Causes and Prevention of Table Grape Berry Collapse

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CSIRO Plant Industry

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CAUSES AND PREVENTION OF TABLE GRAPE BERRY COLLAPSE

Davinder Pal Singh et al.

CSIRO

HAL project number: DG04001

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This is the final report for the project "Causes and prevention of table grape berry collapse". This report describes the results of research into factors contributing to berry collapse of Thompson Seedless, and outlines management practices to moderate the effect of these factors in order to reduce symptoms of berry collapse.

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Media Summary

Berry collapse is an important issue for the table grape industry and to gain a better insight into its cause and the mechanisms involved, the problem has been investigated by CSIRO Plant Industry in collaboration with DPI, Victoria.

This project has focussed on identifying the causes (environmental and or management practices) of berry collapse in order to prevent or effectively reduce crop loss as a result of berry collapse in Thompson Seedless table grapes at harvest.

A key part of the research project involved setting up glasshouse trials to investigate the link between gibberellin (GA) sprays and heat and water stress. Several field trials have also been conducted on properties at Sunnycliffs and Birdwoodton to understand the impact, if any, of girdling (cincturing), water stress and rootstock selection on berry collapse.

The data collected from two glasshouse experiments suggested a strong link between GA sprays and berry collapse when vines were subjected to heat stress. Water stress further increased the incidences of berry collapse under hot glasshouse conditions.

Microscopy was carried out on berries collected from the glasshouse and from field trials to observe the process of collapse throughout the growing season. Extensive microscopy results have suggested that berry collapse is due to cell death. It also suggested that brown striations on berries might be a good indicator of whether or not a berry would succumb to collapse symptoms.

Foliar application of Surround®, which reflects sunlight and reduces sunburn and heat stress, were made and its effect on berry growth and berry collapse symptoms were assessed.

The application of some chemicals known to be involved in plant defence mechanisms (salicylic acid), resulted in the reduction of berry collapse symptoms.

Management practices with the potential to exacerbate berry collapse symptoms, *e.g.* GA sprays and water deficit during hot weather conditions at and around fruit set and early berry growth, need to be avoided to reduce berry collapse.

Technical Summary

Thompson Seedless is the most popular table grape variety on the domestic market. However, incidences of berry collapse in 3 seasons (1997-98, 2000-01 and 2007-08) have resulted in significant crop and financial losses to fresh fruit producers. It is not clear whether the berry collapse is due to heat stress, water stress or management practice(s) alone or in combination.

Based on existing knowledge, a series of studies and trials were conducted over three seasons to investigate the factors contributing to berry collapse for this variety and to develop management options to ameliorate the effect of such factors.

Berry collapse is the necrosis of tissue at the distal end of the berry (i.e. away from the pedicel). Extensive microscopy of the affected tissue suggested that berry collapse symptoms are the result of cell death. During the course of the project, we discovered that berries without any collapse symptoms such as water berries, berries with soft tip (at the distal end) and berries with vertical brown striations also show cell death. Whether these are intermediary symptoms leading to berry collapse or are the result of berry collapse remain to be seen. Striated berries lose water at more than twice the rate of berries without symptoms and have high total soluble solids (TSS or °Brix) suggesting that loss of water through the cuticle is associated with berry cell death.

GA sprays and cincturing are used to enhance berry size at harvest by the table grape industry on a large scale because of consumer demand for bigger fruit. The weather data collected and glasshouse trials suggested that most of the problem is associated with heat stress during the time of GA sprays. The evidence also suggested that water stress contributed to berry collapse under hot weather conditions.

Rootstock and irrigation management trials were also conducted in 2008-09 season to determine any interaction between rootstock, water stress and berry collapse. Although limited berry collapse occurred during that season due to mild weather conditions, data collected suggest that vines on Schwarzmann rootstock had significantly more sun damaged berries, berries with soft tip and brown striations as compared to Ramsey rootstock under water stress conditions. Cinctured vines on both rootstocks have higher incidences of berries with sun damage, soft tip and brown striations as compared to uncinctured vines suggesting a link between rapid growth of the berry and these symptoms.

Subsequently, treatments that can ameliorate heat stress to some extent, *i.e.* application of Surround® (fine clay particles that reflect sun light) resulted in bigger berries and fewer berries with soft tip and brown striations at harvest. Furthermore, the application of Surround® resulted in early véraison. This could be exploited by the table grape industry to market fruit early which may provide a marketing advantage. A large scale trial needs to be conducted to determine maximum efficacy of Surround® or similar products as sprays. The application of some chemicals known to be involved in plant defence mechanisms against pathogens (salicylic acid), also resulted in reduction in incidence of berry collapse at harvest, but only to a limited degree.

Widespread occurrence of berry collapse in Thompson Seedless, particularly in the 2007-08 season, provided an opportunity to identify 'best practice' that may enable growers to minimise the incidence of berry collapse in future seasons.

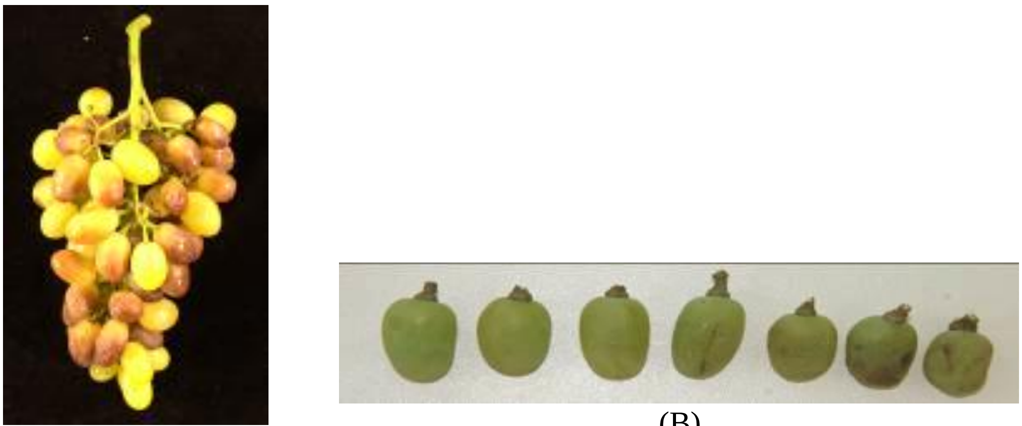
Introduction

Berry collapse is a disorder that has affected Thompson Seedless grapes in three of the last 11 seasons (i.e. 1997/98, 2000/01 and 2007/08). The visual symptoms generally involve discoloration and collapse predominantly at the end of the berry furthest from the pedicel (Figure 1). In seasons when the problem has been serious, brown striations are also observed on collapsed berries. The presence of affected berries on a bunch lowers the visual appeal and hence marketable quality of the bunch as a whole.

Thompson Seedless (*Vitis vinifera* var. Sultana syn. Thompson Seedless) is the most popular table grape variety on the domestic market, making up approximately 35% of exports. The South West region of NSW and North West region of Victoria are a significant part of the Australian table grape industry, accounting for over 85% of Australia's table grape production and the majority of Australia's Thompson Seedless production (Dry and Gregory, 1988). Berry collapse has negatively affected returns to a large number of the region's Thompson Seedless producers.

An initial scoping study (Treeby et al, 2004) documented the development of the symptoms from véraison (berry softening) onwards, suggesting that physiological events occurring between fruit set and berry softening were important. Further, mineral nutrient analyses conducted as part of that study suggested no differences in the mineral levels or mineral gradients between the proximal and distal ends of the collapsed berry (Treeby et al., 2004). The scoping study also noted a putative linkage with heat stress, and gathered some data suggesting that management factors such as the use of GA, cincturing and irrigation may also be important. The physiological events that occur between GA sizing spray application and the appearance of berry collapse are unclear.

The project described in this report, funded by Horticulture Australia Ltd., DPI Victoria and CSIRO, was initiated to further investigate the disorder's symptom development and physiology.



(A) (B)

Figure 1. (A) Collapsed Thompson Seedless grape berries on intact bunch with lighting adjusted to highlight collapsed berries and (B) Progression (left to right) of visual symptoms from véraison onwards.

The overall aim was to identify physiological causes (environmental and/or management practices) which lead to berry collapse in Thompson Seedless. The knowledge generated will serve as a basis to develop strategies to effectively reduce berry collapse of Thompson Seedless grapes.

Materials and methods

Molecular, biochemical and light and electron microscopy techniques were used throughout the investigation to study normal development processes that occur in Thompson Seedless berries. The application of modern table grape production technology such as GA sizing spray was then investigated to determine its effect on berry development processes and the interaction with heat load. The set up of trials and techniques used are explained in detail in the results and discussion section.

Planned activities

1. To develop and test practical agronomic management solutions which may alleviate and/or minimise the incidence of the berry collapse problem in Thompson Seedless table grape vineyards. This may, for example, involve trialling stress related chemicals, integrating climate forecasts with management practices, techniques to alter climate at vine and vineyard scales and developing diagnostic methods for predicting the likelihood of the problem occurring.
2. A working model of the environmental causes of the problem will be tested in the first season, and refined in subsequent seasons as necessary. The hypothesis is that berries are predisposed to berry collapse later in the season by the coincidence of hot daytime temperatures when GA sizing sprays are applied in November. The model will be tested by applying GA when high daytime temperatures are predicted for several days. A comparison will be made with the effect of GA applied when daytime temperatures are more moderate.
3. The regional industry will be targeted through presentations at growers' groups, the Murray Valley Table Grape Growers' Association newsletter and industry journals.

Results and Discussion

1. Physiological mechanisms underlying the development of berry collapse

Berry collapse starts with cell death

The outward symptoms of berry collapse are obvious to the naked eye, but there was no knowledge of what changes had taken place at cellular and tissue structure levels. Further, although the external symptoms became obvious late in the season, it was highly unlikely that changes in the physical appearance of the berries were the result of a sudden deterioration of the berry at that time. In other words, the genesis of the disorder probably started some time before the final symptoms became obvious.

Collapsed and healthy berries were examined to identify what differences in tissue structure might exist, and, on that basis, in the subsequent season (2006-07) examine berries to identify when the first signs of those differences were apparent.

Berry sectioning and vital staining were carried out according to Tilbrook and Tyerman (2008). Horizontally (width wise) or longitudinally (length wise) dissected berries were stained with 4.8 μM Fluorescein diacetate (FDA) solution for 15 minutes. FDA stained berry sections were then visualised with a Zeiss Stermi 2000C (Carl Zeiss, Germany) dissecting microscope at 2.5x magnification under ultraviolet light (mbq52ac, Carl Zeiss, Germany) with a green fluorescent protein filter in place. The images were captured by using a MC 80DX microscope camera (Carl Zeiss, Germany). FDA stains viable cells green.

