KPF).

NB: when the p value (GB x KPF) < 0.05 separation of means is indicated by dissimilar letters and, when p value (GB x KPF) > 0.05 means separation was not conducted.

4.4. Grape Composition Discussion

The response of berry composition to the irrigation treatments, GB and KPF differed between seasons. Harvest date for both seasons was based on the commercial harvest. The target TSS for Shiraz, at this price point in this region, is between 23 and 24 °Brix. In Y2, average TSS at harvest, was higher than in Y1, despite being harvested two weeks earlier. Some of the key drivers of soluble solids accumulation are light, temperature and available water (Dai et al., 2011; Sadras & Petrie, 2011). Sadras and Petrie (2011) found that following the onset of ripening the accumulation of TSS versus thermal time approximates a linear-plateau function. In Y2 of this research, TSS continued to rise with no evidence of a plateau. Others have also found instances where this relationship does not hold (Cooley et al., 2017).

Differences in the onset of ripening combined with severe water stress is likely to have resulted the vines reaching target TSS earlier in Y2 compared with Y1, and hence the earlier harvest date in Y2. In Y2 accumulation of sugars to the berries was adversely affected by water stress. Therefore, water inputs in Y2 has reduced sugar accumulation but also reduced berry weight (discussed in detail in Chapter 3). In addition, the difference in berry size has contributed to seasonal and treatment differences in C_b and P_b.

4.4.1. Irrigation Treatments

Lower yield, yield components (Section 3.3.1 of Chapter 3) and SSB (Figure 4.1) indicate that less sugar was translocated to the berries when less irrigation was applied. This may be attributed to a decrease in assimilation due to water stress, as a result of low available water (cumulative rainfall and irrigation). This is supported by lower SSV observed in Y2 (Figure 4.2), when less water applied; with a similar trend approached conventional levels of significances in Y1 (Figure 4.2). In Y1 and Y2, irrigation treatments had no effect on berry number per cluster or cluster number per vine therefore differences in SSV are likely related to differences in berry weight. This supports the findings of Considine (2004), who found that yield of sugar per vine (equivalent of soluble solids per vine; SSV) was related to berry volume (indicated by berry weigh in this research).

The differences in SSB between the irrigation treatments were established early in the season, with the accumulation of sugar in the berries increasing at a similar rate between véraison and harvest. This indicates that the sensitivity to differences in irrigation water received was greater pre-véraison. The decrease in SSB observed for I_{25} and I_{50} compared with I_{100} reflected the decrease in berry weight evidenced in Chapter 3, with both berry weight and SSB 11% lower for I_{25} and I_{50} compared with I_{100}. Similar proportional decreases in berry weight and SSB

resulted in no impact on TSS. The effects of irrigation were limited to pre-véraison in Y1 due to high rainfall.

Water stress has been linked to earlier ripening in grapevines, with Freeman et al. (1980). They proposed that this may be due to a reduction in vegetative growth with minimal impacts on assimilation, leading to more sugar being partitioned to the fruit. However, in this research, higher TSS and lower SSB were observed, indicating that there has been a reduction in assimilation and/or translocation of sugars to the berries. Lower average berry weight, lower SSB and higher TSS observed in Y1 indicate that the reduction in SSB is less than the reduction in berry size.

In Y1, differences between the irrigation treatments were established at véraison. High rainfall resulted in minimal differences in total water received (irrigation and rainfall) between the irrigation treatments. This may explain why irrigation had no effect on other composition parameters measured in Y1, with all plots receiving enough water such that composition was not affected. In contrast, in Y2 when available water was likely a limiting factor to all treatments, irrigation influenced berry composition more substantially. The decrease in SSB observed for I_{25} compared with I_{100} reflected the decrease in berry weight evidenced in Chapter 3, with a 26% lower berry weight and a 27% lower SSB for I_{25} compared with I_{100}. Therefore, a reduction in berry water content indicated by the higher $B_{\%DW}$ for I_{25} and I_{50} compared with I_{100} has caused the increase in TSS. This change in water content may have been driven by changes in plant water status, with I_{25} and I_{50} plots being under greater water stress than I_{100}. McCarthy (1997) found that Shiraz was most susceptible to water deficits after flowering, with only minor differences in berry weight for water deficits after harvest. Furthermore, McCarthy (1997) found Shiraz to be insensitive to water deficits in the month before harvest. Therefore, it is likely the changes observed due to irrigation were established early in berry development in both years, and that high rainfall prior to harvest has not reversed the impacts of the early season deficits.

Berries are comprised of three major types of tissue (skin, flesh and seeds), with flesh making up the bulk of wine (Kennedy, 2002), at approximately 80% for well-watered Cabernet Sauvignon vines (Roby & Matthews, 2004). Natural variation in berry size limits the ability to use berry size as a factor for determining solute concentration of juice (Roby & Matthews, 2004). In research conducted by Roby and Matthews (2004), seeds accounted for a greater proportion of whole-berry fresh weight across most berry sizes. Late season deficit also increased the proportion of skin (Roby & Matthews, 2004). Results for parameters reported on a per berry basis (SSB, C_b and P_b) trend with berry weight results reported in Chapter 3, thus

are a greater reflection of berry size. Whilst, the reduction in water content and berry size for I_{25} and I_{50} has contributed to the increase in observed C_g and P_g (Figure 4.5 and Figure 4.6). Smaller berries have a larger skin surface area to volume ratio (Kennedy, 2002; Singleton, 1972), contributing to an increase in C_g. However, overall there is less skin on the smaller berries and therefore there is a reduction in C_b for I_{25} and I_{50} (Figure 4.5). Skin and seeds make up a larger portion of smaller berries thus contributing to the increase in P_g for I_{25} and I_{50}, whilst the lack of difference in P_b (Figure 4.6) may be attributed to the timing of the bulk of tannin synthesis, which occurs prior to véraison (Downey et al., 2003).

4.4.2. Glycine Betaine

Higher TSS observed in Y1 (Figure 4.7) may be an indication that GB has resulted in earlier ripening. Although visual observations at the time indicated that there was no impact of GB on véraison. In contrast in Y2, whilst there was an overall significant effect of GB on TSS, the treatments converged over time with no significant effect evident from the third sample date to harvest. It appears that the effects of GB became less evident as a result of low rainfall and little supplementary rainfall following véraison resulting in all treatments becoming water stressed. Abscisic acid (ABA) is a plant hormone associated with grapevine responses to stress. However, it has also been proposed that it plays a role in berry ripening (Wheeler, 2007) in nonclimacteric fruit, such as grapes (Gambetta et al., 2010). In research conducted by Wheeler (2007), ABA levels of Cabernet Sauvignon vines dramatically increased at véraison. Gambetta et al. (2010) identified several novel candidates (genes) involved in the control of nonclimacteric ripening and they also found that some of the patterns of expression correlated with sugar and ABA accumulation during ripening. Increased levels of GB have been observed in response to exogenous ABA application for *Arabidopsis thaliana* (Xing & Rajashekar, 2001) and strawberries (Rajashekar et al., 1999). Therefore, the application of GB may directly or indirectly be involved in one or more of the processes associated with nonclimacteric ripening.

In Y1 of this research, GB reduced number of berries per cluster (refer to Section 3.3.2 of Chapter 3) and SSV (Section 4.3.2), with no observed change in cluster number per vine (refer to Section 3.3.2 of Chapter). In Y2, GB significantly reduced leaf area (refer to Section 5.4.2 of Chapter 5) with no observed change in SSV (Section 4.3.2) or berry number per cluster (Section 3.3.2 of Chapter 3). These results support the findings of Considine (2004), yield of sugar per vine was found to be related to total berry numbers, rather than vigour. However, it should be noted that a measure of vine vigour was not able to be assessed in Y1 to confirm this observation.

At harvest in Y1, GB reduced berry weight by 9% (refer to Chapter 3). This reduction in berry size accounts for the reduction in colour (C_b) and phenolics (P_b) per berry (Section 4.3.2). In Y2, there was an overall effect of GB on berry weight; however, pair-wise comparison shows that the change in berry weight was not significant at harvest. This could explain why GB had no effect on in C_b and P_b as well as C_g and P_g at harvest in Y2. Smaller berries have a larger skin surface area to pulp ratio and seeds may potentially account for a higher proportion of berry weight (Kennedy, 2002), thus potentially influencing C_g and P_g concentrations. However, in this research, the change in berry size was not sufficient to alter C_g or P_g. In research conducted by Walker et al. (2005), improved colour was only observed in their smallest category of berries (0.3 to 0.55 g). Therefore, the larger berry size observed for all treatments in Y1 (1.14 to 2.08 g; Table 3.2) may have contributed to the lack of difference. Furthermore, as discussed in Section 4.4.1 changes in seed number and size also influence berry composition (Roby & Matthews, 2004).

In Y1 and Y2, GB had no effect on $B_{\%DW}$ (Section 4.3.2) indicating that the berries had a similar proportion of water. This further explains why there was no difference in C_g or P_g and indicates that the organic acid content at harvest is proportionate to berry size, hence there was no difference in berry juice pH or TA. Overall, this research supports the findings of Roby and Matthews (2004), who found that berry size played a limited role in final solute concentration of juice (discussed in Section 4.4.1).

This research indicated that there is no economic benefit from applying GB under the conditions observed. Instead, lower yields in Y1 (refer to Chapter 3) and no change to acidity, colour or phenolics is more likely to have resulted in a reduction in economic return from sale of fruit to a winery. Additionally, GB resulted in earlier ripening in Y1, with 0.5 °Brix higher TSS at harvest for GB^+ compared with GB^-. Therefore, if harvest date were to be selected based on a target TSS, then plots with GB applied may need to be harvested earlier than areas without GB applied. Warming temperature have already resulted in trend towards earlier harvest (Webb et al., 2011), thus the potential compression of the growing season to create processing bottlenecks should also be considered. While earlier ripening may not be desirable for wine grapes, there is the potential for higher prices to be received for table grapes that are harvested earlier (Shahak et al., 2008). However, any increase in price would need to be enough to offset any reduction in yield.

4.4.3. Kaolin Particle Film

Water supply, irrigation and rainfall, influenced the vines' response to KPF. Under high rainfall conditions, there was no significant effect of KPF on the measured grape composition parameters. At the time, there was no visual evidence of rainfall washing KPF off the canopy. Although it is possible that whilst the canopy still appeared white some of the coat may have been washed off. This may have contributed to the lack of significant differences due to KPF in Y1. In contrast in Y2, under low rainfall conditions with minimal supplementary irrigation, KPF increased TSS for all sample dates. The increase in TSS observed in Y2 was related to lower water content in the berries, indicated by no difference in SSB and by higher $B_{\%DW}$ observed for KPF^+ at harvest (Section 4.3.3).

Grape cultivar was shown to play a role in grapevine responses to KPF (Glenn et al., 2010). It is possible that this variation is driven by differences the in cultivars' ability to maintain stomatal conductance. Shiraz tends to maintain stomatal conductance more than other varieties (Schultz, 2003), which may mean it is more susceptible to water loss. In research conducted by Glenn et al. (2001), higher water use was reported as a result of an increase in crop load, fruit weight and leaf conductance (g_l). Those authors to concluded that irrigation scheduling may need to be altered to ensure adequate supply of water. In Y2, water inputs (irrigation and rainfall) were not sufficient to meet the crop water requirement with the vines showing signs of severe water stress (refer to Chapter 5). Therefore, when KPF was applied at véraison it is possible that there was an overall increase in g_l, resulting in higher loss of water through transpiration leading to water stress. While, on average, temperatures were warmer than historic averages (Table 2.1), milder maximum temperatures (Table 2.2) may have contributed to this response. This supports the findings of Shellie (2015), who proposed that the effectiveness of KPF may be affected by the onset of cooler ambient temperatures. This response would have been dominant in berries where the water content was lower thus reducing berry fresh weight while increasing TSS.

In Y2, there was a significant interaction of irrigation treatments and KPF on SSV, with the combination of I_{25} and KPF^+ resulting in the lowest SSV and the combination of I_{100} and KPF^- highest SSV (Figure 4.15). Similar observations were observed for cluster weight and yield, further supporting the relationship between SSV and yield components observed by Considine (2004). Analysis of SSB conducted using repeated measures analysis of variance (rANOVA), revealed a similar response for the interaction of irrigation treatments and KPF on overall average SSB in Y1 (Figure 4.16). Additionally, means separation of this data indicated that the combination of I_{25} and KPF^+ significantly reduced overall average SSB compared to I_{25} and KPF^-.

Therefore, this coupled with other observed impacts of the interaction of irrigation treatments and KPF (refer to Table 3.1 of Chapter 3 and Table 4.1) support the recommendations made by Glenn et al. (2001).

The optimum temperature for field-grown grapevines is between 25 and 35 °C (Mullins et al., 1992). Processes, such as photosynthetic activity, are influenced by factors including temperature, light and water availability (Spencer & Peynaud, 1984). Factors, such as cultivar, soil, environmental conditions (i.e. wind speed and humidity) and management practices, have direct and indirect effects on canopy microclimate (Smart, 1985), influencing optimum temperatures for processes, such as photosynthesis. KPF is reported to reduce canopy temperatures (Glenn et al., 1999; Sharma et al., 2015). At ambient temperatures below optimum, a reduction in canopy temperature will reduce net photosynthesis (Medlyn et al., 2002), whilst at ambient temperature above optimum a reduction in canopy temperature increase photosynthesis (Medlyn et al., 2002; Sage & Kubien, 2007) assuming all other inputs are maintained at optimal levels. Therefore, the environmental conditions during the field experiment may have influenced the vines' response to KPF. This will be explored further in Chapter 5 of this thesis.

KPF was applied at véraison, therefore berry growth, after KPF was applied, was driven by the flow of phloem sap to berry as flow of xylem sap into the berry becomes impeded following véraison (Coombe & McCarthy, 2000). In research conducted on the phloem transport system of Douglas-fir trees [*Pseudotsuga menziesii* (Mirb.) Franco], water stress was found to increase phloem glucose and fructose content with no significant effect on sucrose content (Woodruff, 2013). Furthermore, as leaf water potential (ψ_{LEAF}) declines, sieve cell water content also declined leading to a significant increase in sugar molar concentration of the phloem (Woodruff, 2013). In Y2 of this research, applying KPF increased $B_{\%DW}$ and TSS with no change in SSB. Given KPF was applied at véraison, the results presented here combined with those results observed by Wood et al. (2008), supports the theory that applying KPF resulted in increased water stress. Irrigation scheduling may need to be altered when KPF is applied to account for higher water use as recommended by Glenn et al. (2001).

4.4.4. Glycine Betaine and Kaolin Particle Film

There was no significant interaction of GB and KPF on composition parameters measured in Y1 or Y2 (Table 4.1 and Table 4.2). In Y1, there was a significant impact of the interaction of GB and KPF on cluster weight, with the combination of both products resulting in the lowest cluster weight. This indicates that the combined effects of GB and KPF are complex, and that

there is more than just a change in the accumulation of sugar in the berries contributing to the reduction in cluster weight in Y1, when both products are applied. Therefore, the next chapter investigate the impacts of the irrigation treatments, GB and KPF on leaf water potential (ψ_{LEAF}), g_l and canopy growth to further assess this interaction.

4.5. Grape Composition Conclusion

In Y1, early season differences in irrigation water applied and application of GB has reduced SSB and berry weight, with no effect on other compositional parameters measured. In contrast, in Y2 when there was very little rainfall and low supplementary irrigation, all plots became water stressed and the effects of GB became less evident. Similarly, the effect of applying KPF was also influenced by water supply, with KPF increasing TSS and $B_{\%DW}$ under the water stressed conditions in Y2. These observed changes in composition are unlikely to have a positive effect on price paid by wineries. Therefore, will not compensate for any of the yield reductions reported in Chapter 3. It is important to consider that other composition parameters that were not measured in this research may potentially influence the price paid by wineries.

Chapter 5. Impact of Irrigation, Glycine Betaine and Kaolin Particle Film on Canopy Growth, Plant Water Status and Canopy Temperature

5.1. Introduction

This Chapter reports and discusses measurements of canopy growth, plant water status and canopy temperature taken in Y2. Measurements made in Y1 have been excluded from the thesis, as they were unable to be analysed statistically. On several occasions, measurements were stopped due to changes in the consistency of cloud cover. Whilst, on other occasions, it became apparent that the data collection duration and prolonged cloud impacted on the measurements causing unacceptable variability across the data. In Y2, measurements of plant water status and canopy temperatures were conducted on 3 replicates (blocks 1 to 3) to avoid repetition of these issues.

5.2. Materials and Methods

The experiment site, planting material, experimental design, implementation of treatments, sampling procedures and statistical analysis are described in Chapter 2.

Plant water status (Section 5.2.2) and canopy temperature (Section 5.2.3) measurements were taken on two cloudless days during the 2012-13 growing season (Y2) in three replicates (blocks 1 to 3 of the experimental design described in Chapter 2). The first set of measurements were taken on the 7th February 2013 (day of year (DOY) = 38, Y2), 4 days after an irrigation event and 24 h before the next irrigation event, herein referred to as pre-irrigation. The second set of measurements were taken on 14th of February 2013 (DOY = 45, Y2), 24 h after an irrigation event, herein referred to as post-irrigation. The measurements were conducted at E-L growth stage (Coombe, 1995) 36 to 37 for both measurement dates (between véraison and harvest). Measurements of leaf characteristics were taken post-irrigation (Section 5.2.1).

5.2.1. Leaf Characteristics

Post-irrigation (DOY =45, Y2), the newest fully expanded leaf was collected from four randomly selected canes from the northern sample vine in three replicates (blocks 1 to 3 of the experimental design described in Chapter 2). The four leaves from each plot were scanned, and leaf area was analysed using a computer software package (MATLAB 2012b; The MathWorks Inc; Natick, Massachusetts, USA) to determine average leaf size (L_S, cm^2). Two of the leaves were then placed into small paper bags and weighed on a two decimal point

balance, to provide leaf fresh weight (L_{FW}, g) before being placed in an oven at 65 °C. The leaves were then weighed on a two decimal point balance until constant weight was achieved between hourly measurements to determine leaf dry weigh (L_{DW}, g). Leaf percent dry weight ($L_{\%DW}$) was determined from the measurements L_{FW} and L_{DW}, using Equation 5.1.

$$L_{\%DW} = \frac{L_{DW}}{L_{FW}} \times 100 \quad \text{(Equation 5.1)}$$

Specific leaf area (SLA, $cm^2 \cdot g^{-1}$) was determined from the measurements of L_s and L_{DW}, using Equation 5.2.

$$SLA = \frac{L_S}{L_{DW}} \quad \text{(Equation 5.2)}$$

5.2.2. Plant Water Status

Leaf Water Potential

Measurements of ψ_{LEAF} were taken before the sun crossed the horizon and between 1100 and 1300 h AEDT, herein referred to as pre-dawn (ψ_{PD}, MPa) and midday (ψ_{MID}, MPa), respectively. For each replicate, measurements were conducted on two randomly selected fully expanded leaves from the northern sample vine in each plot. Selected leaves were excised from the vine and immediately placed into zip lock plastic bags. The petioles were recut prior to measurements, which were conducted within 60 sec of the leaf's removal from the vine.

Leaf Conductance

At midday, g_l was measured using a porometer (SC-1; Decagon Devices Inc; Pullman, WA, USA). The selected leaves were undamaged and ideally of similar age and size, which was achieved by selecting the fifth fully expanded leaf from two random canes from the northern sample vine in each plot. Larger veins on the leaves were avoided when inserting the leaf into the porometer cup. The porometer was calibrated prior to conducting the measurements.

5.2.3. Canopy Temperature

Infrared Thermal Acquisition

Infrared thermal images (IRTI) were taken using an infrared camera (T640; FLIR® Systems Inc; Wilsonville, Oregon, USA) with a resolution of 640 x 480 pixels and an on-board visual camera with a resolution of 1944 x 2592 pixels. The camera had a measurement range of -40 to 2,000 °C with a thermal sensitivity of 0.04 °C (40 mK) at 30 °C. Images were taken using a 45° lens with a spatial resolution of 1.23 milliradians. IRTI were taken of the vertical canopy plane at a constant distance of 3 m from the canopy and perpendicular to the row direction, for both the

eastern and western sides of the canopy. A single IRTI may be represented as a matrix [$IR_{(m,n)}$; Equation 5.3] with each pixel considered to be an individual temperature (T) measurement in degrees Celsius (°C). Temperature matrices were extracted using a software program (FLIR Tools Version 5.3.15420.1002; FLIR® Systems Inc; Wilsonville, Oregon, USA) and saved as a CSV file.

Infrared Thermal Image Processing

IRTI contain non-relevant areas including areas of sky, soil, grass or non-relevant plant material (Figure 5.1), which needs to be excluded from IRTI analysis (Fuentes et al., 2012). IRTI were analysed and processed using a computer software package (MATLAB R2012b; The MathWorks Inc; Natick, Massachusetts, USA), using custom code developed by Fuentes et al. (2012).

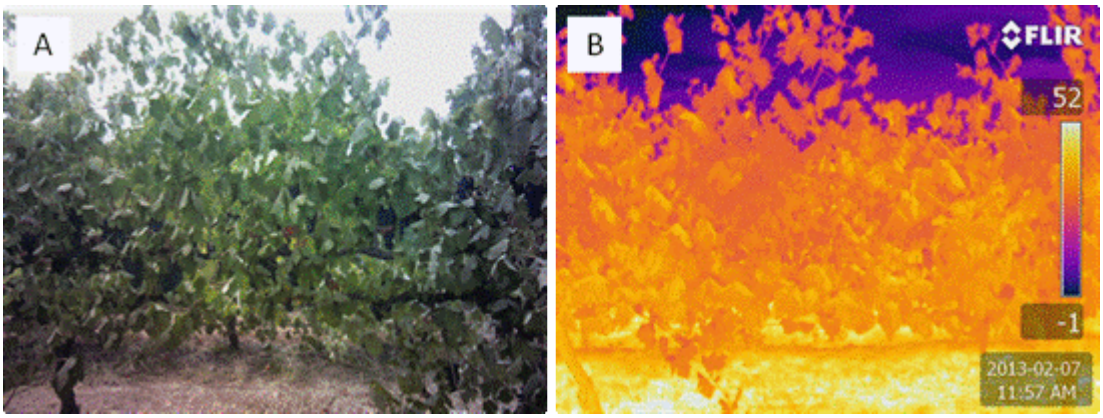


Figure 5.1. (A) Example of a digital image of a grapevine canopy; (B) corresponding infrared thermal image.

The MATLAB code, used within canopy variability in temperatures to mask the images based on manual selection of the canopy minimum (C_{min}) and maximum (C_{max}) temperatures (Fuentes et al., 2012). Regions of sky and cloud cover have no reflection of infrared radiation, thus were successfully masked by C_{min} (Fuentes et al., 2012). C_{max} was determined to mask the ground and other non-relevant areas, such as posts, that are hotter than vine leaves. This was completed using the "thresh_tool.m" graphical user interface (Figure 5.2) developed by Bermis (2005) built into the custom MATLAB code developed by Fuentes et al. (2012). Selection of C_{min} and C_{max} was assisted by a binary image (refer to image titled 'Segmented' in Figure 5.2), which indicated areas higher than the temperature selection bar as white and areas lower than the temperature selection bar as black.

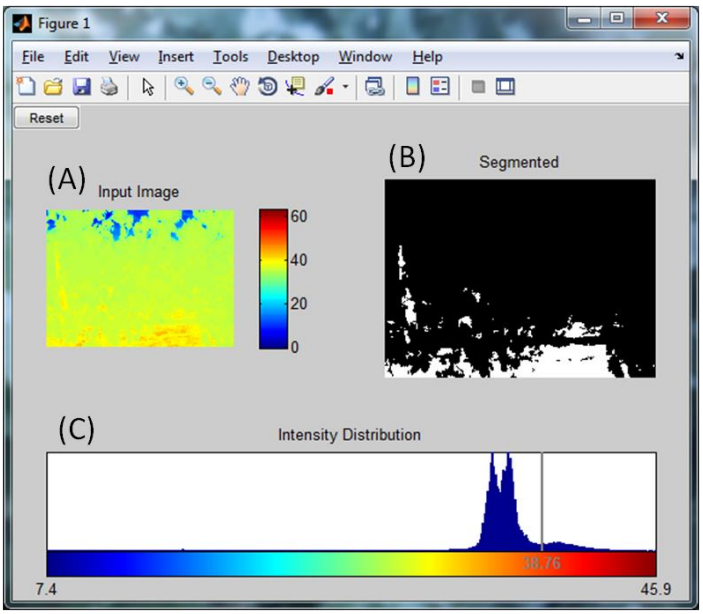


Figure 5.2. The graphical user interface "thresh_tool.m" (Bermis, 2005) used to mask non-leaf material from each image. (A) Original image being processed; (B) binary image with temperature above the temperature slider appearing white; and (C) temperature distribution for the image.

Using the values of C_{min} and C_{max} obtained from the "thresh_tool.m" graphical user interface, a simple filter rule was applied to each image to obtain a filtered matrix (IR_{if}) using Equation 5.4.

$$IR_{if} = C_{max} \geq IR_{(m,n)} \geq C_{min}; \left(IR_{(m,n)} \neq IR_{if}\right) \notin IR_{if} \quad \text{(Equation 5.4)}$$

IR_{if} was obtained by replacing all values in $IR_{(m,n)}$ below C_{min} with "0" and above C_{max} with "0", using Equations 5.5a and 5.5b, after which $IR_{if} = IR_{(m,n)}$.

$$IR_{(m,n)}\left(IR_{(m,n)} \geq C_{max}\right) = 0 \quad \text{(Equation 5.5a)}$$

$$IR_{(m,n)}\left(IR_{(m,n)} \leq C_{min}\right) = 0 \quad \text{(Equation 5.5b)}$$

This enabled data, such as the mean and standard deviation, to be calculated using a "0" exclusion sub-set using Equation 5.6.

$$IR_{if}\left(IR_{if} \cong 0\right) \quad \text{(Equation 5.6)}$$

Analysis of Whole Images

IRTI of the eastern and western side of the vine canopy were analysed for the northern sample vine for each plot in blocks 1 to 3. Whole canopy average canopy temperature (T_C) and whole canopy standard deviation (T_{SD}) were determined using the "0" exclusion set for each of the IRTI.

