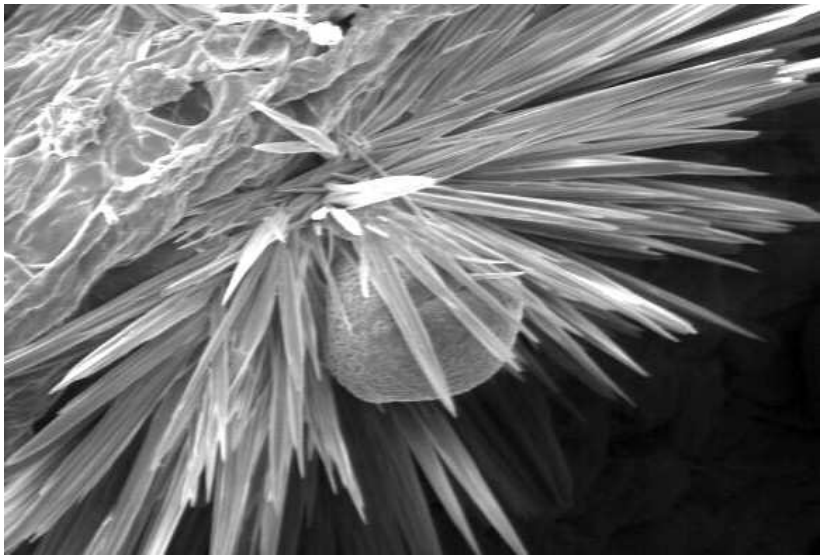
THE UNIVERSITY OF ADELAIDE
AUSTRALIA

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Grape and Wine Research and Development Corporation

Flowering and fruitset of the grapevine



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: UA 04/02

Project Supervisor: Associate Professor Peter Dry
Principal Investigator: Dr Cassandra Collins

Research Organisation: **University of Adelaide**

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GWRDC Final Report

Project No. UA 04/02

Project Supervisor: Associate Professor Peter Dry
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University of Adelaide

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Abstract

This was a pilot project that investigated many aspects of the flowering and fruitset process. For this reason, it has been wide-ranging in its approach, from phenological and climatic studies in the field to studies of gene expression during flowering. The effect of site and season on reproductive performance was studied over four consecutive growing seasons on ten winegrape varieties across a range of climatic regions—the differences between varieties and between sites were greater than the differences between seasons for most variety and site combinations. Flowering time may be accurately predicted by the use of a degree-day model or by the use of an indicator variety such as Ganzin Glory. Fruitset and yield per vine increased in response to both shoot topping and CCC foliar application. Cultural practices, e.g. Mo or CCC foliar sprays, can exert an effect on fruitset via an influence on pollen tube growth, ovule fertilisation or ovule cell morphology. This project has provided an insight into the role of polyamines and ethylene in the flowering and fruitset process.

Executive Summary

This project was originally conceived in consultation with the GWDRC as a pilot project that would investigate many aspects of the flowering and fruitset process. For this reason, this project has been wide-ranging in its approach, from phenological and climatic studies in the field, to anatomical studies of pollination and fertilisation, and studies of gene expression during flowering.

In order to determine the climatic conditions that are beneficial or detrimental to flower development and fruitset, and their frequency, an experiment was set up to study reproductive performance in four consecutive growing seasons (commencing with 2004/05) of ten varieties that are considered, anecdotally, to have poor fruitset, i.e. Cabernet Sauvignon, Chardonnay, Merlot, Nebbiolo, Pinot Noir, Sangiovese, Sauvignon Blanc, Shiraz, Tempranillo and Zinfandel. Vineyard sites were selected across a range of climatic regions from cool (e.g. Adelaide Hills) to warm (McLaren Vale). Reproductive performance was measured in terms of flower number per inflorescence, berry number per bunch, coulure index (CI) and millerandage index (MI)—the latter two are novel indices which were developed during the course of this project and, for the first time, have proved valuable for the quantification of reproductive performance. The differences between varieties and between sites were greater than the differences between seasons for most variety and site combinations. When averaged over all sites there was little effect of season on fruitset. The varieties have been grouped on the basis of their reproductive performance: Group A (Sauvignon Blanc, Pinot Noir and Tempranillo) with low flower number, moderate set, low to moderate berry number (low to moderate CI and MI); Group B (Chardonnay, Shiraz) with low to moderate flower number, moderate set, low to moderate berry number (moderate CI and low to high MI); Group C (Cabernet Sauvignon, Merlot) with moderate flower number, low set, low to moderate berry number (low to high CI and MI); Group D (Nebbiolo) with moderate flower number, moderate set, moderate berry number (moderate CI and MI); Group E (Sangiovese, Zinfandel) with high flower number, high set, high berry number (low to moderate CI and moderate to high MI).

This project aimed to determine a means by which flowering time may be accurately predicted. We have shown that this can be achieved either by the use of a degree-day model or by the use of an indicator variety such as Ganzin Glory. From a grower's standpoint, it is the latter which is likely to be the most cost-effective method. Because the indicator vines will flower up to 2 weeks earlier than Chardonnnay, this is ideal for the optimal timing of any cultural practice that must be applied one to two weeks before flowering, e.g. the CCC foliar spray to improve fruitset. The effectiveness of cultural practices, namely shoot topping and CCC application, on fruitset and other yield

components was investigated on Cabernet Sauvignon, Chardonnay and Tempranillo in two regions. Treatments were applied before and during the flowering period. Fruitset and yield per vine increased in response to treatment, especially when shoot topping was applied from the start of flowering. All varieties at both locations responded to shoot topping to some degree even though none of the seasons could be classified as problematic for fruitset. It is likely that fruitset and thus yield can be improved by cultural practices in all seasons; but the magnitude of the response may be greatest in those seasons when fruitset is limited by climatic conditions or other factors. We recommend that shoot topping be applied every season on these varieties because it is likely to be cost-beneficial. In practice, a CCC foliar spray applied 7 to 10 days prior to flowering (E-L 17 to 18) would be good insurance for Cabernet Sauvignon grown in the Adelaide Hills and in other regions where poor fruitset is a common occurrence.

We have shown that cultural practices such as Mo foliar spray (in the case of Merlot only), CCC foliar spray or shoot topping can exert an effect on fruitset via an influence on pollen tube growth, ovule fertilisation or ovule cell morphology. For example, in the case of Mo on Merlot, pollen tube growth was significantly enhanced by Mo-treatment on Mo-deficient vines. Also, significantly more pollen tubes penetrated the ovules from Mo-treated vines and a higher proportion of ovaries had at least one penetrated ovule. Structural observations revealed that a significantly higher proportion of ovules from Mo-deficient vines were defective—the absence of an embryo sac in those ovules is probably the cause of pollen tube growth inhibition and subsequent poor fruitset.

Previous research showed that yield of Mo-deficient Merlot improved when sodium molybdate was applied as a foliar spray. In this project, experiments were conducted on own-rooted Merlot (clone D3V14) vines in commercial vineyards in the Adelaide Hills (Hills) and at McLaren Vale. The aims were to: a) elucidate the mechanism by which molybdenum affects yield of Merlot; b) to monitor the effects of Mo-treatment on the balance between vine reproductive and vegetative growth; c) to monitor the residual effects of Mo-treatment on growth and yield of Merlot and; d) to determine whether high concentrations of molybdenum are detrimental to yield. Application of Mo foliar spray will only be of benefit to fruitset and yield when vines are deficient in the first instance. Therefore, before application, tissue analysis should be conducted in order to verify this deficiency. Furthermore, we recommend that shoot tips should be used for this purpose in preference to the standard petioles. The critical level of molybdenum for optimal fruitset on own-rooted Merlot is suggested to be 0. 1 mg/kg in the shoot tips at E-L stage 25 (80% flowering).

This project has provided an insight into the role of polyamines and ethylene in the flowering and fruitset process. Real-Time PCR and HPLC analysis showed an interaction between the ethylene and polyamine biosynthesis pathways in the floral developmental process. This increase in the

expression of a gene in the ethylene biosynthetic pathway is a potential indicator of the mechanism which is responsible for the abscission of flowers in Mo-deficient Merlot vines. Also, the corresponding decrease in ACC synthase expression in the Mo sprayed vines is strong evidence that polyamine and ethylene levels are implicated in the restoration of normal berry development. Correspondingly, in the Mo-treated vines, the flowers sampled showed consistently higher levels of many of the polyamine biosynthesis genes when compared with the Mo-deficient control vines. The significant induction of ACC synthase in the Mo-deficient flowers compared with the molybdenum-sprayed flowers indicates a potential shift in the ethylene/polyamine biosynthesis synergies. Flower or fruitlet abscission is a response in plants to developmental or environmental cues and PAs may play a regulatory role in flowering and initial fruitlet abscission.

The presence of calcium crystals in grapevine anthers is reported for the first time. These crystals may act as a defence mechanism to protect the pollen grains and they may also play a role in calcium supply. Because there is little knowledge on the role of calcium in floral development, further research is required.

1. Background

Flowering and fruit set are principal determinants of grapevine yield. Morphological, physiological and environmental factors interact to determine the number of flowers borne on the inflorescence and the percentage of flowers that develop into berries (May 2004). Poor fruit set limits the yield of many varieties in most regions in Australia—for example, most recently, during the 2002/2003 season. Certain varieties are more prone to poor fruit set than others—for example, Merlot, which has increased in area by 14 times since 1990 to become the one of the most important varieties in terms of tonnes crushed for winemaking. Not only does poor set cause a significant economic impact, it is also one of the main causes of the season-to-season variability in many Australian regions, which causes major problems for planning of winery intake and supply to the marketplace.

Adverse climatic conditions during spring are strongly associated with poor set. There is a need to promote Australian research on flowering and fruit set because almost all of the available information has come from regions in other countries with different climatic conditions to Australia. Analysis of long-term climatic records should enable determination of the conditions that are beneficial or detrimental to floral development and set, and their frequency. This will allow the estimation of their likely frequency for a given region, which should lead to improved crop forecasting.

Better methods for prediction of flowering period are required. This can be done by development of a model—suitable for Australian locations with their great variability of temperature in spring—to relate heat summation to phenological development. In turn, this should permit prediction of flowering time at last one week in advance, which would then allow vineyard managers to immediately implement the only effective tool for improving set, ie. shoot tipping (May 2004), should unfavourable conditions be forecast. However, there is no information on the potential effectiveness of this practice in unfavourable seasons.

There is a need for a better understanding of the mechanisms by which floral development and set are controlled and the interactions with environmental and physiological factors. How floral development and set is controlled or at least affected by growth substances may help in avoiding crop loss.

In May 2003, the GWRDC convened a workshop at Tatura, Vic., to identify both the current state of knowledge and potential areas of future research. In collaboration with J. Harvey and D. Glenn of the GWRDC, P. Dry reviewed both the presentations of the workshop and the review by May (2004) and identified priority areas for research.

The literature of flowering and fruitset has been thoroughly reviewed by May (2004). Fruit set was defined by Coombe (1962) as the changeover from the static condition of the flower ovary to the rapidly growing condition of the young fruit. This is a qualitative description and relates to the morphological and physiological changes from ovary to berry. From a viticultural standpoint, fruitset is, in addition, a process that quantitatively determines how many of the ovaries become berries (May 2004). In most crops, initial fruitset is relatively high but fruitlets drop in large numbers some weeks later. However, in the grapevine, this delayed drop does not occur and the proportion of flowers that become berries is determined within one to 2 weeks after flowering (Bessis 1993). It is for this reason that climatic conditions during flowering and fruitset appear to be critical. Fruitset in grapevine is said to be normal at 50% or more and coulure is said to have occurred if it is less than 30% (Bessis 1993). Millerandage, or 'hen and chicken', is also an abnormal condition in which excessive numbers of small berries are mixed with sparse numbers of full-sized berries. Thus both conditions are quantitative deviations from the normal and therefore impact negatively on vine yield.

The causal phenomena leading to coulure and millerandage are: (a) anomalous or defective flower formation, (b) physiological phenomena, (c) environmental factors, (d) pathological interventions (Kozma 1961). There appear to be relatively few varieties with true anomalous or defective flower formation (May 2004) and likewise pathological phenomena do not appear to be important.

By comparison, physiological phenomena play a major role. For example, fruitset is impaired when reserves of carbohydrate and N compounds are in short supply in early spring or when vigorous shoot growth diverts metabolites from the inflorescence. Proline is important for pollen tube growth and acts as a cold protectant for pollen in many plants (Stines et al. 2000). The inflorescence appears to be dependent on stored reserves accumulated during previous growing season (Bennett et al. 2002). Merlot relies more than Pinot Noir on the amount of reserve carbohydrate to satisfy demand during flowering (Zapata et al. 1999, 2001). Is the susceptibility of Merlot to coulure a consequence of this? Availability of Bo, Zn and Mo at appropriate concentrations appears to be important. Application of Mo as a foliar spray increased yield of own-rooted Merlot by decreasing the proportion of chicken berries and thus increasing mean berry weight in the 2002/03 season (Gridley 2003). However, in the following season, Mo application increased yield by increasing the percentage set without any significant effect on mean berry weight (M. Longbottom, Univ. of Adelaide, pers. comm.). Soil water status during flowering is also important: a short period of water stress can lead to coulure (Alexander 1965).

Environmental factors are also very important: there is general agreement that floral development up to and just after anthesis and fruit set are strongly influenced by climate. Temperature appears to have two-fold effect on flowering and set. In a direct manner, it affects development and functioning of sexual parts. Indirectly, growth of the whole vine is affected thereby influencing assimilate supply to the inflorescence (May 2004). Before flowering, temperature affects pollen viability even when assimilate is not limiting. After flowering, any effect of unfavourable weather on set and development must be due to assimilation and supply of carbohydrate (Koblet 1966).

Mean temperatures of less than 15ºC, greater than 32ºC are detrimental (May 2004). Low temperatures will interfere with the development of the ovule after fertilisation to set. Small fruiting Chardonnay vines maintained at 12ºC/9ºC day/night for one week at early flowering had set reduced by one-third to one-half relative to vines at 17ºC/14ºC (Ebadi et al. 1995a). The effect of temperature variability is unknown. May (2004) noted that all information on the effect of temperature on set, with the exception of the work by Ebadi et al. (1995a,b, 1996a,b) has been sourced from other countries with very different climatic conditions to Australia, particularly in terms of temperature variability.

Ovary/berry abscission is due to activation of a preformed abscission layer, mainly just after anthesis (May 2004). What induces or prevents activation of abscission zone leading to shedding of ovary and/or berries? What causes specific tissues to degenerate? Abscisic acid (ABA) and ethylene are known to be involved in the development of abscission in many plant tissues. However, there is a lack of information on the role of both ABA and ethylene in the flowering process and in particular in ovary/berry abscission (May 2004). In addition, polyamines appear to be heavily involved in the process of ovary and fruitlet abscission in many species including grapevine (May 2004). In Merlot, abscission % decreased from 50 to 5 as concentration of endogenous free polyamines increased (Aziz et al. 2001). Inflorescences of varieties with high potential for ovary abscission had low levels of polyamines; those with low potential for abscission had higher concentrations (Paschalidis et al. 2001). Exogenous application of some polyamines increased set and application of inhibitors of polyamine biosynthesis caused decreased set (Aziz et al. 2001). How set is controlled or at least affected by growth substances may help in avoiding crop loss. What is the link between climate and polyamine synthesis? There is no information on the influence of climate on polyamine synthesis in the grapevine (May 2004).