The examination of healthy and collapsed berries (Figure 2A) suggested that the organization of the cells in the flesh of unaffected berries was regular, and the cells were relatively compact. In berries with berry collapse, the cells were less regularly arranged, and the cells appeared to be stretched. In the collapsed part of the berry no viable cells appeared to be present beneath the skin. The collapsed part of the berry appeared to be held together by the berry skin. This observation suggested that collapse was linked to the berry tissue “dismantling” due to cell death, but the primary trigger is unknown.

The extent of the cell death and tissue disassembly can be seen in Figure 2 cross sections of FDA-stained Thompson seedless berries. The flesh of the collapsed berry has almost completely disappeared and the berry is essentially a ‘bag’ of solution containing sugars, organic acids and minerals held together by the berry skin.

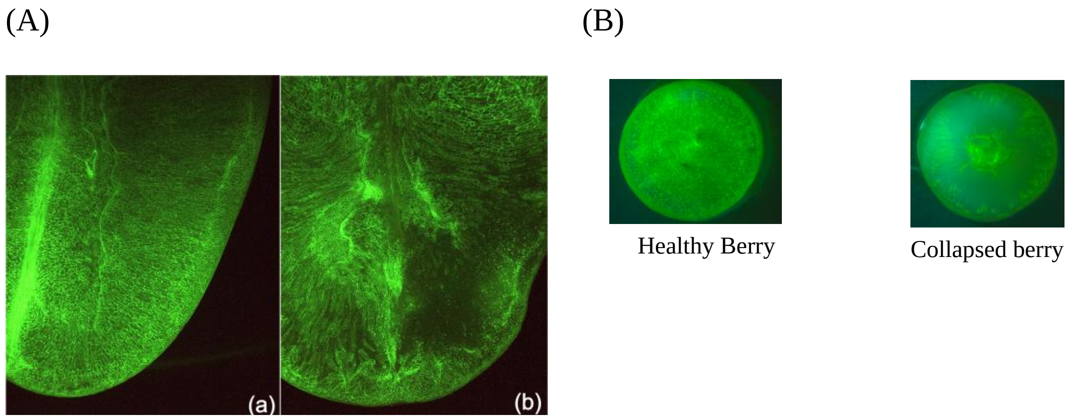


Figure 2. (A) Vertical sections of FDA (Fluorescein diacetate) -stained Thompson seedless berries. (a) Normal berry; (b) berry with symptoms of collapse. (B) Cross sections through the distal end of Thompson Seedless berries stained with FDA showing loss of viable cells in the lower half of the berry. FDA stains viable cells green.

Advanced symptoms of berry collapse have not been observed in January. However, the disappearance of tissue structure was first evident in early January (Figure 3b). It cannot be concluded that this is a normal feature of berry development because other berries collected on the same day showed no evidence of tissue structure disappearance (Figure 3a). The extent of this tissue structure loss in early January was similar in some berries to the extent of tissue structure loss in February (Figure 3c) and March (Figure 3d), when symptoms of berry collapse were seen. In other words, the loss of tissue structure was initiated earlier than mid January, but the loss of tissue structure itself is only part of the problem. Some time later in fruit development, another unidentified event occurs, which, coupled with the existing loss of tissue structure, results in berry collapse. Important questions that arise as a result of these observations are, what causes the lesions to be initiated in the first place; why do these lesions expand; and what are the circumstances that cause some berries to collapse?

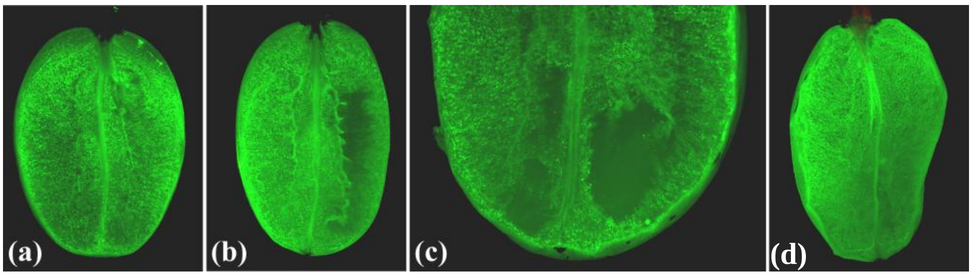


Figure 3. Vertical FDA-stained sections through Thompson Seedless berries sampled during January, February and March, 2007. (a) Berry sampled on January 12, 2007, without any symptoms of tissue loss; (b) berry also sampled on January 12 showing tissue structure loss on the right hand side of the berry; (c) lower half of a berry sampled on February 28, 2007, showing extensive loss of tissue structure in the lower half of the berry; (d) collapsed berry

sampled on March 8 showing tissue structure loss on right hand side of berry adjacent to the collapsed area of the berry.

Cell death, tissue disassembly and berry collapse are not confined to Thompson Seedless. The symptoms have also been observed in Crimson Seedless and American Black Beauty (Figure 4).

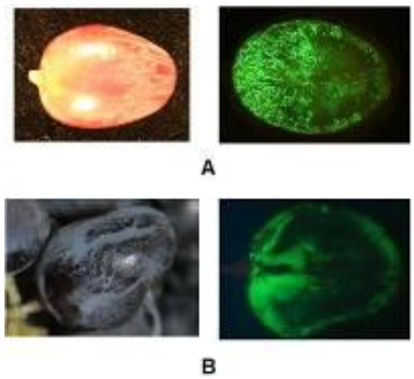


Figure 4. Collapsed Crimson Seedless and Black America. Top images, Crimson Seedless; lower images, American black beauty; left images, intact berries showing collapse; right images, FDA-stained longitudinal sections.

It is unclear from the FDA-stained sections why some of the cells in some berries appeared to have died and ruptured. Death of plant cells can be induced by ozone (Overmyer et al. 2005), aluminum (Pan et al. 2001), pathogens (Hatsugai et al. 2004), heat stress (Zuppini et al. 2006) and UV radiation (Danon et al. 2004). Programmed cell death is an active process that plays a role in plant development and the response to environmental stimuli such as heat stress (Qu et al. 2009). An interesting aside is that despite the fact that the pericarp cells had died, the epidermal cells had not died as well. Clearly, the physiology of the cells in each tissue is sufficiently different to render epidermal cells impervious to or tolerant of the stimulus for cell death.

It is clear that cell death and the disassembly of the grape berry pericarp are a prerequisite for berry collapse, but cell death and tissue disassembly do not automatically mean that berry collapse will take place. However, it seems that although cell death and tissue disassembly may be essential for berry collapse to develop, there is another event later in berry development, that combined with cell death and tissue disassembly, leads to the collapse of berries.

Mature grape berries lack functional stomata, and can only regulate turgor pressure by losing water directly through the epidermis or cuticle layer (Chambers and Possingham, 1963). Scanning electron microscopy (SEM) revealed that the collapsed part (bottom-distal end) of the berry had cracks in the wax layer (Figure 5). These cracks appeared in the cuticle and did not extend into the underlying epidermal layers, as observed when grape berries split (Hiratsuka et al., 1989). It is therefore tempting to speculate that cracks can increase the risk of water loss from the berry due to transpiration (Rosenquist and Morrison, 1998) and hence cause berry collapse. In effect, the berries are not so much collapsing as raisining.

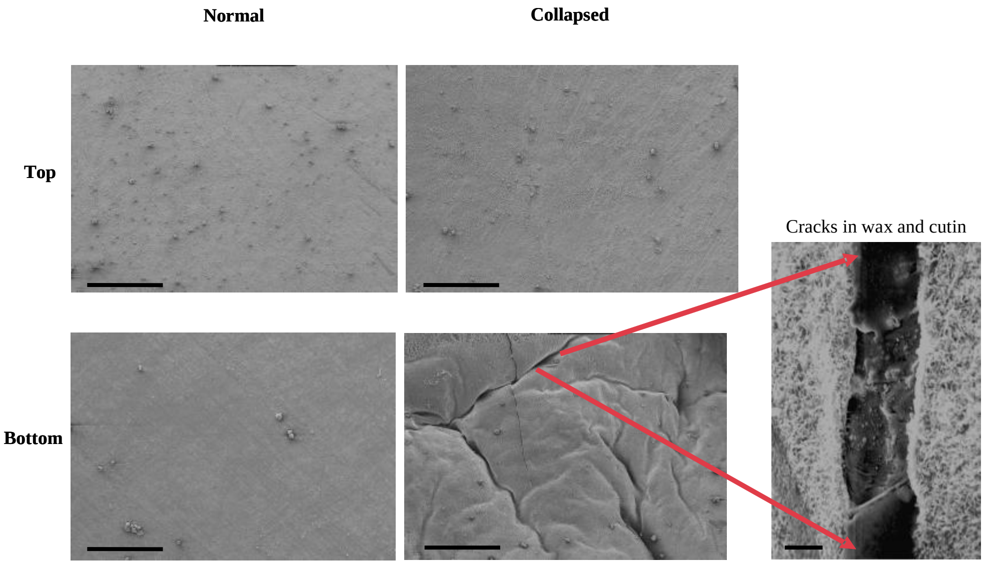
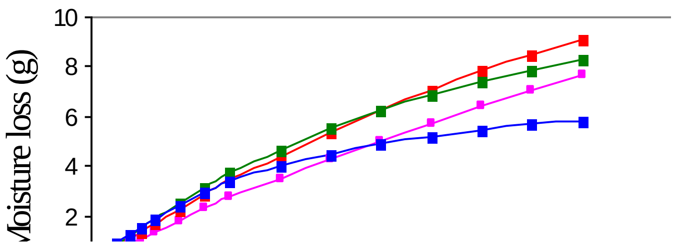


Figure 5. Scanning electron microscopy on the top and bottom half of a healthy berry (A) and a berry showing symptoms of berry collapse (B) at X 80 magnification (_____ 200µm). (C) The cracks in the cuticle (bottom half) of a collapsed berry are shown at higher magnification i.e X 160 (___ 200µm).

The cuticle's prime role is to protect the berry against moisture loss and pathogens (Rosenquist and Morrison, 1998). The fractures or cracks in the cuticle may destroy this protective barrier and expose the berry to disease and environmental stress. In hot weather, there may be increased water loss from the fruit. To test this hypothesis, moisture loss from berries with different visual symptoms (Figure 6A) was measured by incubating representative samples in an oven at 40°C for 120 hours, and weighing every 12 hours. The measurements suggested that collapsed berries, berries with brown striations and berries starting to show symptoms of berry collapse lose moisture 4, 2 and 2.5 times faster, respectively, than healthy berries under control conditions (Figure 6B). It should also be noted that berries with striations and collapsed berries had lower fresh weight (g) and higher total soluble sugars (TSS or °Brix) suggesting a concentration effect due to significant water loss through the cuticle (Figure 6A).

