Improved Frost Management in the Goulburn & Yarra Valleys



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **RT 06/04-1**

Principal Investigator: **Professor Snow Barlow**

Research Organisation: **The University of Melbourne**

Date: **Friday 15th October 2010**

Improved Frost Management in the Goulburn & Yarra Valleys

Sonja Needs[1], Michael Trought[3], Tom C.J. Hill[1], E.W.R. (Snow) Barlow[1]and Gregory M. Dunn[2]

[1]Melbourne School of Land and Environment, The University of Melbourne, Parkville, Victoria, 3010 Australia.

[2]Melbourne School of Land and Environment, The University of Melbourne, Dookie Campus, Victoria, 3647 Australia.

[3]Plant and Food Research Ltd., Marlborough Wine Research Centre, PO Box 845, Blenheim, New Zealand.

July, 2010

Table of Contents

FINAL REPORT to GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION
Table of Contents 3
1. Abstract 4
2. Background....5
3. Aims....5
4. A Review of Frost Recovery Responses 6
4.1 Introduction 6
4.2 Frost in Australian Vineyards 7
4.3 Biology of Frost Damage....11
4.3.1 Role of ice nucleators 11
4.3.2 Nature of cell damage....13
4.3.3. Thawing rate....13
4.4. Freeze avoidance mechanisms....14
4.4.1. Supercooling....14
4.4.2. Freezing point depression (Cryoprotectants) 16
4.4.3. Grapevine responses to frost....16
4.5. Critical damage thresholds....17
4.6. Acclimation/de-acclimation....19
4.7. Frost recovery responses....21
4.6.1. Fertility....21
4.8. Managing frost affected vines 22
4.9. Future Research Priorities....23
4.10. Conclusions....24
4.11. References....26
5. Communication....32
6. Staff engaged in the project....33
7. Budget reconciliation 34

1. Abstract

Spring frosts in October 2006 devastated many vineyards in the South-East states of Australia. The resulting loss of fruit was estimated to be worth A$ 140m. Following the frost growers had to make decisions on the best methods to manage vines. Damage thresholds and recovery responses of grapevines to frost are not well understood for the range of economically important Australian cultivars. The interaction between the timing of frost in relation to vine development, the severity of the frost and individual cultivar responses are important variables which have not been thoroughly investigated in Australia. Here we review the literature to gain an understanding of wine grape management after a spring frost.

Two workshops were organised in conjunction with DPI Victoria Grapecheque) and held in the Yarra Valley (11th June) and Goulburn Valley (12th June). These workshops covered frost prediction, prevention and post frost management, along with a presentation on the biology of frost. Presenters included David Morrison (Bureau of Meteorology), Dr Michael Trought (Marlborough Research Centre), Ms Sonja Needs (Melbourne University) and Dr Tom Hill (University or Melbourne / University of East London). These workshops were well attended, with over 50 in Yarra Valley and over 40 participants at Nagambie. The Yarra Valley workshop was recorded and uploaded to the DPI Victoria website and linked to the GWRDC website and workshop DVDs circulated nationally.

2. Background

Consecutive frosts on 25th and 26th September 2006 and again on October 21 2006 devastated many vineyards in the Goulburn Valley, Strathbogie Ranges and Yarra Valley Wine Regions. While surveys covering many cultivars across the region immediately after the frost events identified the proportion of affected areas, the effects on yield were initially more difficult to quantify and depended on the proportion of secondary shoots that burst and their fertility. This project will allowed Goulburn Valley, Strathbogie Ranges and Yarra Valley grape growers to add significant value to their initial surveys by assessing the recovery responses of key cultivars and comparing these with unaffected vines and vines from areas where frost mitigation strategies such as overhead sprinklers were employed.

Climate change induced global warming is likely to advance grapevine phenolgy, including budburst as was the case in 2006. However, along with elevated temperatures models also predict drier spring conditions for most of Australia's grape growing regions. Drier spring conditions coupled with advanced phenology may expose many of cultivars and regions to increased frost risk.

There is a lack of definitive information on grapevine recovery responses to frost. This is true even for one cultivar, let alone the range of economically important cultivars grown in Australia. The interaction between the timing of the frost event in relation to vine development, the severity of the frost and cultivar responses are important variables. One also has to consider how to best manage the vines to maintain yield in the following season and ensure good canopy structure. This lack of information is an impediment to both the strategic deployment of resources in response to an impending frost events and subsequent management of frost affected grape vines.

3. Aims

This report first addresses climate change related future frost risk, then reviews what is know of recovery responses for a range of wine grape cultivars in order to increase the strategic ability to react to impending frost events and thereby improve the management of frost affected grapevines and finally suggests some recommendations for future frost related research.

4. A Review of Frost Recovery Responses

4.1 Introduction

Frost damage devastated many Australian vineyards during the 2006/07 season causing damage estimated at A$140 million (Anonymous, 2007). Frosts occurred from September (early spring) through until late October 2006 and affected most of South Eastern Australia's wine regions. Some, like the Yarra Valley, experienced two or more frosts over a three week period.

Although global warming projections suggest fewer frost days per year, frost risk may not necessarily be reduced because, as grapevine development is strongly influenced by temperature, buds will burst earlier (Nemani *et al.*, 2001; Hanninen, 1991) and thus become vulnerable to frost damage at an earlier date. In addition predictions of lower rainfall in spring, and associated drier soils, fewer clouds and lower dew points, may increase frost risk at a time when grapevines are most vulnerable (Trought *et al.*, 1999; Jones, 1991). Furthermore, an increase in day–to–day climate variability may lessen any 'positive' impact of mean warming on reducing frost frequency (Rigby and Porporato, 2008). It is worth noting that the warmest spring on record (dataset from 1950 to 2007) occurred in 2006 in southern Australia (Australian Bureau of Meteorology, 2008). Widespread frost damage also occurred in 2003, another relatively hot and very dry year.

Frost management in vineyards can be addressed in several ways. The most obvious is to choose and develop vineyards on sites where frosts are unlikely to occur. Unfortunately, few, if any, frost records are available when establishing a new vineyard site. Some assessments can be made, particularly if the risk can be related to a nearby meteorological station with a long-term record (Trought, 2004). Likewise, an understanding the topography (e.g. slope, proximity to a water mass, shelterbelts etc.) and vineyard management factors (understorey management, cultivar selection, pruning strategy etc.) that influence the susceptibility of a vineyard is invaluable. However, few sites, particularly in cool climate regions, will be entirely free of frost risk. In order to make sensible decisions about the use of frost prevention strategies, it is important for vineyard managers to know the critical temperatures cultivars can tolerate before damage begins. These temperatures are based on weather conditions and the status of the bud or stage of shoot development (Johnson and Howell, 1981), and some variation in critical temperatures among *Vitis vinifera* L. cultivars has been reported (Table 1).

Table 1. Critical temperatures (°C) for woody tissue and dormant buds of different grapevine cultivars. No damage thresholds were given so values shown are assumed to be 100% death (Snyder, 2005; Pudney, 2007; Johnson and Howell, 1981; Pool and Lerch, 2004; Sugar
).

Variety	Woody Tissue	Dormant bud
Grape (*Vitis vinifera*)	-20.6	
French	-23.3	
US	-27.8	
Pinot Noir	-14	-22.6
Chardonnay		-21.7
Viognier		-20.9
Shiraz		-20.4
Concord	-27.3	

Sometimes frost damage cannot be avoided and in these instances it is important to manage the current season's crop, whilst ensuring good canopy structure and yield in the following season. Critical to effective management is an assessment of the extent of frost damage and a quantitative understanding of the recovery responses of cultivars following damage. However, the current lack of quantitative information on how vines recover following a frost makes it difficult to decide how best to manage them to minimise losses in the current and following season, and how to achieve the best cost/benefit outcome.

Here we largely focus on the influence of spring frost events, and describe what is known about the biology of frost damage in grapevines before summarising what is known about critical damage thresholds for cultivars at various stages of development. Studies on grapevine recovery responses are then reviewed, with particular attention being paid to the fertility of regrowth and impacts on carbohydrate reserves in the vine. Finally, implications for post-frost management in vineyards are discussed.

4.2 Frost in Australian Vineyards

Frost is a localised phenomenon which is fairly common in parts of southern Australia (Figs. 1 and 2).