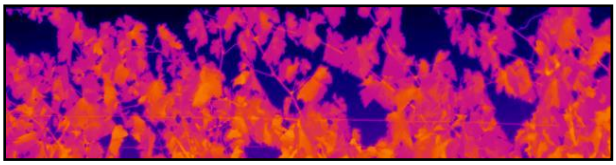
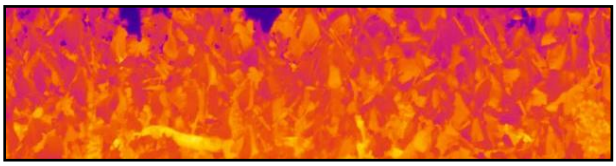
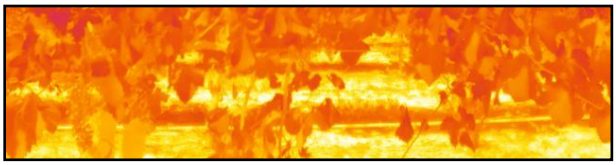
Ambient air temperature (T_A) data was obtained from the weather station (Section 2.2.1 of Chapter 2). The T_A at time each IRTI was captured was estimated based on the assumption that changes in ambient temperature were linear between each of the 10 min measurements. The difference between canopy temperature and ambient temperature was estimated using Equation 5.7.

$$T_C^* = T_C - T_A \qquad \text{(Equation 5.7)}$$

Analysis of Canopy Subsections

In addition to analysis of the whole IRTI for the eastern and western sides of the canopy, analysis was conducted on three sections (top, middle and bottom of the image), to assess differences in canopy temperature and variability in canopy temperature between the three sections of the canopy. IRTI were masked using the values of C_{min} and C_{max} obtained for each image during the whole image analysis (see below) and the masked images were then cropped into three sections based on pixel number (Table 5.1).

Table 5.1. Division of infrared thermal image matrices [$IR_{(m,n)}$] of temperatures (T, °C) of the eastern and western side of the canopy into three sections top (IR_{TOP}), middle (IR_{MID}) and bottom (IR_{BOT}).

$IR_{(m,n)}$ Subdivision	**Example Subsection of Infrared Thermal Image**
$IR_{TOP} = \begin{Bmatrix} T_{1,1} & \cdots & T_{1,n} \\ \vdots & \ddots & \vdots \\ T_{\frac{m}{3},1} & \cdots & T_{\frac{m}{3},n} \end{Bmatrix}$	
$IR_{MID} = \begin{Bmatrix} T_{\frac{m}{3},1} & \cdots & T_{\frac{m}{3},n} \\ \vdots & \ddots & \vdots \\ T_{2\left(\frac{m}{3}\right),1} & \cdots & T_{2\left(\frac{m}{3}\right),n} \end{Bmatrix}$	
$IR_{BOT} = \begin{Bmatrix} T_{2\left(\frac{m}{3}\right),1} & \cdots & T_{2\left(\frac{m}{3}\right),n} \\ \vdots & \ddots & \vdots \\ T_{m,1} & \cdots & T_{m,n} \end{Bmatrix}$	

Average canopy temperature (T_C), canopy temperature standard deviation (T_{SD}), difference between average canopy temperature and ambient temperature (T_C*), minimum canopy temperature (T_{MIN}) and maximum canopy temperature (T_{MAX}) were then calculated for each of the cropped section of the images using custom generated code by Fuentes et al. (2012) as per the whole image analysis above.

Minimum canopy temperature (T_{MIN}) and maximum canopy temperature (T_{MAX}) were determined for each the subsections. The image processing method applied in this research utilised manually C_{min} and C_{max}, with C_{min} selected to mask sky from the top of the image and C_{max} selected to mask the ground and other non-relevant areas, such as posts, from the bottom of the image. Therefore, T_{MIN} was only presented for the bottom and middle subsections of the canopy and T_{MAX} was only presented for the top and middle subsections of the canopy.

5.2.4. Statistical Analysis

Methodology for assessing treatment effects on individual response variables is described in Section 2.7 of Chapter 2.

Non-linear associations between response variables were determined using a computer software package (MATLAB R2012b; The MathWorks Inc; Natick, Massachusetts, USA). The model of best fit and associated parameters was determined from the Curve Fitting Toolbox ("cftool") using non-linear least squares minimisation with the Trust-Region algorithm. The sum of squared errors (SSE) was used to select the model of best fit.

5.3. Justification and Literature for Methodology

This section provides justification and literature related to the plant water status and canopy temperature measurements. Leaf characteristics, plant water status and canopy temperature results are in Section 5.4 (after this section).

5.3.1. Analysis of Plant Water Status

Various measurements of plant water status may be used to assess the effects of differing water supply on a plant (Jones, 2006). Since the development of the pressure bomb (Scholander et al., 1965), leaf water potential (ψ_{LEAF}, MPa) has become common method used in the literature to measure plant water status (Jones, 1990). ψ_{LEAF} can be measured using a pressure chamber, which measures of the pressure required to extract water from the petiole of a leaf, (Scholander et al., 1965); the more pressure required the lower (more negative) the value of ψ_{LEAF} and the greater the stress (Scholander et al., 1965).

Stem water potential (ψ_{STEM}), pre-dawn leaf water potential (ψ_{PD}) and midday leaf water potential (ψ_{MID}) are three measures of plant water status which may be conducted using a pressure bomb (Williams & Araujo, 2002). ψ_{STEM} (also referred to as xylem stem water potential; Jones, 1990) is determined by first covering the leaves in plastic and foil, preventing transpiration, allowing the leaf to equilibrate with the water potential of the stem (Williams & Araujo, 2002). ψ_{PD} is a measure of the leaf water potential before the sun has crossed the

horizon, which said to be in equilibrium with soil water potential (Williams & Araujo, 2002). ψ_{MID} is a measure of leaf water potential at (or as close as possible) to solar noon. In the research conducted by Williams and Araujo (2002), found that ψ_{STEM}, ψ_{PD} and ψ_{MID} were highly correlated for Chardonnay and Cabernet Sauvignon vines. Furthermore, ψ_{STEM}, ψ_{PD} and ψ_{MID} were linearly correlated with carbon dioxide assimilation and stomatal conductance, leading Williams and Araujo (2002) all three measures as viable methods for assessing plant water status in Chardonnay and Cabernet Sauvignon vines.

In this research, ψ_{PD}, ψ_{MID} and leaf conductance (g_l) were chosen as measures of plant water status. Analysis of ψ_{PD} and ψ_{MID} has assisted in comparing the results observed in this research to literature on the effects of KPF other grape varieties, including Viognier (Shellie & Glenn, 2006; Shellie & Glenn, 2008), Merlot (Ou et al., 2010; Shellie, 2015; Shellie & Glenn, 2006; Shellie & Glenn, 2008), Cabernet Sauvignon (Shellie & King, 2013b) and Malbec (Shellie & King, 2013b). Shellie (2006) proposed the ψ_{MID} may enable comparison of vine and berry response to water stress to be conducted independent of growing region. Leaf conductance (g_l) is a measure of stomatal aperture (Jarvis, 1976), which is an indirect measure of the physiological response of plants to water deficits (Jones, 2006). Many photosynthetic parameters were found to be highly correlated with stomatal conductance in C3 plants including grapevines (Medrano et al., 2002).

5.3.2. Extraction of Canopy Temperature from Infrared Thermal Images

Wheaton et al. (2011) compared estimates of canopy temperature that were extracted using three different methods: rapid method (RM), precise method (PM) and auto-method (AM). Each of the methods selected subsection(s) of the image to estimate canopy temperature. Firstly, the RM manually selected a rectangular area containing only canopy (Wheaton et al., 2011). The PM manually selected areas representing six individual (Wheaton et al., 2011). Whilst, the AM was based on image registration (Yang et al., 2009) with the digital image and temperature extraction algorithms (Wang et al., 2010) based on colour identification of light green leaves using Gaussian colour thresholds. Simple linear regression of the three methods (RM, PM and AM; Figure 5.3) revealed strong linear relationships with high coefficients of determination ($R^2 > 0.99$), supporting the use of any of the three methods to estimate canopy temperature.

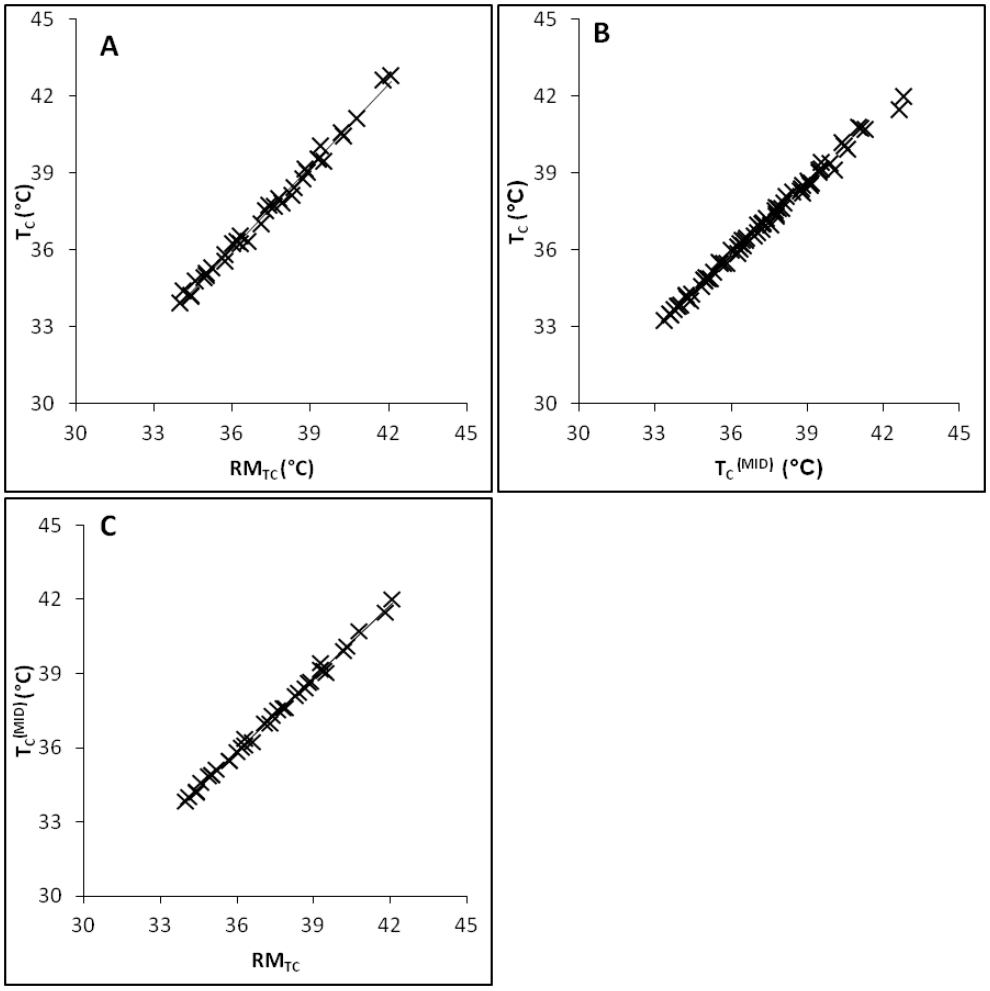


Figure 5.3. Comparison of two semi-automated method (SAM) estimates of canopy temperature (T_C, $T_C^{(MID)}$) and one manual method estimate of canopy temperature (RM_{TC}). (A) T_C and RM_{TC} ($p < 0.001$; $T_C = 1.06 \cdot RM_{TC} - 2.24$; $R^2 = 0.99$; n = 36); (B) T_C and $T_C^{(MID)}$ ($p < 0.001$; $T_C = 0.93 \cdot T_C^{(MID)} + 2.16$; $R^2 = 0.99$; n = 36); and (C) $T_C^{(MID)}$ and RM_{TC} ($p < 0.001$; $T_C = 0.99 \cdot RM_{TC} + 0.36$; $R^2 = 0.99$; n = 36).

This research presented in this thesis used a semi-automated method (SAM) to estimate whole canopy temperature (T_C) and temperature of the middle subsection of the canopy ($T_C^{(MID)}$). Comparison of the estimates from the SAM method and the estimates from the RM was conducted on a subsection (n = 36) of infrared thermal images. A rectangular area of canopy was selected using a computer program [FLIR Tools (version 5.9.16284.1001); FLIR® Systems Inc; Wilsonville, Oregon, USA] interface to obtain average canopy temperature (RM_{TC}). Estimates of T_C and $T_C^{(MID)}$ were obtained using the SAM. Simple linear regression was used to compare canopy temperature estimates (RM_{TC}, Tc and $T_C^{(MID)}$). Simple linear regression between RM_{TC}, T_C and $T_C^{(MID)}$ revealed strong linear relationships with high coefficients of determination between each pair of estimates ($R^2 > 0.99$; Figure 5.3). This supports the use of SAM in this research.

5.4. Plant Water Status, Canopy Temperature and Canopy Growth Results

A summary, of the significant effects of irrigation, GB, KPF and their associated interactions on canopy growth is provided in Table 5.2.

Table 5.2. Significance of the p values for trunk cross-sectional area and leaf characteristic measurements from the general linear analysis of variance (gANOVA) with irrigation treatments (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

	ΔTCA (cm^2)	**Leaf Characteristics**				
		L_S (cm^2)	**L_{FW} (g)**	**L_{DW} (g)**	**$L_{\%DW}$**	**SLA ($cm^2 \cdot g^{-1}$)**
I	ns	ns	ns	ns	ns	ns
GB	ns	0.018 *	ns	ns	ns	ns
KPF	0.026 *	ns	ns	ns	ns	ns
I x GB	ns	ns	ns	ns	ns	ns
I x KPF	ns	ns	ns	ns	ns	ns
GB x KPF	< 0.001 ***	ns	ns	ns	0.018 *	0.046 *
I x GB x KPF	ns	ns	ns	ns	ns	ns

Table 5.3 and Table 5.4 provided a summary of the significant effects of irrigation, GB, KPF and their associated interactions on pre-dawn leaf water potential (ψ_{PD}; Table 5.3), midday leaf water potential (ψ_{MID}; Table 5.3), average canopy temperature (T_C; Table 5.3), canopy and air temperature difference (T_C*; Table 5.3), maximum canopy temperature (T_{MAX}; Table 5.4) and minimum canopy temperature (T_{MIN}; Table 5.4) measured pre- and post-irrigation in the 2012-13 growing season (Y2).

Table 5.3. Significance of the p values for plant water status and canopy temperature measurements measured pre- and post-irrigation in the 2012-13 growing season (Y2) from the general linear analysis of variance (gANOVA) with irrigation treatments (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

	ψ_{PD} (MPa)	ψ_{MID} (MPa)	g_l (mmol·m^{-2}·s^{-1})	Western Side of the Canopy			Eastern Side of the Canopy		
				T_C (°C)	T_C* (°C)	T_{SD} (°C)	T_C (°C)	T_C* (°C)	T_{SD} (°C)
Pre-Irrigation (DOY = 38, Y2)									
I	ns	ns	‡	0.021 *	ns	ns	0.036 *	ns	ns
GB	ns	0.028 *	‡	0.048 *	ns	ns	ns	ns	ns
KPF	ns	ns	‡	0.047 *	ns	ns	ns	ns	ns
I x GB	ns	ns	‡	ns	ns	ns	ns	ns	ns
I x KPF	ns	ns	‡	ns	ns	ns	ns	ns	ns
GB x KPF	ns	ns	‡	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	‡	ns	ns	ns	ns	ns	ns
Post-Irrigation (DOY = 45, Y2)									
I	< 0.001 ***	< 0.001 ***	ns	0.013 *	0.021 *	ns	< 0.001 ***	0.004 **	ns
GB	ns	ns	ns	ns	ns	ns	ns	ns	ns
KPF	ns	ns	< 0.001 ***	ns	ns	ns	ns	ns	ns
I x GB	ns	ns	ns	ns	ns	ns	ns	0.015 *	ns
I x KPF	0.018 *	ns	ns	ns	ns	ns	ns	ns	ns
GB x KPF	ns	0.049 *	ns	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns	ns

‡ Data not recorded

Table 5.4. Significance of the p values for minimum and maximum canopy temperature measurements measured pre- and post-irrigation in the 2012-13 growing season (Y2) from the general linear analysis of variance (gANOVA) with irrigation treatments (I), glycine betaine (GB) and kaolin particle film (KPF) as the fixed model (treatment) terms and block as the random model term.

	Western Side of the Canopy				Eastern Side of the Canopy			
	T_{MIN} (°C)		T_{MAX} (°C)		T_{MIN} (°C)		T_{MAX} (°C)	
	Bottom	**Middle**	**Middle**	**Top**	**Bottom**	**Middle**	**Middle**	**Top**
Pre-Irrigation (DOY = 38, Y2)								
I	0.048 *	0.046 *	ns	ns	ns	ns	0.040 *	ns
GB	0.046	ns	ns	ns	ns	ns	ns	ns
KPF	0.010	0.048	ns	ns	ns	ns	0.031	ns
I x GB	ns	ns	ns	ns	ns	ns	ns	ns
I x KPF	ns	ns	ns	ns	ns	ns	ns	ns
GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns
Post-Irrigation (DOY = 45, Y2)								
I	0.048 *	0.010 **	0.037 *	ns	0.004 **	0.005 **	< 0.001 ***	0.002 **
GB	ns	ns	ns	ns	ns	ns	ns	ns
KPF	ns	ns	0.015 *	0.009 **	ns	ns	ns	ns
I x GB	ns	ns	ns	ns	ns	ns	ns	ns
I x KPF	ns	ns	ns	ns	ns	ns	ns	ns
GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns
I x GB x KPF	ns	ns	ns	ns	ns	ns	ns	ns

More negative values of ψ_{PD} and ψ_{MID} were observed pre-irrigation compared to post-irrigation (Table 5.5). Similar daily minimum temperatures were observed for both measurement days. However, higher daily maximum temperatures were observed pre-irrigation compared with post-irrigation, with daily maximum reached between 1600 and 1700 h AEDT on both measurement days.

Table 5.5. Minimum and maximum daily temperature, pre-dawn leaf water potential (ψ_{PD}, MPa) and midday leaf water potential (ψ_{MID}, MPa) measured pre- and post-irrigation during the 2012-13 growing season (Y2). DOY = day of year.

	DOY, Y2	Temp (°C)		ψ_{PD} (MPa)		ψ_{MID} (MPa)	
		Min	Max	Min	Max	Min	Max
Pre-irrigation	38	21.5	39.1	-1.52	-1.08	-2.06	-1.58
Post-irrigation	45	20.6	32.9	-0.90	-0.46	-1.90	-0.82

There was an asymptotic relationship between ψ_{PD} and ψ_{MID} when the data from all treatments pre- and post-irrigation were pooled together (Figure 5.4).

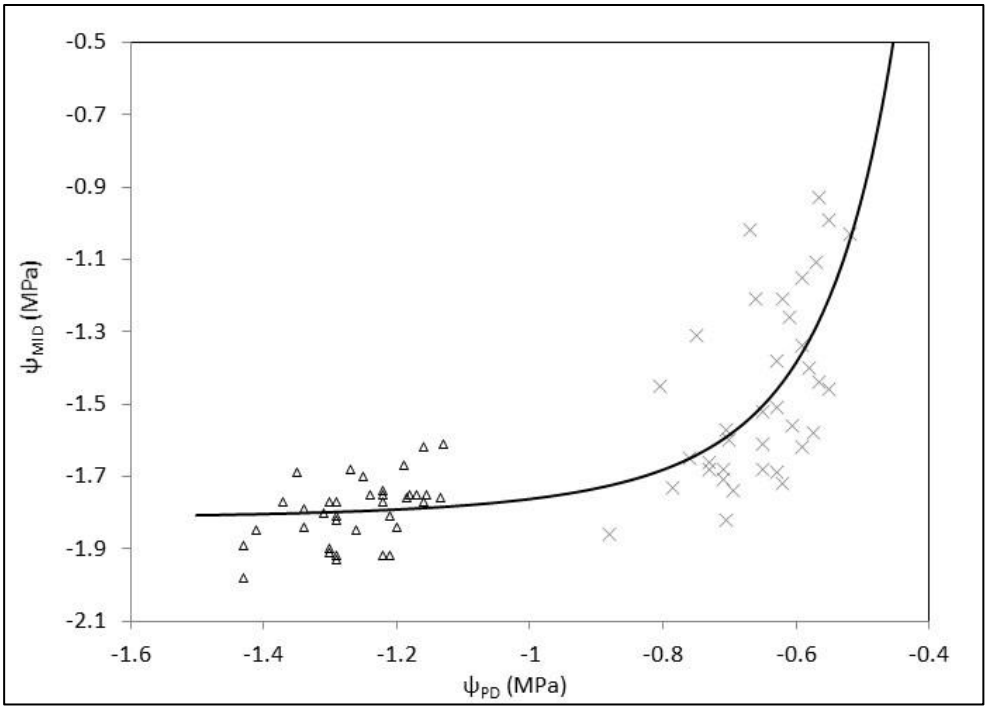


Figure 5.4. Relationship between pre-dawn (ψ_{PD}) and midday (ψ_{MID}) leaf water potential, measured pre- (Δ) and post-irrigation (X). The fitted curve was described by $\psi_{MID} = 0.056(\pm0.010)/\psi_{PD}^4 - 1.818(\pm0.047)$; n = 72.

The 'f(x) = (m/x^4) + c' asymptotic relationship was considered to be the 'line of best fit' based on minimising the sums of squared errors (SSE; Table 5.6).

Table 5.6. Asymptotic relationships between pre-dawn leaf water potential (ψ_{PD}, MPa) and midday leaf-water potential (ψ_{MID}, MPa) measured pre- and post-irrigation in the 2012-13 growing season (Y2); and the associated sums of squared errors (SSE) and coefficient of determination (R^2). Values are reported as means with the 95% confidence intervals in the parentheses. n = 36.

Relationship	Equation	SSE	R^2
f(x) = (m/x) + c	ψ_{MID} = -0.476(±0.095)/ψ_{PD} - 2.190(±0.119)	1.85	0.59
f(x) = (m/x^2) + c	ψ_{MID} = 0.196(±0.037)/ψ_{PD}^2 - 1.934(±0.069)	1.75	0.61
f(x) = (m/x^3) + c	ψ_{MID} = -0.101(±0.019)/ψ_{PD}^3 - 1.855(±0.056)	1.70	0.62
f(x) = (m/x^4) + c	ψ_{MID} = 0.0555(±0.0103)/ψ_{PD}^4 - 1.817(±0.050)	1.68	0.62
f(x) = (m/x^5) + c	ψ_{MID} = -0.0307(±0.0058)/ψ_{PD}^4 - 1.794(±0.048)	1.71	0.62

There was a significant linear relationship between ψ_{MID} and g_l; however, no relationship was found between ψ_{PD} and g_l (Figure 5.5).

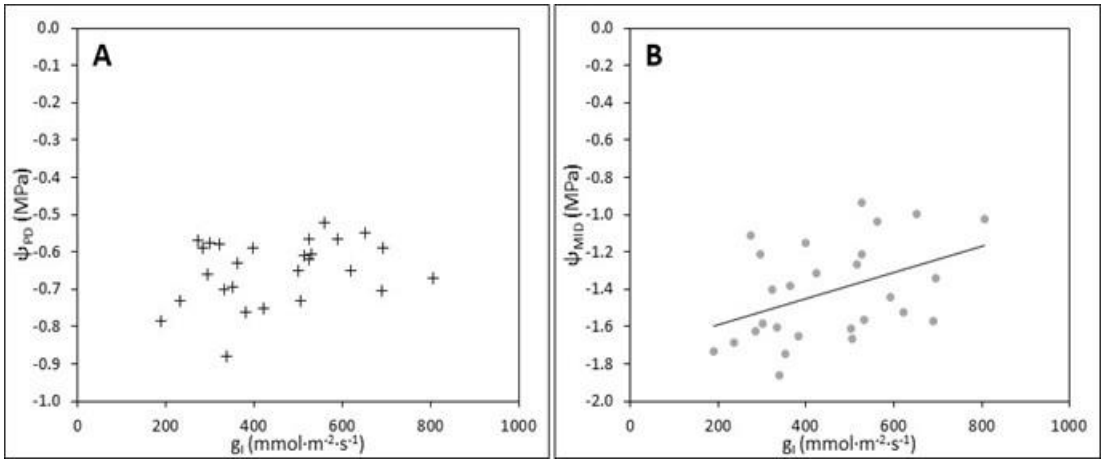


Figure 5.5. Scatter plot between post-irrigation (A) pre-dawn leaf water potential (ψ_{PD}, MPa) and leaf conductance (g_l, mmol·m^{-2}·s^{-1}) relationship not significant ($p > 0.05$; n = 27); and (B) midday leaf water potential (ψ_{MID}, MPa) and g_l ($p < 0.001$; ψ_{MID} = 0.0007(±0.00062)·g_l + 1.72(±0.29); R^2 = 0.1828; n = 27) measured during the 2012-13 growing season (Y2).

Estimated average canopy temperatures were warmer for the bottom and eastern sides of the canopy, with higher variability (T_{SD}) for the bottom of the canopy pre- and post-irrigation (Table 5.7).

Table 5.7. Average canopy temperature (T_C, °C) and standard deviation of the canopy temperature (T_{SD}, °C) for the top, middle and bottom of the eastern and western side of the canopy measured pre- and post-irrigation during the 2012-13 (Y2) growing season[£].