	T1	T2	T3	T4
Berry wt. (g)	4.54	3.05	2.80	1.97
Sugar (°Brix)	23.1	23.3	27.8	29.9

(A)



(B)

Figure 6. (A). Photographs and data of berry weight and sugar concentration, prior to commencement of moisture loss studies, for four classes of berries collected from Thompson Seedless bunches with berry collapse symptoms (ie. T1-healthy, T2- striated, T3- striated with collapse started and T4- collapsed berries). B. Loss of berry weight of the four classes of berries (T1-T4) at 40^{o}C over 120 hours. Berries were weighed at 3-12 hour intervals, depending on the stage of drying.

Furthermore, examination of berries with brown striations under the microscope suggested that the striations are a good indicator of the likelihood of a berry developing symptoms of collapse: in our hands, two out of three berries with brown striations showed cell death/loss of cells (Figure 7). Therefore, it is possible that a berry with brown striations will collapse under extreme environmental conditions (i.e. heat stress and water deficit). However, this will require further investigation.

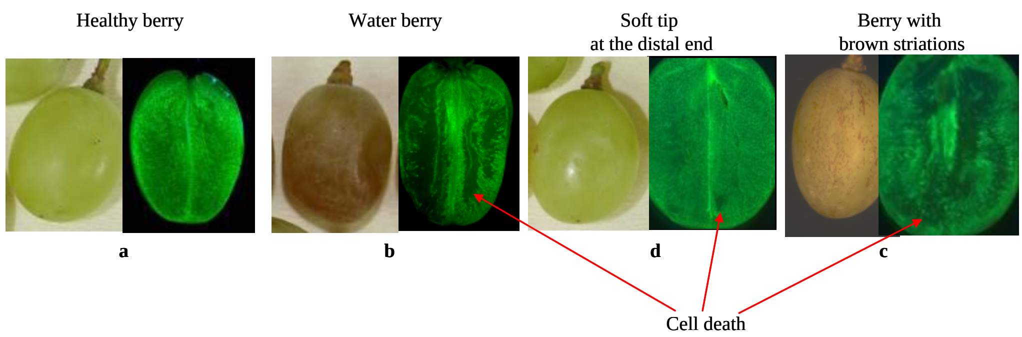


Figure 7. FDA staining of a healthy berry, a waterberry, a berry with soft tip and a berry with brown striations (left to right).

During the course of this project, we discovered that berries without any collapse symptoms such as waterberries and berries with soft tip (at the distal end) also show cell death similar to collapsed berries and berries with vertical brown striations (Figure 7). We do not know much about the development of these symptoms but it is possible that these are intermediary symptoms that lead to berry collapse or are the result of berry collapse. This hypothesis is supported by published data where other research workers have described waterberry, a physiological disorder generally resulting from stem necrosis (Morrison and Iodi, 1990). Waterberry is associated with fruit ripening and most often begins to develop shortly after véraison. The affected berries become watery, soft, and flabby when ripe.

Mitochondrial activity assays

Death of plant cells induced by environmental stimuli, for example heat stress, shares some fundamental processes with animal apoptosis, including release of cytochrome c from mitochondria, activation of specific proteases, cleavage of poly (ADP-ribose) polymerase (PARP) and DNA fragmentation (Qu et al., 2009). How cytochrome c gets to the cytoplasm is a fascinating story that is still unfolding. One of the several mechanisms involved in regulating cell death is accumulation of reactive oxygen species (ROS). In plants, one of the intracellular sources of ROS are the mitochondria (Overmyer, 2003). We hypothesized that in grape berries the normal function of the mitochondria could be perturbed during heat stress through the early accumulation of ROS in other sub-cellular compartments or changes in the plant senescence hormone ethylene, leading to increased production of ROS in the mitochondria. High levels of ROS in mitochondria could trigger release of cytochrome c into cytoplasm and hence activating cell death of grape berries through the action of caspases (proteases) under heat stress conditions.

Mitochondrial activities were measured as oxygen uptake in berries from vines growing in the 200L pots placed in the two glasshouses with mean temperatures of 30 ^{o}C and 38 ^{o}C (see later section on abiotic stress for more detail, page 17). Grape berries were placed in plastic bags on ice and transferred to the laboratory. Crude mitochondria were isolated according to published protocol (Chow et al. 1997). The oxygen content of air saturated water was estimated according to Gilliland et al.

(2003). Respiration rates were measured as oxygen uptake using a Clarke-type (Digital model 10; Rank Brothers, Cambridge, UK) oxygen electrode and expressed as nmol O_2 $min^{-1}g^{-1}$ fresh weight.

The O_2 consumption measurements (Table 1 and Figure 8) suggested that heat stress significantly ($p<0.001$) reduced mitochondrial activity. Oxygen consumption was further reduced in berries treated with GA in both the glasshouses. This reduction in mitochondrial activity may be due to tissue dilution because GA treated berries are significantly bigger (19.9 ±1.0 mm) than untreated control berries (15.8 ± 0.5 mm).

Table 1: Mitochondrial activity of berries sampled from vines growing in a control glasshouse and a heated glasshouse during 2008/09. Control = maximum daytime temperature of 35°C and night time 25°C; Hot = maximum daytime temperature of 45°C and night time 30°C. Values presented are means (n=3), and are significantly different at $p<0.001$.

Glasshouse	GA	O_2 nmol/g Fresh weight
Glasshouse A Control	-	1.963
	+	1.254
Glasshouse B Hot	-	1.065
	+	0.962

Figure 8 provides additional evidence of aberrant mitochondria function throughout berry development as GA- and heat treated berries (Glasshouse B) under water deficit conditions tend to have significantly reduced oxygen consumption as compared to berries under normal temperature conditions (Glasshouse A). Whether this discrepancy, under these growth conditions, is due to accumulation of ROS and release of cytochrome C, remains to be seen.

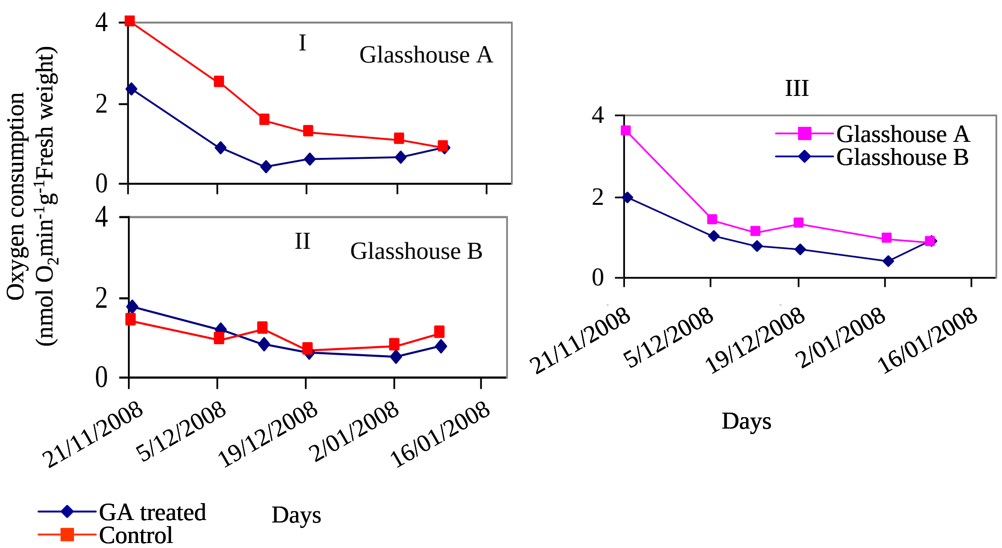


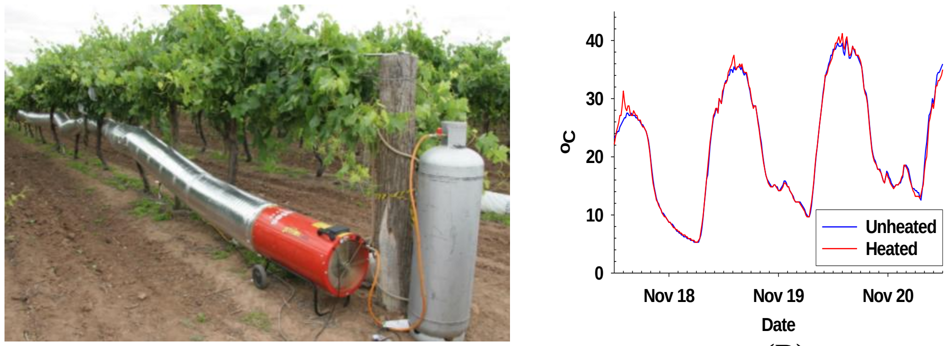
Figure 8. O_2 consumption by mitochondria from control and GA-treated berries. I and II, normal irrigation; III, GA treated berries under water deficit conditions. Glasshouse A (control): maximum daytime temperature of 35°C and night time 25°C; Glasshouse B (heated), maximum daytime temperature of 45°C and night time 30°C.

2. Impact of climate and grower practices on berry collapse

Earlier Investigations

Daily temperature maxima during berry cell division and GA application to increase berry size was the strongest environmental variable linking widespread occurrence of berry collapse in some seasons and not others (Treeby et al., 2002; 2004). Testing this putative linkage required warming the air around some vines during this time and quantifying the incidence of berry collapse on those vines compared to vines that were left unheated.