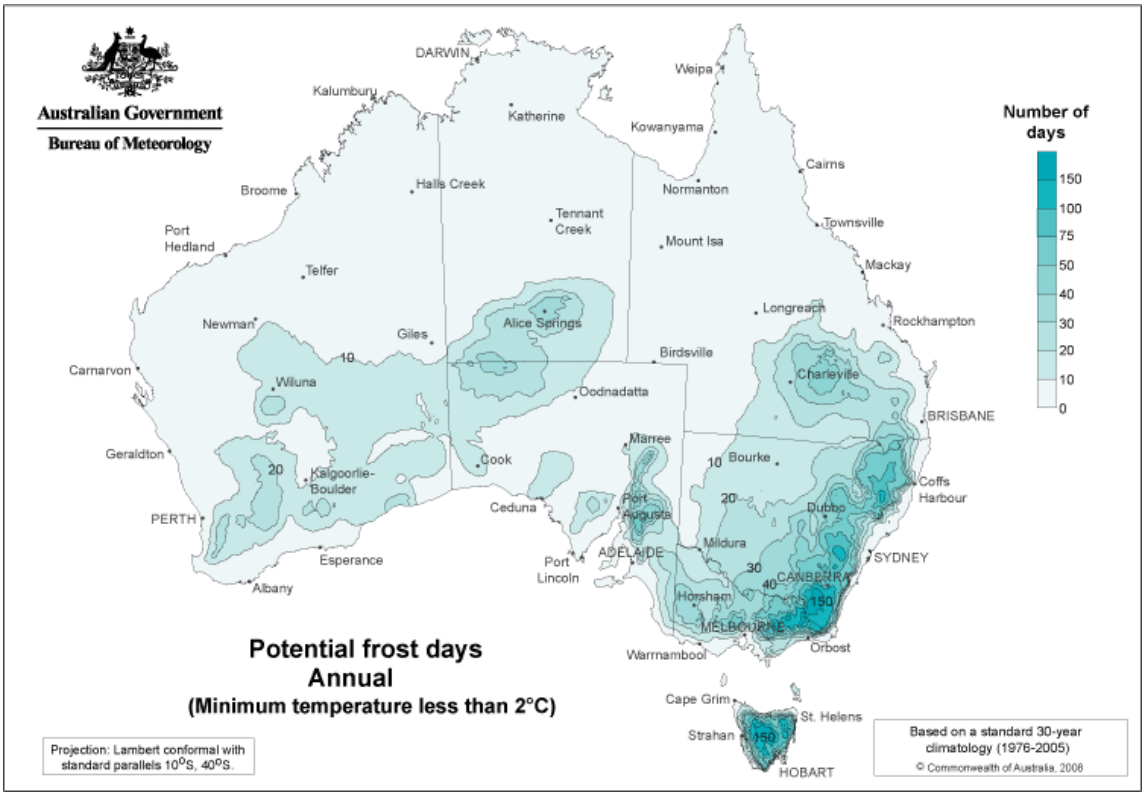


Figure 1. Annual potential frost days for Australia (minimal air temperature less than 2°C). Based on a 30 year climatology (1975-2005) (Burea of Meteorology, 2009)

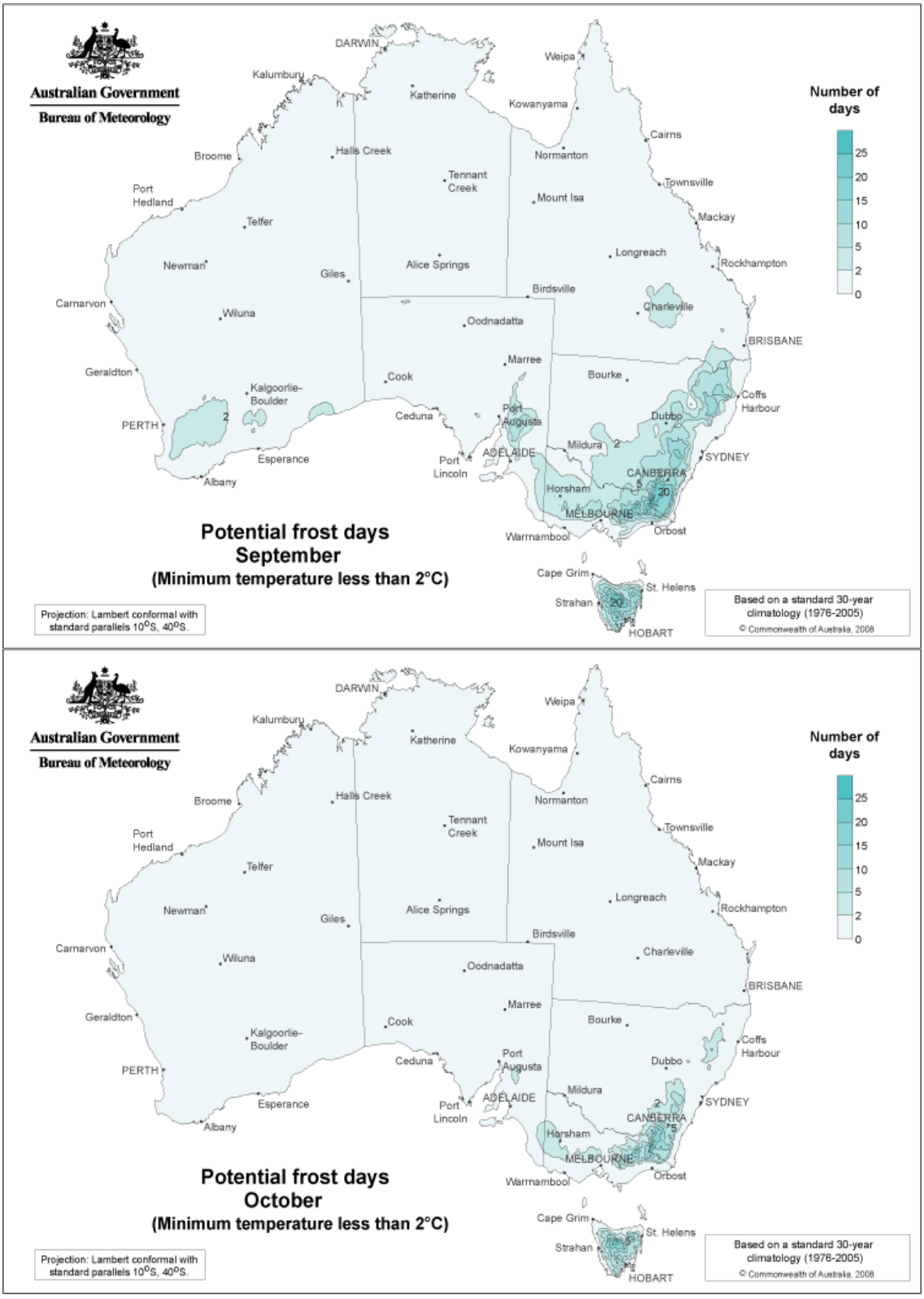


Figure 2. Potential frost days for the spring growing season (minimal air temperature less than 2°C). Based on 30 year climatology (1975-2005). (Bureau of Meteorology, 2009)

Frost forms when the surface temperature falls below freezing point. In Australia, temperature is measured in a Stevenson screen (which is vented to allow air flow whilst mixing it but preventing direct sunlight from heating the thermometer) at a height of about 1.2 m above the ground. Air at ground level is colder than that higher up. As a general rule when the temperature at screen level

is 2.2°C the ground/surface temperature will be around 0°C. (Bureau of Meteorology, 2009) An air frost is recorded when the minimum temperature in the screen falls below 0^{o}C.

Frosts are classed as being either “radiative” or “advective” (Kalma *et al.*, 1992). Radiative frosts result from cooling due to energy loss on clear, calm nights. During the day the ground, and other surfaces such as leaves, fences, buildings, etc, are heated by short wave radiation from the sun. Simultaneously, they are also radiating long wave radiation back to the atmosphere at ~110 W m^{-2}. When incoming radiation is greater than the outgoing the surfaces warm up. On clear nights, with little cloud cover, outgoing radiation exceeds the incoming and the ground looses heat by to the atmosphere. {Snyder, 2000 #87} showed that the net energy loss from the crop on a typical frost night was approximately -18 W m^{-2}. On nights with little or no cloud cover, the lower the air temperature at sunset and the longer the night, the colder the surface gets and the higher the likelihood of a radiation frost. Typically radiation frosts are associated with a developing high pressure zone.

Air cools from the ground up causing an ‘inversion layer’ where the temperature increases with height. As air continues to cool the height of the 0°C isohyet rises, and when the air at approximately 1.2 m reaches 0°C it is referred to as a screen frost (GBMO, 1991). The air continues to cool until sunrise. Since plants also emit heat, the temperature of the vines (trunk, cordon and shoots) is typically 1° to 3°C colder than the air temperature (see Leuning and Cremer, 1988). Cover crops close to the ground may be as much as 4°C colder than air temperature at 1.2 m (McDavitt, 2007; Poling, 2006; Evans, 2000).

Advection frosts, on the other hand, occur when a mass of very cold air moves across the landscape and are associated with windy conditions and daytime temperatures that may be less than 0°C. They can occur on windy nights that are preceded by cold days, but can also occur during the day. Unlike a radiation frost an advection frost has no inversion layer (GBMO, 1991). Advective and radiative frost can occur in combinations. For instance cold advective conditions, which cause air temperatures to drop to close to freezing, may be followed by periods of clear, calm conditions leading to radiative frosts (Synder and de Melo-Abreu, 2005). Also, “micro-scale-advection frosts” can occur when a region is exposed to radiation-type frost conditions and local cold air drainage acts like a mini advection frost (Synder and de Melo-Abreu, 2005). Advection frosts are rarely seen in Australia and most frosts that damage grape growing regions are radiative (Bureau of Meteorology, 2009).