One could argue that, since macroclimate—and thus the occurrence of unfavourable climatic conditions for flowering and set—cannot be controlled, there is minimal opportunity to intervene at the vineyard level. There are a limited number of cultural practices that may be used to improve set. Late pruning resulted in decreased millerandage of Merlot in NZ (Friend et al. 2000)—there

was no effect on total berry number. There is potential for use of shoot tipping (by mechanical or chemical means) in seasons when poor set is likely (or when the impact of poor set is critical for yield, e.g. when there are low bunch numbers) (May 2004). Shoot tip removal causes increased set when climatic conditions are favourable but will it also work when they are not?

2. Project Aims

- To determine the climatic conditions that are beneficial or detrimental to flower development and fruitset, and the frequency of occurrence in Australian regions.
- To determine a means by which flowering time may be accurately predicted.
- To investigate the effectiveness of cultural practices and to determine those most suitable for improving fruitset in seasons of poor set.
- To produce recommendations on practices to improve set.
- To develop an understanding of the mechanisms that induce or prevent the development of abscission zones leading to the shedding of grape flowers/berries.
- To develop an understanding of the role of plant growth regulators in the flowering process and their relationship with environmental factors and cultural practices.

Outputs and Performance Targets 2004-05

Outputs	Performance Targets
1. Information on the relationship between flowering phenology and temperature	Analysis of data from first season- results available July 2005
2. Information of the effectiveness of cultural practices to improve set	Analysis of data from first season- results available July 2005

Outputs and Performance Targets 2005-06

Outputs	Performance Targets
1.Information on the relationship between flowering phenology and temperature	Analysis of data from first season- results available July 2006
2. Information of the effectiveness of cultural practices to improve set	Analysis of data from first season- results available July 2006
3. Information on the mechanisms that induce or prevent the development of abscission zones leading the shedding of grape flowers/ovaries/berries.	Analysis of data from first two seasons- results available July 2006
4. Information on the likely frequency of 'poor set years'	Results available Dec 2006
5. Methods to predict flowering time	Production of information by Dec 2006

Outputs and Performance Targets 2006-07

Outputs	Performance Targets
1.Information on the role of polyamines, ABA and amino acids in the flowering process and their relationship with environmental factors and cultural practices	Production of information by Sep 2007
2.Clear recommendations on cultural practices to improve set	Production of information by Sep 2007
3.Clear recommendations on site selection to improve set	Production of information by Sep 2007

Outputs and Performance Targets 2007-08

Outputs	Performance Targets
1.Climate modelling in viticulture.	Production of information by July 2008
2.Effectiveness of cultural practices to improve set.	Production of information by July 2008
3.Understanding plant growth regulators in the flowering process.	Production of information by July 2008

3. Effect of Site and Season on reproductive performance of ten varieties

3.1 Introduction

In order to determine the climatic conditions that are beneficial or detrimental to flower development and fruitset, and their frequency, a project was set up to study the reproductive performance of all major varieties and some minor varieties that are considered, anecdotally, to have poor fruitset. Accordingly, vineyard sites were selected across a range of climatic regions from cool (the higher parts of the Adelaide Hills) to warm (the Waite Campus of the University of Adelaide at Urrbrae). The range of sites varied by variety and was greater in terms of growing season temperature range for some varieties, e.g. Chardonnay, than others, e.g. Sauvignon Blanc. In all cases, there was a climate recording station either at the site or nearby.

In most studies on fruitset, a count of berry number per bunch has been used to estimate fruitset. In these cases, a sample of bunches has been selected from the vines with the assumption that initial flower number per inflorescence did not vary. However, as May (2004) and others have shown, flower number per inflorescence may vary significantly between inflorescences on the same vine, and from vine to vine. Therefore, it is our contention that the only valid method for determination of fruitset is one that is based on a count of both flower number and berry number on the sampled inflorescences/bunches.

The terminology of fruitset has often been imprecise in the past. In this study, we have distinguished between seeded berries ('hens'), seedless berries ('chickens') and 'live green ovaries' (LGOs). The term 'live green ovary' is preferred, as per May (2004), to the term 'shot berry' which has been frequently used in previous papers. LGOs do not fit the definition of berry, that is "…pericarp consisting of skin enclosing a fleshy or pulpy mass" (Sedgley and Griffin 1989).

May (2004) has stated that fruit set is 'normal' when the bunch framework is filled with berries that have reached full-size. Therefore, *coulure* and *millerandage* are abnormal conditions when the above is not the case. *Coulure* has been defined by May (2004) as "…the excessive shedding of ovaries or very young berries": the end result is a bunch with relatively few 'true' berries (either hens or chickens) plus live green ovaries (LGOs). *Millerandage* has been defined by May (2004) as the condition when "…excessive numbers of small berries are mixed with sparse numbers of full-sized berries". We have adopted the above definitions; however, like Longbottom (2007), we do not consider *millerandage* to be synonymous with the condition known as 'hen and chicken', as stated by May (2004). To our knowledge, the expression of *coulure* and *millerandage* has only been assessed visually in the past.

3.2 Methods and Materials

The details of each site are shown in Table 1.

For all sites, three inflorescences per vine (= ‘sample bunches’) were selected at random on five vines before flowering and labelled for future data collection. Each ‘sample bunch’ was enclosed with a fine mesh bag secured with a plastic tie. After flowering was complete, the bag was removed and flower caps were counted in order to estimate number of flowers per bunch. This method does not influence fruitset (May 2000). Bunch yield components were assessed on each of the ‘sample bunches’ just before the time of commercial harvest. This included a count of seeded berries (‘hens’), seedless berries (‘chickens’) and ‘live green ovaries’ (LGOs), and measurement of bunch weight and rachis weight. Because we do not consider LGOs to be berries, they were excluded from the berry count, and as a consequence, they do not contribute to the measures of fruitset or mean berry weight. Similarly, in the past, chicken berries have often been scored solely on the basis of small size. However, we have found this to be an unreliable indicator because small berries may have seeds; and for this reason in our study, chickens were only determined by dissection. Total bunches per vine were counted just prior to harvest.

Table 3.1. Details of sites used in study.

Region	Site	Location	Elevn m	Variety	Clone	Rootstock	Year planted	Row x vine spacing	Trellis	Prun-ing	Climate station
Adelaide Hills	*Forreston* (ad h for)	4.5 km N Forreston	430	Sauvignon Blanc	F4V6	Own roots	Na	Na	VSP	Cane	On site
	"	"	"	Shiraz	1127	"	"	"	"	Spur	"
	Lobethal (ad h ya)	2 km S Lobethal	350	Sauvignon Blanc	F4V6	Own roots	"	"	VSP	Cane	On site
	"	"	"	Shiraz	1127	"	"	"	"	Spur	"
	Charleston (ad h ch)	1 km E Charleston	410 - 475	Cabernet Sauvignon	G9V3	Own roots	1998	2.75 x 1.5	VSP	Spur	On site
	"	"	"	Pinot Noir	114	"	"	"	"	"	"
	"	"	"	Zinfandel	unknown	"	"	"	"	"	"
				Tempranillo	D8V12	K51-40	"	"	"	"	"
	"	"	"	Chardonnay	I10V1	"	"	"	"	"	"
	"	"	"	Sauvignon Blanc	F4V6	"	"	"	"	cane	"
	Lenswood (ad h lens anl)	4.4 km NE Oakbank	440	Sauvignon Blanc	F4V6	Own roots	Na	Na	VSP	cane	On site
	Lenswood (ad h lens ne)	2.5 km S Lenswood	480 - 500	Sauvignon Blanc	F4V6	"	1995	2.7 x 1.5	VSP	cane	Lenswood Res Stn
		"	"	Pinot Noir	D5V12	"	1995	"	"	spur	"
	Balhannah (ad h jc)	1.6 km SE Balhannah	400	Sauvignon Blanc	F4V6	Own roots	Na	Na	VSP	cane	On site
		"	"	Shiraz	1127	"	"	"	"	Spur	"
	Kuitpo (ad h kui)	5 km Kuitpo	326	Sauvignon Blanc	F4V6	Own roots	1995	2.5 x 1.5	VSP	cane	Kuitpo
		"	"	Shiraz	1127	"	1995	"	VSP	spur	"
McLaren Vale	*West –Hardy* (mcv west h)	3.5 km W McLaren Vale	55	Cabernet Sauvignon	Reynella	Own roots	1999	2.75 x 1.8	VSP	spur	On site
	"	"	"	Shiraz	BVRC12	"	"	"	"	"	"
	"	"	"	Sangiovese	H6V9	"	"	"	"	"	"
	"	"	"	Nebbiolo	11A	"	"	"	"	cane	"
	"	"	"	Tempranillo	D8V12	"	"	"	"	spur	"
	West – Tinlins (mcv west t)	14 km W McLaren Vale	40	Chardonnay	I10V1	Own roots	1995	3.2 x 2.0	VSP	spur	McLaren Vale Visitors Centre
	(mcv west t w44)	"	"	Cabernet Sauvignon	CW44	Own roots	1999	3.2 x 1.8	VSP	Spur	"

	(mcv west t lc10)	"	"	Cabernet Sauvignon	LC10	"	1999	"	"	"	"
	(mcv west t rug)	"	"	Cabernet Sauvignon	CW44	140Ru	1999	"	"	"	"
	(mcv west t 101)	"	"	Cabernet Sauvignon	CW44	101-14	1999	"	"	"	"
		"	"	Shiraz	1654		1997	"	"	"	"
	(mcv west t 101)	"	"	Merlot	D3V14	101-14	1999	"	"	"	"
	(mcv west t sch)	"	"	"	"	Schwarz-mann	1999	"	"	"	"
	(mcv west t rug)	"	"	"	"	140Ru	1999	"	"	"	"
	Seaview (mcv sea)	5.5 km N McLaren Vale	140-150	Cabernet Sauvignon	Unknown	"	1994	3.3 x 2.1	2WV	spur	On site
	"	"	"	Shiraz	Unknown	"	1999	3.4 x 1.8	VSP	"	"
	"	"	"	Shiraz	Unknown	"	1967	3.5 x 1.8	Sprawl	"	"
	East-Gemtree (mcv east)	3 km E McLaren Flat	135	Shiraz	BVRC30	"	1993	3 x 1.5	2WV	spur	McLaren Vale Visitors Centre
	"	"	"	Chardonnay	I10V1	"	1989	"	2WV	"	"
	West-Gemtree (mcv west)	3.5 km W McLaren Vale	35	Chardonnay	I10V1	"	1989	"	Sprawl	"	Hardy weather station on site
Padthaway	Fosters (pad) (m3)	1-2 km W Padthaway centre	37	Cabernet Sauvignon	Unknown	Unknown	1993	3.0 x 2.0	2WV	spur	On site
	" (og)	"	"	"	Unknown	Own roots	1969	3.6 x 2.1	Wide T	"	"
	" (zo2)	"	"	Shiraz	1654	Own roots	1994	3.0 x 2.0	2WV	"	"
	" (zog)	"	"	"	Unknown	Own roots	1970	3.0 x 2.0	2WV	"	"
	" (14)	"	"	Chardonnay	I10V1	Crouchen	1991	3.0 x 2.0	2WV	"	"
	" (ob)	"	"	"	I10V1	Own roots	1981	3.5 x1.8	Single wire	"	"
Adelaide	*Waite*	8 km SE Adelaide	115	Cabernet Sauvignon	G9V3	Own roots	1992	3.0 x 1.8	VSP	spur	BOM Kent Town
	"	"	"	Chardonnay	I10V1	"	"	"	"	"	"

The following bunch yield components and indices of fruitset were calculated as follows:
Total berry number per bunch = number of hen berries per bunch + number of chicken berries per bunch
Fruit set I (%) = total berry number per bunch* 100 / number of flowers per bunch
Fruit set II (%) = hen number per bunch* 100 / number of flowers per bunch
Berry weight (g) = (mean bunch weight (g) - rachis weight (g)) / total berry number per bunch
Fruit yield per vine = mean bunch weight (g) x bunch number per vine
Coulure Index (CI) is an indicator of the proportion of flowers which do not develop into either a berry or an LGO.
Millerandage Index (MI) is an indicator of the proportion of all post-flowering organs that develop into either chickens or LGOs. For both indices, the higher the numerical value, the greater the degree of expression of the condition.

3.3 Results

3.3.1. Effect on flower and berry number, fruit set, CI and MI of site (means of all seasons)

Flower number per inflorescence

Cabernet Sauvignon: Mean of all seasons ranged from 280 to 450. Adelaide Hills (particularly Charleston) and Waite sites were consistently low whereas Padthaway sites (particularly 06) were high. McLaren Vale sites were intermediate but with some variability. Clonal comparison at McLaren Vale West: CW44 was higher than LC10 but difference was only marginal. Rootstock comparison at McLaren Vale West: 140 Ru was higher than 101-14 but difference was only marginal. Some sites have high degree of seasonal variability, eg. Waite (no data collected in 06/07) and Padthaway (no data collected in 04/05).

Shiraz: Mean of all seasons ranged from 135 to 320. All Adelaide Hills sites (particularly Kuitpo) had lower values than McLaren Vale and Padthaway sites. Comparison at Padthaway: zo2 lower than zog. Within McLaren Vale regional comparison: East was lower than West. All had low degree of seasonal variability except Padthaway (zog).

Merlot: Only one site—mean of all seasons ranged from 296 to 326. There was no effect of rootstock and low degree of seasonal variability (4 seasons of data).

Pinot Noir: Two sites in Hills—mean of all seasons ranged from 179 to 190. There was no site effect and a very low degree of seasonal variability (3 or 4 seasons of data).

Tempranillo: Two sites (McLaren Vale West and Hills)—mean of all seasons ranged from 204 to 226. There was no difference between regions and a low degree of seasonal variability (4 seasons of data).

Nebbiolo: One site (McLaren Vale West)—mean of all seasons was 286 with a low degree of seasonal variability (4 seasons of data).

Sangiovese: One site (McLaren Vale West)—mean of all seasons was 360 with a low degree of seasonal variability (4 seasons of data).

Zinfandel: One site (Adelaide Hills Charleston)—mean of all seasons was 371 with a moderate degree of seasonal variability (4 seasons of data).

Chardonnay: Mean of all seasons ranged from 200 to 280. McLaren Vale sites were slightly higher than Padthaway, Hills and Waite. There was a low degree of seasonal variability.

Sauvignon Blanc: All sites in Hills (only Charleston with 4 seasons data): mean of all seasons ranged from 143 to 203 (relatively low range) with minimal effect of site and a very low degree of seasonal variability.

Fruitset

Cabernet Sauvignon (Figure 3.1): Mean of all seasons ranged from 22 to 36. Padthaway and Waite were lower than McLaren Vale and Hills. There was no effect of either clone or rootstock at McLaren Vale West. Padthaway and Hills (particularly Charleston) had high degree of seasonal variability.

Shiraz (Figure 3.2): Mean of all seasons ranged from 30 to 60 (large range). Adelaide Hills Lobethal (ya) and Balhannah (jc) sites were highest, McLaren Vale Seaview the lowest, with others intermediate. Comparison at Padthaway: zo2 was higher than zog but marginal difference. There was a high degree of seasonal variability for many sites.

Merlot (Figure 3.3): Only one site—mean of all seasons ranged from 24 to 39. 140Ru much higher than 101-14; Schwarzmann was intermediate. There was a moderate degree of seasonal variability (4 seasons of data).

Pinot Noir (Figure 3.4): Two sites in Hills—mean of all seasons approximately 40 for both. There was no site effect and a low degree of seasonal variability (3 or 4 of seasons data).

Tempranillo (Figure 3.5): Two sites (McLaren Vale West and Hills)—mean of all seasons ranged from 35 (McLaren Vale West) to 50 (Hills), and a low (McLaren Vale West) to high (Hills) degree of seasonal variability (4 seasons of data).

Nebbiolo (Figure 3.6): One site (McLaren Vale West)—mean of all seasons was 43 with a moderate degree of seasonal variability (4 seasons of data).