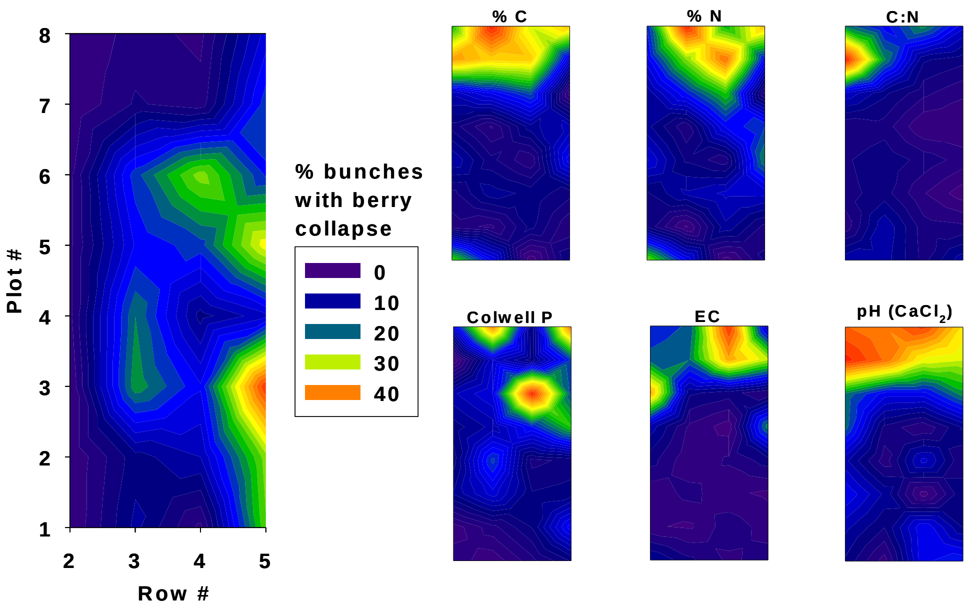
Treeby and colleagues set up a heating trial in season 2006-07 at Euston (Figure 9). Essentially, a gas-fired fan-driven heater was connected to one end of a 40 metre length of lightweight non-ribbed 300 mm air conditioning duct, and another heater was connected to the other end. A 40 mm diameter circle had been cut in the duct, in a straight line, every 400 mm along the duct. The duct was inflated and then suspended *ca* 600 mm from the trellis wire with string every 2 metres and tied to the trunk of every vine with string. The duct was positioned such that the holes were directed toward the middle of the canopy. Using this configuration, only one row could be "heated". Electricity for the fan was supplied by a petrol motor-driven 5 kVA portable generator. Heat was applied daily from about 8 am for about 6 hours from November 8 to November 30. Temperature loggers were placed in the bunch zone of vines in the heated row and of an adjacent non-heated row. The temperature data presented in Figure 2B suggested that the technique employed had only a marginal effect (of the order of 4-6°C) at about noon each day. The incidence of berry collapse was assessed across the patch in February and March, 2007 and no significant difference in berry collapse incidence was observed between non-heated control vines and heated vines. This was not a surprising result because heating fans raised the temperature only marginally and the temperature of heated canopies was in the range of 37-40°C (Figure 2). Temperatures were on average 5-10°C higher (i.e. 40-45°C) during late November, in 3 seasons (1997/98; 2000/01 and 2007/08) when the berry collapse problem was observed, compared to other seasons with no or very low incidences of berry collapse. Therefore, glasshouse trials were conducted in seasons 2007-08 and 2008-09 that enabled the air to be heated in a controlled manner (refer to the section on berry collapse in relation to abiotic stress and GA application).



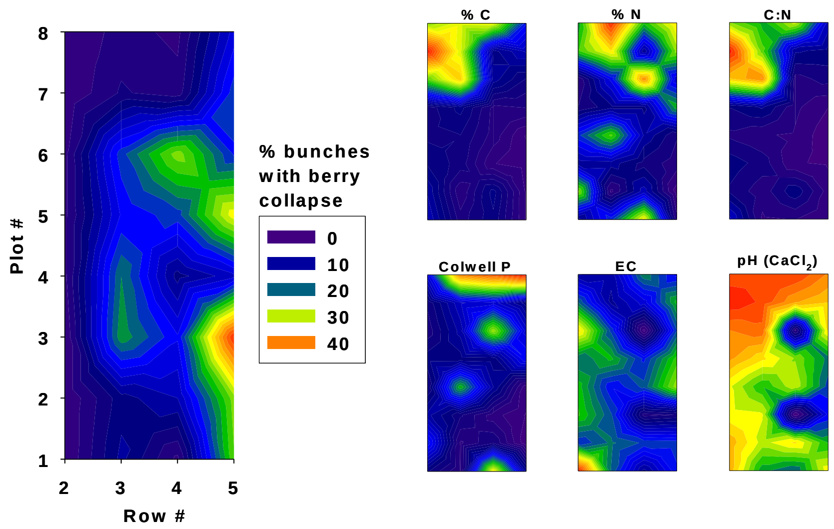
(A) (B)

Figure 9. (A) Image of final gas-fired fan-driven heaters and non-ribbed 300 mm air conditioning duct used to supply heated air to the bunch zone of Thompson Seedless vines at Euston, NSW, during November, 2006. (B) Temperature in the bunch zone of one vine in the heated row and one vine in an adjacent non-heated row in November, 2006 (showing little difference in temperature).

Treeby and colleagues (Researchers at CSIRO, Merbein and DPI Victoria, Irymple) discovered that there was good evidence that at the Euston site there was a spatial component to the incidence of the berry collapse in Thompson Seedless which occurred late in the season (Figure 10). Berry collapse symptoms were observed on vines in a relatively confined area of the Euston site. Visually, there was nothing immediately obvious between vines or soil from affected and non-affected areas. To develop a testable hypothesis, soil samples (at 10-25 and 25-45 cm depths) were collected during 2006-07 season to characterise the site in more detail. Soil parameters such as fertility (i.e. C and N content), texture, and pH were analyzed to determine whether some features of the soil were related to differences in the incidence of berry collapse between various areas of the vineyard. Patterns for pH, fertility, Colwell P (extractable phosphorous in soil) and electrical conductivity (EC), an effective way to map soil texture, in the subsoil did not resemble the pattern for berry collapse (Figure 10).



(A)



(B)

Figure 10. (A) Spatial variability in incidence of berry collapse in Thompson Seedless vines and in soil characteristics at 10-25cm and (B) 25-45 cm depths at the Euston site in 2006-07 season.

Berry collapse in relation to abiotic stress and GA application

While the complexity of programmed cell death makes it difficult to establish direct causes of berry collapse, understanding the primary triggers of berry collapse is important in order to prevent the problem. Treeby *et al*. (2002) suggested that a number of environmental factors and management practices might be contributing to berry collapse. For example heat stress, water stress, soil fertility and texture, rootstock, the use of gibberellic acid (GA) and/or cincturing (also known as girdling) may be implicated. Gibberellins are a class of growth-promoting plant hormones (Swain and Singh 2005), and one in particular, GA_4 is widely applied at the time of rapid berry expansion by table grape producers to increase final berry size, particularly of seedless varieties such as Thompson Seedless.

Our investigation of climatic conditions during seasons when the incidence of berry collapse is widespread suggested a linkage with high temperatures at the time that GA was being applied to improve final berry size. In the 3 seasons (1997/98; 2000/01 and 2007/08) when the incidence of the problem was widespread and severe, temperatures were on average 5-10°C higher during late November and early December compared to those seasons when the incidence was low and sporadic (Figure 11).

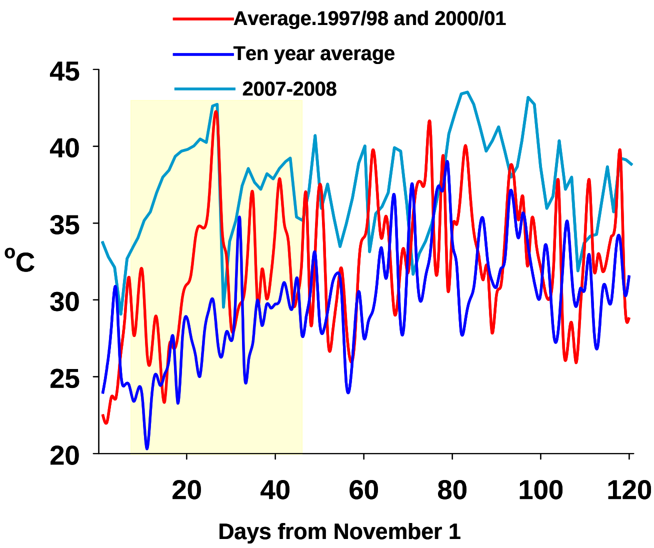


Figure 11. Daily maximum temperatures for Mildura Airport. The data show the high temperatures in November and December (yellow zone), particularly in 2007-08 but also for the mean of seasons, 1997-08 and 2000-01, compared to the ten year average in the period when GA applications would have been applied (ie. day 10 - day 45 from 1st November). Data supplied by the Commonwealth Bureau of Meteorology.

Glasshouse trials were set up to test this putative linkage between high temperature during GA application and berry collapse disorder. In December, 2006, Thompson Seedless vines on Ramsey rootstock were removed by backhoe from a vineyard in Irymple and re-planted in about 0.19 m^3 of potting mix in 200L plastic drums (Figure 12A). The vines were allowed to grow outdoors for the remainder of the 2006/07 season, and at the end of the season, 22 vines had thrown sufficient growth to be of use. In late August, 2007, 11 vines were placed in two glasshouses. Glasshouse temperatures were adjusted from mid November to early December to give daily average temperatures of approximately 25°C (glasshouse A, with minimum and maximum temperatures of 14°C and 35°C) and approximately 35°C (glasshouse B, with minimum and maximum temperatures of 14°C and 50.0°C) in each glasshouse respectively. The same glasshouses were used in the following, 2008/09 season. In that season better temperature control reduced the fluctuations in temperature with minimum and maximum temperatures of 25°C and 35°C in glasshouse A and 30°C and 45°C in glasshouse B. Water stress and well watered irrigation treatments were imposed through to veraison by watering half of the vines in each glasshouse for different periods each day (ie. 5 and 15 minutes, respectively). In addition, half of the available bunches on each vine were dipped three times, a week apart, in 30 ppm GA post berry set (Figure 12B). The treatments were applied for 2 seasons (2007/08 and 2008/09).

Figure 12. Vines established in 0.19 m^3 of potting mix in 200L plastic drums in the glasshouses (A). Dipping of bunches in GA solution (B).

Pre-dawn and mid-day leaf water potentials were measured to ensure that the withholding of water affected vine water relations. As shown in Figure 13, vines in the (hotter) glasshouse B had more negative water potentials as compared to glasshouse A, indicating that heated vines were experiencing a more severe water deficit. Pre-dawn leaf water potential measurements suggested that vines subjected to heat stress and water stress had more negative water potentials and took longer to recover as compared to vines subjected to moderate heat and water stress.