		Pre-Irrigation		Post-Irrigation	
		Western	Eastern	Western	Eastern
	Subsection	°C	°C	°C	°C
T_C	Top	36.1	36.6	30.3	30.6
	Middle	36.6	37.2	31.0	31.4
	Bottom	37.8	38.6	32.6	33.2
T_{SD}	Top	0.77	0.90	1.71	1.46
	Middle	0.71	0.88	1.39	1.43
	Bottom	1.04	1.27	1.81	1.84

[£] To avoid pseudo replication statistical analysis was not performed on this data set.

Post-irrigation, there was a positive linear relationship between T_C and ψ_{PD} ($p \leq 0.006$; Table 5.8) and, T_C and ψ_{MID} ($p < 0.001$; Table 5.8) for both sides of the canopy.

Table 5.8. Relationship between pre-dawn leaf water potential (ψ_{PD}, MPa), midday leaf water potential (ψ_{MID}, MPa) and, the difference between canopy temperature and ambient temperature (T_C, °C); measured post-irrigation during the 2012-13 growing season (DOY = 42, Y2); and associated coefficient of determination (R^2). Values are reported as means with 95% confidence intervals in parenthesis n = 36.

	Equation	R^2	p value
Eastern Side of the Canopy			
ψ_{PD} (MPa)	$T_C = -7.07(\pm5.03)\cdot\psi_{PD} + 27.26(\pm2.31)$	0.19	0.007
ψ_{MID} (MPa)	$T_C = -2.88(\pm1.49)\cdot\psi_{MID} + 27.65(\pm4.57)$	0.31	< 0.001
Western Side of the Canopy			
ψ_{PD} (MPa)	$T_C = -11.0(\pm6.17)\cdot\ \psi_{PD} + 24.27(\pm4.07)$	0.28	< 0.001
ψ_{MID} (MPa)	$T_C = -3.70(\pm1.94)\cdot\ \psi_{MID} + 26.01(\pm2.90)$	0.31	< 0.001

Pre-irrigation, there was no significant linear relationship between T_C^* and ψ_{PD} or ψ_{MID} for either side of the canopy. Post-irrigation, there was no significant linear relationship between T_C* and ψ_{MID} for either side of the canopy. Post-irrigation, there was a significant relationship between T_C* and ψ_{PD} for the western side of the canopy ($p = 0.006$; Table 5.9). However, this relationship was not significant for the eastern side of the canopy ($p > 0.05$).

Table 5.9. Relationship between pre-dawn leaf water potential (ψ_{PD}, MPa), midday leaf water potential (ψ_{MID}, MPa) and canopy temperature (T_C*, °C); measured post-irrigation during the 2012-13 growing season (DOY = 42, Y2); and associated coefficient of determination (R^2). Values are reported as means with 95% confidence intervals in parenthesis. n = 36

	Equation	R^2	p value
Eastern Side of the Canopy			
ψ_{PD} (MPa)			ns
ψ_{MID} (MPa)			ns
Western Side of the Canopy			
ψ_{PD} (MPa)	T_C* = -5.73(±4.00)· ψ_{PD}	0.20	0.006
ψ_{MID} (MPa)			ns

5.4.1. Irrigation Treatments

There was no significant effect of the irrigation treatments on ΔTCA ($p > 0.05$; Table 5.10). In Y2, there was no significant effect of the irrigation treatments on any of the leaf characteristics measured ($p > 0.05$; Table 5.10).

Table 5.10. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on the change in trunk cross-sectional area (ΔTCA, cm^2) and leaf characteristics: leaf sizes (L_S, cm^2), leaf fresh weight (L_{FW}, g), leaf dry weight (L_{DW}, g), leaf percent dry weight ($L_{\%DW}$) and specific leaf area (SLA, $cm^2 \cdot g^{-1}$). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for irrigation treatments (I); ns = not significant ($p > 0.05$).

	Irrigation Treatments			$LSD_{p=0.05}$ (I)	p value (I)
	I_{25}	I_{50}	I_{100}		
Change in Trunk Cross Sectional Area					
ΔTCA	2.04	1.39	2.00	0.81	ns
Leaf Characteristics					
L_S (cm^2)	76.0	70.4	69.9	10.1	ns
L_{FW} (g)	1.79	1.61	1.71	0.31	ns
L_{DW} (g)	0.48	0.42	0.46	0.11	ns
$L_{\%DW}$	26.5	25.9	26.3	2.2	ns
SLA ($cm^2 \cdot g^{-1}$)	164.2	176.3	159.9	42.4	ns

NB: when p value (I) < 0.05 means separation is indicated by dissimilar letters and when p value (I) > 0.05 no means separation was conducted.

Pre-irrigation, there was no significant effect of the irrigation treatments on ψ_{PD} or ψ_{MID} ($p > 0.05$; Table 5.11). Post-irrigation, there was a highly significant effect of the irrigation treatments on ψ_{PD} and ψ_{MID} ($p < 0.001$; Table 5.11). Post-irrigation, there was no significant effect of the irrigation treatments on g_l ($p > 0.05$; Table 5.11). Pre-irrigation, there was a significant effect of the irrigation treatments on T_C of the eastern ($p = 0.036$) and western ($p = 0.021$) sides of the canopy (Table 5.11). However, there was no significant effect of the irrigation treatments on the T_C* or T_{SD} for either side of the canopy ($p > 0.05$; Table 5.11). Post-irrigation, there was a significant effect of the irrigation treatments on T_C and T_C* for the eastern and western sides of the canopy ($p < 0.021$; Table 5.11). However, there was no significant effect of the irrigation treatments on T_{SD} of temperature for either sides of the canopy ($p > 0.05$; Table 5.11).

Table 5.11. Impact of irrigation treatments (I_{25}, I_{50} and I_{100}) on pre-dawn leaf water potential (ψ_{PD}), midday leaf water potential (ψ_{MID}), average canopy temperature (T_C), canopy and air temperature difference (T_C*) and standard deviation of canopy temperature (T_{SD}) measurements for pre-irrigation and post-irrigation in the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for irrigation treatments (I); ns = not significant (p > 0.05).

	ψ_{PD} (MPa)	ψ_{MID} (MPa)	g_l (mmol·m^{-2}·s^{-1})	Western Side of the Canopy			Eastern Side of the Canopy		
				T_C (°C)	T_C* (°C)	T_{SD} (°C)	T_C (°C)	T_C* (°C)	T_{SD} (°C)
Pre-Irrigation (DOY = 38, Y2)									
I_{25}	-1.27	-1.82	‡	38.1_b	3.6	1.29	38.7_b	4.25	1.60
I_{50}	-1.27	-1.80	‡	36.9_{ab}	2.6	1.06	37.5_{ab}	3.19	1.21
I_{100}	-1.23	-1.76	‡	35.8_a	2.73	1.00	36.4_a	3.35	1.23
$LSD_{p=0.05}$ (I)	0.07	0.07	‡	1.57	1.00	0.27	1.73	1.36	0.37
p value (I)	ns	ns	‡	0.021 *	ns (0.095)	ns (0.054)	0.036	ns	ns (0.074)
Post-Irrigation (DOY = 45, Y2)									
I_{25}	-0.72_a	-1.66_a	452	32.4_b	4.06_b	2.04	32.3_b	3.98_{ab}	1.97
I_{50}	-0.65_b	-1.54_a	486	31.7_b	3.67_{ab}	1.18	32.5_b	4.49_b	1.92
I_{100}	-0.59_c	-1.21_b	482	30.3_a	3.95a	1.87	30.8_b	3.42a	1.9
$LSD_{p=0.05}$ (I)	0.04	0.14	128	1.35	0.77	0.21	0.99	0.59	0.23
p value (I)	< 0.001 ***	< 0.001 ***	ns	0.013 *	0.021 *	ns (0.067)	< 0.001 ***	0.004 **	ns

NB: when p value (I) < 0.05 means separation is indicated by dissimilar letters and when p value (I) > 0.05 no means separation was conducted.

‡ Data not recorded

Pre-irrigation, there was a significant effect of irrigation treatments on T_{MIN} for the bottom and middle subsections of the western side of the canopy ($p < 0.048$; Table 5.12). However, there was no significant effect of irrigation treatments on T_{MIN} for the bottom and middles subsections of the eastern side of the canopy ($p > 0.05$; Table 5.12).

Pre-irrigation, there was a significant effect of the irrigation treatments on T_{MAX} of the middle subsection of the eastern side of the canopy ($p = 0.040$; Table 5.12). However, this was not significant for the top subsection of the eastern side of the canopy ($p > 0.05$; Table 5.12). There was no significant effect of irrigation treatments on T_{MAX} for the middle and top subsection of the western side of the canopy ($p > 0.05$; Table 5.12).

Table 5.12. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on minimum temperature (T_{MIN}, °C) of the bottom and middle subsections; and maximum temperature (T_{MAX}, °C) of the middle and top subsections of the canopy measured pre-irrigation during the 2012-13 growing season (Y2). Least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) and are reported for irrigation treatments (I); ns = not significant ($p > 0.05$).

		Irrigation Treatments			$LSD_{p=0.05}$ (I)	p value (I)
		I_{25} °C	I_{50} °C	I_{100} °C		
Eastern Side of the Canopy						
T_{MIN}	**Bottom**	35.69	35.04	34.08	1.46	ns
	Middle	34.76	34.39	33.07	1.43	ns
T_{MAX}	**Middle**	44.17_{b}	41.59_{ab}	40.47_{a}	2.86	0.040
	Top	41.81	39.96	39.20	3.11	ns
Western Side of the Canopy						
T_{MIN}	**Bottom**	35.05_{b}	34.56_{ab}	33.67_{a}	1.10	0.048
	Middle	34.87_{b}	34.14_{ab}	33.17_{a}	1.32	0.046
T_{MAX}	**Middle**	42.34	40.34	39.18	2.57	ns
	Top	41.04	38.98	38.52	2.75	ns

NB: when p value (I) < 0.05 means separation is indicated by dissimilar letters and when p value (I) > 0.05 no means separation was conducted.

Post-irrigation, there was a significant effect of the irrigation treatments on T_{MIN} of the bottom and middle subsections of both side of the canopy ($p < 0.005$; Table 5.13).

Post-irrigation, there was a significant effect of the irrigation treatments on T_{MAX} of the middle subsection of both sides of the canopy ($p < 0.037$; Table 5.13) and the top subsection of the eastern side of the canopy ($p = 0.002$; Table 5.13). However, this trend was not significant for T_{MAX} of the top subsection of the western side of the canopy ($p = 0.075$; Table 5.13).

Table 5.13. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) on minimum temperature (T_{MIN}, °C) of the bottom and middle subsections; and maximum temperature (T_{MAX}, °C) of the middle and top subsections of the canopy measured post-irrigation during the 2012-13 growing season (Y2). Least significant difference at $p = 0.05$ ($LSD_{p=0.05}$) and are reported for irrigation treatments (I); ns = not significant ($p > 0.05$).

		Irrigation Treatments			$LSD_{p=0.05}$ (I)	p value
		I_{25} °C	I_{50} °C	I_{100} °C		I
Eastern Side of the Canopy						
T_{MIN}	**Bottom**	29.78_b	29.89_b	28.18_a	1.03	0.004
	Middle	27.40_b	27.79_b	26.13_a	0.98	0.005
T_{MAX}	**Middle**	39.75_b	40.18_b	37.88_a	1.05	< 0.001
	Top	37.48_b	38.49_b	35.82_a	1.39	0.002
Western Side of the Canopy						
T_{MIN}	**Bottom**	28.30_b	28.04_{ab}	26.83_a	1.24	0.048
	Middle	27.09_b	26.61_b	25.28_a	1.13	0.010
T_{MAX}	**Middle**	40.63_b	39.53_{ab}	38.39_a	1.57	0.037
	Top	39.39	38.61	37.19	1.84	ns

NB: when p value (I) < 0.05 means separation is indicated by dissimilar letters and when p value (I) > 0.05 no means separation was conducted.

5.4.2. Glycine Betaine

There was no significant effect of GB on ΔTCA ($p > 0.05$). In Y2, GB significantly reduced leaf size, with means of 77.2 and 67.0 cm^2 for GB^- and GB^+, respectively ($LSD_{p = 0.05} = 8.3$; $p = 0.018$). However, there was no significant effect of GB on any of the other leaf characteristics measured ($p > 0.05$). There was no significant interaction of the irrigation treatments and GB on ΔTCA ($p > 0.05$; Table 5.14). In Y2, there was no significant interaction of the irrigation treatments and GB on any of the leaf characteristics measured ($p > 0.05$; Table 5.14).

Table 5.14. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on the change in trunk cross-sectional area (ΔTCA, cm^2) and leaf characteristics: leaf sizes (L_S, cm^2), leaf fresh weight (L_{FW}, g), leaf dry weight (L_{DW}, g), leaf percent dry weight ($L_{\%DW}$) and specific leaf area (SLA, $cm^2 \cdot g^{-1}$). Least significant difference at $p = 0.05$ ($LSD_{p = 0.05}$) and are reported for the interaction of irrigation treatments (I) and glycine betaine (GB); ns = not significant ($p > 0.05$).

		Irrigation Treatments			$LSD_{p = 0.05}$ (I x GB)	p value (I x GB)
		I_{25}	I_{50}	I_{100}		
Change in Trunk Cross Sectional Area						
ΔTCA	**GB^-**	2.29	1.19	1.82	1.14	ns
	GB^+	1.79	1.59	2.19		
Leaf Characteristics						
L_S (cm^2)	**GB^-**	78.7	76.4	76.5	14.3	ns
	GB^+	73.2	64.5	63.3		
L_{FW} (g)	**GB^-**	1.82	1.74	1.88	0.31	ns
	GB^+	1.76	1.49	1.55		
L_{DW} (g)	**GB^-**	0.49	0.48	0.51	0.13	ns
	GB^+	0.48	0.37	0.40		
$L_{\%DW}$	**GB^-**	26.5	27.5	27.0	3.2	ns
	GB^+	26.5	24.3	25.7		
SLA ($cm^2 \cdot g^{-1}$)	**GB^-**	169.4	162.8	152.3	47.1	ns
	GB^+	159.0	189.8	167.4		

NB: when p value (I x GB) < 0.05 means separation is indicated by dissimilar letters and when p value (I x GB) > 0.05 no means separation was conducted.

Pre-irrigation there was no significant effect of GB on Ψ_{PD} ($p > 0.05$). However, there was a significant effect of GB on Ψ_{MID}, with means of -1.76 and -1.83 MPa for GB^- and GB^+, respectively ($LSD_{p=0.05} = 0.059$; $p = 0.028$). This response was not significance at $p = 0.05$ for post-irrigation Ψ_{PD}, with means of -0.641 and -0.667 MPa for GB^- and GB^+, respectively ($LSD_{p=0.05} = 0.011$; $p = 0.097$); and post-irrigation Ψ_{MID} with means of -1.42 and -1.52 MPa for GB^- and GB^+, respectively ($LSD_{p=0.05} = 0.112$; $p = 0.068$). Post-irrigation there was no significant effect of GB on g_l ($p > 0.05$).

Pre-irrigation, there was a significant effect of GB on T_C for the western side of the canopy, with means of 36.3 and 37.5 °C for GB^- and GB^+, respectively ($p = 0.048$). However, there was no significant effect of GB on T_C of the eastern side of the canopy ($p > 0.05$). Pre-irrigation, there was no significant effect of GB on T_C or T_{SD} on the eastern or western side of the canopy ($p > 0.05$). Post-irrigation, there was no significant effect of GB on T_C, T_C* or T_{SD} for the eastern or western side of the canopy ($p > 0.05$).

Pre- and post-irrigation there was no significant interaction of the irrigation treatments and GB on Ψ_{PD} or Ψ_{MID} ($p > 0.05$, Table 5.15). Pre-irrigation there was no significant interaction of the irrigation treatments and GB on T_C, T_C* or T_{SD} for the eastern or western side of the canopy ($p > 0.05$; Table 5.15). Post-irrigation, there was a significant interaction of the irrigation treatment and GB on T_C* for the eastern side of the canopy ($p = 0.015$; Table 5.15). However, there was no significant effect of GB on T_C* for the western side of the canopy ($p > 0.05$; Table 5.15). Post-irrigation, there was no significant interaction of the irrigation treatment and GB on T_C or T_{SD} for the eastern or western side of the canopy ($p > 0.05$; Table 5.15).

Pre- and post-irrigation, there was no significant effect of GB on the relationship between T_C and ψ_{PD} or ψ_{MID} for the eastern or western side of the canopy. Pre- and post-irrigation, there was no significant effect of GB on the relationship between ψ_{PD} or ψ_{MID} and T_C* for the eastern or western side of the canopy.

Table 5.15. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on pre-dawn leaf water potential (ψ_{PD}), midday leaf water potential (ψ_{MID}), stomatal conductance (g_l), average canopy temperature (T_C), canopy and air temperature difference (T_C*) and standard deviation of canopy temperature (T_{SD}) measurements for pre-irrigation and post-irrigation in the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction irrigation treatments (I) and glycine betaine (GB); ns = not significant (p > 0.05).

					Western Side of the Canopy			Eastern Side of the Canopy		
		ψ_{PD} (MPa)	ψ_{MID} (MPa)	g_l (mmol·m^{-2}·s^{-1})	T_C (°C)	T_C* (°C)	T_{SD} (°C)	T_C (°C)	T_C* (°C)	T_{SD} (°C)
Pre-Irrigation (DOY = 38, Y2)										
I_{25}	GB^-	-1.24	-1.79	‡	38.2	3.5	1.30	39.0	4.37	1.72
	GB^+	-1.30	-1.85	‡	38.0	3.7	1.28	38.4	4.14	1.48
I_{50}	GB^-	-1.27	-1.78	‡	36.4	2.83	1.02	37.1	3.26	1.07
	GB^+	1.27	-1.83	‡	37.4	2.61	1.10	37.9	3.13	1.36
I_{100}	GB^-	-1.22	-1.71	‡	34.2	2.77	0.77	34.9	2.93	1.00
	GB^+	-1.25	-1.81	‡	37..3	3.19	1.17	37.9	3.76	1.46
$LSD_{p=0.05}$ (I x GB)		0.11	0.10	‡	2.22	1.41	0.38	2.45	1.92	0.53
p value (I x GB)		ns	ns	‡	ns	ns	ns	ns	ns	ns
Post-Irrigation (DOY = 45, Y2)										
I_{25}	GB^-	-0.72	-1.65	370	32.4	4.01	2.07	32.1	3.68_a	1.93
	GB^+	-0.72	-1.66	533	32.4	4.12	2.02	32.5	4.28_{bc}	2.00
I_{50}	GB^-	-0.62	-1.48	498	31.7	3.98	1.76	32.7	4.96_c	2.03
	GB^+	-0.68	-1.60	474	31.6	3.35	1.85	32.3	4.03_b	1.81
I_{100}	GB^-	-0.58	-1.13	449	30.0	3.33	1.89	30.6	3.62_{ab}	1.82
	GB^+	-0.60	-1.30	515	30.6	2.57	1.85	30.9	2.90_a	1.99
$LSD_{p=0.05}$ (I x GB)		0.05	0.19	180	1.91	0.89	0.24	1.24	0.89	0.24
p value (I x GB)		ns	ns	Ns	ns	ns	ns	ns	0.015	ns

NB: when p value (I x GB) < 0.05 means separation is indicated by dissimilar letters and when p value (I x GB) > 0.05 no means separation was conducted.

‡ Data not recorded

Pre-irrigation, there was a significant effect of GB on T_{MIN} for the bottom section of the western side of the canopy with means of 33.97 and 34.88 °C for GB^- and GB^+, respectively ($LSD_{p = 0.05}$ = 0.89; p = 0.046). However, this was not observed T_{MIN} for the middle section of the western side of the canopy (p > 0.05). Pre-irrigation, there was no significant effect of GB on T_{MIN} for the eastern side of the canopy or T_{MAX} for either side of the canopy (p > 0.05). Post-irrigation, there was no significant effect of GB on T_{MIN} or T_{MAX} for either side of the canopy (p > 0.05).

Pre- and post-irrigation, there was no significant interaction of the irrigation treatment and GB on T_{MIN} or T_{MAX} for the eastern or western side of the canopy (p > 0.05; Table 5.16).

Table 5.16. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on minimum temperature (T_{MIN}, °C) of the bottom and middle subsections; and maximum temperature (T_{MAX}, °C) of the middle and top subsections of the canopy measured pre-irrigation during the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p = 0.05}$) and are reported for the interaction irrigation treatments (I) and glycine betaine (GB); ns = not significant (p > 0.05).

			Irrigation Treatments			$LSD_{p = 0.05}$ (I x GB)	p value (I x GB)
			I_{25} °C	I_{50} °C	I_{100} °C		
Eastern Side of the Canopy							
T_{MIN}	Bottom	GB^-	36.22	34.65	33.00	2.07	ns
		GB^+	35.17	35.43	35.17		
	Middle	GB^-	35.05	34.40	32.07	2.03	ns
		GB^+	34.47	34.38	34.07		
T_{MAX}	Middle	GB^-	45.07	40.73	38.00	4.07	ns
		GB^+	43.27	42.45	42.93		
	Top	GB^-	42.73	39.20	37.08	2.54	ns
		GB^+	40.88	40.72	41.32		
Western Side of the Canopy							
T_{MIN}	Bottom	GB^-	35.20	34.15	32.57	1.55	ns (0.079)
		GB^+	34.90	34.97	34.78		
	Middle	GB^-	35.08	35.03	33.70	1.87	ns
		GB^+	34.65	34.63	32.65		
T_{MAX}	Middle	GB^-	42.77	39.67	36.78	3.64	ns
		GB^+	41.92	41.02	41.57		
	Top	GB^-	42.20	40.32	39.32	3.89	ns
		GB^+	39.88	37.65	37.72		

NB: when p value (I x GB) < 0.05 means separation is indicated by dissimilar letters and when p value (I x GB) > 0.05 no means separation was conducted.

Post-irrigation, there was no significant effect of GB on T_{MIN} or T_{MAX} for the eastern or western side of the canopy (p > 0.05).

Post-irrigation, there was no significant interaction of the irrigation treatments and GB on T_{MIN} or T_{MAX} for the eastern or western side of the canopy (p > 0.05; Table 5.17)

Table 5.17. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and glycine betaine (GB^- and GB^+) on minimum temperature (T_{MIN}, °C) of the bottom and middle subsections; and maximum temperature (T_{MAX}, °C) of the middle and top subsections of the canopy measured post-irrigation during the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction irrigation treatments (I) and glycine betaine (GB); ns = not significant (p > 0.05).

			Irrigation Treatments			$LSD_{p=0.05}$ (I x GB)	p value (I x GB)
			I_{25} °C	I_{50} °C	I_{100} °C		
Eastern Side of the Canopy							
T_{MIN}	Bottom	GB^-	29.70	29.88	28.25	1.46	ns
		GB^+	29.87	29.90	28.12		
	Middle	GB^-	27.28	27.65	26.22	1.38	ns
		GB^+	27.52	27.93	26.05		
T_{MAX}	Middle	GB^-	38.97	40.60	37.25	1.49	ns
		GB^+	40.53	39.75	38.50		
	Top	GB^-	37.43	39.67	35.77	1.97	ns
		GB^+	37.52	37.32	35.87		
Western Side of the Canopy							
T_{MIN}	Bottom	GB^-	29.08	27.62	26.58	1.75	ns
		GB^+	27.52	28.47	27.07		
	Middle	GB^-	27.23	26.80	25.13	1.60	ns
		GB^+	26.88	26.42	25.43		
T_{MAX}	Middle	GB^-	40.87	39.53	38.37	2.37	ns
		GB^+	40.38	39.53	38.42		
	Top	GB^-	39.68	38.57	38.33	2.60	ns
		GB^+	38.47	37.45	36.05		

NB: when p value (I x GB) < 0.05 means separation is indicated by dissimilar letters and when p value (I x GB) > 0.05 no means separation was conducted.

5.4.3. Kaolin Particle Film

KPF significantly increased ΔTCA, with means of 1.43 and 2.19 cm^2 for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 0.66; p = 0.026). In Y2, there was no significant effect of KPF on any of the leaf characteristics measured (p > 0.05). There was no significant interaction of the irrigation treatments and KPF on ΔTCA (p > 0.05; Table 5.18). In Y2, there was no significant interaction of the irrigation treatments and KPF on any of the leaf characteristics measured (p > 0.05; Table 5.18).

Table 5.18. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on the change in trunk cross-sectional area (ΔTCA, cm^2) and leaf characteristics: leaf sizes (L_S, cm^2), leaf fresh weight (L_{FW}, g), leaf dry weight (L_{DW}, g), leaf percent dry weight ($L_{\%DW}$) and specific leaf area (SLA, $cm^2 \cdot g^{-1}$). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction irrigation treatments (I) and kaolin particle film (KPF); ns = not significant (p > 0.05).

		Irrigation Treatments			$LSD_{p=0.05}$ (I x KPF)	p value (I x KPF)
		I_{25}	I_{50}	I_{100}		
Change in Trunk Cross Sectional Area						
ΔTCA	**KPF^-**	1.93_{ab}	0.92_a	1.46_{ab}	1.14	ns
	KPF^+	2.15_b	1.87_{ab}	2.54_b		
Leaf Characteristics						
L_S (cm^2)	**KPF^-**	77.0	70.9	68.6	14.3	ns
	KPF^+	74.9	70.0	71.2		
L_{FW} (g)	**KPF^-**	1.77	1.56	1.64	0.44	ns
	KPF^+	1.81	1.66	1.79		
L_{DW} (g)	**KPF^-**	0.46	0.40	0.43	0.16	ns
	KPF^+	0.50	0.45	0.49		
$L_{\%DW}$	**KPF^-**	25.8	25.3	25.6	3.2	ns
	KPF^+	27.3	26.5	27.1		
SLA ($cm^2 \cdot g^{-1}$)	**KPF^-**	173.7	191.9	170.6	47.1	ns
	KPF^+	154.7	160.7	149.2		

NB: when p value (I x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (I x KPF) > 0.05 no means separation was conducted.