4.3 Biology of Frost Damage

When plant tissues freeze, water contained within turns from liquid state to the solid ice state. Water within a cell's protoplast is able to move outward, across the cell membrane, increasing its solute concentration and conferring some protection from freezing. The amount of damage sustained by cells and their ability to recover from a freeze will depend largely on where in the plant's tissue cell water is when it freezes.

4.3.1 Role of ice nucleators

For a plant to sustain damage the water within the plant first needs to freeze. In its pure state, water does not freeze at 0°C. It can cool to a little above -40°C before ice crystals form spontaneously in a process called homogeneoous freezing (Debenedetti, 1996). However, water freezes at just below 0°C in the presence of nucleators that help to align water molecules in a pattern similar to that of ice, catalysing the change from liquid to solid. This is heterogeneous freezing. Most plant tissues are not themselves efficient nucleators, so that in the temperature range of 0°C to roughly -5°C foreign particles provide most nucleation sites (Hirano and Upper, 2000). The most effective natural ice nucleators are several species of ice nucleating bacteria (INB), the most common being *Pseudomonas syringae*, which, by expressing an ice nucleating (IN) protein, can catalyse freezing at up to -1.2°C (Lindow *et al.*, 1989) although the mean for a diverse collection of cultured isolates was -3.5°C (Morris *et al.*, 2008). Lindow (1987) reported a linear relationship between the log of the INB population and the proportion of corn leaves frosted.

Other nucleators include fungi, such as the soil inhabiting fungus *Fusarium avenacium* which can trigger freezing at -2.5°C (Pouleur *et al.*, 1992), pollen, which can nucleate at up to -8°C (Diehl *et al.*, 2001; Diehl *et al.*, 2002; von Blohn *et al.*, 2005), mineral dust, which can be active at -10.5°C (Roberts and Hallett, 1968), and even wind and agitation (eg, sound vibrations from helicopters and wind machines), which can cause supercooled water molecules to collide, compress and freeze (Evans, 2000). However, Hirano and Upper (2000) concluded that in plants "supercooling in the temperature range of 0°C to roughly -5°C is primarily constrained by the presence of ice nucleation active bacteria."

In Australia there has been very limited investigation of the role of INB on crops and, until recently, none into which species and what populations occur on grapevines. In the frost-prone period in 2007 we sampled 4-leaf shoots in two vineyards in Victoria, one of which had suffered 95% shoot death following three consecutive nights of frost. Ice nucleating *Pseudomonas syringae* was isolated from washings of frosted shoots in this vineyard. The same species was also abundant on healthy shoots in the other vineyard which had not experienced frost (Hill and Needs, 2008).

Pseudomonas syringae (typically pv. *syringae*) has been the only INB species isolated from spring buds and shoots on various *V. vinifera* cultivars (Lindow *et al.*, 1978; Luisetti and Gaignard, 1987; Itier *et al.*, 1991; Luisetti *et al.*, 1991; Gaignard and Luisetti, 1993).

There are many strains of *P. syringae* and among those that can ice nucleate there are about half a dozen variants (alleles) of the IN gene. All isolates in the frosted vineyard possessed one allele, *inaV*, and all in the unfrosted vineyard another, *inaQ* (check Hill and Needs, 2008). Two variants in two locations suggests a diversity of IN *P. syringae* across vineyards. The influence of gene variant upon its theoretical maximum ice nucleation temperature is not well known, but is likely to be less important than level of IN protein production and its aggregation (high temperature freezing is triggered by rafts proteins bound together on the cell surface) in response to colony size, nutritional status and physical environment.

To count the INB on shoots we used a direct DNA-based approach, Quantitative Polymerase Chain Reaction (QPCR) of the IN gene. Shoots were firstly shaken in a phosphate buffer to dislodge surface bacteria, then the bacteria were pelleted by centrifugation and their DNA extracted. QPCR was then used to amplify and, in the process, calculate the number of IN genes. Since each bacterium possesses one copy of the gene it also counts the number of bacterial cells. The reaction was designed to detect a broad spectrum of IN gene alleles, and hence strains of INB. Using this method with samples from the frosted vineyard we detected IN bacteria on all 10 Chardonnay shoots tested (five frost killed), 8/10 Shiraz shoots and 5/10 Cabernet Sauvignon shoots (a mix of frost killed and not). The limit of detection in this initial work was only 3000 bacteria per shoot. INB can be present but fail to trigger freezing if few of the bacteria possess large aggregates of the protein. O'Brien and Lindow (1988) tested nine strains of ice nucleating *P. syringae* growing on leaves and found the proportion of cells able to trigger freezing at -5°C under wet conditions varied from 1 in 30 to 1 in 2,500 (mean of 1 in 150). We observed that *inaQ* isolates lost their ability to initiate freezing at -5°C after about two weeks growth in nutrient broth; an illustration of the dynamic nature of this trait. Although there were about twice as many INB (mean of ~200,000 per shoot) on frosted than on unfrosted shoots the difference was not significant due to high variability. Using culturing, Itier *et al.* (1991) and Luisetti *et al.* (1991) recorded a median of roughly 1,000 *P. syringae* per bud or shoot on French vines. This was accompanied by extreme population variability, from ≤300 to 10^8 per bud or shoot, that was irrespective of growth stage. Overall, we found INB on all cultivars tested in Victoria: Cabernet Sauvignon, Chardonnay, Pinot Noir, Sauvignon Blanc and Shiraz.

4.3.2 Nature of cell damage

Ice formation in plant tissue may occur within (intracellular) or external to (extracellular) the cells. Intracellular freezing is where ice forms inside the protoplasm of the cell and extracellular where it forms inside the plant tissue but in the intercellular spaces. There seems to be some confusion as to when these processes occur and which causes damage in grapevines.

Snyder and de Melo-Abreu (2005) summarised that intracellular freezing causes direct damage to the plant cells while extracellular freezing causes indirect damage. Intracellular freezing occurs as a result of rapid cooling whereas extracellular freezing occurs with slower cooling. Natural cooling rates are slow, usually less than -2°C h^{-1}. When cooling rates are faster, such as when evaporative dip occurs when sprinklers are first turned on, intracellular freezing occurs, resulting in fatal damage to the effected tissue (Palta *et al.*, 1993 and Evans 2000).. Microscopic examination (Levitt, 1980; McCully *et al.*, 2004) and laboratory studies by (Palta *et al.*, 1993) showed that during a natural freeze event ice tended to form in extracellular spaces and on the outside of cells. There was no evidence of the cell walls rupturing from ice formation within the cell vacuoles. Rather they were forced to contract by the pressure of the expanding ice on the outside of the cell. In severe cases this contraction caused the cell to collapse (Levitt, 1980). Beck *et al.* (2007) also found that water movement from the cell to the intercellular spaces caused a shrinkage of the whole cell resulting in disintegration of the membrane bi-layer by dehydration. The “leaking” of fluids after a freeze event by the tissues was found to be due to the collapsed cells being unable to reabsorb the fluids (Levitt, 1980), giving rise to the “water soaked” look of frost damaged tissues (Goffinet, 2004).

4.3.3. Thawing rate

In studies conducted by McCully *et al.* (2004) on various species of frost resistant plants, this water was typically reabsorbed by cells when thawed, and the plant regained its original appearance. However, when cells have sustained fatal damage this reabsorption will not occur and cells will, paradoxically, suffer from dehydration. It is important to note that it is not transpiration that causes the dehydration but ice formation in the extracellular spaces, fed by water leached from the cells (Levitt, 1980; Ireland, 2005; Snyder and de Melo-Abreu, 2005; Beck *et al.*, 2004; Beck *et al.*, 2007; Goffinet, 2004).

Although the evidence is not strong, there are claims that the rate of thawing affects the ability of a grapevine to recover from a freeze event. Rapid thawing may not allow the cells to re-absorb the water (Ireland, 2005) because membranes and cytoplasm will rupture and tear from cell walls. To

prevent this thawing must be slow enough that the membrane and cytoplasm expand at the same rate as the cell wall.

4.4. Freeze avoidance mechanisms

Grapevines have two mechanisms they can employ to avoid damage. They are supercooling (freeze avoidance) and freezing point depression (freeze tolerance).

4.4.1. Supercooling

Evans (2000) states that supercooling "is surrounded by a substantial body of myths" and is not clearly understood by grape growers. Supercooling is defined by Levitt (1980) as "the process by which a liquid remains in the liquid state at temperatures well below its crystallization point". Water can remain stably at well below 0°C because while ice is stable and will grow at this temperature it's initial formation, the alignment of water molecules into a sheet of hexagons, is strongly disfavoured by the chaotic motion of the liquid water molecules. INB use the IN protein to catalyse the formation of the seed crystal of ice. It is theorised to fold itself in a switchback fashion to hold water molecules in the correct alignment by bonding them loosely to hydrophilic amino acids (Kajava and Lindow, 1993).