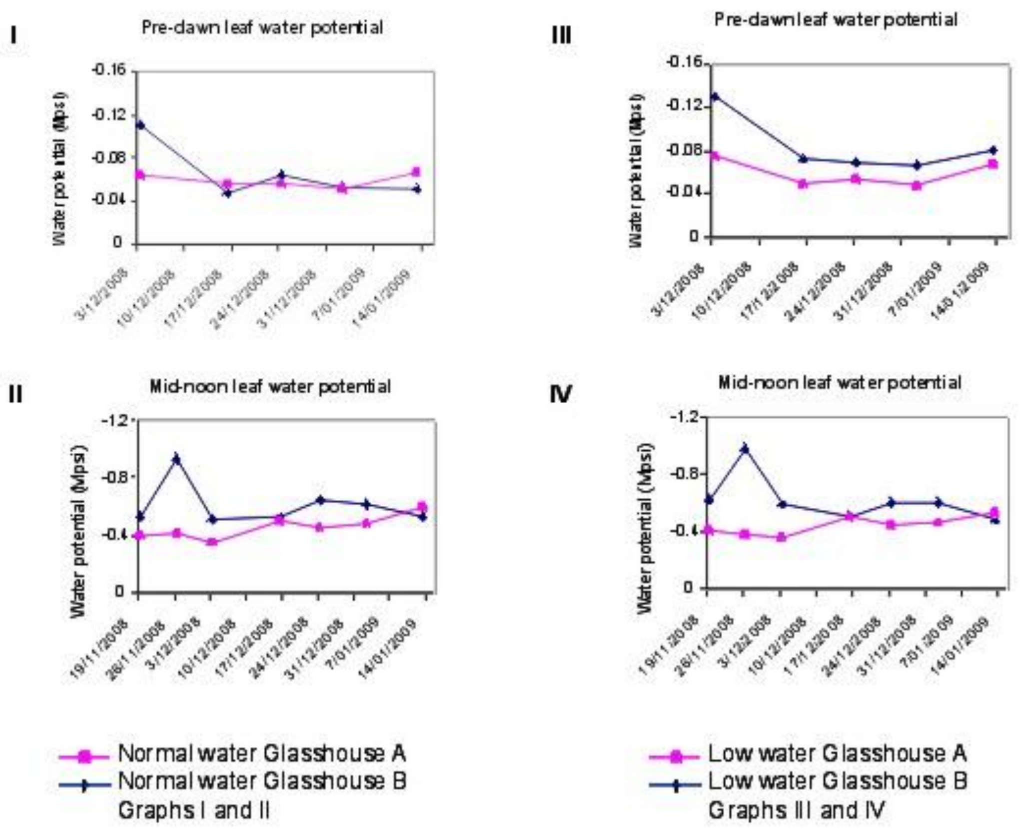


Figure 13. Pre-dawn and mid-day (noon) water potential readings of leaves from the glasshouse trial during season 2008-09. Values are means (n=4). Glasshouse A (control) had maximum daytime temperature of 35°C and night time 25°C and Glasshouse B (heated) had maximum daytime temperature of 45°C and night time 30°C. Normal water- vines were watered for 15 minutes every day; and Low water - vines were watered for 5 minutes every day until véraison.

The data suggest that during the period when Glasshouse B was heated, pre-dawn and mid-day leaf water potentials were more negative for vines in the heated glasshouse compared to the non-heated glasshouse A (Figure13). No significant difference was observed in mid-day (noon) leaf water potential of vines between the two glasshouse conditions and irrigation treatments (Figure 13).

During both seasons, we observed that berries on bunches that had been treated with GA and growing on vines in the heated glasshouse GA showed typical berry collapse symptoms and were more prone to heat damage (Figure 14). The data are summarised in Table 1. Results confirm our previous findings (Treeby et al., 2004) and the hypothesis that GA applications in combination with heat stress caused berry collapse which was further exacerbated if vines were under water stress (Figure 14). The incidence of berries with brown striations was five times greater when GA treated berries were under heat stress (Table 2). This and moisture loss data (Figure 6) suggest that GA was not the primary trigger of berry collapse but it is possible that application of GA increased fracture or splitting in the grape cuticle due to excessive berry expansion leading to water loss especially under high temperature conditions, hence leading to increased incidence of berry collapse.

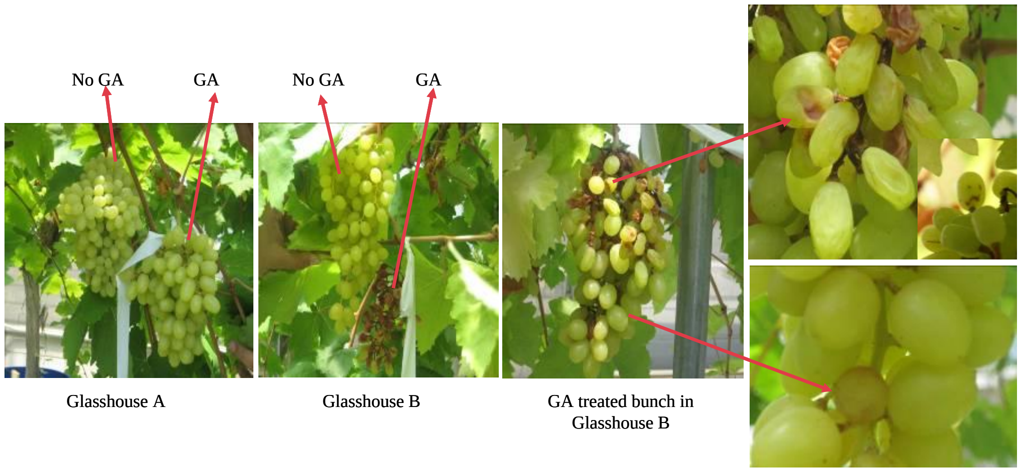


Figure 14. Bunches from glasshouse trials (2007-08 and 2008-09) showing berry collapse in Thompson Seedless in Glasshouse A (control, maximum daytime temperature of 35^{o}C and night time 25^{o}C) and Glasshouse B (heated, maximum daytime temperature of 45^{o}C and night time 30^{o}C).

Table 2. Glasshouse trial showing the effects of heat and water stress at the time of GA application on the number of berries with brown striations, the number of whole soft berries and number of sun damaged and collapsed berries. * indicates significant differences between the treatments (P<0.001); [†]total no. of bunches used for each treatment

Heat	Irrigation	GA	Total no. of bunches†	Total no. of berries	No. healthy berries	No. of berries with brown striations	No. whole soft berries	No. sun damaged berries	No. of collapsed berries	% berries with berry collapse
Control	Control	-	36	701	605	44	16	30	6	0.9
	″	+	26	744	623	73*	9	25	14*	1.9
	Dry	-	31	533	490	13	9	21	0	0.0
	″	+	22	405	359	7	6	23	10*	2.5*
Hot	Control	-	19	895	702	47	2	143	1	0.1
	″	+	16	860	121	127*	5	525*	82*	9.5*
	Dry	-	8	510	418	49	0	43	0	0.0
	″	+	7	311	51	19	1	216*	24*	7.7*

FDA staining and microscopy on berries collected from field and glasshouse showed identical symptoms i.e. loss of tissue, confirming that berry collapse symptoms can be reproduced in the glasshouse (Figure 15).

Figure 15. FDA-stained sections of berries collected from the field and from the glasshouse. White arrow heads indicates cell death.

Rootstock, cincturing, water stress and berry collapse

Field observations early in the project suggested some linkage between rootstock and berry collapse in Crimson Seedless. Crimson Seedless on rootstocks such as (110 Richter, 1103 Paulsen and Dog Ridge) showed higher incidences of berry collapse compared to vines on Teleki and 99 Richter (40-50% vs 20-25% of bunches affected/vine, respectively). The observation was potentially confounded by the fact that the rootstocks were not planted in a randomised pattern, but rather in discrete blocks.

A more suitable site at Sunnycliffs was used in the 2008-09 season to determine any interaction between rootstock, water stress and berry collapse. Two rootstocks, Schwarzmann and Ramsey, were selected to investigate whether the incidence of berry collapse was related to (i) rootstock and (ii) which rootstock was more prone to water stress and hence berry collapse in Thompson Seedless table grapes. A water stress treatment was also imposed because our glasshouse trials indicated that water stress, applied at the time of GA sizing sprays, exacerbated the incidence of berry collapse in GA-treated berries (Table 3). Additionally, it had been demonstrated that xylem hydraulic tensions can change in some varieties of wine grapes at around the time of véraison (Tilbrook and Tyerman, 2008), but whether such changes are in any way linked to onset of berry collapse in Thompson Seedless grape berries is not known.

Cincturing (or girdling) prevents the flow of carbohydrates and hormones from leaves to the roots and lower trunk (Antcliff, 1961; Menzel and Paxton, 1986; Williams, 1980) (Figure 16). Cincturing is normally carried out on mature, well established vines at the time of flowering/GA sizing spray application with the aim of enhancing availability of carbohydrates to the developing berries, rather than for translocation to the roots and lower trunk. Data collected over 2006-07 season from a small scale field trial showed that 24% of the bunches on cinctured vines had collapsed berries compared to 13% on non-cinctured (control) vines at harvest, suggesting that cincturing exacerbates berry collapse. Consequently, a cincturing treatment was also included in the Sunnycliffs trial. The aim was to gather corroborative data on the link between cincturing and berry collapse, and to determine whether berries on water stressed and cinctured vines are more prone to collapse.

Figure 16. Cincturing of mature vine trunk. The procedure involves the removal of a 3-6 mm wide ring of bark cutting through the phloem and cambium layers without damaging the xylem.

The water supply treatments imposed during the time when GA sizing sprays are applied were (i) control (2.5L/hour, twice week) (ii) double (5.0L/hr, twice per week) and (iii) stressed (irrigated once in 3 weeks 2.5L/hr). The irrigation was turned on for 10-12 hours. Wave guide rods/probes with Trase System1 (Soil Moisture Equipment Corp. CA) were used to monitor moisture levels at 30 and 60 cm depth. Leaf water potential measurements were carried out during the period when the water supply treatments were applied.

Although limited berry collapse occurred during this season due to mild weather conditions, data collected suggest that vines on Schwarzmann rootstock had significantly more sun damaged berries, berries with soft tip and brown striations as compared to Ramsey rootstock under water stress conditions (Table 3). These results combined with glasshouse trials further demonstrated that hot weather and water deficit conditions increased berry abnormalities which could potentially lead to berry collapse.

Table 3. Field trial data showing the effect of rootstock, water stress and cincturing at the time of GA application for sizing on the number of berries with brown striations, numbers of whole soft berries and sun damaged berries and % berries with soft tip and brown striations. Twenty bunches per treatment were assessed at the time of harvest. Superscripted letters indicate significant differences between the means within columns ($P<0.001$).

Rootstock	Irrigation	Cincture	Total no. of berries	No. healthy berries	No. of berries soft tips	No. of berries with brown striations	No. whole soft berries	No. sun damaged berries	% berries with soft tip	% berries with brown striations
Ramsey	Control	-	1478	1301	29^{a}	111^{a}	20^{c}	7^{a}	2	8
	″	+	1436	943	97^{b}	212^{b}	11^{b}	161^{d}	7	15
	Dry	-	1886	1464	85^{b}	250^{b}	31^{d}	39^{ab}	5	14
	″	+	1674	1070	237^{e}	258^{b}	8^{a}	104^{c}	14	15
Schwarzmann	Control	-	2614	1936	125^{c}	432^{c}	45^{e}	61^{b}	5	17
	″	+	1997	757	175^{d}	775d	26^{d}	195^{e}	9	39
	Dry	-	2065	1436	151^{c}	409^{c}	44^{e}	20^{a}	17	20
	″	+	2242	796	389^{f}	397^{c}	56^{f}	208^{e}	7	18

Berries from vines on Schwarzmann rootstock generally displayed high incidences of symptoms such as soft tip (at the distal end) and brown striations as compared to berries from vines on Ramsey rootstock (Table 3). Leaves on Schwarzmann rootstock had slightly more negative water potentials (-0.83 ± 0.09 MPa) compared to leaves from Ramsey rootstock (-0.72 ± 0.1 MPa) (Figure 17). The differences between the rootstocks may be associated with differences in root architecture as Schwarzmann generally has a shallow root system compared to Ramsey. Ramsey rootstock, with a deeper root system is potentially able to access water from deeper positions in the soil profile, minimising water stress between the irrigation events.