Pre- and post-irrigation, there was no significant effect of KPF on ψ_{PD} (p > 0.05). Pre-irrigation, the effect of KPF on ψ_{MID} was not significant at p = 0.05, with means of -1.77 and -1.82 MPa for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 0.059; p = 0.058). Post-irrigation the effect of KPF on ψ_{MID} was not significant at p = 0.05, with means of -1.42 and -1.52 MPa for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 0.11; p = 0.068). Post-irrigation, KPF significantly reduced g_l, with means of 577 and 369 $mmol \cdot m^{-2} \cdot s^{-2}$ for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 104; p < 0.001).

Pre-irrigation, there was a significant effect of KPF on T_C for the western side of the canopy measured pre-irrigation, with means of 37.54 and 36.25 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 1.28; p = 0.048). However, this trend was not significant at p = 0.05 for the eastern side of the canopy, with means of 38.2 and 36.8 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 1.41; p = 0.060). Pre-irrigation, there was no significant effect of KPF on TC* or TSD for the eastern or western side of the canopy (p > 0.05). Post-irrigation, there was no significant effect of KPF on T_C, T_C* or T_{SD} for the eastern or western side of the canopy (p > 0.05).

Pre-irrigation, there was no significant interaction of the irrigation treatments and KPF on Ψ_{PD} or ψ_{MID} (p > 0.05; Table 5.19). Post-irrigation, there was a significant interaction of the irrigation treatments and KPF on ψ_{PD}, with the combined effect of I_{25} and KPF^+ resulting in more negative ψ_{PD} values (p = 0.018; Table 5.19). However, there was no significant interaction of the irrigation treatments and KPF on ψ_{MID} or g_l (p > 0.05; Table 5.19). Pre- and post-irrigation, there was no significant interaction of the irrigation treatments and KPF on T_C, T_C* or T_{SD} for the eastern or western side of the canopy (p > 0.05; Table 5.19).

Table 5.19. Impact of irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on pre-dawn leaf water potential (ψ_{PD}), midday leaf water potential (ψ_{MID}), stomatal conductance (g_l), average canopy temperature (T_C), canopy and air temperature difference (T_C*) and standard deviation of canopy temperature (T_{SD}) measurements for pre-irrigation and post-irrigation in the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction irrigation treatments (I) and kaolin particle film (KPF); ns = not significant (p > 0.05).

					Western Side of the Canopy			Eastern Side of the Canopy		
		ψ_{PD} (MPa)	ψ_{MID} (MPa)	g_l (mmol·m^{-2}·s^{-1})	T_C (°C)	T_C* (°C)	T_{SD} (°C)	T_C (°C)	T_C* (°C)	T_{SD} (°C)
Pre-Irrigation (DOY = 38, Y2)										
I_{25}	KPF^-	-1.24	-1.77	‡	38.5	3.87	1.36	39.1	4.45	1.65
	KPF^+	-1.31	-1.87	‡	37.6	3.34	1.22	38.5	4.06	1.55
I_{50}	KPF^-	-1.72	-1.78	‡	37.9	2.83	1.13	38.8	3.73	1.42
	KPF^+	-1.27	-1.82	‡	35.8	2.35	1.00	36.2	2.66	1.00
I_{100}	KPF^-	-1.24	-1.74	‡	36.3	2.77	1.03	36.7	3.24	1.20
	KPF^+	-1.23	-1.78	‡	35.3	3.69	0.91	36.0	3.46	1.26
$LSD_{p=0.05}$ (I x KPF)		0.11	0.10	‡	2.22	1.41	0.38	2.45	1.91	0.53
p value (I x KPF)		ns	ns	‡	ns	ns	ns	ns	ns	ns
Post-Irrigation (DOY = 45, Y2)										
I_{25}	KPF^-	-0.68_b	-1.59	570	32.6	4.24	2.15	32.2	3.85	2.00
	KPF^+	-0.76_a	-1.72	334	32.2	3.89	1.94	32.4	4.11	1.93
I_{50}	KPF^-	-0.66_b	-1.51	603	32.0	3.64	1.84	33.1	4.66	2.03
	KPF^+	-0.64_{bc}	-1.57	369	31.3	3.70	1.77	31.9	4.33	1.85
I_{100}	KPF^-	-0.60_{cd}	-1.15	558	30.7	3.14	1.93	31.2	3.62	1.87
	KPF^+	-0.58_d	-1.28	406	30.0	2.76	1.81	30.3	3.22	1.94
$LSD_{p=0.05}$ (I x KPF)		0.05	0.19	181	1.91	0.89	0.29	1.24	0.84	0.32
p value (I x KPF)		0.018	ns	ns	ns	ns	ns	ns	ns	ns

NB: when p value (I x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (I x KPF) > 0.05 no means separation was conducted.
‡ Data not recorded

Based on the 95% confidence intervals, the asymptotic relationship between ψ_{PD} and ψ_{MID} measured pre- and post-irrigation were significantly different (Table 5.20).

Table 5.20. Impact of kaolin particle film (KPF^- and KPF^+) on the asymptotic relationships between pre-dawn leaf water potential (ψ_{PD}, MPa) and midday leaf-water potential (ψ_{MID}, MPa) measured pre- and post-irrigation in the 2012-13 growing season (Y2); and the associated sums of squared errors (SSE) and coefficient of determination (R^2). Values are reported as means with the 95% confidence intervals indicated in the parentheses.

Treatment	Equation	SSE	R^2
KPF^-	$\psi_{MID} = 0.052(\pm0.016)/\ \psi PD^4 - 1.771(\pm0.080)$	1.01	0.56
KPF^+	$\psi_{MID} = 0.059(\pm0.013)/\ \psi PD^4 - 1.863(\pm0.061)$	0.58	0.71

Post-irrigation, there was a significant effect KPF on the relationship between ψ_{MID} and T_C for the eastern side of the canopy ($p = 0.022$; Table 5.21); reducing the Y-intercept whilst having no significant effect on the slope. Post-irrigation, there was no significant effect of KPF on the relationship between ψ_{PD} and T_C or the relationship between ψ_{MID} and T_C for the western side of the canopy. Post-irrigation, there was no significant effect of KPF on the relationship between ψ_{PD} and T_C* or the relationship between ψ_{MID} and T_C for the eastern or western side of the canopy.

Table 5.21. Impact of kaolin particle film (KPF^- and KPF^+) on the relationship between canopy temperature (T_C, °C) and midday leaf water potential (ψ_{MID}, MPa) measured post-irrigation during the 2012-13 growing season (Y2); and associated coefficient of determination (R^2). Values are reported as means with 95% confidence intervals in parenthesis.

Treatment	Equation	R^2	p value
Midday Leaf Water Potential			
KPF^-	$T_C = -3.25(\pm1.41)\cdot\ \psi_{PD} + 27.56(\pm2.06)$	0.39	0.014
KPF^+	$T_C = -3.25(\pm1.41)\cdot\ \psi_{PD} + 27.56(\pm2.06) - 0.90(\pm0.71)$		

Pre-irrigation, KPF reduced T_{MIN} of the middle subsection on eastern side of the canopy with means of 34.72 and 33.42 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05} = 1.17$; $p = 0.031$) and of the middle subsection of the western side of the canopy with means of 34.61 and 33.52 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05} = 1.08$; $p = 0.048$). Pre-irrigation, KPF reduced T_{MIN} of the bottom subsection of the western canopy with means of 35.04 and 33.82 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05} = 0.89$; $p = 0.031$). However, there was no significant effect of KPF on T_{MIN} for the bottom subsection of the eastern side of the canopy. Pre-irrigation, there was no significant effect of KPF on T_{MAX} for the eastern or western side of the canopy.

Pre-irrigation, there was no significant interaction of irrigation treatments and KPF on T_{MIN} or T_{MAX} for either side of the canopy ($p > 0.05$; Table 5.22).

Table 5.22. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on minimum temperature (T_{MIN}, °C) of the bottom and middle subsections; and maximum temperature (T_{MAX}, °C) of the middle and top subsections of the canopy measured pre-irrigation during the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction irrigation treatments (I) and kaolin particle film (KPF); ns = not significant (p > 0.05).

			Irrigation Treatments			$LSD_{p=0.05}$	p value
			I_{25}	I_{50}	I_{100}	(I x KPF)	(I x GB)
Eastern Side of the Canopy							
T_{MIN}	Bottom	KPF^-	35.87	34.93	34.28	2.07	ns
		KPF^+	35.52	34.15	33.88		
	Middle	KPF^-	35.00	35.40	33.77	2.03	ns
		KPF^+	34.52	33.38	32.37		
T_{MAX}	Middle	KPF^-	45.05	43.60	40.72	4.07	ns
		KPF^+	43.28	39.58	40.22		
	Top	KPF^-	42.75	41.53	39.73	4.40	ns
		KPF^+	40.87	38.38	38.67		
Western Side of the Canopy							
T_{MIN}	Bottom	KPF^-	35.52	35.32	34.28	1.55	ns
		KPF^+	34.58	33.80	33.07		
	Middle	KPF^-	35.08	35.03	33.70	1.87	ns
		KPF^+	34.65	33.25	32.65		
T_{MAX}	Middle	KPF^-	43.20	41.65	39.78	3.64	ns
		KPF^+	41.48	39.03	38.57		
	Top	KPF^-	42.20	40.32	39.32	3.89	ns
		KPF^+	39.88	37.65	37.72		

NB: when p value (I x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (I x KPF) > 0.05 no means separation was conducted.

Post-irrigation, KPF reduced T_{MAX} of middle subsection on eastern side of the canopy, with means of 39.41 and 37.32 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 1.50; p = 0.009); and of the middle subsection the western side of the canopy, with means of 40.38 and 38.65 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 1.37; p = 0.015). Post-irrigation, KPF reduced T_{MAX} of the top subsection of the eastern side of canopy, with means of 38.26 and 36.26 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 1.14; p = 0.001); and of the top subsection the western side of the canopy with means of 39.76 and 38.77 °C for KPF^- and KPF^+, respectively ($LSD_{p=0.05}$ = 0.86; p = 0.026). Post-irrigation, there was no significant effect of KPF on T_{MIN} for the eastern or westerns sides of the canopy (p > 0.05).

Post-irrigation, there was no significant interaction of irrigation treatments and KPF on T_{MIN} or T_{MAX} for either side of the canopy (p > 0.05; Table 5.23).

Table 5.23. Impact of the irrigation treatments (I_{25}, I_{50} and I_{100}) and kaolin particle film (KPF^- and KPF^+) on minimum temperature (T_{MIN}, °C) of the bottom and middle subsections; and maximum temperature (T_{MAX}, °C) of the middle and top subsections of the canopy measured post-irrigation during the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction irrigation treatments (I) and kaolin particle film (KPF); ns = not significant (p > 0.05).

			Irrigation Treatments			$LSD_{p=0.05}$ (I x KPF)	p value (I x KPF)
			I_{25} °C	I_{50} °C	I_{100} °C		
Eastern Side of the Canopy							
T_{MIN}	Bottom	KPF^-	29.35	30.40	28.58	1.46	ns
		KPF^+	30.22	29.38	27.78		
	Middle	KPF^-	27.47	28.23	26.72	1.38	ns
		KPF^+	27.33	27.35	25.55		
T_{MAX}	Middle	KPF^-	39.98	40.90	38.40	1.49	ns
		KPF^+	39.52	39.45	37.35		
	Top	KPF^-	38.47	39.13	37.18	1.97	ns
		KPF^+	36.48	37.85	34.45		
Western Side of the Canopy							
T_{MIN}	Bottom	KPF^-	28.80	28.20	27.05	1.75	ns
		KPF^+	27.80	27.88	26.60		
	Middle	KPF^-	27.15	26.83	25.55	1.60	ns
		KPF^+	26.97	26.38	25.02		
T_{MAX}	Middle	KPF^-	41.27	40.30	39.58	2.37	ns
		KPF^+	39.98	38.77	37.20		
	Top	KPF^-	40.12	39.77	38.33	2.60	ns
		KPF^+	38.47	37.45	36.05		

NB: when p value (I x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (I x KPF) > 0.05 no means separation was conducted.

5.4.4. Kaolin Particle Film and Glycine Betaine

There was a significant interaction of GB and KPF on ΔTCA, with the combination of products resulting in a lower ΔTCA than either product applied alone (p < 0.001; Table 5.24). There was a significant interaction of GB and KPF on $L_{\%DW}$ and SLA with the combination of GB^+ and KPF^- resulting in the lowest $L_{\%DW}$ and SLA (p = 0.018, Table 5.24). In Y2, there was no significant interaction on any of leaf characteristics measured (p > 0.05; Table 5.24).

Table 5.24. Impact of the glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on the change in trunk cross-sectional area (ΔTCA, cm^2) and leaf characteristics: leaf sizes (L_S, cm^2), leaf fresh weight (L_{FW}, g), leaf dry weight (L_{DW}, g), leaf percent dry weight ($L_{\%DW}$) and specific leaf area (SLA, $cm^2 \cdot g^{-1}$). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction glycine betaine (GB) and kaolin particle film (KPF); ns = not significant (p > 0.05).

		Glycine Betaine		$LSD_{p=0.05}$	p value
		GB^-	GB^+	(GB x KPF)	(GB x KPF)
Change in Trunk Cross Sectional Area					
ΔTCA	**KPF^-**	0.78_a	2.74_b	0.93	< 0.001
	KPF^+	2.08_b	1.63_{ab}		
Leaf Characteristics					
L_S (cm^2)	**KPF^-**	79.6	64.8	11.7	ns
	KPF^+	74.9	69.3		
L_{FW} (g)	**KPF^-**	1.83	1.45	0.36	ns
	KPF^+	1.76	1.75		
L_{DW} (g)	**KPF^-**	0.51	0.47	0.13	ns
	KPF^+	0.35	0.48		
$L_{\%DW}$	**KPF^-**	27.4_b	23.7_a	2.6	0.018
	KPF^+	26.6_b	27.3_b		
SLA ($cm^2 \cdot g^{-1}$)	**KPF^-**	159_{ab}	197.9_b	38.5	0.046
	KPF^+	163.5_{ab}	146.3_a		

NB: when p value (GB x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (GB x KPF) > 0.05 no means separation was conducted.

Pre-irrigation, there was no significant interaction of GB and KPF on ψ_{PD} or ψ_{MID} (p > 0.05; Table 5.25). Post-irrigation, there was a significant interaction of GB and KPF on ψ_{MID}, with the combination of GB^+ and KPF^+ resulting in more negative ψ_{MID} values (p = 0.049; Table 5.25) However, post-irrigation there was no significant interaction of GB and KPF on ψ_{PD} (p > 0.05; Table 5.25). This interaction was significant for post-irrigation ψ_{MID} (p = 0.049). The interaction of GB and KPF on post-irrigation g_l was not significant at p = 0.05 (p = 0.076; Table 5.25).

Pre- and post-irrigation, there was no significant interaction of GB and KPF on T_C, T_C* or T_{SD} for either side of the canopy (Table 5.25).

Pre- and Post-irrigation, there was no significant interaction of GB and KPF on T_{MIN} or T_{MAX} for either side of the canopy (p > 0.05; data not presented).

Table 5.25. Impact of glycine betaine (GB^- and GB^+) and kaolin particle film (KPF^- and KPF^+) on pre-dawn leaf water potential ($\psi_{PD'}$, MPa), midday leaf water potential (ψ_{MID}, MPa), stomatal conductance (g_l, $mmol \cdot m^{-2} \cdot s^{-1}$), average canopy temperature (T_C, °C), canopy and air temperature difference (T_C*, °C) and standard deviation of canopy temperature (T_{SD}, °C) measurements for pre-irrigation and post-irrigation in the 2012-13 growing season (Y2). Least significant difference at p = 0.05 ($LSD_{p=0.05}$) and are reported for the interaction glycine betaine (GB) and kaolin particle film (KPF); ns = not significant (p > 0.05).

					Western Side of the Canopy			Eastern Side of the Canopy		
		ψ_{PD} (MPa)	ψ_{MID} (MPa)	g_l ($mmol \cdot m^{-2} \cdot s^{-1}$)	T_C (°C)	T_C* (°C)	T_{SD} (°C)	T_C (°C)	T_C* (°C)	T_{SD} (°C)
Pre-Irrigation (DOY = 38, Y2)										
GB^-	KPF^-	-1.24	-1.79	‡	3.70	2.79	1.13	37.8	3.66	1.42
	KPF^+	-1.26	-1.85	‡	3.55	2.77	0.92	36.2	3.38	1.10
GB^+	KPF^-	-1.26	-1.81	‡	3.81	3.52	1.21	38.6	3.95	1.42
	KPF^+	-1.28	-1.84	‡	3.70	2.82	1.15	37.5	3.40	1.44
$LSD_{p=0.05}$ (GB x KPF)		0.08	0.08	‡	1.57	1.15	0.31	2.00	1.57	0.43
p value (GB x KPF)		ns	ns	‡	ns	ns	ns	ns	ns	ns
Post-Irrigation (DOY = 45, Y2)										
GB^-	KPF^-	-0.64	-1.42_b	496	31.7	3.73	1.95	32.1	4.14	1.97
	KPF^+	-0.65	-1.41_b	382	31.0	3.81	1.87	31.5	4.25	1.89
GB^+	KPF^-	-0.66	-1.41_b	657	31.9	3.61	2.00	32.2	3.95	1.94
	KPF^+	-0.68	-1.63_a	357	31.2	3.10	1.81	31.7	3.53	1.92
$LSD_{p=0.05}$ (GB x KPF)		0.04	0.16	0.076	1.56	0.89	0.24	1.01	0.68	0.26
p value (GB x KPF)		ns	0.049	ns	ns	ns	ns	ns	ns	ns

NB: when p value (GB x KPF) < 0.05 means separation is indicated by dissimilar letters and when p value (GB x KPF) > 0.05 no means separation was conducted.

‡ Data not recorded

5.5. Discussion of Canopy Growth, Plant Water Status and Canopy Temperature

In this research, the effects of the irrigation treatments, GB and KPF on canopy temperature was assessed spatially (differing regions of the canopy) and temporally with respect to irrigation cycle (pre- and post-irrigation). Analysis was conducted on the variability in canopy temperature as well as the minimum and maximum canopy temperature of subsections of the canopy. Light radiation and leaf age also impact on factors, such as photosynthesis (Intrieri et al., 1992; Poni & Intrieri, 2001), influencing canopy temperature. Light microclimates vary spatially within grapevine canopies (Mabrouk & Sinoquet, 1998), impacting on canopy temperature. The top section of the canopy typically comprised unrestrained canes and exposed canes, which were subjected to physical movement as well as being cooled by air movement. Therefore, it is a reasonable hypothesis that the position of the upper canes accounted for a greater proportion of canopy cooling, accounting for the 1.7 to 2.6 °C difference between the top and bottom of the canopy for the eastern and western sides of the canopy pre- and post-irrigation. The middle of the canopy where the canes were supported and secured by the trellis system exhibited more stable temperatures. The bottom of the canopy was characterised by more variable higher temperatures. This region included soil and non-leaf material, such as inter row grass, which may not have been effectively masked. The bottom of the canopy was likely to contain more basal leaves, which tend to have lower rates of photosynthesis and were more exposed to radiant energy from the earth's surface. Similar observations have been observed by Fuentes et al. (2012), who found the top of the canopy had lower average temperatures due to wind and the bottom of the canopy had larger proportion of temperatures closer to the minimum canopy temperature. Additionally, differences in leaf angle may have influenced the variability of canopy temperature between sections (Grant et al., 2016).

The measurements of ψ_{LEAF} and canopy temperatures were conducted towards the end of the season, two to three weeks before harvest. Estimated vapour pressure deficit (VPD) was higher pre-irrigation than post-irrigation, with an estimated VPD during ψ_{PD} measurements of 1.5 and 0.2 kPa for pre- and post-irrigation, respectively. Estimated maximum VPD, during the ψ_{MID} measurements, was approximately 6.0 and 3.8 kPA for pre- and post-irrigation, respectively. Therefore, it is important to note differences in ψ_{LEAF} may be in part attributed to differences in VPD and temperature (Williams & Araujo, 2002). However, irrigation treatments and rainfall over the growing season appear to have had a significant influence on the ψ_{LEAF} values pre- and post-irrigation.

Various thresholds have been cited in the literature classifying the level of stress based on values of pre-dawn (ψ_{PD}) and midday (ψ_{MID}) leaf water potential (Choné et al., 2001; Girona et al., 2006; Grimes & Williams, 1990; Santesteban et al., 2011). Thresholds range from no water deficit to high/severe water deficit, where vegetative growth, berry growth and photosynthesis are inhibited and grape ripening is partially or totally inhibited (Deloire & Heyns, 2011). Based on published ψ_{PD} and ψ_{MID} thresholds classifying the level of stress (Choné et al., 2001; Girona et al., 2005; Grimes & Williams, 1990; Santesteban et al., 2011), the values of ψ_{LEAF} observed in this research indicate all treatments were under severe to very severe water stress towards the end of the Y2 growing season. This was evident pre-irrigation when the time since the last irrigation was the longest. Therefore, consideration should be made for the diurnal changes in water status (i.e. ψ_{PD} versus ψ_{MID}), as well as longer term influences, such as the irrigation cycle and seasonal water deficits. The results presented here provide an example of vines which are under longer term or seasonal water stress, with measurements conducted both pre-irrigation (maximum time since last irrigation) and post-irrigation (minimum time since last irrigation). The effects of irrigation cycle on the plant water status may explain the asymptotic relationship that was observed between ψ_{PD} versus ψ_{MID} when the data was pooled together for pre- and post-irrigation.

5.5.1. Irrigation Treatments

Pre-irrigation, there was a low range of highly negative ψ_{PD} and ψ_{MID} values across all the treatments, indicating it is likely that the vines were approaching their biological limit (Sperry et al., 1998). Hence no significant differences were observed between the irrigation treatment for ψ_{PD} or ψ_{MID}. It is also likely that the vines were not transpiring in the middle of the day with air temperatures above 30 °C. More negative ψ_{MID} values (-2.06 to -1.58 MPa) were observed in this research under high VPD conditions compared with the research conducted by Soar et al. (2006), where values of ψ_{MID} were above -1.3 MPa under the higher VPD conditions.

Application of irrigation and lower VPD improved the plant water status with less negative ψ_{PD} and ψ_{MID} post-irrigation compared with pre-irrigation. Post-irrigation there was a significant effect of the irrigation treatments, with less negative ψ_{PD} and ψ_{MID} values for I_{100} compared with I_{25}. Emergence of treatment differences for irrigation further supports that all treatments were reaching their biological limits pre-irrigation and the less negative values of ψ_{PD} and ψ_{MID} are not just a reflection of differences in VPD. Furthermore, as the vines transpired during the day following the irrigation, some of the treatments approached similar ψ_{MID} to those observed pre-irrigation, indicating that they were still under severe water stress.

Furthermore, consideration of the effects of the irrigation cycle on the plant water status may explain the asymptotic relationship that was observed between ψ_{PD} and ψ_{MID} when the data was pooled together for pre- and post-irrigation (Figure 5.4). The vines tended to approach a minimum value ψ_{MID} (-1.82 MPa; the asymptote), with minimal differences observed between treatments for ψ_{PD} and ψ_{MID} pre-irrigation. This indicates that all treatments were under severe water stress based on ψ_{LEAF} values observed (Choné et al., 2001; Girona et al., 2005; Grimes & Williams, 1990; Santesteban et al., 2011). Temporary relief was received following the irrigation, with less negative values of ψ_{PD} and ψ_{MID} and greater range of values of ψ_{MID} associated with the different irrigation treatments. The vines then quickly became stressed during the day following irrigation. Shiraz has been shown to exhibit anisohydric or near-anisohydric behaviour, with less control of stomatal conductance in response to diminishing water supplies (Schultz, 2003) and changes in VPD (Soar et al., 2006). This may explain lack of response between ψ_{PD} and g_l and the low R^2 value (0.1828) for the relationship between ψ_{MID} and g_l (Figure 5.5). Therefore, the anisohydric behaviour of Shiraz (Schultz, 2003), may have resulted in less control of stomatal conductance in response to the diminishing water supplies observed. It could also explain why the irrigation treatments had no significant effect on g_l.

Measurement of leaf water potential (ψ_{LEAF}) was conducted on two sunlit leaves from the western side of the canopy, whereas IRTI were taken of the whole canopy for the eastern and western sides of the canopy. Pre-irrigation measurements of ψ_{LEAF} indicated that the vines had most likely commenced closing or had closed their stomata, but this did not consider the response on non-sunlit (shaded leaves). The lower average canopy temperature of I_{100} compared with I_{25} on both sides of the canopy pre-irrigation may be due to differences in transpiration across the whole canopy. Alternatively, I_{100} may have resulted in a higher leaf area density increasing the amount of shade in the canopy reduced T_C. Post-irrigation, the irrigation treatments had a significant effect on ψ_{PD}, ψ_{MID}, T_C and T_C*. I_{25} and I_{50} resulted in significantly more negative values of ψ_{PD} and ψ_{MID} and higher T_C and T_C* compared with I_{100}. Therefore, differences in transpiration associated with water stress were likely to have contributed to differences in T_C between the irrigation treatments.

5.5.2. Glycine Betaine

Plots with GB applied were more stressed, indicated by more negative pre-irrigation ψ_{MID} ($p = 0.028$; Section 5.4.2); with similar trend post-irrigation ψ_{PD} ($p = 0.097$; Section 5.4.2) and ψ_{MID} ($p = 0.068$; Section 5.4.2). It is proposed that GB plays a role in osmoregulation (Jolivet et al., 1982; Robinson & Jones, 1986). The more negative value pre-irrigation ψ_{MID} may be indicating

that the vines are able to reach more negative leaf water potentials before they close their stomata. The results presented in Chapter 3 and 4, indicate that there may have been a reduction in biomass accumulation and thus photosynthesis. Firstly, in Y1, under high rainfall conditions, GB resulted in lower berry weight with similar trend approaching significance in Y2 (refer to Figure 3.9 of Chapter 3). Secondly, whilst there was an increase in total soluble solids (TSS, °Brix; refer Figure 4.7 in Chapter 4), there was a reduction in soluble solids per berry (SSB, $g \cdot berry^{-1}$; refer to Figure 4.7 in Chapter 4), indicating a lower accumulation of sugar in the berries. Thirdly, GB resulted in smaller leaves in Y2 and a visible difference in canopy size was observed supporting research conducted by Mickelbart et al. (2006), where individual leaf area of vines was negatively correlated with GB application rate. Finally, there was a negative effect of GB on the change in ΔTCA. This may be an indication that there was a significant change in carbohydrate storage. Further research is required to determine the effects of GB on photosynthesis and biomass accumulation.