(Fuller and Telli, 1999) reported that grapevines at bud burst sustain little damage during frosts warmer than -3°C due to their ability to supercool. Mean critical temperatures at budburst to first leaf were in the range -3.6 – -3.8°C. Similarly, Luisetti *et al.* (1991) and Itier *et al.* (1991) found that from woolly bud to 2–3 leaves separated shoots naturally colonised with IN *P. syringae* froze at -2 – -4°C, and that buds or shoots inoculated with IN *P. syringae* froze ~0.5–1.5°C higher than naturally colonised controls or shoots treated with an antibiotic. Vine tissue itself has an intrinsic ability to supercool (Figure 3). Supercooling allows plants to survive temperatures well below 0°C, but is limited by external and intrinsic ice nucleators along with the size of the tissues being frozen (Hummel and Moore, 1997).

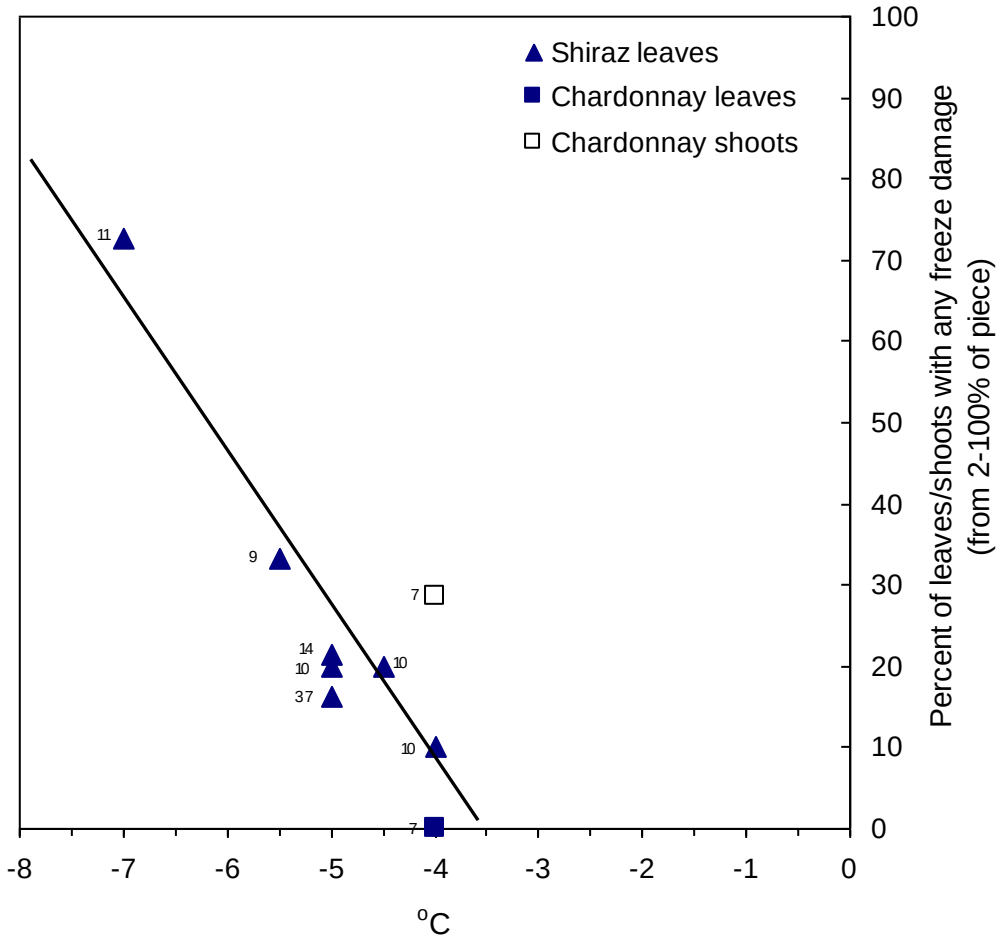


Figure 3. Supercooling ability of vine leaves (6th leaf) and 4-leaf shoots sampled in late spring and early summer, a time when leaf bacteria were naturally low due to rapid plant growth and dry weather. Leaves and shoots were sprayed with deionised water before being placed in plastic bags and incubated for 2 h at the specified temperatures (*N* given for each point).

There can be several degrees difference in critical temperatures between cultivars when dormant buds supercool. Supercooling is more effective in dry conditions (Johnson and Howell, 1981; Itier *et al*., 1991; Powell and Himelrick, 2000; Ireland, 2005) with the temperature to which supercooling operates equivalent of that on tissues without Ice Nucleating Bacteria (Lindow *et al.*, 1978). Further, Itier *et al*. (1991) found that with dry buds the nucleation temp increased from -6 to -4°C between E-L stages 3 to 9 as the *P. syringae* abundance increased from around 100 to 1000 per bud, but that with wet buds spanning the same growth stages there was no pattern and they froze at -2 to -4°C. Johnson and Howell (1981) showed that surface moisture on leaves caused death at around 3°C higher temperatures regardless of phenological stage. Although supercooling can allow plant tissues to cool well below 0°C Carter *et al.*, (1999) found that subsequent damage to flowers (blackcurrants) actually increased if it occurred prior to freezing.

4.4.2. Freezing point depression (Cryoprotectants)

Plant tissues can lower their freezing point by concentrating the solutes dissolved in the cell. (Levitt, 1980) called this process “freeze point depression” and explained that it occurred when plants moved water from within the cell into the extracellular spaces, thus concentrating the solutes within the cells. (Bachmann *et al.*, 1994) state that freeze point depression involves a number of components, including carbohydrates, proteins, enzymes, metabolites and lipids. The concentrations of sugars (carbohydrates) and acids (lipids) within the cells changes while the plants are hardening (acclimating) during late autumn/early winter.

Sugars are effective cryoprotectants which help reduce freeze damage by diluting electrolytes and toxic compounds that accumulate during freezing. Carbohydrate concentration is closely related to preceding air temperatures and will accumulate during winter dormancy (Bachmann *et al.*, 1994, Stushnoff *et al.*, 1993, Palonen, 1999). (Palonen, 1999) reported that an accumulation of sugar during this cold period increased intracellular osmotic potential and lowered the freezing point of the intracellular fluids. A leading component in cold tolerance in plants are the raffinose family oligosaccharides (RFOs), carbohydrates which are able to stabilise the cell membrane during a freeze event (Stushnoff *et al.*, 1993; Bachmann *et al.*, 1994). Another function of RFOs is their ability to allow the plant to be more tolerant of desiccation, not surprising since freeze damage is closely related to dehydration (Brenac *et al.*, 1997).

(Palta *et al.*, 1993) found that membrane lipids also influence cold hardiness. They proposed that the a change from a liquid crystalline to a solid gel state is a leading cause of freeze injury, but that this can be moderated by unsaturated fatty acids which enhance the fluid phase of the membrane. They found in potato, cranberry leaves and pine needles that the more freeze tolerant tissues had increased linoleic acid and decreased palmitic acid levels, and suggested this may be because the melting point of linoleic acid is lower than palmitic acid allowing the plasma membrane to remain more supple during a freeze event.

4.4.3. Grapevine responses to frost

The amount of injury a plant sustains during a frost event depends on a range of factors, including overall pre-freeze environmental conditions such as the minimum temperature, the time it takes to reach the minimum temperature, the dew point (the temperature falls rapidly to the dew point and thereafter more slowly; thus the higher the dew point, the longer it takes to reach a critical temperature) and the time spent below a critical temperature (Trought *et al*., 1999; Evans, 2000; Snyder and de Melo-Abreu, 2005). Other important factors at the time of a frost include the surface moisture and/or status of bud phenology, cultivar differences and ice nucleation events

(Johnson and Howell, 1981; Trought *et al.*, 1999). Whether ice formation has been intracellular or extracellular and the rate of warming after the frost (thawing) may also contribute to frost damage (Beck *et al.*, 2004).

Temperatures prior to a frost may be important. During winter, a short period of warm weather can cause plants to begin de-acclimating, making them much less resistant (Beck *et al.*, 2004). Several studies have investigated cold daytime temperatures preceding spring frosts, to see whether plants were able to respond and thereby lower the temperature at which damage begins. Winkler (1965) suggested that the extent of the damage sustained by grapevines was related to the daytime temperatures preceding a frost, with lower daytime temperatures reducing overnight frost damage. However, Johnson and Howell (1981) found, in controlled environment experiments, that daytime temperatures preceding a spring frost had no effect on frost hardiness of developing buds. Thus, daytime temperatures can be an important factor if there has been a *warm* period. This will be discussed in more detail later.