Cinctured vines on both rootstocks have significantly ($p<0.001$) higher incidences of berries with sun damage, soft tip and brown striations as compared to uncinctured vines (Table 3), suggesting a link between these symptoms and rapid growth of the berry resulting from cincturing of trunks. It is also likely that cincturing has an effect on water relations within the vine as is evident from the leaf water potential measurements (Figure 17). Cinctured vines had slightly more negative leaf water potentials, which in turn can increase berry collapse symptoms. However, this needs to be substantiated in further studies.

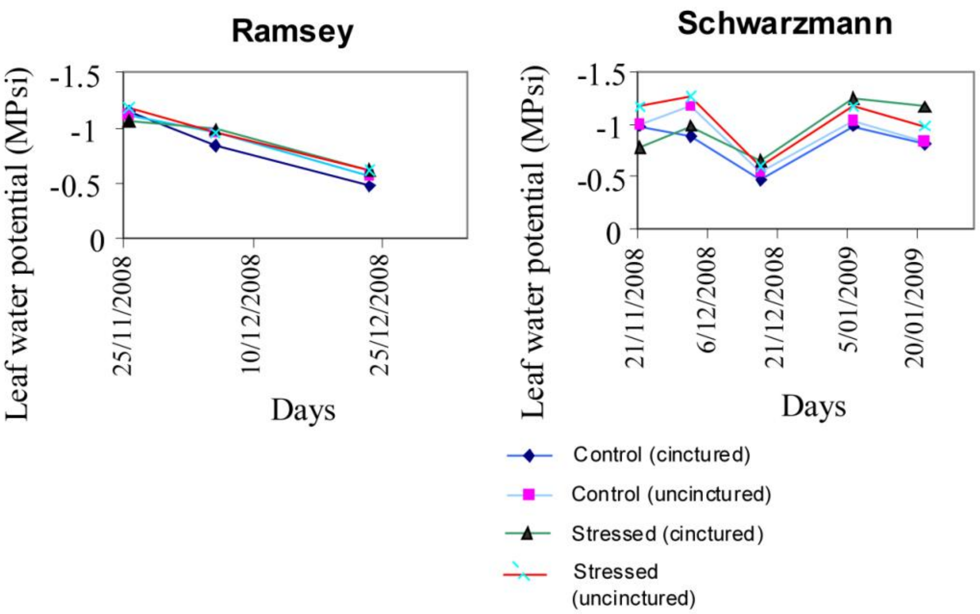


Figure 17. Leaf water potential of Thompson Seedless grafted on two rootstocks (Ramsey and Schwarzmann), which were either cinctured or not cinctured.

3. Potential management strategies to alleviate and/or minimise incidence of berry collapse

Based on our observations and trials carried out during the course of this project, several trials were set up on table grape properties at Sunnycliffs and Birdwoodton with various treatments to further investigate potential to reduce the incidence of berry collapse.

Polyamino acid as a sizing hormone

GA is widely used by table grape growers as a sizing spray. Our glasshouse trials (2007-08 & 2008-09) suggested that GA spray in combination with hot conditions at the time of spray application is possibly involved in the incidence of berry collapse. The market demands larger berry sizes than can be achieved without the use of plant growth regulators, and so we set up a trial with a natural polyamino acid which has been used previously in several horticultural crops to increase fruit set and fruit size. Polyamines have been implicated in various plant growth, physiological and developmental processes (Evans and Malmberg, 1989; Galston and Kaur-Sawhney, 1990). These include stimulation of cell division, response to environmental stresses, regulation of rhizogenesis, embryogenesis, senescence, floral development, and fruit ripening (Kakkar et al., 2000; Walters 2000). Several scientific investigations have suggested that GA activates the production of polyamino acid (Galston and Kaur-Sawhney, 1990). A trial was set up at the Birdwoodton property to investigate whether polyamino acid can be used as a sizing spray. Ten bunches per vine were dipped in 1, 5 or 10 $mgml^{-1}$ putrescine (PA), or 15 ppm GA + 5 $mgml^{-1}$ PA. Thirty ppm GA was used as control. Growth of putrescine and GA treated berries was followed every 15 days until harvest.

Putrescine did not enhance berry growth (Table 4), suggesting that either putrescine does not induce growth in table grape berries or that higher levels of putrescine are required to induce growth, similar to GAs. Analysis of the numbers of berries per bunch with brown striations at harvest indicated that the application of putrescine (10 $mgml^{-1}$) resulted in *ca.* 2.4 berries with brown striations per bunch compared to untreated control (*ca* 30.9 berries) and GA treated (*ca.* 23.3). The reduction in berries with brown striations showed a strong linear response to increasing putrescine concentration (i.e. 30.9, 26.5, 13.8 and 9.1 brown striations for the control, 1 $mgml^{-1}$, 5 $mgml^{-1}$ and 10 $mgml^{-1}$ treatments, respectively. A detailed study is required to investigate whether putrescine (PA) can be developed as sprays to prevent occurrence of brown striations and other symptoms under extreme weather conditions at the time GA sizing sprays.

Table 4: Effect of putrescine on berry characteristics at harvest. Ten bunches were dipped into each treatment solution at flowering time followed by three further treatments, each one week apart. The values are means from 10 bunches collected at harvest.

Berry characteristics	Treatment					
	Control	GA (30ppm)	GA (15ppm) + PA (1mg/ml)	PA (1mg/ml)	PA (5mg/ml)	PA(10mg/ml)
Berry diameter (mm)	14.4	18.4	16.5	13.4	14.7	13.8
Berry wt (g)	2.3	4.9	3.5	1.9	1.9	2.4
°Brix	17.7	15.2	15.3	18.0	18.4	20.7
Waterberries ($P<0.001$)	6.6 [a]	23.5[b]	22.4 [b]	4.6 [a]	14.3 [c]	7.5 [a]
Soft tip (at the distal end) ($P<0.001$)	6.4 [a]	33.0 [a]	19.2 [b]	4.0 [a]	5.4 [a]	0.9 [c]
Brown striations ($P<0.002$)	30.9 [a]	23.2 [a]	12.2 [b]	26.5 [a]	13.8 [b]	9.1 [b]
Berry collapse ($P<0.001$)	0.12[a]	2.00[b]	2.16[b]	0.00[a]	0.00[a]	0.04[a]

Effect of Surround® sprays on berry development under water stress conditions

Surround® (Engelhard) is widely used in apples, apricots, tomatoes, citrus, plums *etc* to reduce plant stress associated with water deficits or excessive heat. Recently, Surround® has been used in wine grapes to reduce water stress related symptoms (Cooley et al., 2008). Surround® treated vines were cooler under high temperature conditions and showed increased vigour, berry size and colour. It has been suggested that Surround® reflects infra red and ultra-violet rays and hence reduces leaf temperature and heat stress. Using the irrigation trial at Sunnycliffs, some canopies on the control, double irrigated and water stressed vines were sprayed with 50 kg Surround®/1000L at the time of flowering followed by two subsequent pre véraison sprays applied at the rate of 5 kg Surround®/1000L (Figure 18) to further investigate the link between heat, water stress and berry collapse. Leaf water potential and temperature (measured with an infra red thermometer) were determined during the period when GA was being applied to size berries

Figure 18. Vine leaves sprayed with Surround®.

Leaf water potential measurements at noon suggested that vines subjected to the stressed irrigation treatment (irrigated once in 3 weeks 2.5L/hr) were under considerable water stress as indicated by higher negative values as compared to leaves from control vines (Figure 19).

Leaves sprayed with Surround® on average had 2-3°C lower surface temperature as compared to control leaves (Table 5).

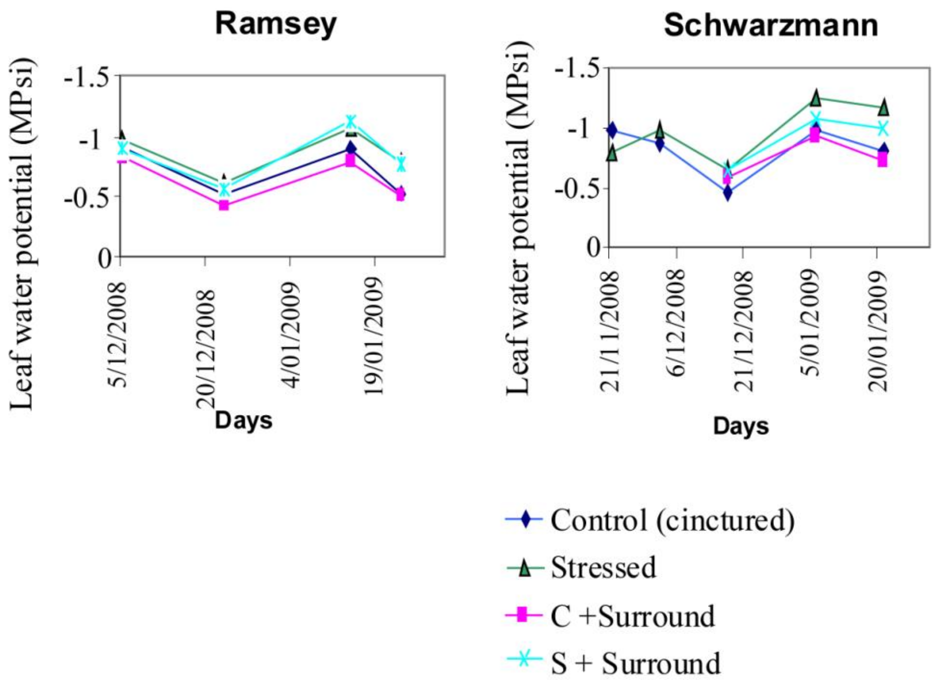


Figure 19. Leaf water potential at noon under control (2.5L/hour, twice in a week) and stressed (irrigated once in 3 weeks 2.5L/hr) irrigation treatments.

Table 5: Effect of Surround® on leaf surface temperature. The values are means (n= 5). * indicates that the means are significantly different between the treatments ($p<0.05$). (Note: due to differences in the time of the day at which measurements were taken, comparisons between the rootstocks are not valid).