Sangiovese (Figure 3.6): One site (McLaren Vale West)—mean of all seasons was 55 with a high degree of seasonal variability (4 seasons of data).

Zinfandel (Figure 3.6): One site (Adelaide Hills Charleston)—mean of all seasons was 56 and a high degree of seasonal variability (4 seasons of data).

Chardonnay (Figure 3.7): Mean of all seasons ranged from 42 to 55. Waite was higher than McLaren Vale East and Padthaway (14). For the comparison at Padthaway: ob was higher than 14. There was a high degree of seasonal variability for Waite, Padthaway (14) and Hills (Charleston).

Sauvignon Blanc (Figure 3.8)
All sites in Hills (only Charleston with 4 seasons data): mean of all seasons ranged from 35 to 53. Lobethal (ya) and Balhannah (jc) were higher than Lenswood (nep), Kuitpo and Forreston. Degree of seasonal variability was high for Balhannah (jc) and Lenswood (nep).

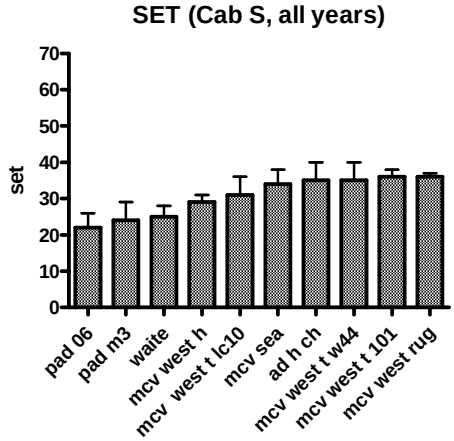


Figure 3.1. Effect of vineyard site of fruitset of Cabernet Sauvignon. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

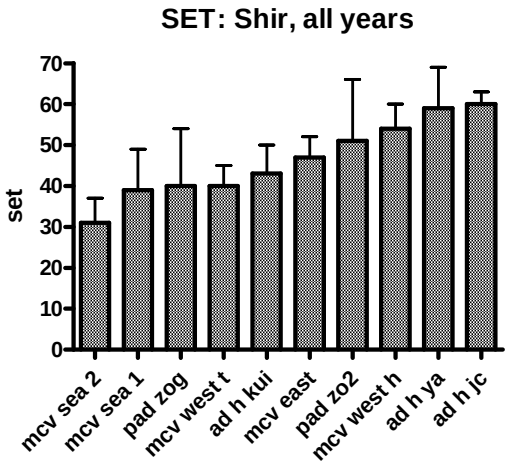


Figure 3.2. Effect of vineyard site of fruitset of Shiraz. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

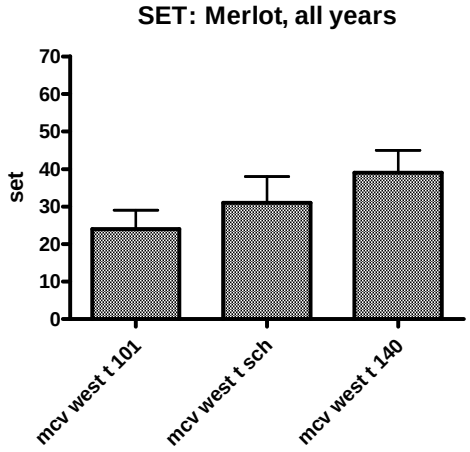


Figure 3.3. Effect of rootstock on fruitset of Merlot at McLaren Vale. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

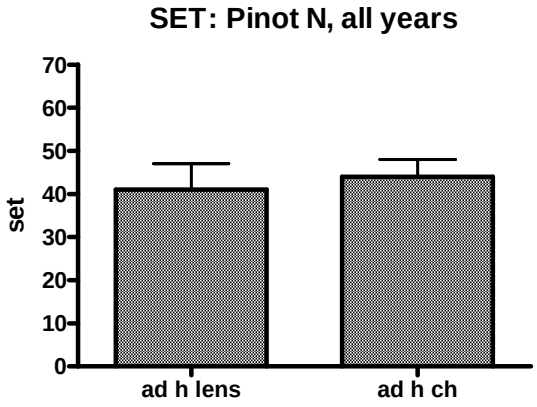


Figure 3.4. Effect of vineyard site on fruitset of Pinot Noir. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

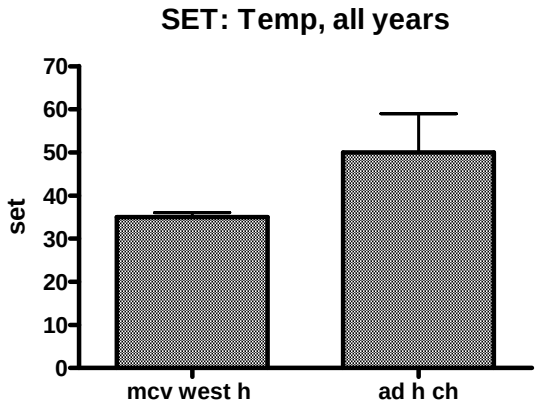


Figure 3.5. Effect of vineyard site on fruitset of Tempranillo. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

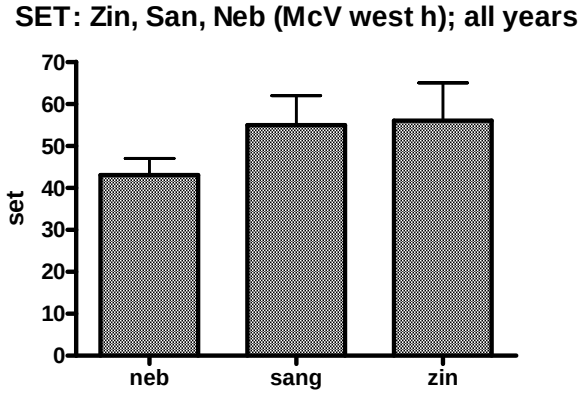


Figure 3.6. Fruitset of Nebbiolo (McLaren Vale), Sangiovese (McLaren Vale) and Zinfandel (Adelaide Hills). Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

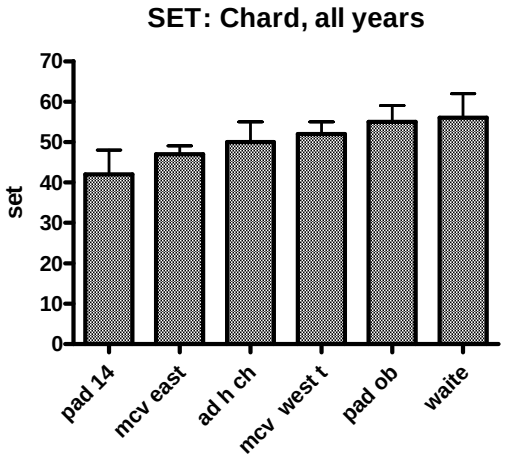


Figure 3.7. Effect of vineyard site on fruitset of Chardonnay. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

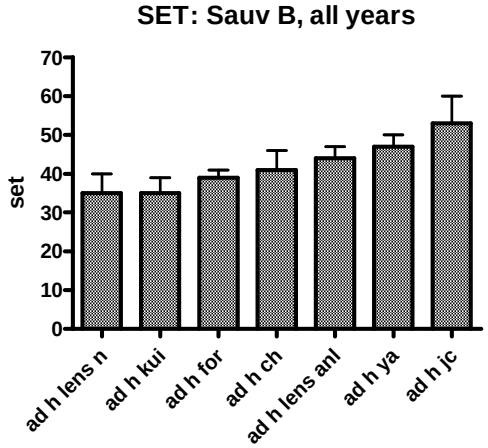


Figure 3.8. Effect of vineyard site on fruitset of Sauvignon Blanc. Means and standard errors of all seasons (2004/05, 2005/06, 2006/07 and 2007/08).

Coulure Index

Cabernet Sauvignon: Mean of all seasons ranged from 2.8 to 6.5 (large range). All McLaren Vale sites were much lower than Padthaway, Waite and Hills. There was no effect of either clone or rootstock at McLaren Vale West. Degree of seasonal variability was low to high. A high CI was associated with low set (except for Hills Charleston).

Shiraz: Mean of all seasons ranged from 1.7 to 6.0 (large range). Adelaide Hills, particularly Lobethal (ya) and Balhannah (jc) sites, were much lower than McLaren Vale Seaview; the others were intermediate. There was variability within McLaren Vale West. Degree of seasonal variability was high for Seaview 2 and Padthaway. High CI was associated with low set.

Merlot: Only one site—mean of all seasons ranged from 4.2 to 5.2. 140Ru was less than 101-14; with Schwarzmann intermediate. Degree of seasonal variability was moderate (4 seasons of data). High CI was associated with low set.

Pinot Noir: Two sites in Hills—mean of all seasons approximately 4.7 for both. There was no site effect and a high degree of seasonal variability (3 or 4 seasons of data).

Tempranillo: Two sites (McLaren Vale West and Hills)—mean of all seasons ranged from 4.0 (Hills) to 5.5 (McLaren Vale West) with a low (McLaren Vale West) to high (Hills) degree of seasonal variability (4 seasons of data).

Nebbiolo: One site (McLaren Vale West)—mean of all seasons was 3.3 with a low degree of seasonal variability (4 seasons of data).

Sangiovese: One site (McLaren Vale West)—mean of all seasons was 3.5 with a moderate degree of seasonal variability (4 seasons of data).

Zinfandel: One site (Adelaide Hills Charleston)—mean of all seasons was 1.7 (relatively low) with a moderate degree of seasonal variability (4 seasons of data).

Chardonnay: Mean of all seasons ranged from 2.8 to 4.6. Waite and McLaren Vale West were lower than McLaren Vale East and Padthaway. For the comparison at Padthaway: ob was higher than 14. Degree of seasonal variability was moderate to high (particularly Padthaway 14 for latter). High CI was associated with low set.

Sauvignon Blanc: All sites in Hills (only Charleston with 4 seasons data). Mean of all seasons ranged from 2.9 to 5.1. Lobethal (ya), Balhannah (jc) and Lenswood (anl) were lower than Lenswood (nep) and Kuitpo. Degree of seasonal variability was high for Kuitpo and Charleston. High CI was associated with low set.

Millerandage Index

Cabernet Sauvignon: Mean of all seasons ranged from 2.5 to 6.0 (large range). The Hills site (Charleston) was less than Padthaway and Waite which were less than all McLaren Vale sites. There was no effect of either clone or rootstock at McLaren Vale West and no difference between Padthaway sites. Degree of seasonal variability was low (Hills Charleston) to high (McLaren Vale West).

Shiraz: Mean of all seasons ranged from 1.7 to 3.5 (moderate range but less than for CI). McLaren Vale (with exception of Seaview 2) and Padthaway were lower than Adelaide Hills. At Padthaway, zo2 was less than zog; and at Seaview, 1 was much less than 2. Degree of seasonal variability was moderate to high.

Merlot: Only one site: mean of all seasons ranged from 3.5 to 5.0. 140Ru was less than Schwarzmann which was less than 101-14, and degree of seasonal variability was moderate to high (4 seasons data).

Pinot Noir: Two sites in Hills—mean of all seasons around 1.8 for both with no site effect and a high degree of seasonal variability (3 or 4 seasons data).

Tempranillo: Two sites (McLaren Vale West and Hills)—mean of all seasons ranged from 1.6 (Hills) to 2.9 (McLaren Vale West) with a high degree of seasonal variability for both (4 seasons data).

Nebbiolo: One site (McLaren Vale West)—mean of all seasons was 3.9 with a low degree of seasonal variability (4 seasons data).

Sangiovese: One site (McLaren Vale West)—mean of all seasons was 3.4 with a high degree of seasonal variability (4 seasons data).

Zinfandel: One site (Adelaide Hills Charleston)—mean of all seasons was 4.9 (relatively high) with a moderate degree of seasonal variability (4 seasons data).

Chardonnay: Mean of all seasons ranged from 1.6 to 3.1 (relatively small range). Padthaway (ob) was lower than McLaren Vale West. Degree of seasonal variability was low to high (particularly Padthaway 14).

Sauvignon Blanc: All sites in Hills (only Charleston with 4 seasons data)—mean of all seasons ranged from 2.5 to 4.0 (relatively small range). Lobethal (ya) and Balhannah (jc) were lower than the rest, with a high degree of seasonal variability for Balhannah. Both Lobethal and Balhannah had low CI and low MI.

Berry number per bunch

Cabernet Sauvignon: Mean of all seasons ranged from 79 to 146 (large range). Waite and Padthaway sites were lowest whereas all McLaren Vale sites were high; Adelaide Hills (Charleston) was intermediate. Clonal comparison at McLaren Vale West: CW44 was higher than LC10 (as for flower number). Rootstock comparison at McLaren Vale West: 140Ru was higher than 101-14 (as for flower number). Some sites had high degree of seasonal variability, eg. Waite (no data collected in 06/07) and Adelaide Hills (Charleston).

Shiraz: Mean of all seasons ranged from 54 to 134 (large range). Kuitpo had much lower values (and low seasonal variability) than all others; McLaren Vale West was higher than all others. Seaview 2 was much lower than 1. There was a high degree of seasonal variability for Seaview 2.

Merlot: Only one site—mean of all seasons ranged from 88 to 129. 140Ru was higher than others and there was a high degree of seasonal variability (4 seasons data).

Pinot Noir: Two sites in Hills—mean of all seasons was approximately 75 for both. There was no site effect, with low to moderate degree of seasonal variability (3 or 4 seasons data).

Tempranillo: Two sites (McLaren Vale West and Hills)—mean of all seasons ranged from 85 (McLaren Vale) to 110 (Hills) with moderate (McLaren Vale) to high (Hills) degree of seasonal variability (4 seasons data).

Nebbiolo: One site (McLaren Vale West)—mean of all seasons was 127 with a moderate degree of seasonal variability (4 seasons data).

Sangiovese: One site (McLaren Vale West)—mean of all seasons was 207 with a high degree of seasonal variability (4 seasons data).

Zinfandel: One site (Adelaide Hills Charleston)—mean of all seasons was 207 with a low degree of seasonal variability (4 seasons data).

Chardonnay: Mean of all seasons ranged from 86 to 145. McLaren Vale West was higher than Padthaway 14 and Hills. Padthaway ob was higher than 14. Degree of seasonal variability was low.

Sauvignon Blanc: All sites in Hills (only Charleston with 4 seasons data). Mean of all seasons ranged from 63 to 95 (relatively low range). JC was higher than Forreston. Degree of seasonal variability was high for JC (2 seasons data).

3.3.2. Effect on fruitset of season (means of all sites)

Cabernet Sauvignon (Figure 3.9): There was no apparent seasonal effect.

Shiraz (Figure 3.10): Data were collected from all sites in 2005/06 and 2006/07. In the 2006/07 season, set (approximately 50%) was slightly higher than in the other 3 seasons which were all similar at approximately 42%.

Merlot (Figure 3.11): Only one site—there were major differences between seasons. 2007/08 was much higher than all others; and 2006/07 had lowest set.

Pinot Noir (Figure 3.12): Two sites in Hills—2004/05 had much lower set than the other seasons which were similar; however, data were only collected from the Lenswood site in that season. For the Lenswood site alone, 2004/05 had lower set than in all other seasons.

Tempranillo (Figure 3.13): Two sites with significant variation for all reproductive indices. Highest set was in 2004/05 and 2005/06 (approximately 50%), lowest in 2007/08 (approximately 30%), with 2006/07 intermediate.

Nebbiolo (Figure 3.14): One site at McLaren Vale West. 2004/05 and 2007/08 were higher than 2005/06 and 2006/07.