Grapevines have been shown to exhibit relatively low levels of GB under non-stressed conditions (Mickelbart et al., 2006) and, to date, accumulation of GB in grapevines in response to stress has only been observed for salinity (Mohammadkhani et al., 2014). The vines' response to GB could be combination of several factors including the method (exogenous), timing (commencing at flowering), frequency of applications (every three to four weeks), amount applied (~20 $mmol \cdot vine^{-1}$ of GB per application) as well as the fact that grapevines are a perennial plant. Most research into GB has focused on genetically engineering the plant to enable it to produce GB or precursors to GB. In this research GB was applied exogenously and therefore the vines were not in control of up-regulating or down-regulating the concentration of GB. GB was applied from flowering in the absence of stress. A different response may have been observed if GB was applied later in the season and/or in the presence of stress. Multiple applications of GB may have resulted in GB levels increasing over the season, which may have potentially become suppressive to the vine growth.

Generation of reactive oxygen species (ROS) is a common plant response to a range of environmental stresses. However, an imbalance between pro-oxidants (ROS and its reaction products) generation and their antioxidants-mediated metabolism and scavenging can lead to a physiological condition called oxidative stress (Anjum et al., 2014). One mode of action proposed for GB, is that it activates the expression of genes for reactive oxygen species (ROS) scavengers (Chen & Murata, 2011), thus potentially it may play a role in stopping or reducing oxidative stress. However, Schopfer and Liszkay (2006) proposed that unstressed plant cells possess the ability to generate a subset of ROS, reactive oxygen intermediates (ROI), and

utilise this ability for a controlled loosening of cell walls, required for cell growth by water uptake. Therefore, in this experiment it is possible that the exogenous application of GB in the unstressed pre-véraison may have resulted in the up-regulation of ROS scavengers, which may have metabolised ROI required for controlled loosening of cell walls. This may explain the reduction in berry weight in Y1 and early in Y2, and the reduction in leaf area observed in Y2 may have been a result of lower levels of ROI in the plant cells, early in the growing season. Further research is required to determine if the effects of the timing and frequency of application on photosynthesis and biomass accumulation.

Pre-irrigation, GB significantly increased T_C for the western side of the canopy. The hypothesis that GB having an osmoregulation role (Jolivet et al., 1982; Robinson & Jones, 1986), where stomata remain open at more negative values of ψ_{LEAF} resulting in a reduction in canopy temperature, was not supported by these findings. Higher average minimum temperatures for the bottom section of the western side of the canopy contributed to the increase in T_C. The bottom section of the western side of the canopy had the least exposure to the sun due to mutual shading from adjoining rows. Therefore, it is possible that whilst most of the canopy has shut down some shaded leaves may still be transpiring, particularly in plots with I_{100} and GB^-.

5.5.3. Kaolin Particle Film

The availability of water appeared to play a crucial role in the vines' response to KPF. In this experiment, the vines were under a season-long severe water stress as discussed above. Values of ψ_{LEAF} tended to be more negative pre- and post-irrigation for plots with KPF applied. Similarly, lower values of g_l were observed post-irrigation for plots with KPF applied. This is further supported by yield data presented in Chapter 3. In Y1, when water was not considered to be a limiting factor in the I_{100} treatment, there was a reduction in average cluster weight at harvest and a decrease in SSB for plots with KPF applied at the lowest irrigation treatment (I_{25}). In Y2, the combined effect of KPF^+ and I_{25} reduced cluster weight and SSB. Also in Y2, there was a significant reduction in berry weight at harvest across all the irrigation treatments. Denaxa et al. (2012) and Roussos et al. (2010) showed that under 'non-irrigated' conditions, KPF resulted higher photosynthetic rates and there was no reported change in yield, which may indicate that whole tree assimilation was not increased. Irrigation was withheld for a total of 7 days for the 'non-irrigated' plots, therefore, the olive trees were not under as severe water stress as the vines in this experiment.

KPF resulted in vines showing signs of more severe water stress. A similar conclusion was made by Glenn et al. (2001) who concluded that irrigation scheduling may need to be modified to ensure adequate supply of water when KPF is applied. The effects of KPF on photosynthesis are unclear; however, results indicate that under water stressed conditions, KPF results in a further reduction in yield, which may indicate a reduction in whole canopy carbon assimilation. Conversely, there was also an increase in the trunk cross-sectional area for plots with KPF applied, which may indicate an increase in whole canopy carbon assimilation. KPF may be linked to changes in transpiration, due to factors such as changes in stomatal density and/or increased light reflection within the canopy. KPF reduced stomatal density in cotton (Subramanian, 1958) and gooseberry seedling (Segura-Monroy et al., 2015). However, for research presented here the treatments were applied at véraison; therefore, it is unlikely that there was a change in stomatal density. Processes, such as photosynthesis, transpiration and canopy growth, are not only affected by water and light, but importantly by canopy temperatures (Spencer & Peynaud, 1984). Increased reflectivity as a result of applying KPF has been linked to reductions in canopy temperatures (Cooley et al., 2008; Glenn et al., 2001).

In this research, the effects of KPF on canopy temperature were assessed spatially (differing regions of the canopy) and temporally with respect to irrigation cycle (pre- and post-irrigation), revealing that KPF had either no effect or it reduced canopy temperature. Reductions in canopy temperature observed by other researchers (Cooley et al., 2008; Glenn et al., 2001), have been linked with increased reflection of light (radiation) off the leaf surface (Cooley et al., 2008; Glenn & Puterka, 2007; Rosati et al., 2007). In this research, the differences and lack of differences in canopy temperature could not be explained by this alone.

The amount of water available to the vines appeared to influence how average, minimum and maximum canopy temperatures were affected by KPF. Pre-irrigation, KPF reduced canopy temperature of the western side of the canopy ($p < 0.05$). A similar response approached conventional levels of significance for the eastern side of the canopy ($p < 0.10$). Pre-irrigation, the vines were under severe water stress, with all plots reaching lower limits of leaf water potential. Therefore, it is likely that the reduction in canopy temperature observed pre-irrigation for plots with KPF applied was due to increased reflection of radiation from the canopy surface, with little or no difference in transpiration.

Post-irrigation, there appeared to be no effect of KPF on T_C. Vines with KPF applied shut down earlier than vines without KPF applied (particularly under lower irrigation treatments). This was supported by the observed reduction in leaf conductance (g_l) for plots with KPF applied. Therefore, canopy temperatures of plots without KPF applied were cooler due to transpiration.

Canopy temperatures of plots with KPF applied were also cooled (despite a reduction in leaf conductance) due to increased reflection of radiation from the canopy surface. It is unclear why there was no significant effect of KPF on T_{MAX} post-irrigation. Whilst it is possible that T_{MAX} values may have been the temperature of a stem or cluster that was not excluded by the 'mask', frequent observations (cross-referencing to the digital images) at the time indicated that this was unlikely to have occurred.

5.5.4. Glycine Betaine and Kaolin Particle Film

Pre- and post-irrigation, there was no significant interaction of GB and KPF on average, minimum or maximum canopy temperature. However post-irrigation, the combined effect of GB and KPF resulted in significantly more negative ψ_{MID} values compared the other treatments. The individual responses for GB and KPF were both significant at p = 0.10, but not at p = 0.05, so incremental effect of both products may have resulted in this significant difference.

The combined effects of GB and KPF resulted in more negative ψ_{LEAF} values, which could be linked to a combination of osmoregulation by GB and higher water loss by KPF. Other factors, such as changes in leaf area density and hence shaded regions and increased reflection of radiation off the canopy by KPF may have influenced canopy temperature. This demonstrates the inherent complexities of assessing the interaction of GB and KPF. It is possible that KPF resulted in a higher water loss through transpiration, with GB allowing each leaf to reach more negative values of ψ_{MID} before shutting down, resulting in the vine returning to stress conditions more rapidly.

5.6. Conclusion

Analyses of presented in this Chapter were conducted in Y2 of this experiment, when all treatments were under severe water stress, particularly pre-irrigation. Analysis of average, minimum and maximum canopy temperature provided some unique insights into vines' response to the irrigation treatments, GB and KPF. GB increased average canopy temperature, which may be linked to less shading in regions of the canopy as a result of a reduction in leaf area density. The amount of water available to the vines appeared to influence how average, minimum and maximum canopy temperatures were affected by KPF. Results presented indicate that changes or lack of changes in canopy temperature (average, minimum and maximum) cannot be explained by increased reflection from the canopy alone; rather they indicate that under certain circumstances KPF may cause changes in transpirational cooling.

Other research has shown that GB and KPF may enable plants to continue to function in the short-term under environmental stresses, such as water deficits and heat stress. However, this research showed that GB and KPF may increase the grapevines susceptibility if the stress conditions are long-term. In the absence of sufficient irrigation and rainfall over the growing season, it is possible that these longer-term impacts of increased water stress may outweigh the purported short-term benefits, such as ameliorating some of the effects of heat stress. This research has demonstrated that care should be taken when applying GB, as GB resulted in the vines showing signs of a stress response. Timing, rate of application and accumulation of GB within the vine may have played a role in this response.

Chapter 6. General Discussion and Conclusion

6.1. Introduction

The principal objective of this research was to investigate the protective capacity of glycine betaine (GB) and kaolin particle film (KPF), individually and combined, on grapevine performance under varying levels of imposed water stress, and their associated interactions on the productive performance and physiology of *Vitis vinifera* L. 'Shiraz' grown under field conditions in North East Victoria, Australia.

More specifically, the objectives of this research were to:

(1) Investigate the effectiveness of a metabolic protectant GB on the physiology and performance of grapevines under water stress conditions;

(2) Investigate the effectiveness of a physical protectant KPF on the physiology and performance of grapevines under water stress conditions;

(3) Determine potential interactions between these protectants on grapevine performance under water stress conditions; and

(4) Discuss the potential value of these protectants under future hotter and drier climatic conditions.

A field experiment was conducted on Shiraz (clone SA16) vines grown at The University of Melbourne', Dookie Campus Vineyard in North East Victoria, Australia (-36.395 °S, 145.703 °E). A randomised complete block design (RCBD) factorial experiment was established in 2011 comprising three irrigation treatments (I_{25}, I_{50} and I_{100}), two GB treatments (GB^- and GB^+) and two kaolin particle film treatments (KPF^- and KPF^+), with six replicates per treatment. Vineyard practice (I_{100}) was a time-date, on-off irrigation strategy with the aim of irrigating for 4 h every 10 to 14 days from mid-November to late December, then every three to six days from late December until just after harvest; I_{50} and I_{25} applied 50 and 25% of the volume of water applied for I_{100} each irrigation event, respectively. Two applications of KPF were applied; the first at véraison and the second one week later. GB applications were applied every three to four weeks from flowering.

The following discussion reviews the outcomes of this research in relation to the literature, it's implications for industry and further research in this area.

6.2. Research Outcomes

6.2.1. Water Stress

In Y1 and Y2, reducing the amount of water applied reduced berry weight and soluble solids per berry (SSB, g·berry^{-1}), contributing to a decrease in cluster weight and yield, despite contrasting rainfall received in the two growing seasons. In Y1, the impact of irrigation treatments was limited to pre-véraison due very high rainfall between véraison and harvest. Whereas in Y2, the impact of irrigation treatments was not limited to pre-véraison, as irrigation accounted for a large portion of water received post-véraison.

In Y1, under the high rainfall conditions, the change in berry weight was likely driven by lower cell division and growth during the first stage of berry growth (Freeman et al., 1979, 1980). This supports work conducted by McCarthy (1997), which showed that Shiraz were more susceptible to water deficits in the period after flowering compared with after véraison. In contrast in Y2, observed leaf water potential values (ψ_{LEAF}, MPa) and canopy temperatures, indicate that all irrigation treatments were under severe water stress and reducing the amount of irrigation water applied also increased berry percent dry weight ($B_{\%DW}$), colour concentration (mg·g^{-1}) and phenolics concentration (P_g, au·g^{-1}). This indicates as the water stress conditions continued after véraison, reducing the amount of irrigation water applied reduced the berry water content.

Water availability (rainfall and irrigation) dominated responses in yield and composition, an observation that has been evidenced extensively (Downey et al., 2006; Freeman et al., 1979; Jones & Tardieu, 1998; McCarthy, 1997; Roby et al., 2004; Roby & Matthews, 2004; Smart, 1985; Vasconcelos et al., 2009). However, water cannot be managed in isolation and consideration must be made for other factors such as heat stress. GB and KPF are two commercially available foliar sprays, which may be used by industry to ameliorate the effects of environmental stresses, such as heat and water stress. Observations related to their effectiveness on commercially grown Shiraz grapevines are discussed below.

6.2.2. Effectiveness of Metabolic Protectant Glycine Betaine

Grapevines have been shown to exhibit relatively low levels of GB under non-stressed conditions (Mickelbart et al., 2006) and accumulation of GB in grapevines in response to stress has only been observed for salinity (Mohammadkhani et al., 2013). Grapevine response to GB may be influenced by factors including the method (exogenous), timing (commencing at flowering), frequency of applications (every three to four weeks), amount applied (~20 mmol·vine^{-1} of GB per application) and the fact that grapevines are a perennial plant. Most

research into GB has focused on genetically engineering the plant to enable it to produce GB or precursors to GB (Huang et al., 2000; Jun et al., 2000; Sakamoto & Murata, 2000). In this research, GB was applied exogenously, therefore the grapevine was not in control of up-regulating or down-regulating GB synthesis.

In the absence of stress, applying GB was found to reduce yield in Y1 (Section 3.3.2). It is possible that a different response may have been observed if GB was applied later in the season and/or in the presence of other stresses, such as heat stress (Ashraf & Foolad, 2007; Jolivet et al., 1982; Sakamoto & Murata, 2000, 2002); or salinity (Jun et al., 2000; Mohammadkhani et al., 2013, 2014; Sakamoto & Murata, 2002). In research conducted by Mäkelä et al. (1998b), exogenous application of GB had no effect on ABA content of tomato plants. Furthermore, ABA has been shown to accumulate prior to GB in drought stressed maize (Zhang et al., 2012). Therefore, it is possible that GB is involved in stress signalling or the mechanisms causing earlier ripening that have been observed under higher levels of ABA. In this research, there was evidence of earlier ripening in both seasons, with GB application resulting in higher total soluble solids (TSS, °Brix) for the first sample date in both years.

The effects of applying GB became less evident as water stress conditions emerged in Y2, with smaller leaf size observed in Y2. This indicates that the timing of application played a role in vines' response to GB. One mode of action proposed for GB (refer to Section 1.5.1 of Chapter 1), is that it activates the expression of genes for reactive oxygen species (ROS) scavengers (Chen & Murata, 2011; Kurepin et al., 2015) thus potentially playing a role in stopping or reducing oxidative stress. Oxidative stress is a physiological condition that occurs when there is an imbalance between generation pro-oxidants and their antioxidants-mediated metabolism and scavenging (Anjum et al., 2014). Generation of ROS is a common plant response to a range of environmental stresses. However, Schopfer and Liszkay (2006) proposed that unstressed plant cells possess the ability to generate a subset of ROS, reactive oxygen intermediates (ROI), and utilise this ability for a controlled loosening of cell walls, required for cell growth by water uptake. Therefore, in this research, exogenous application of GB from flowering may have reduced the levels of ROI required for controlled loosening of cell walls reducing cell division and expansion. This may explain the reduction in berry weight in Y1 and early in Y2, and the reduction in leaf area observed in Y2. Furthermore, a reduction in cell division and expansion prior to véraison may have reduced yield potential, limiting the benefit of applying GB under the water stress conditions experienced from véraison. However, it is important to consider that there may be other unidentified modes of action that have contributed to these observations.

Frequency of application must also be considered. In this research, GB was applied every 3 to 4 weeks from flowering. Therefore, it is possible that endogenous GB accumulated over the growing season, resulted in the negative responses observed here. This supports the findings of Mickelbart et al. (2006) who found that high concentrations of GB can potentially have negative impacts including decreased or ceased leaf growth and severe phytotoxic symptoms in grapevines. It should be noted that, in the research conducted here, there were no visual signs of phytotoxic symptoms.

6.2.3. Effectiveness of Physical Protectant Kaolin Particle Film

Grape variety has been shown to play a role in grapevine responses to applying KPF (Glenn et al., 2010). It is possible that this variation is driven by differences in stomatal conductance (g_l) response to water deficits. Shiraz tends to maintain stomatal conductance under water stress conditions more than other varieties (Schultz, 2003), which may mean Shiraz is more susceptible to water loss. Glenn et al. (2001) reported higher water use as a result of an increase in g_l when KPF was applied, leading the authors to conclude that irrigation scheduling may need to be altered to ensure adequate supply of water. In the research presented here, water availability (irrigation and rainfall) influenced the vines' response to applying KPF. In Y1, under high rainfall conditions, the combined effect of KPF^+ and I_{25} resulted in lower cluster weight; whilst in Y2, under low rainfall conditions, KPF significantly reduced average berry weight for all the irrigation treatments.

Whilst there has been strong agreement that applying KPF increases light reflection, thus reducing canopy temperature (Cooley et al., 2008), there has also been speculation that changes in canopy temperature and reflection of light within the canopy may be related to changes in transpiration. It is possible that applying KPF increased transpiration immediately following irrigation events, resulting in the vines becoming water stressed more rapidly and hence, shutting stomata earlier due to water stress. Additionally, cooler ambient temperatures and/or a reduction in the thickness of the film may have contributed to this response, supporting the recommendations and findings made by Shellie (2015).

Pre-irrigation in Y2, vines in all treatments were reaching biological limits; shutting down stomata due to high vapour pressure deficit (VPD) and low water availability across all treatments. Canopy temperature observations indicate that KPF increased light reflection reducing minimum canopy temperature and average canopy temperature; however, there was no effect of KPF on maximum canopy temperature. Whilst the mechanisms behind this response are unclear, it shows that under some conditions KPF may not reduce maximum

canopy temperature. Consistency of the data and regular cross-referencing with digital images indicate this was not an artefact of the data (i.e. a non-masked cluster). However, future research assessing maximum temperatures under a range of conditions would assist with understanding this observation. In contrast, KPF significantly reduced maximum canopy temperature by approximately 2 °C post-irrigation, with no change to average and minimum canopy temperature. These observations and the lower leaf conductance observed for KPF^+ post-irrigation support the theory that KPF resulted in the vines returning to stress conditions more rapidly, thus closing stomata and reducing transpirational cooling. However, further research is required to determine the mechanisms behind this response.

6.2.4. Interaction of Glycine Betaine and Kaolin Particle Film

Under non-stressed conditions in Y1, applying GB appears to have resulted in the vines exhibiting a stress response, with the impact becoming less evident under water stressed conditions in Y2. In contrast the response to KPF was influenced water stress with KPF making the vines more susceptible to the stress. The combined effect of GB^+ and KPF^+ may have caused a response similar to KPF^+ under water stressed conditions. This is evidenced by the combined effect of GB^+ and KPF^+ that resulted in lower average bunch weight in Y1 and more negative values of Ψ_{MID} post-irrigation in Y2. However, interpretation of the interaction of GB and KPF on the change in trunk cross-sectional area (ΔTCA, cm^{-2}) was complicated by the impacts of the irrigation treatments seasonality. Independently applying GB or KPF increased ΔTCA; however, the combined effect of GB^+ and KPF^+ was not significantly different from applying no products. Results presented here indicate there is the potential for an interaction of GB and KPF. It is therefore strongly recommended that these compounds are not used together under non-stressed conditions until analysis of this complicated response is better understood particularly around “mode of action”, and across different growing conditions.

6.3. Industry Applications

Climate change and climate variability remain a major challenge for the wine industry globally and particularly under Australian conditions. This research will provide industry with an indication of how Shiraz grapevines respond to the application of GB and KPF under both high and low water availability conditions. Valuable observations were made on the yield, physical and physiological responses under varying conditions of water availability, and how vines responded based on the analysis of canopy temperature. Uncharacteristically low maximum temperatures were observed during the experimental period (refer to Section 2.2.2 of Chapter 2). Therefore, it is not possible to draw definitive conclusions regarding the effectiveness of

these commercially available stress ameliorants under heat stress conditions. Further research under heat stress conditions is needed.

In this research, the amount of irrigation water applied varied with treatments; however, frequency and duration of irrigation events remained the same. Observations in Y2, under severe water stress conditions may provide an indication of the potential value of applying GB and/or KPF under a future drier climate. Under low water availability, it may be possible to alter the frequency or timing of the irrigation treatments instead of the amount applied. Firstly, all plots were extremely water stressed in Y2, with highly negative values pre-irrigation for ψ_{LEAF} and no observed difference between the irrigation treatments, whereas post-irrigation, the I_{100} plots had less negative values of ψ_{LEAF}. Therefore, it may be more beneficial to apply a larger amount of water less frequently, possibly in preparation for very hot days. Secondly, even under the high rainfall conditions in Y1, differences in berry weight were observed between the irrigation treatments. Therefore, it may be more beneficial to prioritise irrigation water allocations to pre-véraison to maximise berry size. However, the author recognises that for some vineyards this might present a significant challenge due to several factors including (1) pumping times being limited by access to off-peak electricity rates; (2) capacity of the irrigation system; and (3) availability of water for irrigation. Furthermore, alteration of one management practice may have flow on effects for other management practices, such as nutrition and fertilisation.

Water availability (rainfall and irrigation) dominated treatment responses in yield and composition. Importantly the novel observations presented here, demonstrated both GB and KPF have different impacts across different environmental conditions and show that there is a potential for interactions that could compromise commercial production. Future research should focus on understanding the mechanisms behind these negative responses to assist in developing application guidelines for KPF and GB.

In summary, the results observed in this research indicate that neither product showed sufficient improvement in yield or quality to justify their use of GB and/or KPF in Shiraz vineyards in North East Victoria. Furthermore, it is recommended that these GB and KPF are not used together until analysis of this complicated response is better understood particularly around the ‘mode of action’, and across different growing conditions.

6.4. Recommendations for Further Research

The research presented here identified many gaps for further research to better understand the effectiveness of metabolic protectant GB and physical protectant KPF in ameliorating environmental stresses, such as heat and water stress. These are summarised below.

6.4.1. Analysis under Heat Stress Conditions

Severe heat stress conditions were not experienced in this research. Therefore, determining the potential value of applying GB and/or KPF under a future hotter and drier climate was limited. Field experiments assessing the impacts of climate events, such as heatwaves, have their inherent challenges; evidenced by the low number of days above 35 °C research (Figure 2.4 in Chapter 2). Artificial heating of leaves and fruit could be used to examine the effects of GB and KPF on heat stress. Two approaches that can be used to artificially heat part or the whole of the canopy *in situ* are: (1) a temperature-controlled chamber (Racsko & Schrader, 2012; Schrader et al., 2001); or (2) a large-scale open top-system (Sadras et al., 2012b). The temperature-controlled chamber is an electrical heating device (Schrader et al., 2001) in which attached fruit or leaves can be enclosed under full sunlight (Racsko & Schrader, 2012). The large-scale open top-system artificially heats the whole canopy under full sunlight by circulating hot water through a series of panels located under the canopy (Sadras et al., 2012b). To assess the effects of temperature, independently of radiation, fruit and leaves could be artificially shaded using filters or a block out. The artificial heating systems may be used to simulate heatwaves, and measurements of leaf and fruit temperature may provide some insights as to the potential value of the two products. A combination of infrared thermal imaging and contact measurements (canopy sensors and thermocouples; Moffat, 2013) could be used to assess temporal and spatial changes in leaf and fruit temperatures.

6.4.2. Analysis of Hormones and By-products of Stress

This research showed that applying GB every three to four weeks from flowering under non-stressed conditions has the potential to result in stress like responses, including lower yield and smaller leaf size. In summary, three possible causes of this response have been proposed: (1) GB may be directly or indirectly involved in the stress signalling pathway(s) activated by ABA causing stress induced symptoms (i.e. smaller leaves); (2) GB may up-regulating ROS scavengers reducing ROI content required for cell division; and (3) plants may respond to the accumulation of high levels of endogenous GB. Analysis of the various proposed causes may be conducted by assessing endogenous levels of various compounds and hormones or by assessing the up-regulation and down-regulation of various genes. Endogenous levels of GB

may be assessed using methodology described by Mickelbart et al. (2006). Analysis of ABA may be conducted using molecular probes to assess the expression of genes associated with ABA synthesis and catabolism using methodology similar to Speirs et al. (2013). These methods could also be used to assess why the application of KPF to grapevines under water deficits increased susceptibility to water stress. Additionally, exogenous application of ABA could be used to assess potential interactions between ABA and KPF.

6.4.3. Analysis of Flavour Compounds

Management strategies, such as deficit irrigation, are often aimed at reducing excessive vegetative growth (Collins, 2006). Excessive vegetative growth is often associated with poor fruit exposure (Dokoozlian & Kliewer, 1995a), which may have negative implications such as lower grape quality (Dokoozlian & Kliewer, 1995a; Smart & Robinson, 1991). In this research, there is evidence to suggest GB reduced canopy size, whilst KPF increased light reflection within the canopy. Distinct flavour differences were noticed, particularly where GB was applied, by the lead author and other researchers assisting with field work. Therefore, it would be beneficial to analyse other composition parameters, such as flavonoids, to assess if there is a positive effect of GB, KPF or the interaction of GB and KPF on flavour, and hence grape quality. Alternatively, small-scale wine production could also provide insights on the effects of the various treatments on the sensory attributes; however, these facilities were not available at the time of this research.