Irrigation	Surround®	Rootstock	
		Ramsey	Schwarzmann
Control	-	35.0°C	27.4°C
Control	+	*32.8°C	*24.9°C
Stressed	-	36.7°C	29.5°C
Stressed	+	*35.0°C	*27.1°C

Véraison (berry softening) is used as a marker to determine the progress of berry ripening (Coombe, 1992). The berry size and sugar accumulation (°Brix) data throughout berry development (Figure 20) indicated that berries on vines, for both rootstocks, treated with Surround® matured early. The difference in maturity between grapes on vines treated with Surround® and grapes on control vines was 1.5-3.0 °Brix, which amounts to 1-2 weeks in terms of the time taken to accumulate soluble solids at 2 °Brix per week.

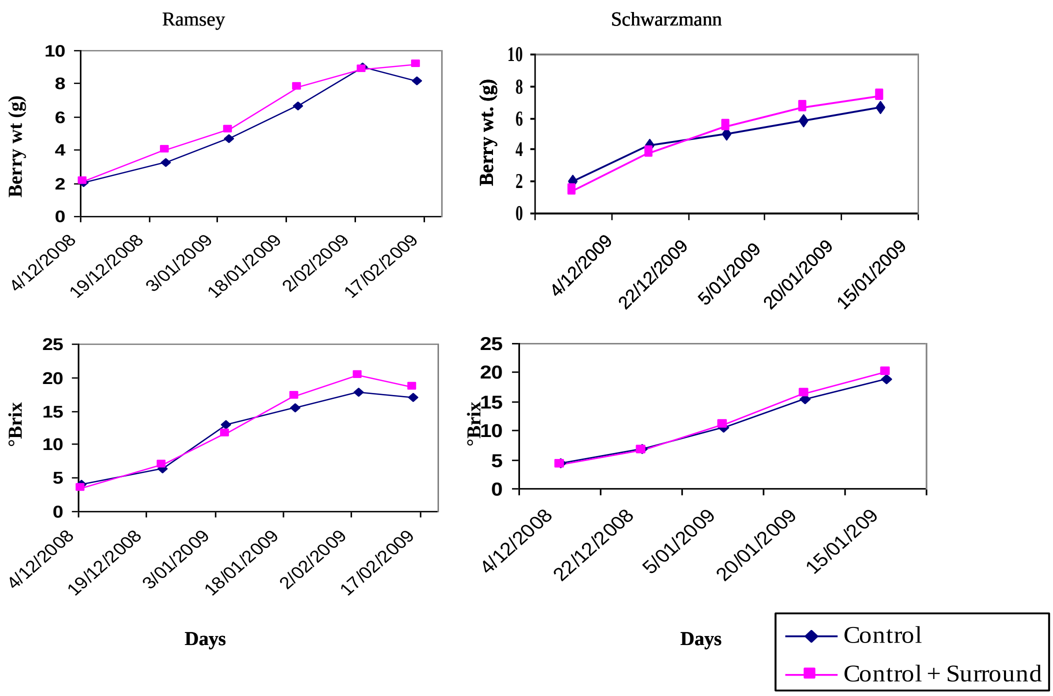


Figure 20. Impact of irrigation treatment and Surround® spray on berry fresh weight (g) and sugar levels, i.e. °Brix during berry development.

At harvest bunches on Ramsey rootstock produced bigger berries and had significantly ($P<0.004$) more fresh weight (ca 39%) than that of Schwarzmann rootstock (Table 6). This is not very surprising considering that Ramsey rootstock generally has deeper root systems and that vines are more vigorous compared to Schwarzmann rootstock. On both rootstocks, Surround® significantly reduced the number of berries with brown striations in the stressed treatments. As the development of berries with brown striations is a good indicator of potential berry collapse, the results indicates that application of Surround® may be used to reduce berry collapse associated with water and canopy stress. This requires verification in seasons when the incidence of berry collapse is high or in controlled glasshouse studies.

Table 6: Field trials showing the effect of water stress and Surround® spray at the time of GA sizing sprays on berry characteristics at harvest time. Letters indicate significant differences between the treatments in the column (0.05<P<0.001). The values are means of 5 bunches per vine per treatment.

Rootstock	Irrigation	Sun damage	Waterberries	No. of berries with brown striations	Berry diameter (mm)	Berry fresh weight (g)	°Brix
Ramsey	Control	8.70 [a]	0.25 [a]	4.25 [a]	21.46 [a]	8.16 [a]	17.08 [a]
	Control + Surround®	8.53 [a]	0.42 [a]	5.26 [a]	19.96 [b]	9.14 [b]	18.52 [b]
	Stressed	12.6 [b]	0.65 [b]	12.50 [b]	19.89 [b]	8.39 [a]	17.71 [a]
	Stressed + Surround®	9.30 [a]	0.55 [a]	7.95 [a]	20.52 [c]	8.61 [a]	18.20 [b]
Schwarzmann	Control	9.75 [a]	0.70 [b]	23.10 [c]	18.26 [d]	5.87 [c]	19.58 [c]
	Control + Surround®	7.60 [c]	0.25 [a]	22.45 [c]	19.97 [b]	7.33 [d]	18.84 [b]
	Stressed	5.76 [d]	1.95 [c]	33.62 [d]	19.28 [b]	6.37 [ce]	15.68 [d]
	Stressed + Surround®	6.65 [d]	5.30 [d]	23.20 [c]	19.38 [b]	7.26 [d]	18.54 [b]

Salicylic acid (SA) sprays to reduce incidence of berry collapse

Plant hormones such as jasmonic acid (JA) and salicylic acid (SA) are involved in plant defense mechanisms against pathogen attack and hence cell death (Farmer et al., 1992). In the 2006-07 and 2007-08 season, field trials were set up at Euston to investigate the impact of JA, SA and BION (an activator of plant defense mechanisms marketed by Syngenta, Australia) on the incidence of Thompson Seedless berry collapse. Data collected over two seasons, 2006-07 and 2007-08, showed that SA reduced the berry collapse symptoms by 25%, indicating that SA pre-treatment could enhance SA-related defence gene activation and potentially protect vines/berries from heat stress. JA did not have any impact on berry collapse incidence (Table 7). We do not know the mechanism of SA mediated reduction of berry collapse symptoms. However it is tempting to hypothesize that SA application reduces berry collapse symptoms by inducing gene expression of heat shock proteins (HSPs). It has been shown previously that SA application reduced chilling injury in tomato (Ding et al., 2001) and heat stress in mammals (Jurivich et al., 1992) by increasing expression of genes encoding HSPs. The role of HSPs in berry collapse can be investigated by conducting either western blotting by using anti-antibodies against HSPs or reverse transcriptase-polymerase chain reaction (RT-PCR) conducted by using HSP specific primers on RNAs isolated from SA treated berries. The other possible role of SA could be induction of the non-classical pathway of respiration i.e. alternative oxidase pathway (Chivasa and Carr, 1998). The mitochondrial alternative oxidase (AOX) catalyses the O_2-independent oxidation of ubiquinol, limiting the mitochondrial generation of ROS (Overmyer, 2003) and hence reduced cell death and berry collapse.

BION treated bunches showed higher incidences of berry collapse compared to control untreated bunches suggesting that BION might be triggering the cell death or causing berry collapse via some unknown mechanism. In the future, BION can be used as a tool to determine the biochemical and molecular reasons leading to berry collapse.

Table 7: Effect of SA, JA and BION on incidence of berry collapse.

Treatment	Healthy berries/bunch	Collapsed berries/bunch	Total no. of berries/bunch	Bunches with collapsed berries
Control	119.7 ± 27.5	4.6 ± 1.1	150.7 ± 34.6	13/19
BION (0.4mM)	110.2 ± 25.3	11.1 ± 2.5	144.5 ± 33.2	15/19
Jasmonic acid (1.0mM)	135.7 ± 31.1	4.5 ± 1.0	152.4 ± 35.0	14/19
Salicylic acid (0.1mM)	112.7 ± 23.5	2.9 ± 0.6	133.2 ± 27.8	11/20

Conclusions and recommendations

The microscopic symptoms suggest that berry collapse is associated with loss of tissue structure. It seems that heat stress may be triggering cell death. The glasshouse trials (2007-08 and 2008-09) suggested that berry collapse symptoms can be reproduced in the glasshouse as is confirmed by microscopic and visual symptoms and confirms a link between GA and heat stress at the time of GA sizing spray applications. Furthermore, under normal weather conditions with temperatures up to 35°C, vines can tolerate water stress without any further increase in the incidence of berry collapse. However, the results suggest that management practices need to be changed under high temperature (i.e. up to 40-45°C) conditions at the time GA application in order to reduce berry collapse symptoms.

1. Industry recommendations

The scientific studies and observation of grower practices, particularly in season 2008 have identified a range of management practices to assist growers to minimise the incidence of berry collapse. These include:-

- Careful attention to water management to minimise stress during shoot and berry development. This should include early spring irrigations to fill the soil profile and provide buffering during hot weather conditions and scheduling to maintain soil moisture at or near field capacity during berry development to offset vine transpiration and soil water evaporation, estimated to be a total of 36-54 L per day per vine. Eliminating water stress appears to be a critical factor to decrease canopy and fruit temperatures by transpirational cooling.
- Complete ground surface irrigated, eg. with undervine sprinklers.
- Maintenance of an established cover crop with potential beneficial effects on vineyard floor micro-environment. It is also likely that higher volumes of water, applied to maintain the growth of the cover crop may provide extra moisture buffering capacity to the vine under high stress conditions (ie. high temperatures and low humidity).
- Adoption of vineyard cooling techniques such as ‘misters’ to reduce temperatures and increase the humidity within the vineyard.
- Adoption of large, wide V trellises which would contribute to reduced water loss through soil water evaporation, a result of increased shading and less reflection of heat from the soil surface, and with this the likelihood of lower canopy temperatures.
- The use of moderate vigour, drought sensitive rootstocks such as Schwarzmann for the production of Thompson Seedless should be avoided. Thompson Seedless vines grafted on Schwarzmann had higher levels of berry collapse than vines grafted on Ramsey, particularly under conditions of water stress.
- Cincturing, which has been shown to increase berry collapse and related symptoms, should not be used if hot conditions are predicted during berry development.

- Growers should consider avoiding the use of GA sizing sprays during predicted heat wave conditions.
- Positioning of plastic vine covers above the vine canopy (i.e. not in contact with the canopy) to ensure adequate air circulation and ventilation.