Sangiovese (Figure 3.15): One site at McLaren Vale West. 2007/08 was higher than the other seasons.

Zinfandel (Figure 3.16): One site (Adelaide Hills Charleston). 2006/07 was higher than 2005/06 and 2007/08.

Chardonnay (Figure 3.17): Relatively small differences between seasons—2004/05 had slightly higher set than 2007/08.

Sauvignon Blanc (Figure 3.18): All sites were in the Hills (data for Balhannah (jc), Lobethal (ya), Lenswood (anl) and Forreston were collected only in 2005/06 and 2006/07). 2007/08 was lower

than the other seasons which were similar (however, this is not a fair comparison because Kuitpo and Charleston were the only sites used in this season and Kuitpo had low set in every season).

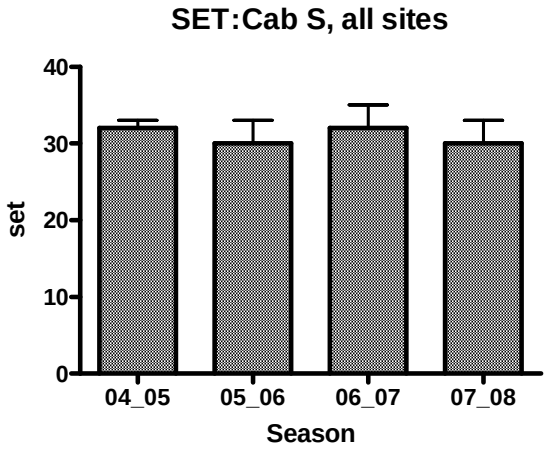


Figure 3.9. Effect of season on fruitset of Cabernet Sauvignon. Means and standard errors of all sites.

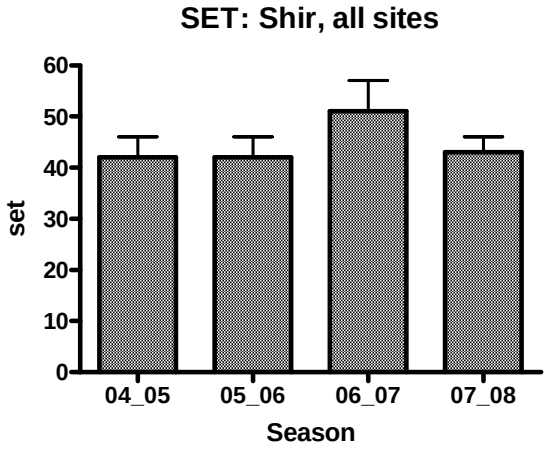


Figure 3.10. Effect of season on fruitset of Shiraz. Means and standard errors of all sites.

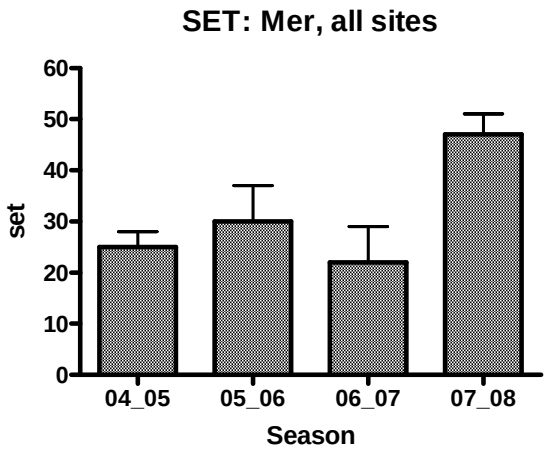


Figure 3.11. Effect of season on fruitset of Merlot. Means and standard errors of all rootstocks (McLaren Vale).

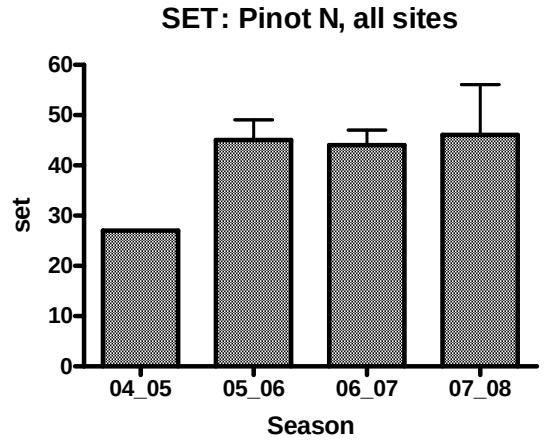


Figure 3.12. Effect of season on fruitset of Pinot Noir. Means and standard errors of all sites.

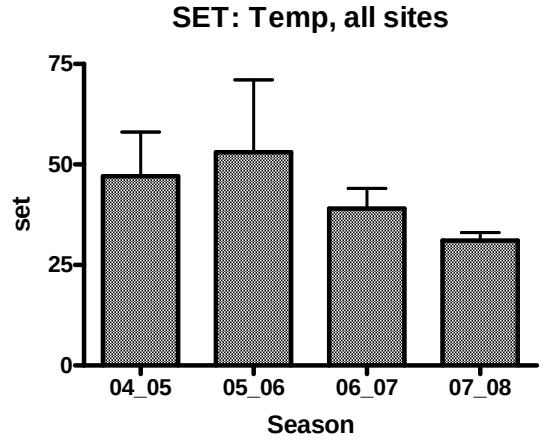


Figure 3.13. Effect of season on fruitset of Tempranillo. Means and standard errors of all sites.

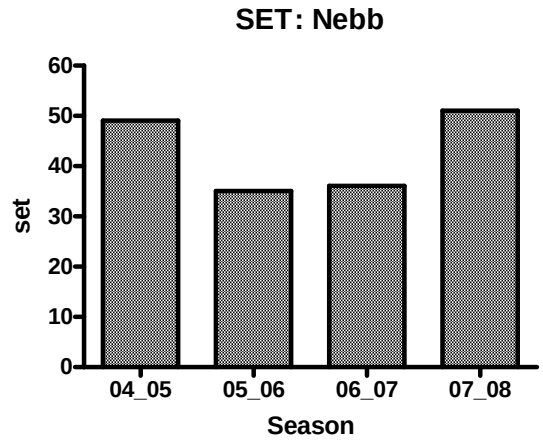


Figure 3.14. Effect of season on fruitset of Nebbiolo at a single site at McLaren Vale.

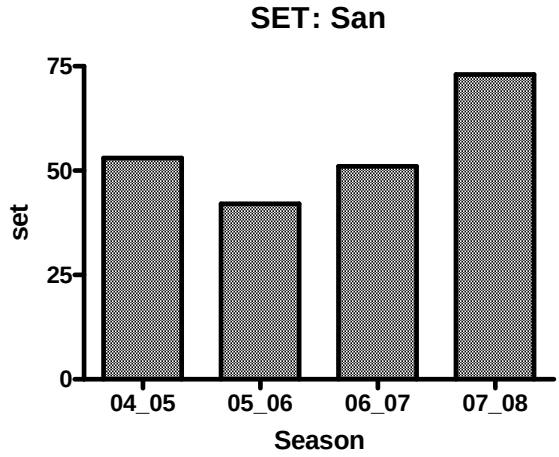


Figure 3.15. Effect of season on fruitset of Sangiovese at a single site at McLaren Vale.

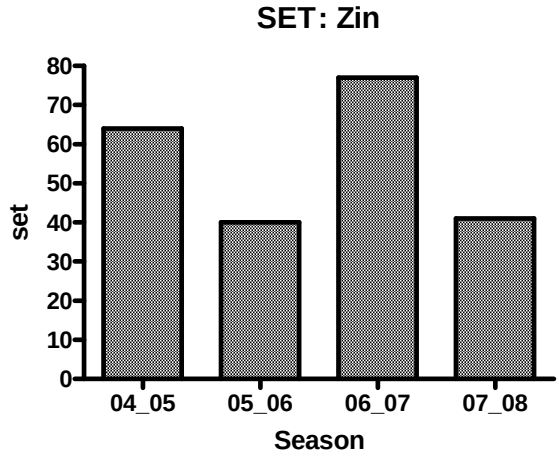


Figure 3.16. Effect of season on fruitset of Zinfandel at a single site at Charleston (Adelaide Hills).

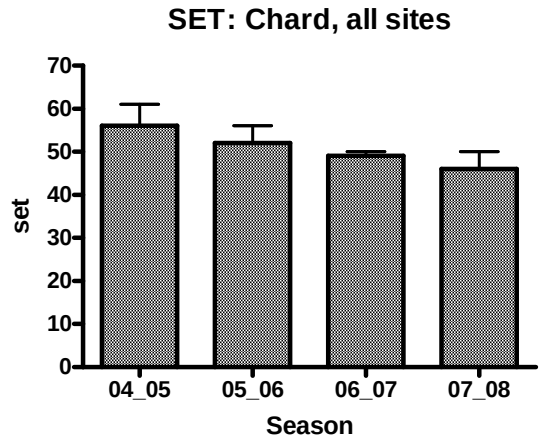


Figure 3.17. Effect of season on fruitset of Chardonnay. Means and standard errors of all sites.

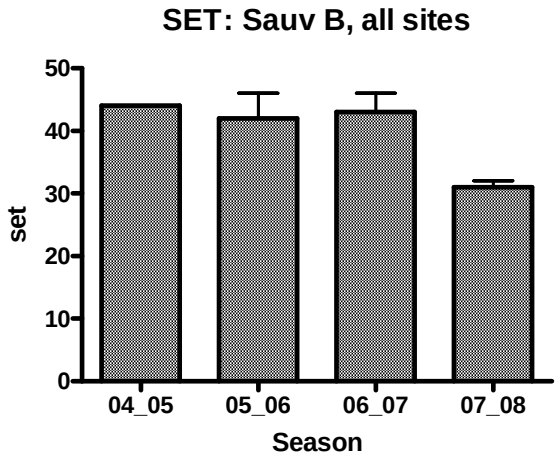


Figure 3.18. Effect of season on fruitset of Sauvignon Blanc. Means and standard errors of all sites.

3.3.3. Effect on fruitset of variety (averaged over all sites and seasons)

Cabernet Sauvignon and Merlot had consistently lower set than all other varieties, irrespective of site. For Cabernet Sauvignon: both inter-season and inter-site variability was very low. For Merlot: inter-season variability was low. At the other extreme, both Sangiovese and Zinfandel had higher set than other varieties; together with high variability for both site and season. Sauvignon Blanc, Tempranillo, Pinot Noir, Nebbiolo, Shiraz and Chardonnay had intermediate values, with low to moderate site and season variability (Figure 3.19).

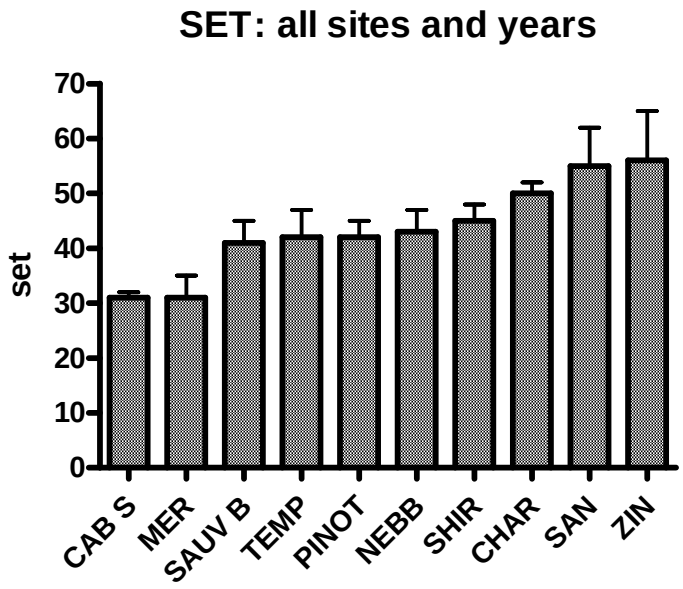


Figure 3.19. Fruitset of all varieties. Means and standard errors of all sites and seasons.

3.3.4. Effect of region and site: summaries by variety (Table 3.2)

Cabernet Sauvignon had a high degree of seasonal variability for all parameters. Berry number per bunch ranged from low to moderate due to a combination of moderate to high flower number but low set (at all sites in most seasons). Both CI and MI were low to high, depending on site. The Waite site had low berry number due to a combination a moderate flower number and low set, associated with high CI and moderate MI. Padthaway was similar to Waite except that flower number was higher. In the Hills, low berry number was the result of low flower number (as for Waite) but better set (also high CI but lower MI than Waite). For McLaren Vale, there was some variability between sites. Flower number was moderate to high, set was low but berry number was moderate (the highest of all regions). This region also had the lowest CI and highest MI. There was no significant effect of either clone or stock on any parameter except for berry number.

Shiraz had a high seasonal variability for some parameters. Berry number per bunch ranged from low to moderate due to a combination of low to moderate flower number and moderate set (but some sites in Hills may be high). CI was low to high (very seasonal) and MI low to moderate. In the Hills, there were 3 sites: Lobethal (ya), Balhannah (jc) and Kuitpo: the first two were similar in most respects and different to Kuitpo. The Hills sites had the lowest flower numbers (particularly Kuitpo) but the highest set (for Lobethal and Balhannah): this resulted in a moderate berry number (associated with the lowest CI and highest MI). Kuitpo had a low berry number due to a very low flower number. At Padthaway, there were differences between the 2 sites: flower number was higher than for the Hills, with intermediate values of set, berry number and CI, but low MI. For McLaren Vale, there was a large degree of variation between sites. Flower number was moderate

for most sites with moderate set (except Seaview): this resulted in moderate berry number. Most sites had moderate CI and low MI. Seaview 2 had lower berry number than Seaview 1: although there was no difference in flower number, both had the lowest set all of regions/sites (and highest CI) but Seaview 2 had lower set than Seaview 1 (and much higher MI). Also Seaview 2 had very high seasonal variability. There was a large range of CI across all sites as for Cabernet (but also a larger geographical range than all other varieties except for Cabernet and Chardonnay).

Merlot had just one site with 3 rootstocks. Berry number per bunch ranged from low to moderate due to a combination of moderate flower number and generally low set. CI was moderate to high (very seasonal) and MI moderate (but high in some seasons). Set and berry number were highest for 140Ru, lowest for 101-14, and intermediate for Schwarzmann, with no differences for flower number. CI and MI were inversely related to set and berry number, being highest for 101-14, lowest for 140Ru and intermediate for Schwarzmann.

Pinot Noir had 2 sites, both in the Hills. There was no difference between sites for any parameter and seasonal variability was moderate. Berry number per bunch was low due to a combination of low flower number and moderate set. CI was moderate and MI low.

Tempranillo: there were 2 sites, one at McLaren Vale and one in the Hills (Charleston). Berry number per bunch was low to moderate due to a combination of low flower number and moderate set. CI was moderate to high and MI low. There was no effect of site on flower number. Berry number in the Hills was higher due to higher set than McLaren Vale (associated with lower CI and lower MI than McLaren Vale). The Hills had more seasonal variation.

Nebbiolo had one site at McLaren Vale. Berry number per bunch was moderate due to a combination of moderate flower number and moderate set. Both CI and MI were moderate.

Sangiovese had one site at McLaren Vale. Berry number per bunch was high due to a combination of high flower number and high set. Both CI and MI were moderate.

Zinfandel had one site at Charleston in the Hills. Berry number per bunch was high due to a combination of high flower number and high set. CI was low and MI was high.