4.5. Critical damage thresholds

Howell *et al.*, (2006) have drawn a distinction between injury and damage which will be used hereafter. Damage is defined as death to the cell, organ or whole vine. Injury however occurs when only part is damaged. For example, if frost kills a newly emerging shoot but the bud remains viable, then the shoot is damaged (dead) but the bud is only injured. Critical temperature is defined by Young (1966) and Johnson and Howell (1981) as “the lowest temperature a bud (or shoot) can endure for 30 min or less without injury”.

It is also important to note that critical temperatures are not air temperature but the temperature of the plant surface, which can be 1 to 3°C lower than air temperature. (Leuning and Cremer, 1988, Trought *et al.*, 1999). For vineyard managers it is imperative to be aware that a predicted overnight low of 0°C (air temperature) could mean that the vines are in fact at a critical temperature of -2°C. In dry conditions, understanding the relationship between air temperature and the humidity (dew point) is crucial to making decisions on frost protection (Poling, 2006). When predicting potential frost risk three factors need to be taken into account. 1. The temperature at the start of the cooling period. 2. The rate of cooling, which is faster before the dew point and 3. The length of the dark period. Experiments by Howell and Shaulis (1980) and supported by work from Hamman *et al.* (1990) it was shown that there was a 2-6°C difference in shoot temperature between exposed canes and shaded canes. Also, light brown shoots were 4°C warmer than darker shoots and horizontal shoots were cooler than vertical shoots

Studies can be separated into those that involve either effects on buds before budburst or effects on shoots and/or inflorescences after budburst. Although the critical temperatures at which damage may occur to grapevines varies with phenological stage and cultivar, it is generally accepted that freeze resistance decreases as shoots age, with damage to newly emerged shoots and inflorescences occurring at around –2°C (see the section on Supercooling. It is impossible to determine a precise relationship between air temperatures and damage in grapevines as bud temperature and ability to supercool varies from bud to bud (Snyder and de Melo-Abreu (2005). Is it worth also noting that in practice air temperatures can vary significantly over quite short distances. As a result, the critical temperature in practice will depend on this factor as well.

A complicating factor is that methods used to determine the critical temperature vary between experimenters. Stergios and Howell (1973) tested several methods for evaluating the critical temperature (T_c) and injury to plants. These included; visual scoring, photosynthesis rates, water potential gradients and electrolyte leakage. Murray *et al.* (1989) suggested that all of these methods have drawbacks and that the electrolyte leakage test is the most reliable for distinguishing death after a few days in frosted plant tissues. For exasmple, cucumber plants that had undergone chilling and did not show visible signs of damage still suffered water loss and electrolyte leakage when put into warm growth cabinets (Minchin and Simon 1973). It is worth noting that while these cells recovered, cells in plants that had visible signs of wilting were not, when put back into warm growth chambers. That several methods are used to establish critical temperatures, on different cultivars, and on different ranges of phenological stages may explain the inconsistent results among studies. Even so, Wilson (2001) stated that while opinions on critical temperatures for freezing injury vary, there are clear differences between species, tissues and stages of growth, with the overall critical temperature depending on the amount of hardening that the plant tissues have undergone (see also Snyder and de Melo-Abreu, 2005).

Table 2. Critical temperature (LT_c) values (°C) for grapevines. Damage thresholds imply that 30 minutes at the indicated temperature is expected to cause 0, 10, 50 and 90 percent kill of the plant part affected (Snyder, 2005; Pudney, 2007; Johnson and Howell, 1981; Pool and Lerch, 2004; Sugar, 2003).

Variety	**Pinot Noir**	**Concord**	**Pinot Noir**	**Concord**	**Concord**	**Grape (*V. vinifera*)**
% damage threshold	LT_0	LT_{10}	LT_{50}	LT_{50}	LT_{90}	Unspecified
Woody tissue			-14		-27.3	-20.6

First swell (woolly)		-10.6		-4.9	-19.4	-3.5
Late (full) swell		-6.1	-3.4 to -	-3.8	-12.2	-1.1
Bud burst	-1	-3.9	-2.2 to -	-3.3	-8.9	
First leaf	-1	-2.8	-2		-6.1	-1.1
Second leaf	-1	-2.2	-1.7		-5.6	-0.5
Third leaf		-2.2			-3.3	
Fourth leaf	-0.6	-2.2	-1.2		-2.8	0

Critical temperatures vary greatly between cultivars (Tables 1 and 2) and while they appear higher for *V. vinifera* than the US species *V. riparia*, there have been no studies that suggest reasons for the differences. However, there have been numerous studies on other plant species investigating the differences between their cultivars. For a comparison of three Red Raspberry cultivars found that the cultivar with the lowest cold-hardiness had much lower concentrations of soluble carbohydrate and sucrose, suggesting that carbohydrate reserves over winter correlate with cold-hardiness (Palonen, 1999). Bachmann *et al.* (1994) and Stushnoff *et al.* (1993) also found that higher sugar concentration was correlated with greater freeze tolerance. By contrast, a comparison of 11 strawberry cultivars found no conclusive evidence for the differences in cultivar freeze tolerance (Hummel and Moore, 1997). However, they did find that flower size affects the plants ability to super-cool with smaller flowers able to tolerate lower temperatures. This has also been found with other species such as blackcurrant (Carter *et al.*, 1999), citrus (Yelenosky, 1988), peach (Andrews *et al.*, 1986; Jimenez and Diaz, 2003) and red and black currants (Warmund *et al.*, 1991). Delayed budburst/flowering (Carter *et al.*, 1999) can also contribute to a cultivar's ability to withstand, or avoid, freeze damage, and has been observed in many grapevine cultivars.

Much authors have remarked that little information is known about critical temperatures in grapevines, and that what has been reported is variable. As discussed earlier, this is due to several factors, including experimental methods, the phenology and growing conditions of the plants, the cultivar type and ice nucleators. Further research is needed to identify the critical temperatures for important *V. vinifera* cultivars in Australia, as much of the work to date has been on American hybrids.

4.6. Acclimation/de-acclimation

As stated earlier, during Autumn vine tissues change to increase the plant's cold hardiness (generally temperatures down to -15 °C). These events are associated with continental climates,

rather than frost events, and so is referred to as acclimation. Several changes occur, including altered/modified concentrations of solutes in the plant's cells, and membranes becoming less resistant to water movement, allowing it to diffuse more freely into extracellular spaces and back. Acclimation is photoperiod-dependant. Light treatment experiments by Beck *et al.*, (2007) found that short day length promotes hardening, with the rate peaking at -0.7°C per day. Over winter tissues are dormant and the xylem vessels empty of water, and therefore extremely tolerant of low temperatures: down to -15°C for most *V. vinifera* species and up to -40°C for *V. riparia* (Levitt, 1980) The primary buds are the least cold tolerant (Levitt, 1980) and therefore the most susceptible to winter freeze injury. Interestingly Mansfield and Howell (1981) found that leaves play a vital role in acclimation by producing photosynthates and other hormonal components which aid cold hardiness. In experiments on defoliation stresses they found that cold hardiness in primary buds was greatly reduced by early defoliation in Autumn, suggesting that management practices which effect the leaves of grapevines during the growing season may have an effect on the vines ability to acclimate. Wample and Bary (1992) stated that it is a common belief that a failure to remove fruit will negatively effect the vines cold hardiness by affecting the carbohydrate storage. However, they showed that this is not the case and that late harvesting or even failure to remove fruit had little to no effect on soluble carbohydrate, startch storage and cold hardiness.

When daytime temperatures begin to warm during Spring grapevines can rapidly de-acclimate. The xylem vessels fill with water causing an internal positive pressure and sap "bleeding" from cut surfaces, and begin respiring. This can results in vines becoming increasingly vulnerable; with water in the xylem there is the opportunity for ice nucleation within plant water during a frost (Goffinet, 2004, Snyder and de Melo-Abreu, 2005). Pool and Lerch, (2004) state that during sunny late winter or early spring days grapevine trunks and woody tissues can heat up to several degrees warmer than the surrounding air temperature, initiating deacclimation. Deacclimation is less photoperiod-dependant than acclimation (Beck *et al.*, 2004) and occurs when spring temperatures exceed 10°C for 1 week. Alternatively, 24-48 hours at 25°C will induce it (Stushnoff *et al.*, 1993). In addition to xylem filling, during deacclimation changes in concentrations of carbohydrates and unsaturated fatty acids increase the potential for freeze damage (Stushnoff *et al.*, 1993). Deacclimation is irreversible. Once the process has begun the plant will continue to move away from the dormant state, making it extremely susceptible to late winter and early spring frosts at a time which they would normally be dormant. Susceptibility to premature deacclimation is a major concern in a time of changing climate. For example, it is a serious problem with apricot in Northern China (Meng *et al.*, 2007).