6.4.4. Analysis of Assimilation and Transpiration

The mode(s) of action resulting in KPF increasing grapevines stress response to water deficits remains unclear. It is possible that applying KPF to a variety with anisohydric or near anisohydric stomatal response has resulted in an increase in transpiration rates. This may have accelerated the rate of which the vines began shutting down (closing stomata), evidenced by the lower gl and analysis of canopy temperature, discussed above. Analysis of diurnal whole canopy assimilation and transpiration may assist in understanding this relationship, assisting industry in the development of appropriate application methodologies. Additionally, analysis of another wine grape variety (e.g. Cabernet Sauvignon with very different stomatal behaviour to Shiraz – isohydric vs anisohydric behaviour) under the same environmental conditions, may improve the current understanding of the effects of stomatal behaviour on the grapevine response to products such as GB and KPF.

6.4.5. *Analysis of Alternative Application Methodology*

Alternative methods of exogenously applying GB need to be explored which may include altering amount, frequency and timing of applications. For example, it may be beneficial to apply GB reactively in response to a heat stress event, as this research has shown the potential for a negative response under non-stressed conditions. In addition to exploring differing amounts, frequency and timing of applications for KPF, it would also be beneficial to explore different application methodologies, such as only spraying part of the canopy (i.e. the main fruiting zone).

6.4.6. *Analysis over Multiple Growing Seasons*

Given the perennial nature of grapevines, the effects of water stress in one season can impact on the yield of future seasons, even when full irrigation is restored (Petrie et al., 2004). High rainfall in the month prior to harvest in Y1 limited the ability of this research to assess the effects of water stress and the potential alleviating (or not) effects of GB and/or KPF on subsequent seasons yield and yield components. Analysis of the effects of GB and/or KPF over three or more seasons would provide unique insights on the effects of these products on future seasons yield and potentially explain the effects of GB and KPF on ΔTCA observed in this research.

6.5. Concluding Remarks

Climate change and climate variability remain a major challenge for the wine industry globally and particularly under Australian conditions. Environmental and management decisions can have a significant effect on the total water available for the vines, which subsequently affect the grapevines' response to management strategies, such as GB and KPF, employed to reduce the impacts of environmental stresses such as heat stress.

GB has commonly been associated with reducing the effects of various environmental stresses. However, this research has found evidence of GB causing symptoms commonly associated with environmental stress (i.e. smaller leaves and lower yield). These effects were not alleviated by high rainfall prior to harvest in Y1 and, in Y2 when water availability after véraison remained low these effects became less evident. It is proposed by this research that GB may play a vital role in limiting cell division during the first stage of berry growth through the up regulation of ROS scavengers.

In contrast, KPF response has been shown to be influenced by water stress with KPF appearing to make the vine more susceptible to the stress. These finding are important as they indicate

that the use of these compounds to ameliorate the impacts of environmental stresses, such as heat stress, needs to be carefully assessed along-side predicted grapevine water availability. Shiraz is an economically important variety in Australia making up approximately half of red wine grape production. There has been limited research evaluating the effects of KPF under deficit and non-deficit conditions particularly in Australia. Therefore, the findings presented in this thesis will be beneficial to growers in Australia. Furthermore, this research demonstrated there is the potential for complicated interactions between the irrigation treatments, GB and KPK; and caution needs to be exercised before applying both products.

Chapter 7. References

Abou-Khaled, A., Hagan, R. M., & Davenport, D. C. (1970). Effects of kaolinite as a reflective antitranspirant on leaf temperature, transpiration, photosynthesis, and water-use efficiency. *Water Resources Research, 6*(1), 280-289.

Adeyemi, O., Grove, I., Peets, S., Domun, Y., & Norton, T. (2018). Dynamic neural network modelling of soil moisture content for predictive irrigation scheduling. *Sensors, 18*(10), 3408.

Agboma, P., Jones, M., Peltonen-Sainio, P., Rita, H., & Pehu, E. (1997). Exogenous glycinebetaine enhances grain yield of maize, sorghum and wheat grown under two supplementary watering regimes. *Journal of Agronomy and Crop Science, 178*(1), 29-37.

Ahmad, R., Lim, C. J., & Kwon, S.-Y. (2013). Glycine betaine: a versatile compound with great potential for gene pyramiding to improve crop plant performance against environmental stresses. *Plant Biotechnology Reports, 7*(1), 49-57.

Allakhverdiev, S., Feyziev, Y. M., Ahmed, A., Hayashi, H., Aliev, J. A., Klimov, V., Murata, N., & Carpentier, R. (1996). Stabilization of oxygen evolution and primary electron transport reactions in photosystem II against heat stress with glycinebetaine and sucrose. *Journal of Photochemistry and Photobiology B: Biology, 34*(2-3), 149-157.

Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Crop evapotranspiration – guidelines for computing crop water requirements – FAO irrigation and drainage paper 56. *FAO, Rome, 300*(9), D05109.

Anjum, N. A., Gill, S. S., Khan, I., & Gill, R. (2014). Environmental change, and plant amino acids and their derivatives – an introduction. In N. A. Anjum, S. S. Gill, & R. Gill (Eds.), *Plant adaptation to environmental change: significance of amino acids and their derivatives*: CABI.

Aro, E.-M., Virgin, I., & Andersson, B. (1993). Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta, 1143*(2), 113-134.

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany, 59*(2), 206-216.

Atkin, O. K., Loveys, B. R., Atkinson, L. J., & Pons, T. L. (2005). Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Experimental Botany, 57*(2), 267-281.

Australian Bureau of Statitics. (1995). Australian wine grape industry, 1995; Cat. No. 1329.0. Retrieved 20th November, 2018 from http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1329.01995?OpenDocument

Australian Bureau of Statitics. (2006). Australian wine grape industry, 2005; Cat. No. 1329.0. Retrieved 20th November, 2018 from http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1329.02005?OpenDocument

Australian Bureau of Statitics. (2015). Vineyards, Australia, 2014-15; Cat. No. 1329.0.55.002. Retrieved 20th November, 2018 from http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1329.0.55.0022014-15?OpenDocument

Australian Bureau of Statitics. (2016). Agricutlural commodities, Australia, 2014-15; Cat. No. 7121.0. Retrieved 20th November, 2018 from

http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/7121.02014-15?OpenDocument

Avenant, J. H. (1994). The effect of hail netting on the performance of table grapes in the summer rainfall regions of South Africa. *Proceedings of the International Symposium on Table Grape Produciton, 1994*, 227-229.

Avenant, J. H. (1995). The effect of hail netting on the performance of table grapes *Vitis vinifera* (L.) in the summer rainfall region. *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie, 14*(3), 85-91.

Barber, J., & Andersson, B. (1992). Too much of a good thing: light can be bad for photosynthesis. *Trends in Biochemical Sciences, 17*(2), 61-66.

Berli, F. J., Moreno, D., Piccoli, P., Hesphanhol-Viana, L., Silva, M. F., Bressan-Smith, R., Cavagnaro, J. B., & Bottini, R. (2010). Abscisic acid is involved in the response of grape (*Vitis vinifera* L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. *Plant, Cell and Environment, 33*(1), 1-10.

Bermis, R. (2005). Thresholding tool (https://au.mathworks.com/matlabcentral/fileexchange/6770-thresholding-tool/content/thresh_tool.m). from MATLAB Central File Exchange

Berry, J., & Bjorkman, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology, 31*(1), 491-543.

Besaratinia, A., Yoon, J.-I., Schroeder, C., Bradforth, S. E., Cockburn, M., & Pfeifer, G. P. (2011). Wavelength dependence of ultraviolet radiation-induced DNA damage as determined by laser irradiation suggests that cyclobutane pyrimidine dimers are the principal DNA lesions produced by terrestrial sunlight. *The Journal of the Federation of American Societies for Experimental Biology, 25*(9), 3079-3091.

Boari, F., Donadio, A., Schiattone, M. I., & Cantore, V. (2015). Particle film technology: a supplemental tool to save water. *Agricultural Water Management, 147*, 154-162.

Boulton, R. (1980). The general relationship between potassium, sodium and pH in grape juice and wine. *American Journal of Enology and Viticulture, 31*(2), 182-186.

Box, G. E. P. (1950). Problems in the analysis of growth and wear curves. *Biometrics, 6*(4), 362-389.

Bramley, R. G. V., Ouzman, J., & Boss, P. K. (2011). Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes. *Australian Journal of Grape and Wine Research, 17*(2), 217-229.

Brillante, L., Belfiore, N., Gaiotti, F., Lovat, L., Sansone, L., Poni, S., & Tomasi, D. (2016). Comparing kaolin and pinolene to improve sustainable grapevine production during drought. *PloS One, 11*(6), 1-19.

Brodie, G. I. (2008). *Innovative wood drying: applying microwave and solar technologies to wood drying*: VDM Publishing.

Brown, B. A., Cloix, C., Jiang, G. H., Kaiserli, E., Herzyk, P., Kliebenstein, D. J., & Jenkins, G. I. (2005). A UV-B-specific signaling component orchestrates plant UV protection. *Proc Natl Acad Sci U S A, 102*(50), 18225-18230.

Bussotti, F., Desotgiu, R., Pollastrini, M., & Cascio, C. (2010). The JIP test: a tool to screen the capacity of plant adaptation to climate change. *Scandinavian Journal of Forest Research, 25*(S8), 43-50.

Caprio, J., & Quamme, H. (2002). Weather conditions associated with grape production in the Okanagan Valley of British Columbia and potential impact of climate change. *Canadian Journal of Plant Science, 82*(4), 755-763.

Cartechini, A., & Palliotti, A. (1995). Effect of shading on vine morphology and productivity and leaf gas exchange characteristics in grapevines in the field. *American Journal of Enology and Viticulture, 46*(2), 227-234.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., Carvalho, I., Faria, T., & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany, 89*(7), 907-916.

Chen, T. H., & Murata, N. (2008). Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science, 13*(9), 499-505.

Chen, T. H. H., & Murata, N. (2011). Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant, Cell and Environment, 34*(1), 1-20.

Choné, X., van Leeuwen, C., Dubourdieu, D., & Gaudillère, J. P. (2001). Stem water potential is a sensitive indicator of grapevine water status. *Annals of Botany, 87*(4), 477-483.

Christensen, L. P., Dokoozlian, N. K., Walker, M. A., & Wolpert, J. A. (2003). *Wine grape varieties in California*: University of California Agriculture and Natural Resources Publication 3419.

Clingeleffer, P. R., Martin, S., Dunn, G., & Krstic, M. (2001). *Crop development, crop estimation and crop control to secure quality and production of major wine grape varieties : a national approach* Wayville, SA, Australia. Retrieved from: https://publications.csiro.au/rpr/pub?list=BRO&pid=procite:e3b20ec6-9806-471c-a8f1-939515a1f626

Collins, D. A., Della-Marta, P. M., Plummer, N., & Trewin, B. C. (2000). Trends in annual frequencies of extreme temperature events in Australia. *Australian Meteorological Magazine, 49*(4), 277-292.

Collins, M. (2006). *Physiological responses of field grown Shiraz grapevines to partial rootzone drying and deficit irrigation.* (Doctor of Philosophy), The University of Melbourne, Melbourne Retrieved from https://minerva-access.unimelb.edu.au/handle/11343/39175

Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Beureau of Meteorology (BOM). (2015a). Australian climate influences. Retrieved 8th January, 2017 from https://www.climatechangeinaustralia.gov.au/en/climate-campus/climate-system/australian-climate-influences/

Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Beureau of Meteorology (BOM). (2015b). Climate change in Australia. Information for Australia's natural resource management regions: technical report. Retrieved 28th July, 2018 from https://www.climatechangeinaustralia.gov.au/en/publications-library/technical-report/

Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Beureau of Meteorology (BOM). (2015c). Climate variability and climate change. Retrieved 28th July, 2018 from https://www.climatechangeinaustralia.gov.au/en/climate-campus/climate-system/variability-vs-change/

Considine, J. A. (2004). Grapevine productivity and yield components: a case study using field vines of Zante currant. *Australian Journal of Grape and Wine Research, 10*(2), 108-115.

Cooley, N., Glenn, D., Clingeleffer, P., & Walker, R. (2008). The effects of water deficit and particle film technology interactions on Cabernet Sauvignon grape composition. *Acta Horticulturae, 792*, 193-200.

Cooley, N., Higgins, J., Holmes, M., & Attridge, T. (2001). Ecotypic differences in responses of *Arabidopsis thaliana* L. to elevated polychromatic UV-A and UV-B+A radiation in the natural environment: a positive correlation between UV-B+A inhibition and growth rate. *Journal of Photochemistry and Photobiology B: Biology, 60*(2-3), 143-150.

Cooley, N., Holmes, M., & Attridge, T. (2000a). Growth and stomatal responses of temperate meadow species to enhanced levels of UV-A and UV-B+ A radiation in the natural environment. *Journal of Photochemistry and Photobiology B: Biology, 57*(2-3), 179-185.

Cooley, N., Truscott, H., Holmes, M., & Attridge, T. (2000b). Outdoor ultraviolet polychromatic action spectra for growth responses of *Bellis perennis* and *Cynosurus cristatus*. *Journal of Photochemistry and Photobiology B: Biology, 59*(1-3), 64-71.

Cooley, N. M., Clingeleffer, P. R., & Walker, R. R. (2017). Effect of water deficits and season on berry development and composition of Cabernet Sauvignon (*Vitis vinifera* L.) Grown in a hot climate. *Australian Journal of Grape and Wine Research, 23*(2), 260-272.

Coombe, B. G., & McCarthy, M. G. (2000). Dynamics of grape berry growth and physiology of ripening. *Australian Journal of Grape and Wine Research, 6*(2), 131-135.

Cottrell, T. E., Wood, B. W., & Reilly, C. C. (2002). Particle film affects black pecan aphid (Homoptera: Aphididae) on pecan. *Journal of Economic Entomology, 95*(4), 782-788.

Crippen, D. D., & Morrison, J. C. (1986). The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *American Journal of Enology and Viticulture, 37*(4), 235-242.

Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen, C., Vivin, P., & Delrot, S. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *American Journal of Enology and Viticulture, 62*(4), 413-425.

Dal Santo, S., Tornielli, G. B., Zenoni, S., Fasoli, M., Farina, L., Anesi, A., Guzzo, F., Delledonne, M., & Pezzotti, M. (2013). The plasticity of the grapevine berry transcriptome. *Genome Bbiology, 14*(6), 1-18.

De Langre, E. (2008). Effects of wind on plants. *Annual Review of Fluid Mechanics, 40*, 141-168.

Deloire, A., Carbonneau, A., Wang, Z., & Ojeda, H. (2004). Vine and water: a short review. *OENO One, 38*(1), 1-13.

Deloire, A., & Heyns, D. (2011). The leaf water potentials: principles, method and thresholds. *Wynboer, 265*, 119-121.

Denaxa, N.-K., Roussos, P. A., Damvakaris, T., & Stournaras, V. (2012). Comparative effects of exogenous glycine betaine, kaolin clay particles and Ambiol on photosynthesis, leaf sclerophylly indexes and heat load of olive cv. Chondrolia Chalkidikis under drought. *Scientia Horticulturae, 137*, 87-94.

Department of Science (Queensland Government). (2016). SILO data formats. Retrieved 5th May 2018 from https://legacy.longpaddock.qld.gov.au/silo/data_format.html

Dinis, L.-T., Bernardo, S., Conde, A., Pimentel, D., Ferreira, H., Félix, L., Gerós, H., Correia, C., & Moutinho-Pereira, J. (2016a). Kaolin exogenous application boosts antioxidant capacity and phenolic content in berries and leaves of grapevine under summer stress. *Journal of Plant Physiology, 191*, 45-53.

Dinis, L.-T., Ferreira, H., Pinto, G., Bernardo, S., Correia, C., & Moutinho-Pereira, J. (2016b). Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation. *Photosynthetica, 54*(1), 47-55.

Dokoozlian, N. K., & Kliewer, W. M. (1995a). The light environment within grapevine canopies. I. Description and seasonal changes during fruit development. *American Journal of Enology and Viticulture, 46*(2), 209-218.

Dokoozlian, N. K., & Kliewer, W. M. (1995b). The light environment within grapevine canopies. II. Influence of leaf area density on fruit zone light environment and some canopy assessment parameters. *American Journal of Enology and Viticulture, 46*(2), 219-226.

Doraiswamy, P. C., & Rosenberg, N. J. (1974). Reflectant induced modification of soybean canopy radiation balance. I. Preliminary tests with a kolinite reflectant. *Agronomy Journal, 66*(2), 224-228.

Downes, R. G. (1949). *A soil, land-use and erosion survey of parts of the counties of Moira and Delatite, Victoria*: Commonwealth Scientific and Industrial Research Organization.

Downey, M. O., Dokoozlian, N. K., & Krstic, M. P. (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, 57*(3), 257-268.

Downey, M. O., Harvey, J. S., & Robinson, S. P. (2003). Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research, 9*(1), 15-27.

Dragolovich, J., & Pierce, S. K. (1994). The role and regulation of methylamines in the response of cells to osmotic stress. In K. Strange (Ed.), *Cellular and Molecular Physiology of Cell Volume Regulation* (pp. 123-132). Boca Raton, Florida: CRC Press Inc.

Eagleson, P. S. (1978). Climate, soil, and vegetation: 1. Introduction to water balance dynamics. *Water Resources Research, 14*(5), 705-712.

Edwards, E. J., Smithson, L., Graham, D. C., & Clingeleffer, P. R. (2011). Grapevine canopy response to a high-temperature event During deficit irrigation. *Australian Journal of Grape and Wine Research, 17*(2), 153-161.

Egri, A., Horváth, A., Kriska, G., & Horváth, G. (2010). Optics of sunlit water drops on leaves: Conditions under which sunburn is possible. *New Phytologist, 185*(4), 979-987.

Feller, U., Crafts-Brandner, S. J., & Salvucci, M. E. (1998). Moderately high temperatures inhibit ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) activase-mediated activation of RuBisCO. *Plant Physiology, 116*(2), 539-546.

Fernandez, G. C. J. (2007). Design and analysis of commonly used comparative horticultural experiments. *HortScience, 42*(5), 1052-1069.

Figueiredo, A., Fortes, A. M., Ferreira, S., Sebastiana, M., Choi, Y. H., Sousa, L., Acioli-Santos, B., Pessoa, F., Verpoorte, R., & Pais, M. S. (2008). Transcriptional and metabolic profiling of grape (*Vitis vinifera* L.) leaves unravel possible innate resistance against pathogenic fungi. *Journal of Experimental Botany, 59*(12), 3371-3381.

Fila, G., Ghashghaie, J., Hoarau, J., & Cornic, G. (1998). Photosynthesis, leaf conductance and water relations of *in vitro* cultured grapevine rootstock in relation to acclimatisation. *Physiologia Plantarum, 102*(3), 411-418.

Findlay, N., Oliver, K. J., Nil, N., & Coombe, B. G. (1987). Solute accumulation by grape pericarp cells: IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. *Journal of Experimental Botany, 38*(4), 668-679.

Fischer, G., Almanza-Merchán, P. J., & Ramírez, F. (2012). Source-sink relationships in fruit species: a review. *Revista Colombiana de Ciencias Hortícolas, 6*(2), 238-253.

Foletta, S. G. (2006). *The effect of particle film technology, crop load and leaf removal on yield and fruit composition of Vitis vinifera L. cv. Shiraz.* (Bachelor of Agricultural Science (Honours)), The University of Melbourne, Retrieved from https://eds-b-ebscohost-com.ezp.lib.unimelb.edu.au/eds/detail/detail?vid=0&sid=57439620-4191-48c9-a859-bfa194edc200%40pdc-v-sessmgr02&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=melb.b3189864&db=cat00006a

Fowles, G. W. A. (1992). Acids in grapes and wines: a review. *Journal of Wine Research, 3*(1), 25-41.

Freeman, B. M., Lee, T. H., & Turkington, C. R. (1979). Interaction of irrigation and pruning level on growth and yield of Shiraz vines. *American Journal of Enology and Viticulture, 30*(3), 218-223.

Freeman, B. M., Lee, T. H., & Turkington, C. R. (1980). Interaction of irrigation and pruning level on grape and wine quality of Shiraz vines. *American Journal of Enology and Viticulture, 31*(2), 124-135.

Frohnmeyer, H., & Staiger, D. (2003). Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiology, 133*(4), 1420-1428.

Fuentes, S., De Bei, R., Pech, J., & Tyerman, S. (2012). Computational water stress indices obtained from thermal image analysis of grapevine canopies. *Irrigation Science, 30*(6), 523-536.

Gallant, A. J. E., Hennessy, K. J., & Risbey, J. (2007). Trends in rainfall indices for six Australian regions: 1910-2005. *Australian Meteorological Magazine, 56*(4), 223-241.

Gambetta, G. A., Matthews, M. A., Shaghasi, T. H., McElrone, A. J., & Castellarin, S. D. (2010). Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. *Planta, 232*(1), 219-234.

Gargiulo, A. A. (1991). Quality and quantity: are they compatible? *Journal of Wine Research, 2*(3), 161-181.

Gifford, R. M., & Evans, L. (1981). Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology, 32*(1), 485-509.

Gindaba, J., & Wand, S. J. E. (2005). Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. *HortScience, 40*(3), 592-596.

Girija, C., Smith, B., & Swamy, P. (2002). Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). *Environmental and Experimental Botany, 47*(1), 1-10.

Girona, J., Gelly, M., Mata, M., Arbones, A., Rufat, J., & Marsal, J. (2005). Peach tree response to single and combined deficit irrigation regimes in deep soils. *Agricultural Water Management, 72*(2), 97-108.

Girona, J., Mata, M., del Campo, J., Arbonés, A., Bartra, E., & Marsal, J. (2006). The use of midday leaf water potential for scheduling deficit irrigation in vineyards. *Irrigation Science, 24*(2), 115-127.

Glenn, D., & Puterka, G. (2004). Particle film technology: an overview of history, concepts and impact in horticulture. *Acta Horticulturae, 636*, 509-511.

Glenn, D. M. (2012). The mechanisms of plant stress mitigation by kaolin-based particle films and applications in horticultural and agricultural crops. *HortScience, 47*(6), 710-711.

Glenn, D. M. (2016). Effect of highly processed calcined kaolin residues on apple productivity and quality. *Scientia Horticulturae, 201*, 101-108.

Glenn, D. M., Cooley, N., Walker, R., Clingeleffer, P., & Shellie, K. (2010). Impact of kaolin particle film and water deficit on wine grape water use efficiency and plant water relations. *HortScience, 45*(8), 1178-1187.

Glenn, D. M., Drake, S., Abbott, J. A., Puterka, G. J., & Gundrum, P. (2005). Season and cultivar influence the fruit quality response of apple cultivars to particle film treatments. *HortTechnology, 15*(2), 249-253.

Glenn, D. M., Prado, E., Erez, A., McFerson, J., & Puterka, G. J. (2002). A reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury in apple. *Journal of the American Society for Horticultural Science, 127*(2), 188-193.

Glenn, D. M., & Puterka, G. J. (2005). Particle films: a new technology for agriculture. *Horticultural Reviews, 31*, 1-44.

Glenn, D. M., & Puterka, G. J. (2007). The use of plastic films and sprayable reflective particle films to increase light penetration in apple canopies and improve apple color and weight. *HortScience, 42*(1), 91-96.

Glenn, D. M., Puterka, G. J., Drake, S. R., Unruh, T. R., Knight, A. L., Baherle, P., Prado, E., & Baugher, T. A. (2001). Particle film application influences apple leaf physiology, fruit yield, and fruit quality. *Journal of the American Society for Horticultural Science, 126*(2), 175-181.

Glenn, D. M., Puterka, G. J., Vanderzwet, T., Byers, R. E., & Feldhake, C. (1999). Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. *Journal of Economic Entomology, 92*(4), 759-771.

Gokbayrak, Z., Dardeniz, A., & Bal, M. (2008). Stomatal density adaptation of grapevine to windy conditions. *Trakia Journal of Sciences, 6*(1), 18-22.

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., & Simal-Gándara, J. (2015). Wine aroma compounds in grapes: a critical review. *Critical Reviews in Food Science and Nutrition, 55*(2), 202-218.

Gonzalez-Dugo, V., Durand, J.-L., & Gastal, F. (2010). Water deficit and nitrogen nutrition of crops. a review. *Agronomy for Sustainable Development, 30*(3), 529-544.

Grant, O. M., Ochagavía, H., Baluja, J., Diago, M. P., & Tardáguila, J. (2016). Thermal imaging to detect spatial and temporal variation in the water status of grapevine (*Vitis vinifera* L.). *The Journal of Horticultural Science and Biotechnology, 91*(1), 43-54.

Grape and Wine Research and Development Corporation (GWRDC). (2005). *Annual report 2004-5* Wayville, SA, Australia Retrieved from: https://parlinfo.aph.gov.au/parlInfo/search/display/display.w3p;orderBy=customrank;page=0;query=Grape%20and%20Wine%20Research%20and%20Development%20Corporation%20Reports%202004;rec=0;resCount=Default

Gratani, L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in Botany, 2014*, 1-17.

Greenhouse, S. W., & Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika, 24*(2), 95-112.

Greer, D. H., & Weedon, M. M. (2014). Does the hydrocooling of *Vitis vinifera* cv. Semillon vines protect the vegetative and reproductive growth processes and vine performance against high summer temperatures? *Functional Plant Biology, 41*(6), 620-633.

Greer, D. H., Weedon, M. M., & Weston, C. (2011). Reductions in biomass accumulation, photosynthesis *in situ* and net carbon balance are the costs of protecting *Vitis vinifera* 'Semillon' grapevines from heat stress with shade covering. *AoB Plants, 2011*, 1-13.

Greer, D. H., & Weston, C. (2010). Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Functional Plant Biology, 37*(3), 206-214.