Chardonnay was characterised by high seasonal variation for most sites. Berry number per bunch was low to moderate due to a combination of low to moderate flower number and mostly moderate set. CI was moderate and MI was low. McLaren Vale West had slightly higher flower number than Padthaway, Waite or the Hills, and combined with moderate set, this resulted in the highest

berry number. McLaren Vale East had slightly lower set and thus fewer berries than West. Waite had the highest set, but berry number was intermediate because flower number was relatively low. It also had the lowest CI and relatively low MI. The Hills had low flower number and moderate set resulting in moderate berry number. Padthaway was similar to the Hills, but with higher CI. There was high seasonal variability for both set and CI for most sites.

Sauvignon Blanc had sites only in the Hills. Berry number per bunch was low due to a combination of low flower number (for all sites with little difference between sites and low seasonal variability) and mostly moderate set. CI was moderate to high and MI was low to moderate. Balhannah (jc) had the highest berry number due to highest set. Lobethal (ya) was similar to Balhannah. Forreston had the lowest berry number due to relatively low set (high MI). There was high seasonal variability for set for some sites.

Table 3.2. Summary of all seasons and sites

Flower number per inflorescence*: low = <250, moderate = 250 to 350, high = >350
Fruitset: low = <35, moderate = 35 to 50, high = >50
Coulure Index (CI) and Millerandage Index (MI): low = <3.0, moderate = 3.0 to 5.0, high = >5.0
Berry number per bunch*: low = <100, moderate = 100-150, high = >150

Variety	Flower #	Fruitset	CI	MI	Berry #
Cabernet Sauvignon	Moderate (Hills, McLaren Vale, Waite) to high (Padthaway, McLaren Vale)	Low (but moderate in some seasons)	Low (McLaren Vale) to moderate(McLaren Vale) to high (Padthaway, Hills, Waite)	Low (Hills) to moderate (Padthaway, Waite, McLaren Vale) to high (McLaren Vale)	Low (Waite, Padthaway, Hills) to moderate (McLaren Vale)
Shiraz	Low (Hills, Padthaway) to moderate (Padthaway, McLaren Vale)	Moderate (but some Hills sites may be high)	Low (Hills) to moderate (Padthaway, Hills, McLaren Vale) to high (McLaren Vale	Low to moderate (Hills)	Low (Hills) to moderate
Merlot [@]	Moderate	Low	Moderate to high	Moderate (high in some seasons)	Low to moderate
Pinot Noir	Low	Moderate (seasonal effect)	Moderate (high in some seasons)	Low	Low
Tempranillo	Low	Moderate	Moderate to high (low in some seasons at McLaren Vale)	Low (moderate in some seasons at McLaren Vale)	Low (McLaren Vale) to moderate (Hills)
Nebbiolo [@]	Moderate	Moderate	Moderate	Moderate	Moderate
Sangiovese [@]	High	High (seasonal effect)	Moderate	Moderate (low in some seasons)	High
Zinfandel [$]	High	High (“)	Low	High	High
Chardonnay	Low (Padthaway, Hills) to moderate (McLaren Vale)	Moderate (most) to high (Waite) but depends on season)	Moderate (high in some seasons at Padthaway)	Low to moderate	Low (Padthaway) to moderate (Padthaway, Hills, McLaren Vale)
Sauvignon B [$]	Low	Moderate (low in some seasons)	Moderate to high (Hills)	Low (Hills) to moderate	Low

* Absolute values
[$] Hills sites only
[@] McLaren Vale sites only

3.3.5. Relationship between flower number per inflorescence and berry number per bunch

There was a strong relationship between flower number and berry number for all sites and seasons for Merlot (r^2 = 0.6453**), Chardonnay (r^2 = 0.5581***) and Cabernet Sauvignon (r^2 = 0.506***). However, for Tempranillo (r^2 = 0.24), Sauvignon Blanc (r^2 = 0.1416), Pinot Noir (r^2 = 0.06) and Shiraz (r^2 = 0.02), the relationship was weak to very weak.

3.4 Discussion

This study has confirmed the desirability of flower counts for the accurate determination of fruitset. It is one of few studies where fruitset has been calculated using flower number per inflorescence/bunch as denominator in the formula and not simply inferred from counts of berry number. For example, it is clear that variation in flower number from season to season may be significant, particularly for Cabernet Sauvignon and the high variability of total berry number for some varieties (e.g. Merlot, Chardonnay, Cabernet Sauvignon) may be as much a consequence of variation in flower number as variation in fruitset.

Furthermore, the relationship between berry number and fruitset may not be sufficiently robust to justify the use of berry number as an 'index' of fruitset. Certain varieties have a reputation for "poor fruitset" that has been inferred from relatively low berry number per bunch, eg Tempranillo, Sauvignon Blanc, Pinot Noir and Chardonnay (in some locations). However, this study has demonstrated that, compared with other varieties, all of these varieties tend to have moderate set—and higher than that for Cabernet Sauvignon and Merlot which tend to have consistently low set, but moderate to high flower number.

The value of the new indices CI and MI has been more than satisfactorily demonstrated in this study. Rather than description of grapevine reproductive performance in a subjective and imprecise manner, these indices provide a means by which performance may be quantified; and furthermore, the individual expressions of *coulure* and *millerandage* may be separated. There appear to be significant differences between the varieties in this study with regard to expression of both CI and MI. For CI: Zinfandel had low values; Sangiovese, Pinot Noir, Chardonnay and Nebbiolo had moderate values; Cabernet Sauvignon and Shiraz had low to high values; and Sauvignon Blanc, Tempranillo and Merlot had moderate to high values. In the case of some varieties, notably Cabernet Sauvignon and Shiraz, both seasonal and regional variation was high. Interestingly, CI for Cabernet Sauvignon in the Hills was high but for Shiraz it was low. For MI: Pinot Noir and Tempranillo had low values; Cabernet Sauvignon, Shiraz, and Sauvignon Blanc had low to moderate values; Nebbiolo, Merlot and Sangiovese had moderate values; and Zinfandel had high

values. In the case of Zinfandel, this variety has a reputation for *millerandage*, and these data quantitatively support that qualitative observation.

Chicken numbers were relatively low, both in absolute terms and proportionally, for all varieties. There is a lack of data in previous papers and, furthermore, it is likely that chicken numbers have been over-estimated in many cases because they have been classified solely on the basis of size, for example, in papers by May (2000) and Friend and Trought (2007). Also, chicken berries contribute very little to bunch weight; for example, just 0.9% and 2.0% of total bunch weight for Cabernet Sauvignon and Chardonnay respectively. Furthermore, LGOs make up an even smaller proportion at less than 1% of total bunch weight. On average, an individual chicken berry weighs from 0.06 g to 0.15 g, and an LGO from 0.006 g to 0.009 g for Cabernet Sauvignon and Chardonnay. LGO numbers were much higher than chickens, both in absolute terms and proportionally. LGO (or 'shot berry') counts have rarely been documented in the past: one of the few exceptions is Friend and Trought (2007) who found that the proportion of LGOs ranged from 4 to 35% over three seasons for Merlot, which is comparable with our results. We strongly recommend that LGOs should not be included in counts of berry number per bunch because they do not fit the definition of a 'berry' (Sedgley and Griffin 1989). Furthermore, for winegrapes, LGOs do not contribute to the yield of juice per vine, and thus potential wine volume. For all varieties, seasonal variation of hen number was low compared with both chickens and LGOs. This suggests that the proportion of hens stays relatively constant from season to season whereas the ratio of chickens to LGOs is more variable.

In summary, we propose that varieties may be grouped on the basis of reproductive performance. For example:

Group A: low flower number, moderate set, low to moderate berry number (low to moderate CI and MI)
Sauvignon Blanc, Pinot Noir and Tempranillo

Group B: low to moderate flower number, moderate set, low to moderate berry number (moderate CI and low to high MI)
Chardonnay, Shiraz

Group C: moderate flower number, low set, low to moderate berry number (low to high CI and MI)
Cabernet Sauvignon, Merlot

Group D: moderate flower number, moderate set, moderate berry number (moderate CI and MI)
Nebbiolo

Group E: high flower number, high set, high berry number (low to moderate CI and moderate to high MI)
Sangiovese, Zinfandel

From the point of view of our research, it was unfortunate that, for the regions and the four seasons studied, there was not a repetition of the climatic events that occurred during the spring of 2000 that were associated with the poor set of that season. In that season, lower than average temperature conditions occurred in most SA regions during the flowering and set period. In fact, it was the experiences of that season which was partly responsible for the creation of our project.

Preliminary investigation of the temperature data collected during the four seasons has indicated that, for most varieties, there was not an apparent relationship between the temperature regime during the flowering/set period and the reproductive performance at a particular site. For this reason, much more detailed analysis of our data set will be necessary in order to attempt to explain, for some varieties, why certain sites consistently had lower set than others, irrespective of season. For example, for Cabernet Sauvignon, why did the McLaren Vale sites consistently have better set than those at Padthaway? For Shiraz, why did the Balhannah and Lobethal sites in the Adelaide Hills consistently have better set than those at the Seaview sites at McLaren Vale? In a few cases, there does appear to have been a seasonal effect: for example, for Merlot at the single site at McLaren Vale, the 2007/08 season had higher set than the other three seasons. However, in this particular case, it seems that temperature difference is not the explanation. At the time of writing this report, this detailed analysis had not been completed due to a lack of funding.

4. Phenology and prediction of flowering time.

4.1 Introduction

Models which relate crop development to readily-available weather data are useful management and research tools (Nix 1981). Such models may be used for management of environmental conditions (Buchanan et al. 1979). Research has indicated that cold and rainy weather may inhibit flowers opening, especially at temperatures below 15°C - 17°C (May 2004). Ideal conditions for flowering are when humidity is low and temperatures and sunlight are high and therefore thought to aid cap fall. The influence of sunlight is not fully understood but radiant heating may cause a rise in temperature and therefore aid in the flowering process (May, 2004).

To better understand the effects that different weather conditions have on the flowering process we investigated the timing of phenological growth stages for different varieties in the three different regions in which we assessed the effect of site and season on reproductive performance (Chapter 3). While other studies have looked at heat degree days in terms of harvest and ripening, our studies focused on the period from budburst to flowering. Some of the questions we have aimed to answer are: do varieties grown in Australia vary greatly in the number of heat degree days required for flowering? how do the number of days between bud burst and flowering relate to the accumulated heat degree days for the same period? does a shorter period from budburst and flowering equate to higher or lower fruitset?

A simpler method for predicting flowering time may be to use an early flowering variety as an indicator. This may be particularly useful for vineyards where cultural practices are regularly applied before flowering to control/improve fruitset. We investigated the use of early flowering Ganzin Glory vines for this purpose.

4.2 Materials and Methods

4.2.1. Phenological data

Using the modified Eichorn-Lorenz system (Coombe 1995) phenological growth stages were recorded fornightly at all the sites described in Table 3.1. Phenological data was collected from ten different varieties: Cabernet Sauvignon, Shiraz, Sauvignon Blanc, Pinot Noir, Merlot, Chardonnay, Tempranillo, Nebbiolo, Sangiovese, and Zinfandel. Degree days (or degree hours) have been adopted by viticulturists to indicate the heat requirements of specific developmental stages. A base

temperature of 10°C has been generally accepted for grapevines (Winkler et al. 1974; William et al. 1985) and has been adopted so far in this study.

4.2.2. Ganzin Glory Vines

Ganzin Glory (ARG9) was grafted to a number of different vines and varieties in the vineyards where we were collecting phenological data (McLaren Vale and the Adelaide Hills). T-bud grafts were used in December 2004 and are shown in Figure 4.1 below. Phenology was recorded fortnightly from budburst through to flowering for all vines grafted with Ganzin Glory. These assessments were then related to phenological data collected from the corresponding varieties and sites.



Figure 4.1. Ganzin Glory (ARG9) grafted onto Chardonnay vines, McLaren Vale, South Australia; (a) T-bud graft of Ganzin Glory to Chardonnay, December 2004 (b) budburst, August 2005 (c) before flowering, October 2005.

4.3 Results

4.3.1. Accumulated heat degree days

Using the data collected in the experiment described in chapter 3, accumulated heat degree days were calculated from budburst to flowering. This information tells us what the heat requirement is for different varieties in different regions and therefore allows us to better predict flowering time. An example of these data for Cabernet Sauvignon in the Adelaide Hills has been included. Note: we were also able to use data collected by the vineyard owner to add to our data set. For Cabernet Sauvignon vines in the Adelaide Hills the number of accumulated heat degree days from bud burst to flowering ranged from 220 to 245 (Table 4.1). The number of days from bud burst to flowering varied from season to season as well as percentage fruitset (Table 4.1).

Table 4.1 Example of the number of accumulated heat degree days and the number of days from bud burst to flowering between budburst and flowering for Cabernet Sauvignon in the Adelaide Hills from 2001-2006.

Year	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Accumulated heat degree days from budburst to flowering	220	230	241	225	245	243
Number of days from budburst to flowering	84	60	59	67	64	42
% Fruitset	n/a	n/a	n/a	34.1	41.4	29.4

4.3.2. Ganzin Glory vines

On average Ganzin Glory vines flowered two weeks earlier than Chardonnay and three - four weeks earlier than other varieties such as Shiraz and Cabernet Sauvignon, regardless of whether the season was considered "poor" or "good" in terms of fruitset (Table 4.2). Ganzin Glory vines consistently flowered earlier over three seasons in all locations.

Table 4.2 Flowering dates for Ganzin Glory vines grafted to Chardonnay, Shiraz and Cabernet Sauvignon vines and for the same varieties without grafting; 2005-2007.

Variety and Location	**Flowering Date**		
Year	**2005**	**2006**	**2007**
Ganzin Glory, McLaren Vale	4-Oct	25-Sep	2-Oct
Chardonnay, McLaren Vale	23-Oct	9-Oct	17-Oct
Ganzin Glory, McLaren Vale	5-Oct	27-Oct	3-Oct
Shiraz, McLaren Vale	2-Nov	23-Oct	10-Nov
Ganzin Glory, Adelaide Hills	28-Oct	3-Nov	n/a
Cabernet Sauvignon, Adelaide Hills	24-Nov	24-Nov	n/a
Ganzin Glory, Waite Campus	8-Oct	22-Sep	1-Oct
Chardonnay, Waite Campus	28-Oct	5-Oct	15-Oct

4.4 Discussion/Conclusions

The number of accumulated heat degree days from flowering to fruitset was consistent for all the varieties assessed in this study. The number of days between budburst and flowering varied

between seasons and was closely related to fruit set. When fruitset was relatively “poor” in susceptible varieties such as Cabernet Sauvignon and Merlot, the number of days from budburst and flowering was significantly greater than the average. The increase in number of days during this period is an indication of lower than average temperatures. Furthermore, when the length of this period was shorter than the average (associated with higher temperatures), fruitset of Cabernet Sauvignon was also reduced.

While the use of accumulated heat degree days to predict flowering time appears to be reliable, as an indicator the use of Ganzin Glory may be a valuable, simple and inexpensive tool for growers who regularly need to apply a cultural practice to improve fruitset.

These data are currently being used to develop a model that will improve our understanding of the relationship between phenology and climatic events, and the weather during this critical stage of reproductive development. These results will form the basis for a publication that will be submitted to the Australian Journal of Grape and Wine Research. We are also preparing an industry publication to outline how the ten different varieties varied in phenological development over a four year period.

5. Response to Cultural Practices

5.1 Introduction

Cultural practices have been used to improve fruitset of grapevines since the nineteenth century (May 2004). They include late pruning, tipping (also known as pinching) and topping of shoots, cincturing and spray application of growth regulators. Tipping was defined by Coombe (1959) as removal of the apical 8 cm or less of shoot whereas topping is removal of 15 cm or more. Tipping is normally a manual operation whereas topping can be mechanised, particularly where canopies have vertically positioned shoots, e.g. a VSP trellis.