4.7. Frost recovery responses

Following a frost, a significant proportion of the vine may be damaged. However, in time, regrowth will generally occur from undamaged buds on the vine. The degree of injury to the vine will depend on the timing, degree and proportion of damage to various parts of the vine. Understanding the relative contribution that different buds on the vine make to regrowth and yield is important in predicting the vine's recovery. Two important aspects must be considered: the extent to which damage is likely to affect yield and fruit maturity in the current season; and growth, development and productivity in the following season. In some cases, it may be better to sacrifice some poor production in the current season to ensure that production is not compromised in the next.

4.6.1. Fertility

A compound bud, comprising a primary latent bud and two secondary latent buds (often referred to secondary and tertiary buds), develops in the axil of leaves in the growing season. Over winter these remain dormant and, initially, are subtended/covered/protected/enclosed by a common scale giving the appearance of being just one bud in the axil of the foliage shoot. In viticulture, this compound bud is referred to as the 'eye' or 'node'. It contains axillary buds of the second (the primary latent bud) and third orders (secondary latent buds) with regard to the lateral shoot.

In laboratory tests, Andrews *et al.* (1984) found that within a bud the primary buds are the least temperature hardy followed by the secondary and tertiary buds. This was explained by the proportion of water contained within them; the amount of cooling they could tolerate being directly, inversely proportional to their volume of water. (Wolpert and Howell, 1985) reported that there were also differences in grapevine tissue hardiness as a function of their position (basal vs apical). This is described in more detail by (Goffinet, 2004).

Primary latent buds give rise to primary shoots in the following season. These comprise the vine's vegetative growth as well as carrying the crop. Secondary latent buds do not normally go on to form shoots, except where there is some damage to the primary latent bud prior to budburst (eg. primary bud-axis necrosis or mite damage) or when the primary shoot itself is damaged after budburst by frost or hail, for example. When they do form shoots, these shoots can be identified by observing their phyllotaxy which departs at about 90º from that of primary shoots. According to Pratt (1974), shoots formed from secondary latent buds (first secondary latent bud to form) usually bear inflorescences, whereas shoots that develop from tertiary (second secondary latent bud to form) buds tend to bear none. On the other hand, Mullins *et al.* (1992) stated that both these buds

remain small and seldom contain inflorescence primordia. Such an apparent contradiction may reflect varietal differences in the material studied.

Post frost management will thus be governed by the fruiting habit of the variety frosted. Cultivars grown in Australia tend to bear well with short spur pruning. (Winkler, 1965) stated that these will produce almost a full crop after a frost event when the latent secondary buds shoot, however these secondary shoots are still vulnerable to any further frosts. Seyedbagheri and Fallahi, (1994) from Wilson (2001) found that when the primary bud is destroyed the secondary buds can bear 50-70% the yield of the primary shoots. (Winkler, 1965) also stated that varieties with fruitful basal buds will produce 25-50% of normal yield from basal buds left on the old parts of the vine, buds that have been dormant for some time (latent buds/water shoots) or buds left on the vine from careless pruning. In experiments by (Friend *et al.*, 2006) on Chardonnay, in New Zealand, it was found that while 63% of primary shoots had two bunches only 39% of secondary shoots had two bunches. Similar percentages of primary and secondary shoots had one bunch. Interestingly there was no significant difference between bunch and berry weights in bunches from primary and secondary shoots. The potential productivity may also depend on the proportion of primary buds that developed originally. Where bud break (shoots per node retained after pruning) has been low, there are presumably a greater number of primary buds left to grow after a frost event, hence yields may be little affected.

It is important to note that the secondary crop will need more time to ripen.
In some varieties and areas the secondary crop may not ripen after a late frost event. Also, where there is crop from both primary and secondary shoots significant variability in fruit maturity may result. This causes issues with harvesting and other vineyard management decisions (eg. botrytis vulnerability). However, the reduced secondary crop may mature and ripen faster than the undamaged primary crop (Trought *et al.* 1999).

4.8. Managing frost affected vines

There are some inconsistencies in the literature as to the best management practice post frost. Winkler (1933) stated that once frost damage has occurred frosted shoots should be removed or they will reduce growth and productivity of secondary buds. On the other hand, (Kasimatis and Kissler, 1974) were not able to gain an effect on growth and productivity of secondary buds by removal of frosted shoots. This may be due to a number of factors that must be taken into account, including severity of damage, cultivar type, pruning regime, secondary shoot fruitfulness, phenological stage and economics.

Again, while Lider (1965) found no significant difference between hand stripping damaged shoots compared with no treatment on spur pruned Folle Blanc and cane pruned Cabernet Sauvignon and Riesling, Winkler (1965) found yield was increased on spur pruned Malaga and Tokay when frosted shoots were removed. He noted that the auxiliary buds produced unfruitful lateral shoots if the frosted shoots were not removed. Trought *et al.* (1999) also reported that higher yields were obtained by removing the damaged shoots from frosted vines 10-14 days after it occurred, and by removing all shoots in severely frosted vines.

When considering post frost management, even if there is little to no crop, (Trought et al., 1999) says that growers must consider whether there might be excessive shoot numbers growing on damaged vines. Excessive shoot numbers may result in inadequate and thin shoot growth, resulting in low productivity. To ensure that sufficient growth occurs a reduction of node number retained after a frost may be necessary to secure a few “good” shoots rather than a large number of small shoots. It has been observed that shoot growth following a frost can be relatively slow, as much of the carbohydrate reserves, necessary for rapid and uniform shoot growth will have been utilized in the initial shoot development before the frost. Additional fertilization may be appropriate to encourage additional growth.

Howell *et al.* (2006) explained that although the economic impact of frost is generally regarded as simply the loss of income from reduced yields, growers also need to decide whether they have enough crop (or potential crop from secondary shoots) to continue the normal program of management or to opt for maintenance only. “Making such hard decisions immediately after the frost event can be unwise” (Howell *et al.*, 2006, p.7) After a frost it is important that growers undertake a rigorous review of the potential productivity of the vineyard. This will include assessing the number of undamaged inflorescences (delaying this assessment for several days will enable the damaged flowers to abort). In addition, the extent of the frost in the district can be evaluated. If the frost has been widespread, even quite low yields may have particular value in maintaining fruit and wine supply.

4.9. Future Research Priorities

Long term predictions for frost occurrence and severity are difficult. The Bureau of Meteorology is able to give growers a few days notice, warning before a frost event occurs. However, it is up to individual growers to determine the severity of the frost within their individual vineyards and to implement management to counteract the effects of the frost. The seemingly random nature of frost makes it a difficult area to research, given that a vineyard may be severely effected one year and be completely untouched another. Obtaining consecutive years data may not always be

possible. To date the majority of research carried out on frost has been from Europe, America and New Zealand. Frosts in Australia are infrequent, only a few degrees below zero and only for a short period of time. In the past these frosts have generally been managed with overhead sprinklers, However with fluctuating and uncertain water supplies facing few growers in Australia have the water available for this method of frost protection. Also, most vineyards are now drip irrigated and lack the irrigation infrastructure for overhead sprinkler frost control The drought itself has been a factor in the past few years with drier springs being a factor leading to damaging frosts within Australian wine regions. For this reason there seems to be a need for research into post frost management. The influence of time of frost on regrowth from secondary shoots; how long do they get from the time of re-shoot to harvest and the influence of retained node number and the consequence of multiple shoots arising from lateral buds.

An important challenge for post frost management is when the vineyard is injured rather than damaged. It is easy to decide what to do if everything is dead, but assessing the injury if there is varying degrees of damage or damage in patches across a vineyard makes management more challenging. If there is fruit remaining from primary shoots mixed in with that from secondary shoots what effect does this have on fruit variability? How does this vary with variety?

There has been extensive research published on Ice Nucleating Bacteria (INB), but very little on the role of INB causing frost damage on plants. There are numerous chemical sprays available for grapevines that claim to be frost protectants' which inhibit or kill INB, however there is no independent research to support which of these chemicals work or by what mechanism they inhibit the INB. These putative frost prevention sprays need to be objectively and independently tested.

There are is substantial uncertainty surrounding winter hardiness, acclimation and deacclimation. Further research into the physiology surrounding grapevine acclimation and deacclimation and the reasons for different cultivars being more frost resistant than others would benefit growers and also assist grapevine breeding programs.

Clear information on secondary shoot fruitfulness for the economically important Vitis Vinifera species is urgently needed

4.10. Conclusions

Australia's southern wine regions have suffered three damaging frosts in the last decade. Grapevines are able to survive by either supercooling or through freeze point depression. Ice

nucleating bacteria aggravate frost damage in vines by impeding supercooling, but the centrality of their role, and what control measures might be used to limit their impact, is not yet known. Growers can aid their vines in supercooling by ensuring protection measures such as water and wind machines are engaged early before supercooling begins to prevent a snap freeze. There are still gaps in our understanding of freeze point depression and what can be done to aid vines in this natural defence. Current research points to the building of carbohydrate reserves before dormancy to aid acclimation. Several studies allude to the possibility that thawing rate affects a grapevine's ability to recover, but as yet there has been no direct study examining this and the possibility to exploit it.