2. Scientific recommendations

Opportunities identified for further research during the project include:-

- Further studies to enhance understanding of grape berry responses to applications of GA and interactions with abiotic stresses (temperature and water) be undertaken and include impacts on berry anatomy and cell wall composition.
- Confirm identification of early and effective visual indicator(s) of berry collapse, for example, brown striations
- Development of management practices to reduce incidence of brown striations, water berry and berries with soft tip at the distal end. A detailed study needs to be carried out to elucidate whether these symptoms are related to, and cause of, berry collapse.
- Development of effective treatments to minimise berry collapse, eg. Surround® and salicylic acid (SA), based on glasshouse trials where extreme weather conditions (i.e. deficit irrigation, heat stress) can be applied and verified
- Develop an understanding of the mechanism of the grape berry heat shock response to provide a model to investigate 'cross talk' between programmed cell death and plant hormones such as GA and SA signal transduction pathways
- Berry collapse studies be extended to other key varieties, e.g. Crimson Seedless, where related symptoms have been noted

3. Evaluation report

The project, "Causes and prevention of table grape berry collapse", aimed to identify factors contributing to berry collapse of Thompson Seedless and develop management practices to moderate the effect of these factors in order to reduce symptoms of berry collapse. Both of these objectives have been met. The project has shown that berry collapse in Thompson Seedless is associated with the interaction of high temperatures during the early stages of berry development and the application of GA to increase berry size. The problem is exacerbated by water stress and cincturing. Both the scientific studies and anecdotal evidence, collected when berry collapse was severe in season 2008, have provided a basis to develop 'best grower practices' to minimise the problem. These practices largely involve (1) improved irrigation management to avoid water stress, maintain leaf function and to promote transpirational cooling of the canopy and fruit, (2) application of techniques to improve the environment within the vineyard; for example, reduce temperatures and increase the humidity, ie. full ground cover irrigation, maintenance of a cover crop and misters, and (3) avoidance of cincturing and application of Surround® to the canopy to reduce leaf temperature.

Two major difficulties were faced in the conduct of the project. Firstly, the problem of berry collapse in Thompson Seedless only occurred in one season, i.e. 2008. Hence studies to identify causes and develop management practices to minimise berry collapse in Thompson Seedless were very difficult to implement in most seasons. This problem was overcome by the use of very large potted vines grown under glasshouse conditions in the 2008 and 2009 season. This created major issues with glasshouse space, operation of the glasshouse facilities beyond design specifications including the use of supplemental heating at critical time points and low bunch numbers due to the low fruitfulness of Sultana in the glasshouse environment. Furthermore, opportunities were taken in season 2008 to re-focus the project when berry collapse was a very significant problem in Thompson Seedless across the district and collect information on grower practices that reduced the problem and utilise fruit samples to undertake scientific studies to describe both the development and symptoms of berry collapse. Secondly, it is unfortunate that the project did not attract a successful PhD candidate. The PhD position was advertised three times in capital city newspapers and on websites. Universities were also contacted directly. However, despite significant effort to advertise availability of the position, the studentship was unable to be successfully filled. Hence a science graduate, with well developed laboratory skills and the capacity, under direction, to address the milestones relating to developing an understanding of the causes of berry collapse in Thompson seedless, which would have been part of the PhD study, was appointed as a technical officer to the project. This appointment ensured all project milestones were met. It also provided some flexibility to re-focus the project and address the more practical aspects which identified best management practices to minimise the problem in the high incidence season (2007/08) and undertake detailed field studies when the project was extended in season 2008/09.

Technology transfer

Technology transfer has involved development of educational material for distribution to growers, grower presentations, publication of results in industry journals, field demonstrations, presentations at steering committee meetings and information sessions with industry stakeholders.

1. Educational material

Educational material on Thompson Seedless berry collapse was distributed to all growers in the form of 'flyer' included with the Vine magazine in September 2008. It contained key information on the known causes and 'best grower practices' to minimise the problem of berry collapse in Thompson Seedless. The distribution of the 'flyer' was timed so that growers had the most up-to-date information prior to the sensitive period when GA application would be undertaken for berry thinning (i.e. during flowering) and berry sizing (i.e. post-flowering). The 'flyer' is included below. In addition, a grower information sheet has been developed to summarise management strategies to minimise the incidence of berry collapse. It is included as an appendix to this report (appendix 1).

Thompson Seedless Berry Collapse: September 2008 update

CSIRO Plant Industry

In 2007-08, widespread berry collapse in Thompson Seedless had a devastating impact on the table grape industry with massive losses in the Robinvale, Sunraysia and Riverland districts. Variations in the incidence of the problem has provided the researchers investigating the problem from CSIRO Plant Industry and the Department of Primary Industries, Victoria, with an opportunity to identify 'best practices' that may enable growers to minimise the incidence of berry collapse in future seasons.

The problem

Thompson Seedless Berry Collapse has been linked to high temperatures (and low humidity) during early berry development, particularly around the time of gibberellin (GA) application to increase berry size. The problem has been shown to be exacerbated by both water stress and cincturing. It leads to development of striated necrotic (dead) tissue on the berry surface followed by loss of internal cell structure and apparent loss of moisture from the berry.

Key features of vineyard management on properties where berry collapse was minimal included (Figure 2):

- Complete ground surface irrigated, eg. with undervine sprinklers.
- Careful attention to water management to minimise stress during shoot and berry development. This included significant irrigation in early spring to fill the soil profile and provide a buffer against stress during the early stages of berry development which coincides with the period when vines reach full canopy size and have highest transpiration rates. Application of GA promotes shoot growth and berry development and hence, would further contribute to increased vine transpiration. Based on an estimated maximum vine canopy size of 30 m^2 and published information on vine transpiration for sultanas (ie. 1.0-1.5 L per m^2 of canopy per day) it is estimated that water requirements to eliminate stress during berry development would be about 36-54 L per day per vine, allowing 20% for soil evaporation losses. Eliminating water stress appears to be a critical factor to decrease canopy and fruit temperatures by transpirational cooling, potentially in the order of 2-8 $^{\circ}C$.

(B)

Figure 1

A. Bunch showing typical berry collapse at harvest.

B. Berry showing striations associated with necrotic tissue which occurs just after veraison and is the first sign of a major problem.

- Maintenance of an established cover crop with potential beneficial effects on vineyard floor micro-environment. It is also likely that higher volumes of water, applied to maintain the growth of the cover crop may have provided extra moisture buffering capacity to the vine under high stress conditions (ie. high temperatures and low humidity).
- Adoption of large, wide V trellises which would contribute to reduced water loss through soil water evaporation, a result of increased shading and less reflection of heat from the soil surface, and with this the likelihood of lower canopy temperatures.
- In some cases, the use of misters situated above the canopy to reduce canopy temperatures and increase humidity under high temperature conditions.
- Positioning of plastic vine covers above the vine canopy (ie. not in contact with the canopy) to ensure adequate air circulation and ventilation.

In conclusion, a number of vineyard management practices have been identified which should enable Thompson Seedless growers to minimise losses from berry collapse if high temperatures occur during and subsequent to the period when GA is applied to enhance berry size. In particular, attention should be given to irrigation management to minimise water stress during the critical berry development period. Growers should also consider avoiding cinturing if high temperatures are forecast.

CSIRO Plant Industry

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Figure 2

Vineyard with minimal problems of berry collapse based on a wide V-trellis showing maintenance of cover crop (left) and structure supporting the plastic vine covers above the canopy (right).

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2. Grower presentations

Singh, D.P., Treeby, M., Pitt, K. and **Clingeleffer, P.** (16-17 September, 2009). Berry collapse in Thompson Seedless grape. Presentation at the 12th Australian Table Grape Technical Conference held in Mildura

Singh, D.P. (24th October, 2009) Causes and prevention of berry collapse at Sunnycliffs. STGGA Field Walk.

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4. Publication

Singh, D.P., Treeby, M., Pitt, K. and Clingeleffer' P. (2009). Thompson Seedless berry collapse. 12th Australian Table Grape Technical Conference Proceedings. 16-17 September, 2009.

5. Posters

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Nguyen, T., Pitt, K., Krstic, M., and Treeby, M. Berry collapse development. ADFA Field day, Mildura, 2007.

Pitt, K., Nguyen, T., T a grower information sheet has been developed to cover management strategies to minimise the incidence of berry collapse. It is included as an appendix to this report. reeby, M. and Krstic, M. Berry collapse - does it strike twice? ADFA Field day, Mildura, 2007.

Nguyen, T., Pitt, K., Krstic, M., and Treeby, M. Table grape berry collapse. ADFA Field day, Mildura, 2006.

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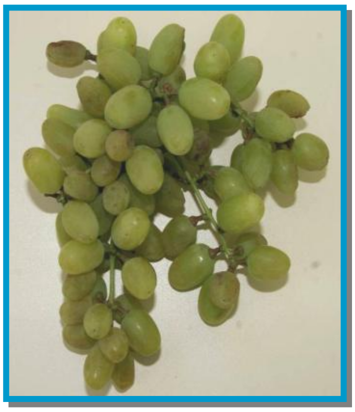
Appendix

1. Grower information sheet

A grower information sheet has been developed which summarises management strategies to minimise the incidence of berry collapse. It includes a brief overview of the causes, factors to be considered during vineyard development and key management practices to be considered during crop development (see attached below).

Thomson Seedless Berry Collapse :- Grower Best Practice

CSIRO Plant Industry



Berry Collapse in Thompson Seedless is associated with cell death and loss of tissue structure within the berry. It has been linked to high temperatures during early berry development and the use of gibberellin (GA) to increase berry size. The problem is compounded by water stress and cincturing.

Grower practices to minimise the incidence of berry collapse

Vineyard development

- Use high vigour, deep rooted rootstocks such as Ramsey. Avoid moderate vigour, drought sensitive rootstocks such as Schwarzmann
- Install large, wide V-trellises to increase shade and reduce water loss through soil water evaporation
- Develop systems to improve the micro-climate in the vineyard (ie. reduce temperature and increase humidity)
    - systems that irrigate the complete ground surface (e.g. under-vine sprinklers)
    - develop and maintain cover crops, to minimise direct solar radiation to soil surface, but taking care to avoid potential negative impacts on soil moisture status
    - install 'misters' for use during heat waves

Vineyard management

- Careful attention to water management to minimise stress during shoot and berry development
    - early spring irrigations to fill the soil profile and provide buffering during hot weather
    - schedule to maintain soil moisture at or near field capacity during berry development to offset vine transpiration and soil water evaporation, estimated to be a total of 35-55 L per day per vine
- Avoid GA sizing sprays during predicted heat wave conditions (i.e. > 35^{o}C)
- Do not cincture if heat wave conditions are predicted
- Consider the use of particle film products such as Surround® to reduce canopy temperature during the early stages of berry development
- Position plastic vine covers above the vine canopy to ensure adequate air circulation and ventilation

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