Both tipping and topping are known to improve fruitset if applied during flowering, but experimental responses in the past have been varied (Coombe 1959, 1962, 1970; Koblet 1966; Brown et al. 1988; May 2004; Guerra 2006). CCC (Cycocel, 2-chlorethyltrimethyl-ammonium chloride) is a growth retardant that has been used in commercial viticulture to improve fruitset and yield. Bunch dips or canopy sprays have increased berry number per bunch by up to 20%. The optimal timing was 2 to 3 weeks prior to flowering.

The aim of this research was to investigate the separate effects of shoot topping and CCC foliar application on fruit set and other yield components of Chardonnay and Cabernet Sauvignon at different sites and in different seasons. Both varieties are known to have poor fruit set in some seasons, particularly in the Adelaide Hills region (Ebadi et al. 1995a, May 2004). Tempranillo was also included as a shoot topping experiment because it is said to have poor set; however, to our knowledge, this has not been verified in Australia.

5.2 Materials and Methods

Field experiments were established in a commercial vineyard at Charleston (34.95 deg S, 138.81 deg E) in the Adelaide Hills region and in an experimental vineyard on the Waite Campus of the University of Adelaide (34.97 deg S, 138.63 deg E). The own-rooted Cabernet Sauvignon (clone G9V3), Chardonnay (clone I10V1) and Tempranillo (clone unknown) vines at both sites were cordon-trained and spur-pruned, trained using vertical shoot positioning (VSP) and drip irrigated. Row x vine spacing was 2.7 m x 1.5 m at Charleston and 3.0 m x 1.8 m at Waite. All vines were older than 6 years at the start of experimentation. The node number per vine was set at 45 to 50 for all experiments. Flowering of Chardonnay at the Waite site commenced in mid to late October (mean daily temperature in October is 15.7ºC) and flowering of all above varieties at the

Charleston site commenced from early to late November (mean daily temperature in November is 15.4ºC).

5.2.1. CCC experiment

This was conducted at Charleston on Cabernet Sauvignon for three seasons (2004/05, 2005/06, 2006/07). Either water (plus non-ionic wetter) (= 'Control') or chlormequat (2-chlorethyltrimethyl-ammonium chloride; Sigma Aldrich) (CCC) at a concentration of 77 g/L in water were thoroughly applied to the whole canopy with a hand-held sprayer with 2 L capacity and adjustable nozzle (Hills Garden Sprayer) at an approximate rate of 400 mL per vine. The treatments were applied to the same vines in each season at two different spray times based on phenological stages using the modified Eichorn-Lorenz system (Coombe 1995): approximately two weeks before flowering at E-L 16 (2/11/2004, 9/11/2005, 2/11/2006) and one week later (before flowering) at E-L 17 to 18 (9/11/2004, 16/11/2005, 9/11/2006). A randomised block design with 5 replicates per treatment and single vine plots was used.

5.2.2. Shoot topping experiments

Manual shoot topping was done so as to simulate a mechanical hedging operation: the distal 10-15 cm of each vertical shoot was removed on one occasion. Shoots were topped at particular phenological stages viz. pre-flowering (E-L 15 and E-L 17); start of flowering (E-L 19); 50% cap fall (E-L 23); capfall complete (E-L 26) and set (E-L 27). The same vines were used for each treatment in every season and shoot topping was compared with an untreated control. Table 5.1 shows the phenological stages and corresponding calendar dates at which shoot topping was applied for every variety/site/season combination. Because the Cabernet Sauvignon vines used at Charleston were removed by the vineyard owner in winter 2007, this experiment was moved to another block 500 m distant. The vines in this block were clone LC10 on own roots, planted in 1999 with 2.7 m x 1.8 m spacing, VSP trellis and pruned to 44 nodes per vine.

For all CCC or shoot topping experiments, three inflorescences per vine (= 'sample bunches') were selected at random before flowering and labelled for future data collection. Each 'sample bunch' was enclosed with a fine mesh bag secured with a plastic tie. After flowering was complete, the bag was removed and flower caps were counted in order to estimate number of flowers per bunch. This method does not influence fruitset (May 2000). Bunch yield components were assessed on each of the 'sample bunches' just before the time of commercial harvest. This included a count of seeded berries ('hens'), seedless berries ('chickens') and 'live green ovaries' (LGOs), and measurement of bunch weight and rachis weight.

Two way ANOVA analysis was performed using Genstat, version 10 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

Table 5.1. Shoot topping experiments: timing by phenological stage and corresponding calendar date.

Variety	Site	Season	E-L stages [1]					
			15	17	19	23	26	27
Chardonnay	Adelaide Hills	2004/05	27 Oct	9 Nov	23 Nov	-	2 Dec	10 Dec
Chardonnay	Adelaide Hills	2005/06	-	17 Nov	21 Nov	23 Nov	27 Nov	5 Dec
Chardonnay	Adelaide Hills	2006/07	-	2 Nov	7 Nov	12 Nov	18 Nov	21 Nov
Chardonnay	Waite	2004/05	-	15 Oct	18 Oct	27 Oct	10 Nov	15 Nov
Chardonnay	Waite	2005/06	-	12 Oct	24 Oct	30 Oct	4 Nov	11 Nov
Cabernet Sauvignon	Adelaide Hills	2006/07	-	-	11 Nov	-	23 Nov	-
Cabernet Sauvignon	Adelaide Hills	2007/08	-	-	-	29 Nov	-	-
Tempranillo	Adelaide Hills	2005/06	-	17 Nov	27 Nov	5 Dec	9 Dec	13 Dec

[1] Coombe (1995)

5.3 Results

5.3.1. Effect of shoot topping

Chardonnay, Adelaide Hills

For this variety, fruitset varied across the three seasons: 2004/05 could be classified as 'moderate' for fruitset (50% for the control vines), 2005/06 as 'good' (62% for control) and 2006/07 as 'poor' (42% for control) in relative terms. Flower number per inflorescence was variable from season to season and ranged from 161 to 252 for the control. CI for controls was 2.2 in the 'good' season, 3.4 in 'poor' and 3.9 in 'moderate' and MI for controls was 2.9 in the 'good' season, 4.6 in 'poor' and 2.6 in 'moderate'.

Shoot topping applied at E-L 19 or later increased fruitset relative to the control in every season (Figure 5.1). The E-L 19 treatment had the highest fruitset in all seasons. The average increase for fruitset over three seasons for E-L 19 relative to the control was 26%, with the best response in the 'poor' season (30% increase), the least in the 'good' season (24% increase) and an intermediate response in the 'moderate' season (27% increase).

Shoot topping at all times decreased CI in the 'good' season but not in the other seasons: the average CI over three seasons for E-L 19 was 1.8 compared with 3.2 for the control. By comparison, shoot topping reduced MI in both 'moderate' and 'poor' seasons but not in the 'good' season: the average MI over three seasons for E-L 19 was 2.6 compared with 3.4 for the control.

Similarly, shoot topping increased yield relative to the control in both the 'moderate' and 'poor' seasons, but not in the 'good' season (Figure 5.2). As for fruitset, shoot topping at E-L 19 produced the highest average yield increase relative to the control (38%): the response was best in the 'poor' season (68%), least in the 'good' season (16%) and intermediate in the 'moderate' season (41%).

The main driver of yield was bunch weight because bunch number was not significantly affected by shoot topping. Bunches of E-L 19 vines were heavier than controls in all seasons: 12% more in the 'good' season, 41% in the 'moderate' and 48% in the 'poor' (with an average of 25% over the three seasons). There was no significant treatment effect on flower number, total berry number per bunch, berry weight or rachis weight.

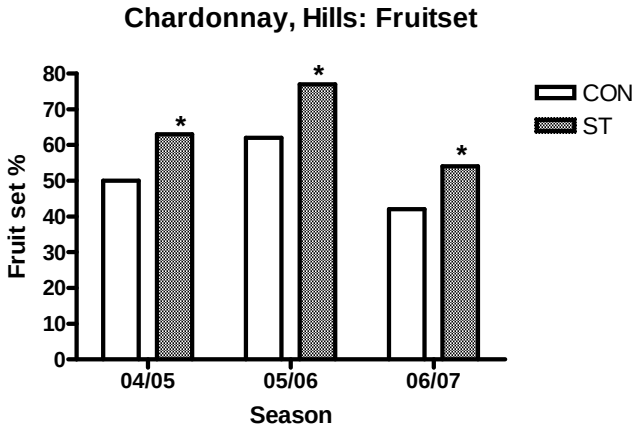


Figure 5.1. Effect of shoot topping (at EL19) on fruitset of Chardonnay, Adelaide Hills: 2004/05, 2005/06 and 2006/07. * indicates treatment is significantly different to control in that season ($p<0.05$).

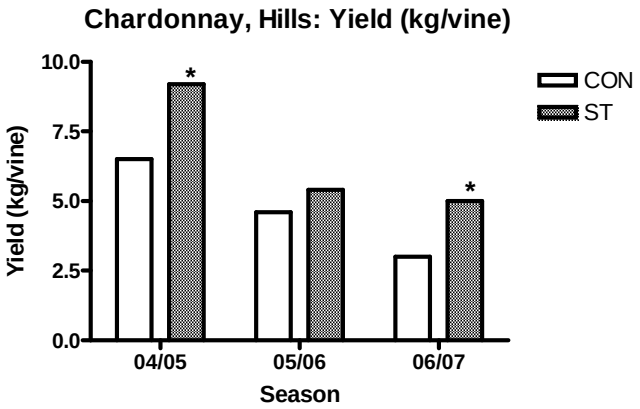


Figure 5.2. Effect of shoot topping (at EL19) on yield (kg/vine) of Chardonnay, Adelaide Hills: 2004/05, 2005/06 and 2006/07. * indicates treatment is significantly different to control in that season ($p<0.05$).

Chardonnay, Waite

At this site, fruitset was relatively good in both seasons: for example, fruitset of control vines was 74% in 2004/05 and 58% in 2005/06. Also CI and MI were relatively low in both seasons.

There was no effect of shoot topping on fruitset, CI or MI in the first season (Figure 5.3). However, in the second season, shoot topping at E-L 23 increased fruitset and decreased CI relative to the control, but MI was only reduced by shoot topping at E-L 27. For CI, E-L 23 averaged 1.0 over the two seasons compared with 2.4 for the control. In neither season was there a significant effect on flower number, hen number, berry weight or rachis weight.

E-L 23 was the only shoot topping treatment that significantly increased yield: a 45% increase relative to the control in the second season (Figure 5.4). This yield response was due to a combination of more bunches per vine (11% more than the control) and heavier bunches (30% more) due to increased fruitset and total berry number per bunch (32% and 56% respectively more than the control).

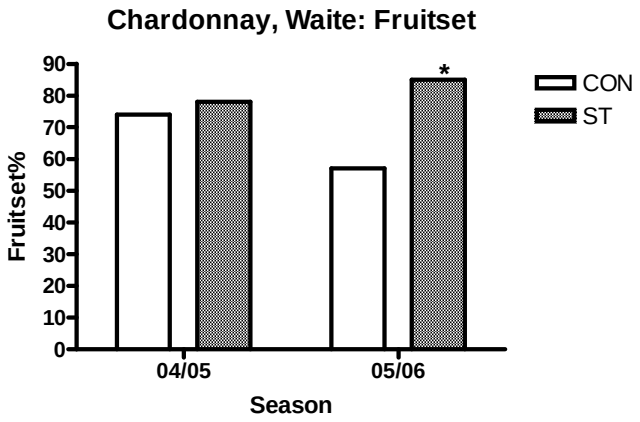


Figure 5.3. Effect of shoot topping (at EL 23) on fruitset of Chardonnay, Waite: 2004/05 and 2005/06. * indicates treatment is significantly different to control in that season ($p<0.05$).

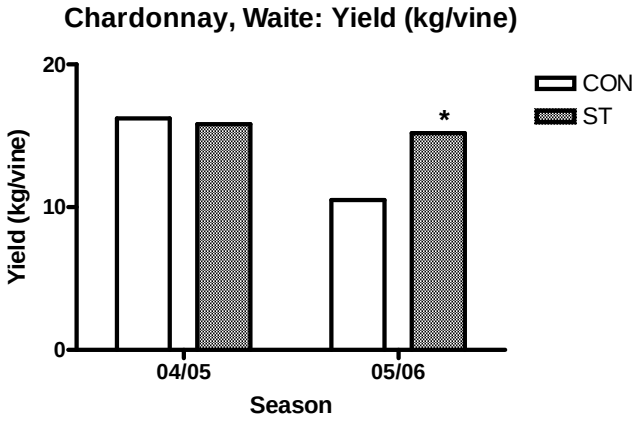


Figure 5.4. Effect of shoot topping (at EL 23) on yield (kg/vine) of Chardonnay, Waite: 2004/05 and 2005/06. * indicates treatment is significantly different to control in that season ($p<0.05$).

Cabernet Sauvignon, Adelaide Hills

The 2006/07 season was characterised by poor fruitset of Cabernet Sauvignon in the Adelaide Hills: fruitset of control vines was 28% and CI was relatively high at 6.9. Although fruitset in the following season was better (37% for control), it was still relatively poor, as is typical for this variety.

Shoot topping improved fruitset in both seasons, irrespective of the timing of the operation. For example, fruitset was 20% to 33% higher than the control in 2006/07; and 24% higher in 2007/08 (Figure 5.5). Both treatment times increased the number of hen berries and decreased the number of chickens in 2006/07; but there was no treatment effect in the following season. Better fruitset for E-L 26 in 2006/07 resulted in increased total berry number (47% increase) and bunch weight (46%

increase). Shoot topping decreased CI in both seasons, and MI in just the first season. There was no significant effect of treatment on flower number, LGO number, berry weight or pruning weight.

In 2006/07, shoot topping increased fruit yield per vine (48% and 97% relative to the control for E-L 19 and E-L 26 respectively) due to a combination of heavier bunches and more bunches per vine (21% and 35% relative to the control for E-L 19 and E-L 26 respectively) (Figure 5.6). However, there was no effect of shoot topping on berry number, bunch weight, bunch number or fruit yield in 2007/08.

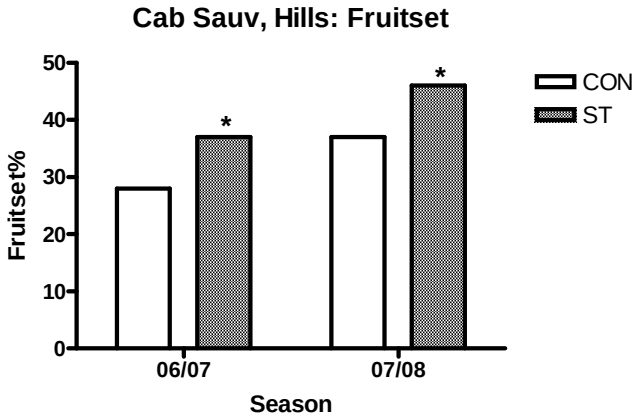


Figure 5.5. Effect of shoot topping (at EL 26 in 2006/07 and at EL 23 in 2007/08) on fruitset of Cabernet Sauvignon. * indicates treatment is significantly different to control in that season ($p<0.05$).

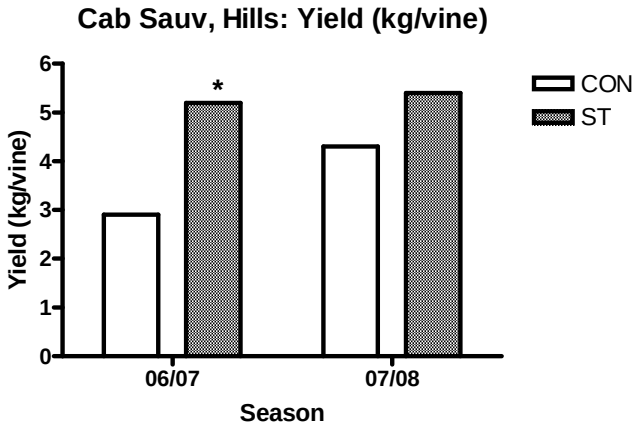


Figure 5.6. Effect of shoot topping (at EL 26 in 2006/07 and at EL 23 in 2007/08) on yield (kg/vine) of Cabernet Sauvignon. * indicates treatment is significantly different to control in that season ($p<0.05$).