Extensive research has been done on critical temperatures for different plant species. For vines, however, the information is variable and comparisons complicated by the different methods of measurement used. While there have been several reliable studies on grapevines very little has been on the economically important Australian cultivars. Critical temperatures for *V. vinifera* appear higher than for *V. riparia* but no studies clearly explain the observed differences. The range of critical temperatures depends on several factors including phenological stage, cultivar and ice nucleators present. Generally, damage to exposed inflorescence and newly emerged shoots occurs at around –2°C, with freeze resistance decreasing as shoots age.

Primary buds are the least temperature hardy followed by the secondary and tertiary buds. When a primary bud is killed by frost the secondary latent bud will often survive and shoot. The literature is not clear about the yield of these secondary shoots and the quality of the fruit. Secondary shoot fruitfulness will be largely dependant on the cultivar, but also pruning regime and post frost management will impact upon it.

After a frost, management options need to be carefully considered. Factors which will determine management will include: severity of the damage, cultivar type, pruning regime, secondary shoot fruitfulness, phonological stage and economics. There is not an easy answer to post frost management and as yet there has not been a study completed which gives a clear indication to what management growers should implement for current.

4.11. References

ABS (2008) Wine production down, sales up: ABS. [http://www.abs.gov.au/ausstats/abs@.nsf/mediareleasesbytitle/3746147D4B331B06CA2573DE00786719?OpenDocument] Accessed: 15 June 2008

ANDREWS, P. K., PROEBSTING, E. L. & GROSS, D. C. (1986) Ice nucleation and supercooling in freeze sensitive peach and sweet cherry tissues. *American Soc. Hort. Sci*, 111, 232-236.

ANDREWS, P. K., SANDIDGE, C. R., III & TOYAMA, T. K. (1984) Deep Supercooling of Dormant and Deacclimating Vitis Buds. *Am. J. Enol. Vitic.*, 35, 175-177.

ANONYMOUS (2007) Australia had 11th warmest year in 2006. The Age. January 3, 2007

BACHMANN, M., MATILE, P. & KELLER, F. (1994) Metabolism of the Raffinose Family Oligosaccharides in Leaves of Ajuga reptans L. (Cold Acclimation, Translocation, and Sink to Source Transition: Discovery of Chain Elongation Enzyme). *Plant Physiol.*, 105, 1335-1345.

BECK, E. H., FETTIG, S., KNAKE, C., HARTIG, K. & BAHATTARAI, T. (2007) Specific and unspecific responses of plants to cold and drought stress. *Journal of Biosciences,* 32, 501-510.

BECK, E. H., HEIM, R. & HANSEN, J. (2004) Plant resistance to cold stress; Mechanisms and environmental signals triggering frost hardening and dehardening. *Journal of Biosciences,* 29, 449-459.

BUREAU OF METEOROLOGY (2009) Annual and monthly potential frost days. http://www.bom.gov.au/jsp/ncc/climate_averages/frost/index.jsp

BRENAC, P., HORBOWWICZ, M., DOWNER, M., DICKERMAN, A. M., SMITH, M. E. & OBENDORF, R. L. (1997) Raffinose accumulation related to desication tolerance during maize seed development and maturation. *Journal of Plant Physiology,* 150, 481-488.

CARTER, J., BRENNAN, R. & WISNIEWSKI, M. (1999) Low-temperature Tolerance of Blackcurrant Flowers. *HortScience,* 34, 855-859.

EVANS, R. G. (2000) The art of protecting grapevines from low temperature Injury. *ASEV 50th Anniversary Meeting.* Seattle, Washington.

FRIEND, A. P., TROUGHT, M. C. T. & CREASY, G. L. (2006) Impact of a spring frost event on the fruitfulness of Chardonnay Grapevines. (Poster). *6th International Cool Climate Symposium.* Christchurch NZ.

FULLER, M. P. & TELLI, G. (1999) An investigation of the frost hardiness of grapevine (*Vitis vinifera*) during bud break. *Ann. appl. Biol,* 135, 589-595.

GOFFINET, M. C. (2004) Anatomy of grapevine winter Injury and recovery. . *Cornell University Grape Pages.* Cornell University.

GREAT BRITAIN. METEOROLOGICAL OFFICE. & LEWIS, R. P. W. 1991, *Meteorological glossary / Meteorological Office ; [edited by R.P.W. Lewis]* HMSO, London

HILL, T. (2008) Wine and frozen water don't mix: quantification of ice nucleating bacteria on grapevines. Melbourne, University of Melbourne.

HOWELL, G. S., POOL, R. M. & TROUGHT, M. C. T. (2006) Managing cold, frost and other weather related grapevine damage – a review. . *International Cool Climate Symposium.* Christchurch NZ.

HUMMEL, R. L. & MOORE, P. P. (1997) Freeze Resistance of Pacific Northwest Strawberry Flowers. *J. Amer. Soc. Hort. Sci.,* 122, 179-182.

IRELAND, W. (2005) *Frost and Crops: Frost Prediction and plant Protection. ,* New Zealand, W Ireland.

JIMENEZ, C. M. & DIAZ, J. B. R. (2003) A Statistical Model to Estimate Potential Yields in Peach before Bloom. *J. Amer. Soc. Hort. Sci.,* 128, 297-301.

JOHNSON, D. E. & HOWELL, G. S. (1981a) The effect of grape morphology and cultivar on the phenological development and critical temperatures of primary buds on grape canes. . *Journal of American Society of Horticultural Science,* 106, 545-549.

KALMA, J. D. PERERA, H. WOOLDRIDGE S. A. and STANHILL G. (2000) Seasonal changes in the fraction of global radiation retained as net all-wave radiation and their hydrological implications. *Hydrological Sciences—Journal—des Sciences Hydrologiques,* 45(5) 653

KASIMATIS, A. N. & KISSLER, J. J. (1974) Responses of Grapevines to Shoot Break-Out Following Injury by Spring Frost. *Am. J. Enol. Vitic.,* 25, 17-20.

LEUNING, R. & CREMER, K. W. (1988) Leaf temperatures during radiation frost Part I. Observations. *Agricultural and Forest Meteorology,* 42, 121-133.

LEVITT, J. (1980) *Responses of plants to environmental stresses. Chilling freezing and high temperature stresses.,* New York, NY, Acad. Press.

LIDER, J. V. (1965) Some Responses of Grapevines to Treatment for Frost in Napa Valley. *Am. J. Enol. Vitic.,* 16, 231-236.

LINDOW, S. E., ARNY, D. C., UPPER, C. D. & BARCHET, W. R. (1978) The role of bacterial ice nuclei in frost injury to sensitive plants. IN LI, P. H. & SAKAI, A. (Eds.) *Plant Cold Hardiness and Freezing Stress - Mechanisims and Crop Implications.* New York, Academic Press.

MANSFIELD, T. K. & HOWELL, G. S. (1981) Response of soluble solids accumulation, fruitfullness, cold resistance, and onset of bud growth to differential defoliation stress at veraison in concord grapevines. *Am. J. Enol. Vitic.,* 32, 200-205.

MCCULLY, M. E., CANNY, M. J. & HUANG, C. X. (2004) The Management of Extracellular Ice by Petioles of Frost-resistant Herbaceous Plants. *Annals of Botany* 94, 665-674.

MCDAVITT, B. (2007) MetService feature article: Frost. [http://www.metservice.co.nz/public/learning/2007-september.html] Acessed: 23 August 2008

MENG, Q., LIANG, Y., WANG, W., DU, S., LI, Y. & YANG, J. (2007) Study on Supercooling point and freezing point in floral organs of apricot. *Agricultural Sciences in China,* 6, 1330-1335.

MINCHIN, A. & SIMON, E. W. (1973) Chilling Temperature in Cucumber Leaves in Relation to Temperature. *Journal of Experimental Botany,* 24, 1231-33.

MORRIS, C., GEORGAKPOULOS, D. & SANDS, D. (2004) Ice nucleation active bacteria and their potential role in precipitation. *Journal de Physique IV France,* 121, 87-103.

MULLINS, M.G., BOUQUET, ALAIN. AND WILLIAMS, L.E. (1992) Biology of the Grapevine, Cambridge University Press, Cambridge.

MURRAY, M. B., CAPE, J. N. & FOWLER, D. (1989) Quantification of frost damage in plant tissues by rates of electrolyte leakage. *New Phytologist,* 113, 307-311.

NEMANI, R.R., WHITE, M.A, CAYAN, D.R., JONES, G.V., RUNNING, S.W AND COUGHLAN, J.C. (2001) Asymmetric climatic warming improves California vintages. *Climate Research*, Nov. 22, 19(1):25-34.

PALONEN, P. (1999) Relationship of Seasonal Changes in Carbohydrates and Cold Hardiness in Canes and Buds of Three Red Raspberry Cultivars. *J. Amer. Soc. Hort. Sci.*, 124, 507-513.