Greer, D. H., Weston, C., & Weedon, M. (2010). Shoot architecture, growth and development dynamics of *Vitis vinifera* cv. Semillon vines grown in an irrigated vineyard with and without shade covering. *Functional Plant Biology, 37*(11), 1061-1070.

Grimes, D. W., & Williams, L. E. (1990). Irrigation effects on plant water relations and productivity of Thompson Seedless grapevines. *Crop Science, 30*(2), 255-260.

Gunning-Trant, C., & Shafron, W. (2010). *Australian wine grape production projections to 2011-12* Retrieved from: http://www.wineecoreports.com/upload/internet/GUNNING-TRANT_SHAFRON_Australian-wine-grape-production-projections-2013%E2%80%9314.pdf

Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *The American Journal of Medicine, 91*(3), S14-S22. doi:10.1016/0002-9343(91)90279-7

Hardie, W. J., & Considine, J. A. (1976). Response of grapes to water-deficit stress in particular stages of development. *American Journal of Enology and Viticulture, 27*(2), 55-61.

Hardy, P. J. (1968). Metabolism of sugars and organic acids in immature grape berries. *Plant Physiology, 43*(2), 224-228.

Harris, J., Kriedemann, P., & Possingham, J. (1968). Anatomical aspects of grape berry development. *Vitis, 7*(106), 19.

Hawker, J. S. (1969). Changes in the activities of enzymes concerned with sugar metabolism during the development of grape berries. *Phytochemistry, 8*(1), 9-17.

Hoare, T. (2016). Increasing irrigation efficiency - technology to save water without risking yield or quality. *Wine & Viticulture Journal, 31*(4), 51-53.

Hochberg, U., Degu, A., Fait, A., & Rachmilevitch, S. (2013). Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. *Physiologia Plantarum, 147*(4), 443-452.

Holgate, A. (2000). *Berry colour index assay-application and interpretation of the assay as an objective measure of red wine grape quality in a commercial winery.* Paper presented at the 5th International Symposium on Cool Climate Viticulture and Oenology Melbourne, Australia

Howell, G. S. (2001). Sustainable grape productivity and the growth-yield relationship: a review. *American Journal of Enology and Viticulture, 52*(3), 165-174.

Huang, J., Rozina, H., Adam, L., Rozwadowski, K. L., Hammerlindl, J. K., Keller, W. A., & Selvaraj, G. (2000). Genetic engineering of glycinebetaine production towards enhancing stress tolerance in plants. *Plant Physiology, 122*, 747-756.

Hudson, B. D. (1994). Soil organic matter and available water capacity. *Journal of Soil and Water Conservation, 49*(2), 189-194.

Iland, P., Dry, P., Proffitt, T., & Tyerman, S. (2011). *The grapevine: from the science to the practice of growing vines for wine*. Adelaide: Patrick Iland Wine Promotions

Iland, P., Ewart, A., Sitters, J., Markides, A., & Bruer, N. (2000). *Techniques for chemical analysis and quality monitoring during winemaking* Campbelltown, SA, Australia: Patrick Iland Wine Promotions.

Intrieri, C., Poni, S., Silvestroni, O., & Filippetti, I. (1992). Leaf age, leaf position and photosynthesis in potted grapevines. *Advances in Horticultural Science, 6*(1), 23-27.

Iriti, M., Rossoni, M., Borgo, M., Ferrara, L., & Faoro, F. (2005). Induction of resistance to gray mold with benzothiadiazole modifies amino acid profile and increases proanthocyanidins in grape: primary versus secondary metabolism. *Journal of Agricultural and Food Chemistry, 53*(23), 9133-9139.

Isbell, R., & the National Committee on Soil and Terrain. (2016). *The Australian soil classification* (2nd ed.). Clayton South, VIC, Australia: CSIRO Publishing.

Jackson, D. I., & Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality – a review. *American Journal of Enology and Viticulture, 44*(4), 409-430.

Jarvis, C., Barlow, E., Darbyshire, R., Eckard, R., & Goodwin, I. (2017). Relationship between viticultural climatic indices and grape maturity in Australia. *International Journal of Biometeorology, 61*(10), 1849-1862.

Jarvis, P. G. (1976). The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society B: Biological Sciences, 273*(927), 593-610.

Jeffrey, S. J., Carter, J. O., Moodie, K. B., & Beswick, A. R. (2001). Using spatial interpolation to construct a comprehensive archive of Australian climate data. *Environmental Modelling and Software, 16*(4), 309-330.

Jifon, J. L., & Syvertsen, J. P. (2003). Kaolin particle film applications can increase photosynthesis and water use efficiency of 'Ruby Red' grapefruit leaves. *Journal of the American Society for Horticultural Science, 128*(1), 107-112.

Jin, P., Zhang, Y., Shan, T., Huang, Y., Xu, J., & Zheng, Y. (2015). Low-temperature conditioning alleviates chilling injury in loquat fruit and regulates glycine betaine content and energy status. *Journal of Agricultural and Food Chemistry, 63*(14), 3654-3659.

Jolivet, Y., Larher, F., & Hamelin, J. (1982). Osmoregulation in halophytic higher plants: the protective effect of glycine betaine against the heat destabilization of membranes. *Plant Science Letters, 25*(2), 193-201.

Jones, H. G. (1990). Physiological aspects of the control of water status in horticultural crops. *HortScience, 25*(1), 19.

Jones, H. G. (1992). *Plants and microclimate: a quantitative approach to environmental plant physiology* (2nd ed.). Cambridge: Cambridge University Press.

Jones, H. G. (1999). Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. *Plant, Cell and Environment, 22*(9), 1043-1055.

Jones, H. G. (2004). Irrigation scheduling: advantages and pitfalls of plant-based methods. *Journal of Experimental Botany, 55*(407), 2427-2436.

Jones, H. G. (2006). Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany, 58*(2), 119-130.

Jones, H. G., & Tardieu, F. (1998). Modelling water relations of horticultural crops: a review. *Scientia Horticulturae, 74*(1-2), 21-46.

Jovanovic, B., Smalley, R., Timbal, B., & Siems, S. (2017). Homogenized monthly upper-air temperature data set for Australia. *International Journal of Climatology, 37*(7), 3209-3222.

Jun, H. R., Adam, L. H., Rozwadowski, K. L., Hammerlineli, J. L., Keller, W. A., & Selvaraj, G. (2000). Genetic engineering of glycinebetaine production towards enhancing stress tolerance in plants. *Plant Physiology, 122*, 747-756.

Jurik, T. W. (1986). Temporal and spatial patterns of specific leaf weight in successional northern hardwood tree species. *American Journal of Botany, 73*(8), 1083-1092.

Kataria, S., Jajoo, A., & Guruprasad, K. N. (2014). Impact of increasing ultraviolet-B (UV-B) radiation on photosynthetic processes. *Journal of Photochemistry and Photobiology B: Biology, 137*, 55-66.

Kato, Y., & Sakamoto, W. (2009). Protein quality control in chloroplasts: a current model of D1 protein degradation in the photosystem II repair cycle. *The Journal of Biochemistry, 146*(4), 463-469.

Keever, G. J., & Cobb, G. S. (1985). Irrigation scheduling effects on container media and canopy temperatures and growth of 'Hershey's Red'azalea. *HortScience, 20*(5), 921.

Keller, M. (2005). Deficit irrigation and vine mineral nutrition. *American Journal of Enology and Viticulture, 56*(3), 267-283.

Keller, M. (2010). Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research, 16*, 56-69.

Keller, M. (2015). *The science of grapevines: anatomy and physiology*. London, UK; San Diego, CA, USA; Waltham, MA, USA; and Oxford, UK: Academic Press.

Keller, M., Romero, P., Gohil, H., Smithyman, R. P., Riley, W. R., Casassa, L. F., & Harbertson, J. F. (2016). Deficit irrigation alters grapevine growth, physiology, and fruit microclimate. *American Journal of Enology and Viticulture, 67*(4), 426-435.

Keller, M., Zhang, Y., Shrestha, P. M., Biondi, M., & Bondada, B. R. (2015). Sugar demand of ripening grape berries leads to recycling of surplus phloem water via the xylem. *Plant, Cell and Environment, 38*(6), 1048-1059.

Kennedy, J. (2002). Understanding grape berry development. *Practical Winery and Vineyard, 4*, 1-5.

Kjelgren, R. (2009). *Isohydric and anisohydric water use considerations in species selection for the great green wall of Africa*. Paper presented at the Great Green Wall of Africa Conference, Dakar, Senegal.

Kliewer, W. M., & Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *American Journal of Enology and Viticulture, 23*(2), 71-77.

Krasnow, M. N., Matthews, M. A., Smith, R. J., Benz, J., Weber, E., & Shackel, K. A. (2010). Distinctive symptoms differentiate four common types of berry shrivel disorder in grape. *California Agriculture, 64*(3), 155-159.

Kriedemann, P. E., & Goodwin, I. (2003). *Regulated deficit irrigation and partial rootzone drying: an overview of principles and applications*. In A. Currey (Ed.), *Irrigation Insights Number 4*. Retrieved from https://www.wineaustralia.com/getmedia/50a58da5-ecc5-4aa1-ad06-8e3c87e38de9/NPI-01-01-RECV-27-10-03-PRD-irrigation-insights

Krstic, M., Clingeleffer, P., Dunn, G., Martin, S., & Petrie, P. (2005). *Grapevine growth and reproduction: an overview*. Paper presented at the Transforming Flowers to Fruit, Proceedings ASVO Conference, Mildura, Victoria.

Kurepin, L. V., Ivanov, A. G., Zaman, M., Pharis, R. P., Allakhverdiev, S. I., Hurry, V., & Hüner, N. P. A. (2015). Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. *Photosynthesis Research, 126*(2-3), 221-235.

Ladyman, J. A., Hitz, W. D., & Hanson, A. D. (1980). Translocation and metabolism of glycine betaine by barley plants in relation to water stress. *Planta, 150*(3), 191-196.

Lai, C. G., Rix, G. J., Foti, S., & Roma, V. (2002). Simultaneous measurement and inversion of surface wave dispersion and attenuation curves. *Soil Dynamics and Earthquake Engineering, 22*(9-12), 923-930.

Lebon, E., Dumas, V., Pieri, P., & Schultz, H. R. (2003). Modelling the seasonal dynamics of the soil water balance of vineyards. *Functional Plant Biology, 30*(6), 699-710.

Lebon, G., Duchene, E., Brun, O., & Clément, C. (2005). Phenology of flowering and starch accumulation in grape (*Vitis vinifera* L.) cuttings and vines. *Annals of Botany, 95*(6), 943-948.

Lebon, G., Wojnarowiez, G., Holzapfel, B., Fontaine, F., Vaillant-Gaveau, N., & Clément, C. (2008). Sugars and flowering in the grapevine (*Vitis vinifera* L.). *Journal of Experimental Botany, 59*(10), 2565-2578.

Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *The American Journal of Clinical Nutrition, 81*(1), 330S-335S.

Levitt, J. (1980). Physiological ecology: a series of monographs, texts, and treatises. In T. T. Kozlowski (Ed.), *Volume I - Chilling, freezing and high temperature stresses* (2nd ed., pp. 597-599). New York, USA; London, UK; Toronto, Canada; Sydney, Australia; and San Francisco, USA: Academic Press.

Lobell, D. B., Cahill, K. N., & Field, C. B. (2007). Historical effects of temperature and precipitation on California crop yields. *Climatic Change, 81*(2), 187-203.

Loveys, B. R., Dry, P. R., Stoll, M., & McCarthy, M. G. (2000a). Using plant physiology to improve the water use efficiency of horticultural crops. *Acta horticulturae*(1).

Loveys, B. R., Dry, P. R., Stoll, M., & McCarthy, M. G. (2000b). Using plant physiology to improve the water use efficiency of horticultural crops. *Acta Horticulturae, 537*(2), 187-197.

Lund Research Ltd. (2018). Repeated measures ANOVA. Retrieved 10 October 2018 from https://statistics.laerd.com/statistical-guides/repeated-measures-anova-statistical-guide.php

Mabrouk, H., & Sinoquet, H. (1998). Indices of light microclimate and canopy structure of grapevines determined by 3D digitising and image analysis, and their relationship to grape quality. *Australian Journal of Grape and Wine Research, 4*(1), 2-13.

Mäkelä, P., Jokinen, K., Kontturi, M., Peltonen-Sainio, P., Pehu, E., & Somersalo, S. (1998a). Foliar application of glycinebetaine – a novel product from sugar beet – as an approach to increase tomato yield. *Industrial Crops and Products, 7*(2-3), 139-148.

Mäkelä, P., Peltonen-Sainio, P., Jokinen, K., Pehu, E., Setala, H., Hinkkanen, R., & Somersalo, S. (1998b). Effect of foliar applications of glycinebetaine on stomatal conductance, abscisic acid and solute concentrations in leaves of salt-or drought-stressed tomato. *Australian Journal of Plant Physiology, 25*, 655-663.

Mäkelä, P., Peltonen-Sainio, P., Jokinen, K., Pehu, E., Setälä, H., Hinkkanen, R., & Somersalo, S. (1996). Uptake and translocation of foliar-applied glycinebetaine in crop plants. *Plant Science, 121*(2), 221-230.

Marsh, I., & Shaw, B. (2000). *Australia's wine industry: collaboration and learning as causes of competitive success* Retrieved from: https://www.nswbusinesschamber.com.au/NSWBC/media/Policy/Thinking%20Business%20Reports/Older%20Reports/Australia-s-Wine-Industry-Collaboration-and-Learning-as-Causes-of-Competitive-Success.pdf

Martinelli, F., Basile, B., Morelli, G., d'Andria, R., & Tonutti, P. (2012). Effects of irrigation on fruit ripening behavior and metabolic changes in olive. *Scientia Horticulturae, 144*, 201-207.

Martínez-Lüscher, J., Morales, F., Delrot, S., Sánchez-Díaz, M., Gomès, E., Aguirreolea, J., & Pascual, I. (2013). Short-and long-term physiological responses of grapevine leaves to UV-B radiation. *Plant Science, 213*, 114-122.

Martorell, S., Medrano, H., Tomàs, M., Escalona, J. M., Flexas, J., & Diaz-Espejo, A. (2015). Plasticity of vulnerability to leaf hydraulic dysfunction during acclimation to drought in grapevines: an osmotic-mediated process. *Physiologia Plantarum, 153*(3), 381-391.

Matthews, M. A., & Anderson, M. M. (1988). Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. *American Journal of Enology and Viticulture, 39*(4), 313-320.

Matthews, M. A., Anderson, M. M., & Schultz, H. R. (1987). Phenologic and growth responses to early and late season. *Vitis, 26*, 147-160.

May, P. (2004). *Flowering and fruitset in grapevines*. Adelaide, Australia: Phylloxera and Grape Industry Board of South Australia in association with Lythrum Press.

Mayr, C. M., Geue, J. P., Holt, H. E., Pearson, W. P., Jeffery, D. W., & Francis, I. L. (2014). Characterization of the key aroma compounds in Shiraz wine by quantitation, aroma reconstitution, and omission studies. *Journal of Agricultural and Food Chemistry, 62*(20), 4528-4536.

McCarthy, M. (1997). The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research, 3*(3), 2-8.

McCarthy, M. G., & Coombe, B. G. (1999). Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport? *Australian Journal of Grape and Wine Research, 5*(1), 17-21.

McCarthy, M. G., Loveys, B. R., Dry, P. R., & Stoll, M. (2002). *Regulated deficit irrigation and partial rootzone drying as irrigation management techniques for grapevines* Retrieved from: http://www.fao.org/3/y3655e/y3655e11.htm#k

McCree, K. J. (1972). Test of current definitions of photosynthetically active radiation against leaf photosynthesis data. *Agricultural Meteorology, 10*, 443-453.

McRae, J. M., & Kennedy, J. A. (2011). Wine and grape tannin interactions with salivary proteins and their impact on astringency: a review of current research. *Molecules, 16*(3), 2348-2364.

Medlyn, B. E., Dreyer, E., Ellsworth, D., Forstreuter, M., Harley, P. C., Kirschbaum, M. U. F., Le Roux, X., Montpied, P., Strassemeyer, J., Walcroft, A., Wange, K., & Loustau, D. (2002). Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant, Cell and Environment, 25*(9), 1167-1179.

Medrano, H., Escalona, J. M., Bota, J., Gulías, J., & Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany, 89*(7), 895-905.

Melis, A. (1999). Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage *in vivo*? *Trends in Plant Science, 4*(4), 130-135.

Mickelbart, M. V., Chapman, P., & Collier-Christian, L. (2006). Endogenous levels and exogenous application of glycinebetaine to grapevines. *Scientia Horticulturae, 111*(1), 7-16.

Moffat, T. (2013). *Sensor technology to assess grape bunch temperature variability in Vitis vinifera L. cv. Shiraz.* (Master of Agriculture Science), Stellenbosch University, Stellenbosh. Retrieved from https://scholar.sun.ac.za/handle/10019.1/80102

Mohammadkhani, N., Heidari, R., Abbaspour, N., & Rahmani, F. (2013). Comparative study of salinity effects on ionic balance and compatible solutes in nine Iranian table grape (*Vitis vinifera* L.) genotypes. *Journal of International des Sciences de la Vigne et du Vin, 47*(2), 99-114.

Mohammadkhani, N., Heidari, R., Abbaspour, N., & Rahmani, F. (2014). Comparative study of salinity effects on ionic balance and compatible solutes in nine Iranian table grape (*Vitis vinifera* L.) genotypes. *Journal of Plant Nutrition, 37*(11), 1817-1836.

Mohanty, P., Allakhverdiev, S. I., & Murata, N. (2007). Application of low temperatures during photoinhibition allows characterization of individual steps in photodamage and the repair of photosystem II. *Photosynthesis Research, 94*(2-3), 217-224.

Montgomery, J., & Hornbuckle, J. (2010). Water matters: piloting IrriSat SMS technology in the Gwydir Valley. *The Australian Cottongrower, 31*(3), 18.

Montgomery, J., Hornbuckle, J., Hume, I., & Vleeshouwer, J. (2015). *IrriSAT – weather based scheduling and benchmarking technology*. Paper presented at the 17th ASA Conference, Hobart, Australia.

Moreshet, S., Cohen, Y., & Fuchs, M. (1979). Effect of increasing reflectance on yield, growth, and physiological behavior of a eryland cotton crop 1. *Crop Science, 19*(6), 863-868.

Mori, K., Goto-Yamamoto, N., Kitayama, M., & Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany, 58*(8), 1935-1945.

Mullins, M. G., Bouquet, A., & Williams, L. E. (1992). *Biology of the grapevine*. Cambridge, United Kingdom: Cambridge University Press.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Environment, 25*(2), 239-250.

Murata, N., Mohanty, P., Hayashi, H., & Papageorgiou, G. (1992). Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. *FEBS Letters, 296*(2), 187-189.

National Climate Centre. (2009). *The exceptional January-February 2009 heatwave in South-Eastern Australia* Retrieved from: http://www.bom.gov.au/climate/current/statements/scs17c.pdf

Nicholls, N. (2004). The changing nature of Australian droughts. *Climatic Change, 63*(3), 323-336.

Nicholls, N., & Collins, D. (2006). Observed climate change in Australia over the past century. *Energy and Environment, 17*(1), 1-12.

Nishikawa, M., Hashida, M., & Takakura, Y. (2009). Catalase delivery for inhibiting ROS-mediated tissue injury and tumor metastasis. *Advanced Drug Delivery Reviews, 61*(4), 319-326.

Novoplansky, A., & Goldberg, D. E. (2001). Effects of water pulsing on individual performance and competitive hierarchies in plants. *Journal of Vegetation Science, 12*(2), 199-208.

Ojeda, H., Deloire, A., & Carbonneau, A. (2001). Influence of water deficits on grape berry growth. *Vitis, 40*(3), 141-145.

Olarte Mantilla, S. M., Collins, C., Iland, P. G., Johnson, T. E., & Bastian, S. E. P. (2012). Review: Berry Sensory Assessment: concepts and practices

for assessing winegrapes' sensory attributes. *Australian Journal of Grape and Wine Research, 18*(3), 245-255.

Ollé, D., Guiraud, J. L., Souquet, J. M., Terrier, N., Ageorges, A., Cheynier, V., & Verries, C. (2011). Effect of pre- and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. *Australian Journal of Grape and Wine Research, 17*(1), 90-100.

Ou, C., Du, X., Shellie, K., Ross, C., & Qian, M. C. (2010). Volatile compounds and sensory attributes of wine from cv. Merlot (*Vitis vinifera* L.) grown under differential levels of water deficit with or without a kaolin-based, foliar reflectant particle film. *Journal of Agricultural and Food Chemistry, 58*(24), 12890-12898.

Oukarroum, A., El Madidi, S., & Strasser, R. (2012). Exogenous glycine betaine and proline play a protective role in heat-stressed barley leaves (*Hordeum vulgare* L.): a chlorophyll a fluorescence study. *Plant Biosystems – An International Journal Dealing with all Aspects of Plant Biology, 146*(4), 1037-1043.

Papageorgiou, G. C., & Murata, N. (1995). The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving photosystem II complex. *Photosynthesis Research, 44*(3), 243-252.

Park, E.-J., Jeknic, Z., & Chen, T. H. (2006). Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. *Plant and Cell Physiology, 47*(6), 706-714.

Payne, R. (2014). *Genstat ANOVA and design: a guide to ANOVA and design in Genstat®*. VSN International Hertfordshire, UK Retrieved from: http://cdn.vsni.co.uk/downloads/genstat/release17/doc/AnovaGuide.pdf

Pellegrino, A., Gozé, E., Lebon, E., & Wery, J. (2006). A model-based diagnosis tool to evaluate the water stress experienced by grapevine in field sites. *European Journal of Agronomy, 25*(1), 49-59.

Pereira, L. S., Oweis, T., & Zairi, A. (2002). Irrigation management under water scarcity. *Agricultural Water Management, 57*(3), 175-206.

Pérez, F. J., Meza, P., Berti, M., & Pinto, M. (2000). Effect of carbon source and sucrose concentration on growth and hexose accumulation of grape berries cultured *in vitro*. *Plant Cell, Tissue and Organ Culture, 61*(1), 37-40.

Petrie, P. R., Cooley, N. M., & Clingeleffer, P. R. (2004). The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera* L.) in the current and following season. *Australian Journal of Grape and Wine Research, 10*, 203-215.

Poni, S., & Intrieri, C. (2001). Grapevine photosynthesis: effects linked to light radiation and leaf age. *Advances in Horticultural Science, 15*(1), 5-15.

Potters, G., Pasternak, T. P., Guisez, Y., & Jansen, M. A. K. (2009). Different stresses, similar morphogenic responses: integrating a plethora of pathways. *Plant, Cell and Environment, 32*(2), 158-169.

Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J., & Jansen, M. A. K. (2007). Stress-induced morphogenic responses: growing out of trouble? *Trends in Plant Science, 12*(3), 98-105.

Pratt, C. (1971). Reproductive anatomy in cultivated grapes – a review. *American Journal of Enology and Viticulture, 22*(2), 92-109.

Pudney, S., Nicholas, P. R., & Skewes, M. (2004). Irrigaiton management: scheduling. In N. Nicholls (Ed.), *Soil, Irrigation, Nutrition (Grape Production Series No. 2)*. Adelaide, South Australia: Hyde Park Press.

Puterka, G. J., Glenn, D. M., Sekutowski, D. G., Unruh, T. R., & Jones, S. K. (2000). Progress toward liquid formulations of particle films for insect and disease control in pear. *Environmental Entomology, 29*(2), 329-339.

Quick, W., Chaves, M., Wendler, R., David, M., Rodrigues, M., Passaharinho, J., Pereira, J., Adcock, M., Leegood, R., & Stitt, M. (1992). The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. *Plant, Cell and Environment, 15*(1), 25-35.

Racsko, J., & Schrader, L. E. (2012). Sunburn of apple fruit: historical background, recent advances and future perspectives. *Critical Reviews in Plant Sciences, 31*(6), 455-504.

Raison, J. K., Pike, C. S., & Berry, J. A. (1982). Growth temperature-induced alterations in the thermotropic properties of *Nerium oleander* membrane lipids. *Plant Physiology, 70*(1), 215-218.

Rajashekar, C., Zhou, H., Marcum, K., & Prakash, O. (1999). Glycine betaine accumulation and induction of cold tolerance in strawberry (*Fragaria* X *ananassa* Duch.) plants. *Plant Science, 148*(2), 175-183.

Rathinasabapathi, B., Burnet, M., Russell, B. L., Gage, D. A., Liao, P.-C., Nye, G. J., Scott, P., Golbeck, J. H., & Hanson, A. D. (1997). Choline monooxygenase, an unusual iron-sulfur enzyme catalyzing the first step of glycine betaine synthesis in plants: prosthetic group characterization and cDNA cloning. *Proceedings of the National Academy of Sciences, 94*(7), 3454-3458.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). *Handbook of enology, volume 1: the microbiology of wine and vinifications* (Vol. 1). West Sussex, England: John Wiley & Sons.

Ritchie, J. (1981). Soil water availability. *Plant and Soil, 58*(1-3), 327-338.

Robinson, J., & Smart, R. (1999). Yield. In J. Robinson (Ed.), *The Oxford Companion to Wine*. New York, USA: Oxford.

Robinson, S., & Jones, G. (1986). Accumulation of glycinebetaine in chloroplasts provides osmotic adjustment during salt stress. *Functional Plant Biology, 13*(5), 659-668.

Roby, G., Harbertson, J. F., Adams, D. A., & Matthews, M. A. (2004). Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. *Australian Journal of Grape and Wine Research, 10*(2), 100-107.

Roby, G., & Matthews, M. A. (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, 10*(1), 74-82.

Rogiers, S. Y., Hatfield, J. M., Jaudzems, V. G., White, R. G., & Keller, M. (2004). Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. *American Journal of Enology and Viticulture, 55*(2), 121-127.