Tempranillo, Adelaide Hills

Shoot topping was trialled on this variety because growers in the Adelaide Hills have claimed that its characteristically low berry number per bunch is a consequence of poor fruitset. Yield components were measured in both 2004/05 and 2005/06 and shoot topping was applied only in the second season.

Control vines had much lower yield in the second season than the first (76% reduction) due to a combination of fewer bunches per vine, and lower bunch weight. The latter was mainly due to fewer flowers per inflorescence (by 51%) because there were no seasonal differences in fruitset. Therefore, yield variability of Tempranillo may be more a consequence of variation in both flower number and inflorescence number from season to season, rather than any major variation in fruitset.

Shoot topping increased fruitset and decreased CI if applied at E-L 19 or later. The best response was achieved at E-L 23 with 57% increase in fruitset relative to the control. There was no significant effect of treatment on flower number, berry weight, bunch number or hen number. All shoot topping treatments applied later than E-L 17 increased total berry number per bunch and bunch weight: the best response was achieved at E-L 19 with increases of 101% and 67% respectively relative to the control. Also, shoot topping at E-L 19 and E-L 23 increased the number of chickens, the number of LGOs and MI.

Yield increased in response to shoot topping, mainly as a result of the increase in bunch weight: the best response was achieved at E-L 26 with an increase of 84% relative to the control.

5.3.2. Effect of CCC

Cabernet Sauvignon consistently has poor fruitset in the Adelaide Hills: fruitset of control vines ranged from 25% to 34% over the three seasons of experimentation, and bunches were loose with a high proportion of LGOs. CI was high (from 5.5 to 6.1) and MI was moderate to high (from 2.9 to 4.9).

Application of a CCC foliar spray one week before flowering (= ‘1 week’) increased fruitset relative to the control in all three seasons whereas the application at two weeks before flowering (= ‘2 weeks’) did so only in the final season (Figure 5.7). Overall, 1 week treatment produced better fruitset than the 2 weeks: the average increase relative to the control over the 3 seasons was 58% and 39% for 1 week and 2 weeks respectively.

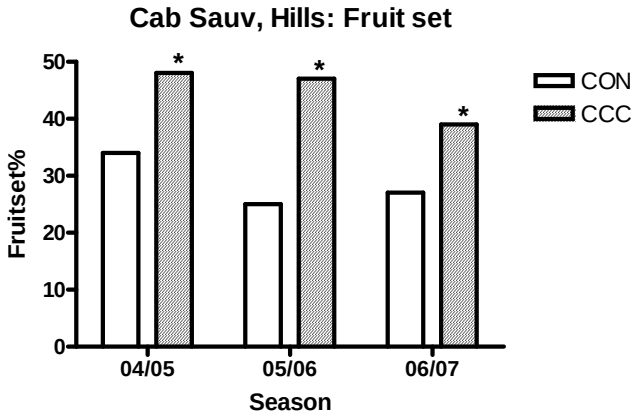


Figure 5.7. Effect of CCC application (at one week before flowering) on fruitset of Cabernet Sauvignon: 2004/05, 2005/06 and 2006/07. * indicates treatment is significantly different to control in that season ($p<0.05$).

Similarly the 1 week treatment reduced CI in the first two seasons, averaging 4.0 over three seasons compared with 5.9 and 5.2 for control and 2 weeks respectively. CCC application at either time reduced MI in the final season, averaging 3.7, 2.7 and 3.1 over three seasons for control, 2 weeks and 1 week respectively.

Although both CCC treatments increased total berry number per bunch relative to the control (average of 25% and 39% for 2 weeks and 1 week respectively), this was significant only in 2004/05. This was due to an increased number of hen berries (average of 28% and 37% for 2 weeks and 1 week respectively) because there was no effect of treatment on the relatively low number of chicken berries. LGOs were much more plentiful than chicken berries in every season.

There was no significant effect of CCC treatment on flower number, rachis weight, berry weight or bunch weight. The response of pruning weight to CCC was not consistent over the three seasons. There was no significant effect of CCC treatment on bunch number per vine except in the final season (2006/07) when the 1 week treatment had 26% more bunches than the control.

On average, fruit yield per vine averaged 23% and 26% higher than the control for 2 weeks and 1 week respectively, but for each CCC treatment this was significant in only one out of three seasons (Figure 5.8).

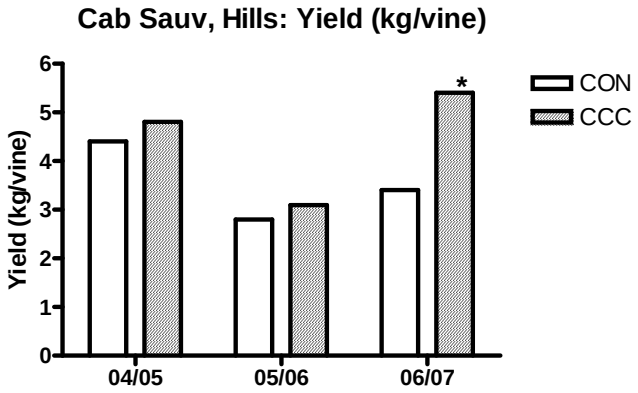


Figure 5.8. Effect of CCC application (at one week before flowering) on yield (kg/vine) of Cabernet Sauvignon: 2004/05, 2005/06 and 2006/07. * indicates treatment is significantly different to control in that season ($p<0.05$).

5.4 Discussion

Response to shoot topping application

All varieties at both locations responded to shoot topping to some degree. For fruitset, the magnitude of the improvement ranged from 20% to 57% relative to the control. The best result was achieved for Waite Chardonnay (in the second season) and Tempranillo with an increase of approximately 50% relative to the control. For yield per vine, the magnitude of the improvement ranged from 16% to 97% relative to the control. This is consistent with Guerra (2006) who reported that yield of Malbec increased by up to 84% in response to shoot tipping.

In terms of the timing of the operation, depending on variety and location, any time between the start of flowering (E-L 19) and end of flowering (E-L 26) is likely to achieve a positive response. For Chardonnay, E-L 19 timing was consistently best at the Adelaide Hills site whereas slightly later at E-L 23 was best for the Waite. In reality, the time between these two stages is relatively short (mean of 8 and 4 days for Waite and Adelaide Hills respectively), and for commercial application it may be necessary to span these two stages.

Shoot topping had no significant effect on flower number per inflorescence for any variety in the season in which shoot topping was applied. The magnitude of the improvement in berry number per bunch ranged from 17 to 101% in our study. This is greater than that reported in the literature; for example, Coombe (1959, 1962, 1970) reported 10 to 30% increase for a range of varieties that are susceptible to poor fruitset. In our study, although there was generally a significant effect of shoot topping on fruitset, this was not necessarily the case for berry number, perhaps because berry

number is a function of both fruitset and initial flower number. Increased berry number in response to shoot topping was mainly due to increased hen number because chicken numbers were low, and as a result, their contribution to bunch weight was negligible. Shoot topping increased bunch weight, in agreement with Guerra (2006). For all varieties and sites, the increase relative to the control ranged from 12% to 67%, and this was mainly due to increased berry number.

In general, both CI and MI decreased in response to shoot topping. In the case of Adelaide Hills Chardonnay, the mean CI over three seasons for the E-L 19 treatment was 1.8 compared with 3.2 for the control, and mean MI was 2.6 compared with 3.4 for the control.

All varieties at all sites responded to shoot topping to some degree and the degree of response was very much dependent on the base level of fruitset which in turn is a function of both variety and the conditions during flowering and fruitset. For example, the yield response of Adelaide Hills Chardonnay ranged from 16 to 68%: shoot topping at E-L 19 produced the highest average yield increase relative to the control in every season and the response was best in the 'poor' season (68%), least in the 'good' season (16%) and intermediate in the 'moderate' season (41%). This confirms the view of May (2004) that topping may not be effective if applied under conditions that are not limiting to fruitset.

Response to CCC application

Cabernet Sauvignon consistently has poor fruitset in the Adelaide Hills and this was the case for each of the three seasons of our study: fruitset of control vines ranged from 25 to 34%, with associated high values of CI and moderate to high values of MI. The application of CCC as a foliar spray to Cabernet Sauvignon in the Adelaide Hills at 1 to 2 weeks prior to flowering (E-L 16 to 18) increased fruitset and consequently yield. The application at 1 week produced the best result in every season (average 58% increase relative to the control).

The improved fruitset of the 1 week treatment correlated with decreased values of both CI and MI. The former was due to an increased number of hens because there was no consistent effect on number of chickens or LGOs. The lower MI value was a function of an increased number of hens in real terms because there was no change in the number of chickens (which was low in every season).

Previous reports have indicated increases in berry number per bunch from 20% to almost 100% in response to foliar application of CCC (Coombe 1967, 1970; Brown et al. 1988). In our study the average increase over three seasons was 25% to 39%. The 1 week application produced a better result than the 2 weeks, and this is in agreement with Brown et al. (1988). However, it is important

to note that the 2 weeks treatment also produced commercially acceptable results, in agreement with Coombe (1967).

Yield increase in response to CCC averaged 23% and 26% for 2 weeks and 1 week applications, with no significant effect on berry weight. The best performance of any CCC treatment was in the final season when the 1 week treatment produced 59% higher yield than the control due to a combination of better fruitset (and heavier bunches due to more berries per bunch, mainly hens) and more bunches per vine.

5.5 Conclusions

This study investigated the effectiveness of shoot topping and CCC application on fruitset and other yield components with Cabernet Sauvignon, Chardonnay and Tempranillo. Treatments were applied before and during the flowering period. Fruitset and yield per vine increased in response to treatment, especially when shoot topping was applied between E-L stages 19 and E-L 23, and when CCC was applied one week before flowering. All varieties at both locations responded to shoot topping to some degree: for fruitset, the magnitude of the improvement ranged from 20% to 57% relative to the control, and from 16% to 97% for yield per vine. Fruitset and thus yield can be improved by cultural practices in all seasons; but the magnitude of the response is greatest in those seasons when fruitset is limited by climatic conditions or other factors. Neither berry number per bunch nor the visual appearances of the bunch are reliable indicators of fruitset performance. Optimal timing of these cultural practices is critical to maximise the response.

All varieties at both locations responded positively to shoot topping to some degree. Therefore, we recommend that shoot topping be applied every season on these varieties because it is likely to be cost-beneficial, particularly in ‘poor’ seasons. Shoot topping can be mechanised, does not require chemical input and the optimal timing is easily determined. With respect to the latter, there is also a window of opportunity of at least 4 days in the Adelaide Hills. In practice, a CCC foliar spray applied 7 to 10 days prior to flowering (E-L 17 to 18) would be good insurance for Cabernet Sauvignon grown in the Adelaide Hills and in other regions where poor fruitset is a common occurrence. CCC is registered for use on Cabernet Sauvignon in Australia.

6. Influence of cultural practices on fruit set, ovule morphology and pollen tube development

6.1 Introduction

Previous studies have shown that the main seasonally-variable determinants of yield are bunch number, berry number, and berry weight (Clingeleffer et al. 1997; Martin et al. 2000). Two critical stages in berry development (and hence determination of berry number) are pollination and fertilisation as they affect fruitset. Fruitset occurs immediately after fertilisation when rapid development of the ovary wall prevents the formation of an abscission layer. The number of flowers that set in grapevines is affected by many factors such as the environment and the availability of nutrients (Skene 1969; Coombe 1970, 1973b; Dunn 2003) and these have been described previously in this report. When poor fruitset is a problem, previous research has shown that cultural practices and the application of growth regulators can be used to increase fruitset in grapevines (Weaver et al. 1965; Skene 1969; Coombe 1970; Weaver 1972; Roubelakis and Kliewer 1976).

As described in Chapter 5, cultural practices such as shoot topping and 2(chloroethyl)trimethylammonium chloride (CCC) may be successfully used to control grapevine fruitset (Weaver et al. 1965; Skene 1969; Coombe 1970). CCC inhibits shoot growth and it has been suggested that organic nutrients are diverted from the shoot tip to the developing ovary (Roubelakis and Kliewer 1976).

For this study we also investigated the plant growth regulator (2-chloroethyl) phosphonic acid (ethephon). Ethephon is converted to ethylene and has been shown to cause flower and fruit abscission in grapevines (Weaver and Pool 1969; Mainland and Nesbitt 1974; Lane and Flora 1979; Phatak et al. 1980). However, a study by Mannini et al. (1981) reported that that pre-flowering and flowering applications of ethephon to the vegetative part of the vine resulted in promotion of fruitset. This study aimed to investigate the effectiveness of shoot topping and two different plant growth regulators on reproductive development and how this relates to abscission, fruitset and resulting berry development.

6.2 Materials and Methods

Cabernet Sauvignon, Chardonnay and Tempranillo vines in a vineyard at Charleston, South Australia were used for this study. Material was collected from the shoot topping (Chardonnay and

Tempranillo) and CCC (Cabernet Sauvignon) experiments described in Chapter 5. A further experiment on Cabernet Sauvignon vines was also established. Foliar spray applications of ethephon at concentrations of both 100mg/L and 50mg/L were applied 2 and 1 week/s before flowering and at the start of flowering. As in the other experiments described in Chapter 5, fruitset and bunch weights were measured for each of the treatments as a measure of reproductive performance. Twenty flowers from 5 inflorescences from each treatment were collected from all experiments during the flowering period to (a) assess the morphology of the ovaries and ovules and (b) assess pollen tube development.

6.2.1. Ovule Morphology

Ovary samples were fixed overnight in 3 % glutaraldehyde in 0.025M phosphate buffer, pH 7.2, for a minimum of 48h at 0-4ºC. After fixation samples were put through an alcohol dehydrations series: methoxy-ethanol, ethanol, propanol and butanol. Samples were left for a minimum of 2 h in each alcohol, then infiltrated overnight in a 1:1 mixture of butanol: glycol methacrylate (GMA). Over 4 days samples were then infiltrated with two changes of 100% GMA. Bud sections were then embedded in GMA in gelatine capsules and polymerised at 60ºC.

Embedded ovaries were trimmed and filed to expose the longitudinal sections (LS) for different PBN severity ratings. Sections 3-4 µm thick were made with an ultra-microtome (Reichert-Jung 2050 supercut) and stained with periodic acid-Schiff's reagent (PAS) and 0.5 % Toluidine blue O (TBO) in 50 mM sodium acetate, pH 4.5 (O'Brien and McCully, 1981). Sections were mounted using microscope slide media (Surgipath, Sub-X mounting medium) and examined using an Olympus BH2 light microscope and micrographs taken using a Nikon TE300 inverted Microscope at magnifications from 4X to 40X.

6.2.2. Pollen tube growth

Flowers were harvested, fixed in Carnoys fluid (absolute ethanol:chloroform: acetic acid, 6:3:1) for 24h, and stored in 70% ethanol at 48°C. Fixed flowers were hydrated through 50% and 30% ethanol to distilled water, 30 min for each, softened with 0.8.M NaOH for 6.h at room temperature, and washed in running water overnight. Ovules were stained with 0.1% aniline blue (Martin, 1959) in alkaline phosphate buffer (pH 11.5) for 1h, mounted in 80% glycerol, and observed under UV light using a Zeiss photomicroscope (Axiophot) equipped with a filter set of exciter filter 395-440, interference beam splitter FT 460, and barrier filter LP 470. The number of pollen tubes in the upper and lower style, and pollen tubes penetrating the ovules were recorded. Pollen was considered to have germinated if the pollen tube extended to a length equal to at least twice the diameter of the grain.