PALTA, J. P., WEISS, L. S., HARBARGE, J. F., BAMBERG, J. B. & STONE, J. M. (1993) Moelcular mechanisms of freeze-that injury and cold acclimation in herbaceous plants: merging physiological and genetic approaches. IN JACKSON, M. B. & BLACK, C. R. (Eds.) *Interacting stresses on plants in a changing climate.* Berlin, Springer-Verlag.

POLING, E. B. (Ed.) (2006) North Carolina Winegrape Grower's Guide - Spring Frost Control. [http://www.cals.ncsu.edu/hort_sci/fruit/winegrapes/winegrapes0.pdf] Accessed: October 2007

POOL, R. M. & LERCH, S. (2004) Managing cold injured vines; what we learned in 2003. *Cornell Grape Pages.* [http://www.nysaes.cornell.edu/hort/faculty/pool/Managing%20WI%20Vynds/Manage%20WI%20Vineyard%20What%20we%20learned%20in%202003.htm] Accessed: May 2007

POWELL, A. A. & HIMELRICK, D. G. (2000) Principles of freeze protection for fruit crops. *Alabama Cooperative Extension System.* [http://www.aces.edu/department/peaches/freeze.html] Accessed: February 2008

PRATT, C. (1974) Vegetative Anatomy of Cultivated Grapes--A Review *Am. J. Enol. Vitic.*. 25: 131-150

PUDNEY, S (2007) Frost Protection in Vineyards and volumetric allocations in the South East, DWLBC Report 2007/07, Government of South Australia, through Department of Water, Land and Biodiversity Conservation, Mount Gambier.

RIGBY, J. R., AND A. PORPORATO (2008), Spring frost risk in a changing climate, *Geophys. Res. Lett.*, 35, L12703

SNYDER, R. L (2000) Principles of Frost Protection, (Long version – Quick Answer *FP005*) University of California

SNYDER, R. L. & DE MELO-ABREU, J. P. (2005) *Frost Protection: fundamentals, practice, and economics.* Food and Agriculture Organization of the United Nations. [http://www.fao.org/docrep/008/y7223e/y7223e00.htm#Contents] Accessed: 3 December 2007

STERGIOS, B. G. & HOWELL, G. S. (1973) Evaluation of viability tests for cold stressed plants. *J. Amer. Soc. Hort. Sci.,* 98, 325-330.

STUSHNOFF, C., REMMELE, R. L. J., ESSENSEE, V. & M, M. (1993) Low temperature induced biochemical mechanisms: Implications for cold acclimation and de-acclimation. IN JACKSON, M. B. & BLACK, C. R. (Eds.) *Interacting Stresses on Plants in a Changing Climate.* Berlin, Springer-Verlag.

SUGAR, D., GOLD, R., LOMBARD, P. & GARDEA, A. (2003) Strategies for frost protection. . IN HELLMAN, E. W. (Ed.) *Oregon Viticulture.* Oregon State University.

TROUGHT, M. C. T., HOWELL, G. S. & CHERRY, N. (1999) Practical Considerations for Reducing Frost Damage in Vineyards. *Report to New Zealand Winegrowers.* New Zealand.

WAMPLE, R. L. & BARY, A. (1992) Harvest Date as a Factor in Carbohydrate Storage and Cold Hardiness of Cabernet Sauvignon Grapevines. *J. Amer. Soc. Hort. Sci.,* 117, 32-36.

WARMUND, M., GEORGE, M. & TAKEDA, F. (1991) Supercooling in Floral Buds of `Danka' Black and `Red Lake' Red Currants. *J. Amer. Soc. Hort. Sci.,* 116, 1030-1034.

WFA (2007) Winemakers' Federation of Australia vintage report. . Adelaide, National Wine Centre.

WILSON, S. (2001) Frost Management in Cool Climate Vineyards. . *Final report to Grape and Wine Research & Development Corporation.* .

WINKLER, A. J. (1933) The treatment of frosted grape vines. . *Proc. Am Soc Hort. Sci,* 30, 253-257.

WINKLER, A. J. (1965) *General Viticulture,* Berkeley, University of California Press.

WISNIEWSKI, M., FULLER, G., GLENN, D. M., PALTA, J. P., CARTER, J., GUSTA, L., GRIFFITH, M. & DUMAN, J. (2001) Factors involved in ice nucleation and propagation in plants: an overview based on new insights gained from the use of infrared thermography. *ICEL. AGR. SCI.* , 14, 41-47.

WOLPERT, J. A. & HOWELL, G. S. (1985) Cold Acclimation of Concord Grapevines. I. Variation in Cold Hardiness Within the Canopy. *Am. J. Enol. Vitic.,* 36, 185-188.

YELENOSKY, G. (1988) Capacity of citrus flowers to supercool. *HortScience,* 23, 365-367.

YOUNG, R. H. (1966) Freezing points and lethal temperatures of citrus leaves. *Proc. Am. Soc. Hort. Sci.* , 272-279

L, G. S. (1985) Cold Acclimation of Concord Grapevines. I. Variation in Cold Hardiness Within the Canopy. *Am. J. Enol. Vitic.,* 36, 185-188.

YELENOSKY, G. (1988) Capacity of citrus flowers to supercool. *HortScience,* 23, 365-367.

YOUNG, R. H. (1966) Freezing points and lethal temperatures of citrus leaves. *Proc. Am. Soc. Hort. Sci.* , 272-279.

5. Communication

Two highly suceesful workshops were organised in conjunction with DPI Victoria Grapecheque) and held in the Yarra Valley (11th June, 2008) and Goulburn Valley (12th June 2008). These workshops covered frost prediction, prevention and post frost management, along with a presentation on the biology of frost. Presenters included David Morrison (Bureau of Meteorology), Dr Michael Trought (Marlborough Research Centre), Ms Sonja Needs (Melbourne University) and Dr Tom Hill (University or Melbourne / University of East London). These workshops were well attended, with over 50 in Yarra Valley and over 40 participants at Nagambie. The Yarra Valley workshop was recorded and uploaded to the DPI Victoria website and linked to the GWRDC website and DVDs were circulated nationally.

Results of the experiments that were set up immediately post the 2006 frosts were presented to various forums, including the frost workshops sponsored by this project, and were included in the post graduate subject Advanced Viticulture Techniques. These experiments characterised frost recovery responses, including yield and canopy responses in the current and following season and analysed the economics of a range of post frost management treatments.

An experimental program begun during the project to characterise post frost yield responses of our most economically important *Vitis vinifera* varieties has been completed by Ms Sonja Needs and will form a chapter of her Masters thesis, due for submission in

6. Staff engaged in the project

Sonja Needs[1],
Michael Trought[3]
Tom C.J. Hill[1]
E.W.R. (Snow) Barlow[1]
Gregory M. Dunn[2]

[1]Melbourne School of Land and Environment, The University of Melbourne, Parkville, Victoria, 3010 Australia.
[2]Melbourne School of Land and Environment, The University of Melbourne, Dookie Campus, Victoria, 3647 Australia.
[3]Plant and Food Research Ltd., Marlborough Wine Research Centre, PO Box 845, Blenheim, New Zealand.

7. Budget reconciliation

END OF PROJECT
FINANCIAL STATEMENT
Statement of Receipts and Expenditure

At the conclusion of each Project, the GWRDC requires a Statement of Receipts and Expenditure of GWRDC funds received in relation to the Project.

Project Title:	Improved frost management in the Goulburn/Yarra Valleys and Strathbogie Ranges

GWRDC Project Number	Project Start Date	Project End Date
RT 06/04-1	1/05/2007	30/04/2009

Financial Year (e.g. 2006/07)	**(a)** ***Funds brought forward from previous year***	**(b)** **#Approved GWRDC Budget (full year)**	**(c)** **Total budget available (a)+(b)**	**(d)** **Actual expenditure ($)**	**(e)** **Difference ($) (c)-(d)**
Year 1: 2006/07	**n/a**	20,000.00	20,000.00	20,000.00	0.00
Year 2: 2007/08	0.00	20,000.00	20,000.00	20,000.00	0.00
Year 3: 20__/__	0.00		0.00		0.00
Year 4: 20__/__	0.00		0.00		0.00
Year 5: 20__/__	0.00		0.00		0.00
TOTAL		40,000.00		40,000.00	**0.00**

* "Funds brought forward" refers to any unspent GWRDC funds from the previous year and approved by the GWRDC to be spent the following year (i.e. column *(e)* from previous year).
# "Approved GWRDC Budget" is the funding amount specified in the Project Agreement, including any approved Variations after Project commencement.

Was funding expended as approved in the Project Agreement? Yes √ No ☐

Has the cash and/or in-kind funding from Contributing Agencies been received and applied to the Project as approved in the Project Agreement? Yes √ No ☐

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative)  ……

…… …… …… ……

Name: Gregory Mark Dunn *Title:Dr* *Date:19th October 2010*