Rosati, A., Metcalf, S. G., Buchner, R. P., Fulton, A. E., & Lampinen, B. D. (2007). Effects of kaolin application on light absorption and distribution, radiation use efficiency and photosynthesis of almond and walnut canopies. *Annals of Botany, 99*(2), 255-263.

Rose, L. (2007). *Creating our future – new varieties and styles.* Paper presented at the 13th Australian Wine Industry Technical Conference.

Roussos, P. A., Denaxa, N.-K., Damvakaris, T., Stournaras, V., & Argyrokastritis, I. (2010). Effect of alleviating products with different mode of action on physiology and yield of olive under drought. *Scientia Horticulturae, 125*(4), 700-711.

Ruan, Y.-L., Patrick, J. W., Bouzayen, M., Osorio, S., & Fernie, A. R. (2012). Molecular regulation of seed and fruit set. *Trends in Plant Science, 17*(11), 656-665.

Rusjan, D., Korošec-Koruza, Z., & Veberič, R. (2008). Primary and secondary metabolites related to the quality potential of table grape varieties (*Vitis vinifera* L.). *European Journal of Horticultural Science, 73*(3), 124.

Sadras, V., Montoro, A., Moran, M., & Aphalo, P. (2012a). Elevated temperature altered the reaction norms of stomatal conductance in field-grown grapevine. *Agricultural and Forest Meteorology, 165*, 35-42.

Sadras, V., Reynolds, M., De la Vega, A., Petrie, P., & Robinson, R. (2009). Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. *Field Crops Research, 110*(3), 242-250.

Sadras, V. O., Bubner, R., & Moran, M. A. (2012b). A large-scale, open-top system to increase temperature in realistic vineyard conditions. *Agricultural and Forest Meteorology, 154-155*, 187-194.

Sadras, V. O., & Petrie, P. R. (2011). Quantifying the onset, rate and duration of sugar accumulation in berries from commercial vineyards in contrasting climates of Australia. *Australian Journal of Grape and Wine Research, 17*(2), 190-198.

Sage, R. F., & Kubien, D. S. (2007). The temperature response of C3 and C4 photosynthesis. *Plant, Cell and Environment, 30*(9), 1086-1106.

Sakamoto, A., & Murata, N. (2000). Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of stress tolerance. *Journal of Experimental Botany, 51*(342), 81-88.

Sakamoto, A., & Murata, N. (2002). The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant, Cell and Environment, 25*(2), 163-171.

Salvucci, M. E., & Crafts-Brandner, S. J. (2004a). Inhibition of photosynthesis by heat stress: the activation state of RuBisCO as a limiting factor in photosynthesis. *Physiologia Plantarum, 120*(2), 179-186.

Salvucci, M. E., & Crafts-Brandner, S. J. (2004b). Relationship between the heat tolerance of photosynthesis and the thermal stability of RuBisCO activase in plants from contrasting thermal environments. *Plant Physiology, 134*(4), 1460-1470.

Santesteban, L. G., Miranda, C., & Royo, J. B. (2011). Suitability of pre-dawn and stem water potential as indicators of vineyard water status in cv. Tempranillo. *Australian Journal of Grape and Wine Research, 17*(1), 43-51.

Saxton, K., Rawls, W. J., Romberger, J., & Papendick, R. (1986). Estimating generalized soil-water characteristics from texture 1. *Soil Science Society of America Journal, 50*(4), 1031-1036.

Scarlett, N. (2009). Reducing berry raisining in the vineyard: a case study in small-scale, on-farm research. *Australian and New Zealand Grapegrower and Winemaker, 550*, 25-28.

Scarlett, N., Needs, S., Howell, K., & Cooley, N. M. (2011). Berry desiccation: developing pre-emptive methods to adapt to heat spikes and dry conditions. *Australian and New Zealand Grapegrower and Winemaker, 567*, 20-23.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D., & Hemmingsen, E. A. (1965). Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. *Science, 148*(3668), 339-346.

Schopfer, P., & Liszkay, A. (2006). Plasma membrane-generated reactive oxygen intermediates and their role in cell growth of plants. *Biofactors, 28*(2), 73-81.

Schrader, L. E., Zhang, J., & Duplaga, W. K. (2001). Two types of sunburn in apple caused by high fruit surface (peel) temperature. *Plant Health Progress, 10*, 1-5.

Schultz, H. B., & Lider, L. A. (1964). Modification of the light factor and heat load in vineyards. *American Journal of Enology and Viticulture, 15*(2), 87-92.

Schultz, H. R. (2003). Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant, Cell and Environment, 26*(8), 1393-1405.

Segura-Monroy, S., Uribe-Vallejo, A., Ramirez-Godoy, A., & Restrepo-Diaz, H. (2015). Effect of kaolin application on growth, water use efficiency, and leaf epidermis characteristics of *Physalis peruviana* L. seedlings under two irrigation regimes. *Journal of Agricultural Science and Technology, 17*(6), 1585-1596.

Shahak, Y., Gussakovsky, E. E., Cohen, Y., Lurie, S., Stern, R., Kfir, S., Naor, A., Atzmon, I., Doron, I., & Greenblat-Avron, Y. (2004a). ColorNets: a new approach for light manipulation in fruit trees. *Acta Horticulturae, 636*, 609-616.

Shahak, Y., Gussakovsky, E. E., Gal, E., & Ganelevin, R. (2004b). ColorNets: crop protection and light-quality manipulation in one technology. *Acta Horticulturae, 659*, 143-151.

Shahak, Y., Ratner, K., Giller, Y. E., Zur, N., Or, E., Gussakovsky, E. E., Stern, R., Sarig, P., Raban, E., Harcavi, E., Doron, I., & Greenblat-Avron, Y. (2008). Improving solar energy utilization, productivity and fruit quality in orchards and vineyards by photoselective netting. *Acta Horticulturae, 772*, 65-72.

Shams, M., Yildirim, E., Ekinci, M., Turan, M., Dursun, A., Parlakova, F., & Kul, R. (2016). Exogenously applied glycine betaine regulates some chemical characteristics and antioxidative defence systems in lettuce under salt stress. *Horticulture, Environment, and Biotechnology, 57*(3), 225-231.

Sharma, R., Reddy, S. V. R., & Datta, S. (2015). Particle films and their applications in horticultural crops. *Applied Clay Science, 116*, 54-68.

Shellie, K. (2015). Foliar reflective film and water deficit increase anthocyanin to soluble solids ratio during berry ripening in Merlot. *American Journal of Enology and Viticulture, 66*(3), 348-356.

Shellie, K., & Glenn, D. (2006). Wine grape response to kaolin particle film under deficit and well-watered conditions. *Acta Horticulturae, 792*, 587-591.

Shellie, K., & Glenn, D. M. (2008). Wine grape response to foliar particle film under differing levels of preveraison water stress. *HortScience, 43*(5), 1392-1397.

Shellie, K. C. (2006). Vine and berry response of Merlot (*Vitis vinifera* L.) to differential water stress. *American Journal of Enology and Viticulture, 57*(4), 514-518.

Shellie, K. C., & King, B. A. (2013a). Kaolin-based foliar reflectant and water deficit influence Malbec leaf and berry temperature, pigments, and photosynthesis. *American Journal of Enology and Viticulture, 64*(2), 223-230.

Shellie, K. C., & King, B. A. (2013b). Kaolin particle film and water deficit influence red winegrape color under high solar radiation in an arid climate. *American Journal of Enology and Viticulture, 64*(2), 214-222.

Siebert, T. E., Wood, C., Elsey, G. M., & Pollnitz, A. P. (2008). Determination of rotundone, the pepper aroma impact compound, in grapes and wine. *Journal of Agricultural and Food Chemistry, 56*(10), 3745-3748.

Singleton, V. L. (1972). Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. *American Journal of Enology and Viticulture, 23*(3), 106-113.

Sirvydas, A. P., Kučinskas, V., Kerpauskas, P., & Nadzeikienė, J. (2011). Theoretical modeling of temperature pulsations in plant leaf which are caused by leaf swing with respect to the sun. *Journal of Environmental Engineering and Landscape Management, 19*(3), 251-259.

Smart, R., & Robinson, M. (1991). *Sunlight into wine: a handbook for winegrape canopy management*. Underdale, SA, Australia: Winetitles.

Smart, R. E. (1985). Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. *American Journal of Enology and Viticulture, 36*(3), 230-239.

Smart, R. E., & Sinclair, T. R. (1976). Solar heating of grape berries and other spherical fruits. *Agricultural Meteorology, 17*(4), 241-259.

Soar, C. J., & Loveys, B. R. (2007). The effect of changing patterns in soil-moisture availability on grapevine root distribution, and viticultural implications for converting full-cover irrigation into a point-source irrigation system. *Australian Journal of Grape and Wine Research, 13*(1), 2-13. doi:doi:10.1111/j.1755-0238.2007.tb00066.x

Soar, C. J., Speirs, J., Maffei, S., Penrose, A., McCarthy, M. G., & Loveys, B. (2006). Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. *Australian Journal of Grape and Wine Research, 12*(1), 2-12.

Song, J., Shellie, K. C., Wang, H., & Qian, M. C. (2012). Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*Vitis vinifera* L.). *Food Chemistry, 134*(2), 841-850.

Sparvoli, F., Martin, C., Scienza, A., Gavazzi, G., & Tonelli, C. (1994). Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). *Plant Molecular Biology, 24*(5), 743-755.

Speirs, J., Binney, A., Collins, M., Edwards, E., & Loveys, B. (2013). Expression of ABA synthesis and metabolism genes under different irrigation strategies and atmospheric VPDs is associated with stomatal conductance in grapevine (V*itis vinifera* L. cv Cabernet Sauvignon). *Journal of Experimental Botany, 64*(7), 1907-1916.

Spencer, A., & Peynaud, E. (1984). *Knowing and making wine*. New York, United States of America: A Wiley. Interscience Publication.

Sperry, J., Adler, F., Campbell, G., & Comstock, J. (1998). Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell and Environment, 21*(4), 347-359.

Subramanian, S. (1958). The effect of anti-transpirants on the yield of cotton. *The Madras Agricultural Journal, 79*(8), 427-430.

Swanson, C. A., & El-Shishiny, E. D. H. (1958). Translocation of sugars in the Concord grape. *Plant Physiology, 33*(1), 33.

Taiz, L., & Zeiger, E. (2002). *Plant physiology* (3rd Edition ed.). Sunderland, Massachusetts, United States of America: Sinauer Associates.

Teranishi, M., Nakamura, K., Morioka, H., Yamamoto, K., & Hidema, J. (2008). The native cyclobutane pyrimidine dimer photolyase of rice is phosphorylated. *Plant Physiology, 146*(4), 1941-1951. doi:10.1104/pp.107.110189

Tezara, W., Mitchell, V., Driscoll, S., & Lawlor, D. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature, 401*(6756), 914.

Tilbrook, J., & Tyerman, S. D. (2008). Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. *Functional Plant Biology, 35*(3), 173-184.

Timbal, B., & Drosdowsky, W. (2013). The relationship between the decline of Southeastern Australian Rainfall and the strengthening of the subtropical ridge. *International Journal of Climatology, 33*(4), 1021-1034.

Tramontini, S., van Leeuwen, C., Domec, J.-C., Destrac-Irvine, A., Basteau, C., Vitali, M., Mosbach-Schulz, O., & Lovisolo, C. (2013). Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. *Plant and Soil, 368*(1-2), 215-230.

Vasconcelos, M. C., Greven, M., Winefield, C. S., Trought, M. C., & Raw, V. (2009). The flowering process of *Vitis vinifera*: a review. *American Journal of Enology and Viticulture, 60*(4), 411-434.

Vatandoost, S., Davarynejad, G., & Tehranifar, A. (2017). Impact of kaolin particle film on light extinction coefficient and radiation use efficiency of pistachio (*Pistachia vera*). *AgroLife Scientific Journal, 6*(2), 214-218.

Vivin, P., Castelan, M., & Gaudillère, J. (2001). A source/sink model to simulate seasonal allocation of carbon in grapevine. *Acta Horticulturae, 584*, 43-56.

Volokitin, A., & Persson, B. N. (2007). Near-field radiative heat transfer and noncontact friction. *Reviews of Modern Physics, 79*(4), 1291.

Volschenk, H., Van Vuuren, H., & Viljoen-Bloom, M. (2006). Malic acid in wine: Origin, function and metabolism during vinification. *South African Journal of Enology and Viticulture, 27*(2).

VSN International. (2015). Genstat reference manual (release 18). In *Part 3 procedures*. Hemel, Hempstead, UK: VSN International.

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: an overview. *Environmental and Experimental Botany, 61*(3), 199-223.

Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., Kerridge, G. H., Rühl, E. H., & Nicholas, P. R. (2005). Shiraz berry size in relation to seed number and implications for juice and wine composition. *Australian Journal of Grape and Wine Research, 11*(1), 2-8.

Wand, S. J. E., Theron, K. I., Ackerman, J., & Marais, S. J. S. (2006). Harvest and post-harvest apple fruit quality following applications of kaolin particle film in South African orchards. *Scientia Horticulturae, 107*(3), 271-276.

Wang, X., Yang, W., Wheaton, A., Cooley, N., & Moran, B. (2010). Automated canopy temperature estimation via infrared thermography: a first step towards automated plant water stress monitoring. *Computers and Electronics in Agriculture, 73*(1), 74-83.

Waterhouse, A. L. (2002). Wine phenolics. *Annals of the New York Academy of Sciences, 957*(1), 21-36.

Watt, A. M., Dunn, G. M., May, P. B., Crawford, S. A., & Barlow, E. W. R. (2008). Development of inflorescence primordia in *Vitis vinifera* L. cv. Chardonnay from hot and cool climates. *Australian Journal of Grape and Wine Research, 14*(1), 46-53.

Watt, M., Silk, W. K., & Passioura, J. B. (2006). Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Annals of Botany, 97*(5), 839-855.

Wear, J. I., & Patterson, R. (1962). Effect of soil pH and texture on the availability of water-soluble boron in the soil 1. *Soil Science Society of America Journal, 26*(4), 344-346.

Webb, L., Watt, A., Hill, T., Whiting, J., Wigg, F., Dunn, G., Needs, S., & Barlow, E. (2009). *Extreme heat: managing grapevine response. Documenting regional and inter–regional variation of viticultural impact and management input relating to the 2009 heatwave in SE Australia* Melbourne, Australia Retrieved from: https://www.researchgate.net/profile/Leanne_Webb/publication/276936661_Extreme_heat_managing_grapevine_response_based_on_vineyard_observations_from_the_2009_heatwave_across_south-eastern_Australia/links/02e7e53b9e2f816630000000/Extreme-heat-managing-grapevine-response-based-on-vineyard-observations-from-the-2009-heatwave-across-south-eastern-Australia.pdf

Webb, L., Whiting, J., Watt, A., Hill, T., Wigg, F., Dunn, G., Needs, S., & Barlow, E. W. R. (2010). Managing grapevines through severe heat: a survey of growers after the 2009 summer heatwave in South-Eastern Australia. *Journal of Wine Research, 21*(2-3), 147-165.

Webb, L. B., Whetton, P. H., & Barlow, E. W. R. (2011). Observed trends in winegrape maturity in Australia. *Global Change Biology, 17*(8), 2707-2719.

Wheaton, A. D., Cooley, N. C., Dunn, G. M., Goodwin, I., Wang, X., Moran, B., & Yang, W. (2011). Use of thermal imagery to detect water stress during berry ripening in *Vitis vinifera* L. 'Cabernet Sauvignon'. *Acta Horticulturae, 889*.

Wheeler, S. F. (2007). *The role of abscisic acid in grape berry development.* (Doctor of Philosophy), The University of Adelaide, School of Agriculture and Wine. Retrieved from https://digital.library.adelaide.edu.au/dspace/bitstream/2440/57767/8/02whole.pdf

Williams, L. E., & Araujo, F. J. (2002). Correlations among predawn leaf, midday leaf, and midday stem water potential and their correlations with other measures of soil and plant water status in *Vitis vinifera*. *Journal of the American Society for Horticultural Science, 127*(3), 448-454.

Winetitles Media Pty Ltd. (2016). Wine industry statistics – exports. Retrieved 10th January 2017 from http://winetitles.com.au/statistics/exports.asp

Winkel, T., & Rambal, S. (1993). Influence of water stress on grapevines growing in the field: from leaf to whole-plant response. *Functional Plant Biology, 20*(2), 143-157.

Winkler, A. J., Cook, J. A., Kliewer, W. M., & Lider, L. A. (1974). *General viticulture*. Berkley, Los Angeles, USA and London, UK: University of California Press.

Wood, C., Siebert, T. E., Parker, M., Capone, D. L., Elsey, G. M., Pollnitz, A. P., Eggers, M., Meier, M., Vössing, T., Widder, S., Krammer, G., Sefton, M. A., & Herderich, M. J. (2008). From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *Journal of Agricultural and Food Chemistry, 56*(10), 3738-3744.

Woodruff, D. R. (2013). The impacts of water stress on phloem transport in Douglas-fir trees. *Tree Physiology, 34*(1), 5-14.

Xing, W., & Rajashekar, C. (2001). Glycine betaine involvement in freezing tolerance and water stress in *Arabidopsis thaliana*. *Environmental and Experimental Botany, 46*(1), 21-28.

Yang, W., Wang, X., Wheaton, A., Cooley, N., & Moran, B. (2009). *Automatic optical and IR image fusion for plant water stress analysis*. Paper presented at the 12th International Conference on Information Fusion, Seattle, WA, USA.

Yang, X., Liang, Z., & Lu, C. (2005). Genetic engineering of the biosynthesis of glycinebetaine enhances photosynthesis against high temperature stress in transgenic tobacco plants. *Plant Physiology, 138*(4), 2299-2309.

Yildirim, E., Ekinci, M., Turan, M., Dursun, A., Kul, R., & Parlakova, F. (2015). RRoles of glycine betaine in mitigating deleterious effect of salt stress on lettuce (*Lactuca sativa* L.). *Archives of Agronomy and Soil Science, 61*(12), 1673-1689.

Young, P. R., Eyeghe-Bickong, H. A., du Plessis, K., Alexandersson, E., Jacobson, D. A., Coetzee, Z., Deloire, A., & Vivier, M. A. (2016). Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. *Plant Physiology, 170*(3), 1235-1254.

Zhang, L., Gao, M., Hu, J., Zhang, X., Wang, K., & Ashraf, M. (2012). Modulation role of abscisic acid (ABA) on growth, water relations and glycinebetaine metabolism in two maize (*Zea mays* L.) cultivars under drought stress. *International Journal of Molecular Sciences, 13*(3), 3189-3202.

Zotarelli, L., Dukes, M., & Morgan, K. (2010). *Interpretation of soil moisture content to determine soil field capacity and avoid over-irrigating sandy soils using soil moisture sensors* University of Florida Retrieved from: https://pdfs.semanticscholar.org/4c52/2b8638c84516e81b3baca2250bc530ca25ca.pdf

Chapter 8. Appendices

8.1. Climate for the Dookie Region

Table 8.1. Extrapolated climate averages for The University of Melbourne's Dookie Campus Vineyard in North East Victoria, Australia (-36.395 °S, 145.703 °E) from 1986 to 2012 (Jeffrey et al., 2001), for rainfall, temperature, reference evapotranspiration (ET_o) and relative humidity obtained from the SILO website (www.longpaddock.qld.gov.au/silo).

	Rainfall (mm)	Temperature (°C)			ET_o (mm)	Avg Relative Humidity (%)
		Avg	Avg Max	Avg Min		
Jan	39	22	29	14	184	58
Feb	44	21	29	14	153	61
Mar	41	18	25	11	125	65
Apr	38	14	21	8	75	71
May	51	10	16	5	42	77
Jun	57	8	12	3	28	80
July	64	7	11	3	29	81
Aug	54	8	13	3	44	78
Sept	51	10	16	5	68	75
Oct	48	13	20	7	107	70
Nov	48	17	24	10	140	65
Dec	41	19	27	12	171	60
Annual	**575**				**1165**	

8.2. Meteorological Data for the Dookie Region

Table 8.2. Environmental conditions for The University of Melbourne's Dookie Campus Vineyard in North East Victoria, Australia (-36.395 °S, 145.703 °E) during the experiment. The 2011-12 (Y1) and 2012-13 (Y2) growing seasons are highlighted in grey. Data collected from Dookie Campus weather station, except for ET_o, which was obtained from the SILO website (www.longpaddock.qld.gov.au/silo).

	Rainfall (mm)	Temperature (°C)					ET_o (mm)	Avg Relative Humidity (%)
		Avg	Min	Avg Min	Max	Avg Max		
2011-Sept	56.5	12.3	2.5	6.7	28.8	17.9	74.5	62.5
2011-Oct	20.6	15.5	4.8	9.9	30.8	21.3	106.5	60.5
2011-Nov	72.4	19.6	8.4	13.4	35.4	25.9	134.6	56.5
2011-Dec	62.1	20.3	6.5	13.4	34.3	27.0	171.5	49.8
2012-Jan	48.4	23.5	8.3	16.2	39.1	30.4	186.1	44.3
2012-Feb	148.6	22.0	10.3	15.7	35.5	28.8	134.5	53.5
2012-Mar	148.7	17.8	7.8	12.7	29.1	23.2	99.2	64.1
2012-Apr	22.1	15.4	4.3	9.9	29.4	21.7	70.1	65.1
2012-May	18.5	10.9	1.5	6.3	21.0	16.1	39.1	68.6
2012-Jun	26.2	9.1	-1.4	5.0	16.8	13.4	27.7	73.1
2012-Jul	58.3	8.9	0.0	5.5	15.7	13.0	29.5	74.9
2012-Aug	41.7	9.0	0.7	5.1	19.1	13.3	42.2	70.8
2012-Sept	21.4	11.9	2.4	6.7	26.6	17.5	69.7	63.5
2012-Oct	43.6	14.5	4.1	8.1	32.4	21.0	111.6	55.5
2012-Nov	20.3	19.7	6.8	12.4	39.9	27.2	150.1	47.3
2012-Dec	31.8	21.5	8.7	13.9	38.2	28.7	178.5	42.6
2013-Jan	3.6	23.0	8.0	14.0	43.0	32.0	207.7	51.0
2013-Feb	57.2	23.1	8.5	15.4	37.5	30.8	157.2	56.0
2013-Mar	20.4	19.2	4.5	12.2	33.5	26.1	127.7	65.0
2013-Apr	7.1	14.8	2.5	8.2	26.5	21.4	78.2	70.0

8.3. Glycine Betaine and Kaolin Particle Film Calculations

Table 8.3. Calculations for application rate for glycine betaine (GB).

GreenStim™ Label Information and Calculations		
Application Rate	4 kg·ha^{-1}	
Active Constituent	glycine betaine (GB)	
Active Constituent (GB)%	97%	
Spray Volume	400 L·ha^{-1}	
GreenStim™ Concentration	C_{GS}	$= \frac{\mathbf{4\ kg \cdot ha^{-1}}}{\mathbf{400\ L \cdot ha^{-1}}}$ $= \mathbf{0.01\ kg \cdot L^{-1}}$
GB Chemical Formula	$C_5H_{11}NO_2$	
Molecular Weights	C: 12.011 (x5) H: 1.008 (x11) N: 14.007 (x1) O: 15.999 (x2)	
Molecular Weight of	MW_{GB}	$= \mathbf{117.15\ g \cdot mol^{-1}}$ $= \mathbf{0.11715\ kg \cdot mol^{-1}}$
GB Concentration	C_{GB}	$= \mathbf{0.01\ kg{\cdot}L^{-1}} \times \mathbf{97\%}$ $= \mathbf{0.0097\ kg \cdot L^{-1}}$ $= \frac{\mathbf{0.0097\ kg \cdot L^{-1}}}{\mathbf{0.11715\ kg \cdot mol^{-1}}}$ $= \mathbf{0.0828\ mol \cdot L^{-1}}$ $= \mathbf{82.8\ mM}$
Vine Spacing	1.8 x 3.4 m	
Vines per Hectare	1634 vines·ha^{-1}	
Spray Volume per Vine	V_{vine}	$= \frac{\mathbf{400\ L{\cdot}ha^{-1}}}{\mathbf{1634\ vines{\cdot}ha^{-1}}}$ $= \mathbf{0.2445\ L{\cdot}vine^{-1}}$
Amount of GB per Vine	GB (mol)	$= \mathbf{0.2445\ L \cdot vine^{-1}} \times \mathbf{82.8\ mM}$ $= \mathbf{0.02024\ mol \cdot vine^{-1}}$ $= \mathbf{20.24\ mmol{\cdot}vine^{-1}}$

Table 8.4. Calculations for application rate for kaolin particle film (KPF)

Surround®WP Label Information and Calculations		
Application Rate	50 kg·ha^{-1}	
Active Constituent	Processed kaolin ('kaolin')	
Active Constituent (Kaolin)%	95%	
Spray Volume	1000 L·ha^{-1}	
Surround®WP Concentration	C_{Kaolin}	$= \frac{\mathbf{50\ kg \cdot ha^{-1}}}{\mathbf{1000\ L \cdot ha^{-1}}}$ $= \mathbf{0.05\ kg \cdot L^{-1}}$
Kaolin Concentration	C_{Kaolin}	$= \mathbf{0.05\ kg{\cdot}L^{-1}} \times \mathbf{95\%}$ $= \mathbf{0.0475\ kg \cdot L^{-1}}$
Vine Spacing	1.8 x 3.4 m	
Vines per Hectare	1634 vines·ha^{-1}	
Spray Volume per Vine	V_{vine}	$= \frac{\mathbf{1000\ L{\cdot}ha^{-1}}}{\mathbf{1634\ vines{\cdot}ha^{-1}}}$ $= \mathbf{0.612\ L{\cdot}vine^{-1}}$
Amount of Active Constituent per Vine	Kaolin (g)	$= \mathbf{0.612\ L \cdot vine^{-1}} \times \mathbf{0.0475\ kg \cdot L^{-1}}$ $= \mathbf{0.02907\ kg \cdot vine^{-1}}$ $= \mathbf{29.1\ g \cdot vine^{-1}}$