6.3 Results

As mentioned previously, CCC and shoot topping has been shown to improve fruitset which also corresponded with an increase in mean bunch weight. The reverse is observed when ethephon (which breaks down to ethylene) is applied at the start of flowering, one week before flowering and two weeks before flowering (Figures 6.1 and 6.2).

Using detailed sections with both staining and light microscopy techniques we assessed cell morphology in the ovaries and ovules of flowers that were either shoot topped, received a CCC application or an ethephon application. A significantly higher proportion of ovaries and ovules displayed “healthy” cell morphology when shoot topping and CCC was applied compared with those that had received either an ethephon treatment or were control vines. Those that received an ethephon treatment had significantly higher proportions of “deformed” ovules (Figure 6.3). Table 6.1 shows the proportions of healthy ovules in the different treatments applied.

Using fluorescence microscopy techniques we also assessed pollen tube growth in the style of ovaries and the number of ovules that were fertilised. A similar response in pollen tube development was observed with a significantly greater number of pollen tubes in the ovaries of inflorescences from vines that had been either shoot topped or treated with CCC compared with those treated with ethephon (Table 6.2). This also corresponded to an increase in the number of fertilised ovaries (Table 6.2).

Regardless of the number of pollen tubes in the upper style, the maximum found in the lower style was rarely more than two. The highest number was recorded for shoot topping applied at the start of flowering where up to three pollen tubes were observed. Ovule penetration by a pollen tube always occurred via the micropyle and generally in only one of the four ovules. The highest percentage of pistils that showed pollen tube penetration of the ovules occurred when CCC was applied one and two weeks before flowering and shoot topping was applied at the start of flowering (Table 6.2).

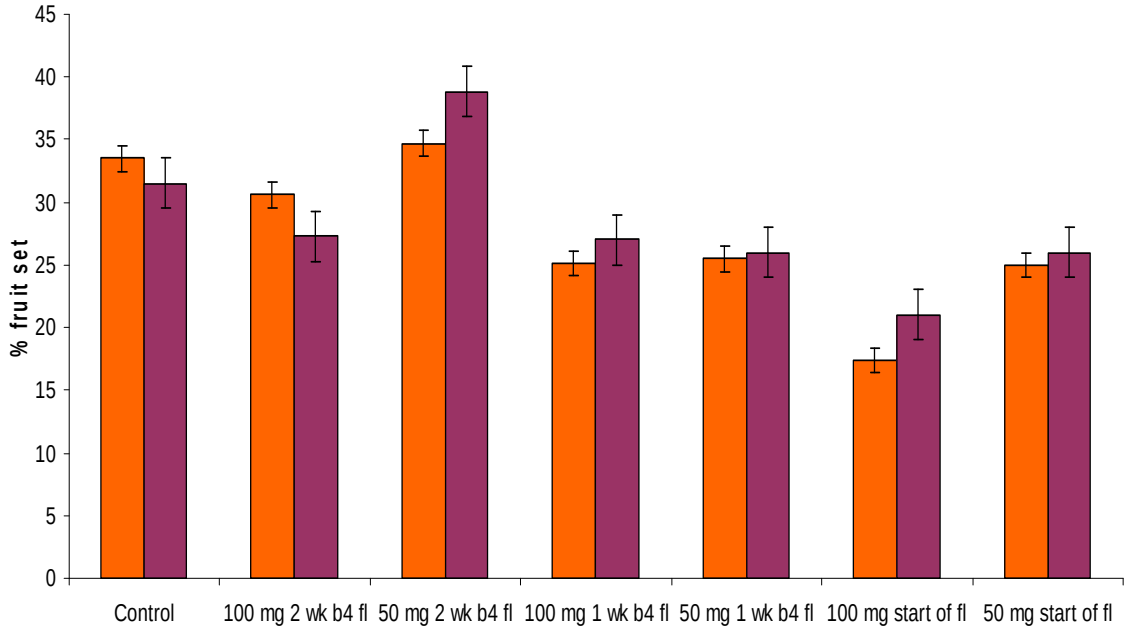


Figure 6.1. Mean percentage fruitset for Cabernet Sauvignon vines treated with different concentrations and application times of ethephon at Charleston, SA. Bars represent standard errors.

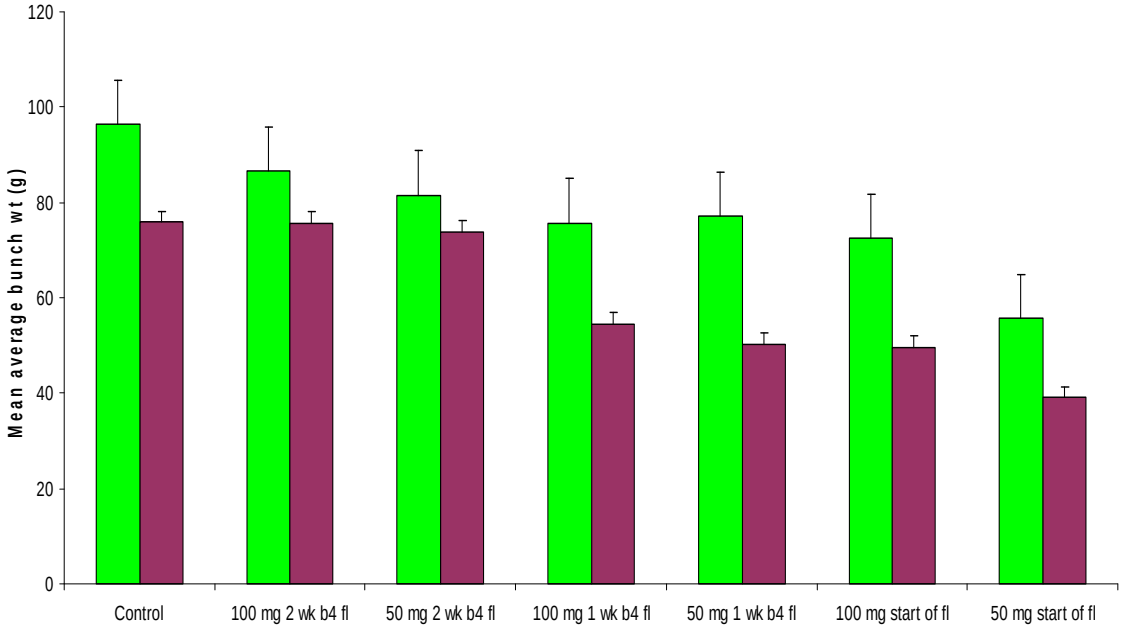


Figure 6.2. Mean bunch weight for Cabernet Sauvignon vines treated with different concentrations and application times of ethephon at Charleston, SA. Bars represent standard errors.

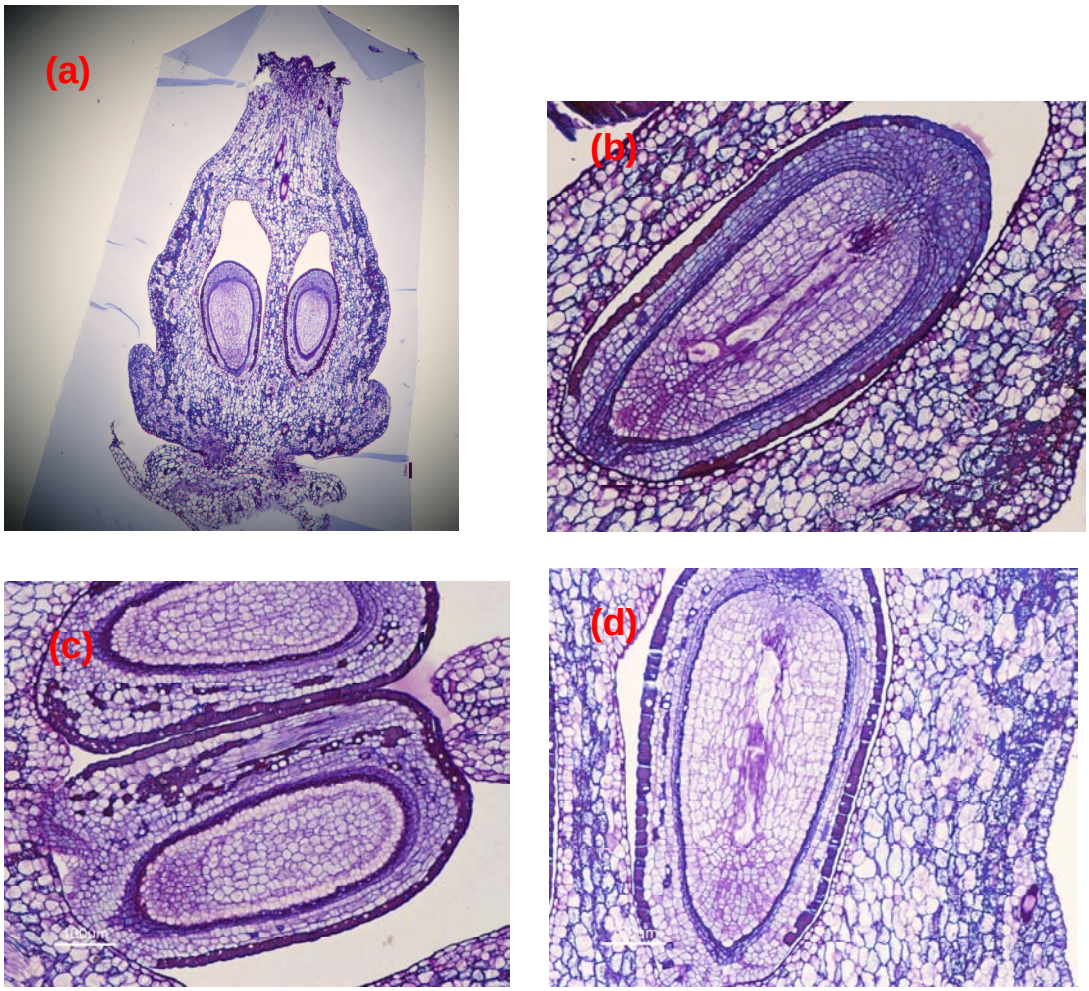


Figure 6.3. Longitudinal sections of ovaries and ovules of Cabernet Sauvignon vines: (a-b) examples of “healthy” ovaries and ovules and (c-d) examples of “deformed” ovules.

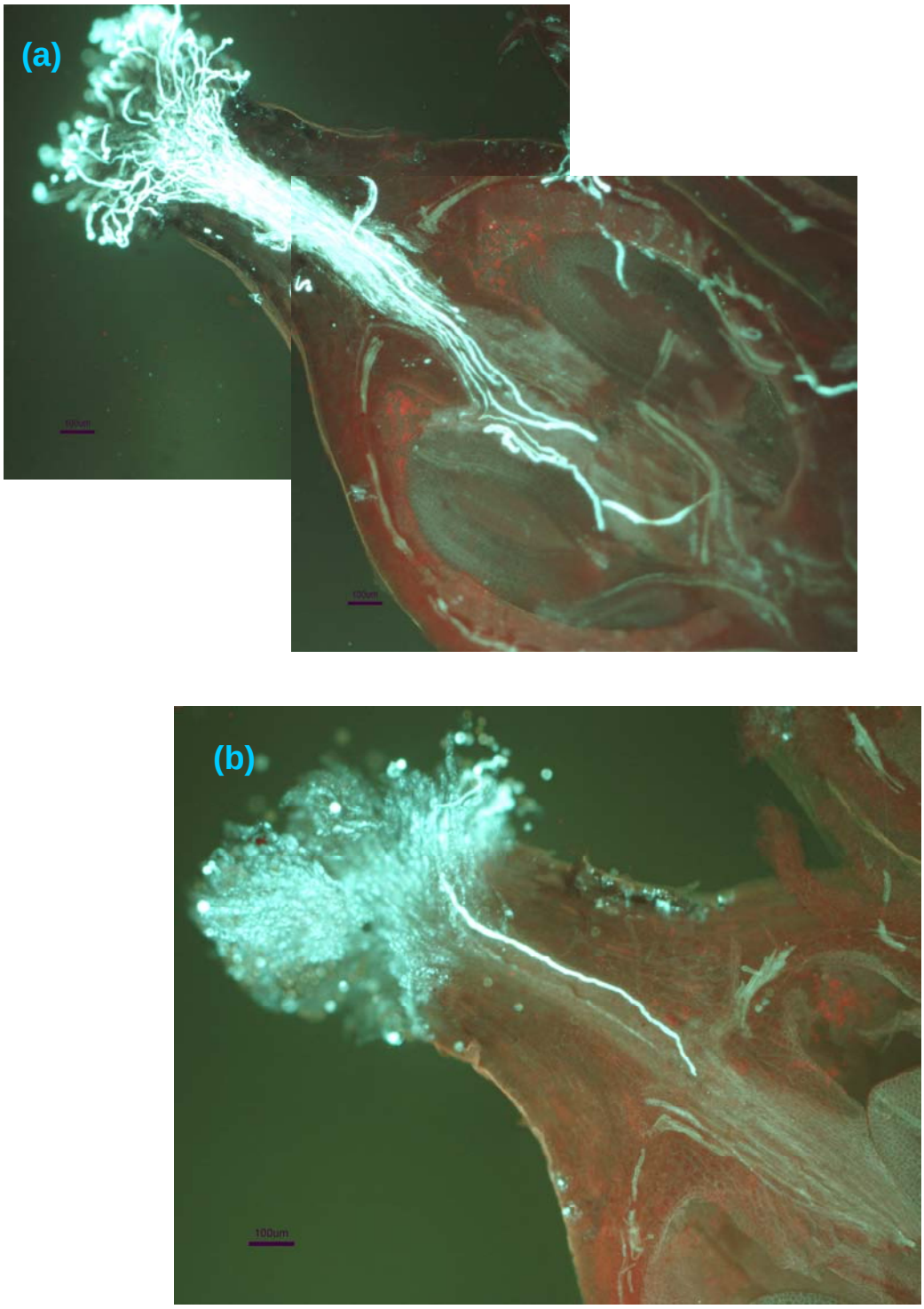


Figure 6.4. Pollen tube growth in the grapevine flowers of Cabernet Sauvignon vines sprayed with (a) CCC and (b) ethephon.

Table 6.1. Percentage of ovaries displaying healthy cell morphology in Chardonnay and Tempranillo vines that were shoot topped and in Cabernet Sauvignon vines that were sprayed with CCC or ethephon (± standard error).

Treatment and timing	% Healthy cell morphology
Shoot topping application - Chardonnay	
Control - no shoot topping	71.3 ± 1.21
Before flowering (EL Stage 17)	63.5 ± 2.34
Start of flowering (EL Stage 19)	83.1 ± 3.10
Shoot topping application - Tempranillo	
Control - no shoot topping	72.6 ± 2.15
Before flowering (EL Stage 17)	65.2 ± 3.45
Start of flowering (EL Stage 19)	82.9 ± 1.89
Chlormequat application	
Control - no chlormequat	78.4 ± 2.56
Two weeks before flowering	85.6 ± 2.98
One week before flowering	86.7 ± 3.58
Ethephon application	
Control - no ethephon	77.5 ± 3.67
Two weeks before flowering 100 mg/L	72.5 ± 4.15
Two weeks before flowering 50 mg/L	71.9 ± 2.57
One week before flowering 100 mg/L	65.2 ± 1.23
One week before flowering 50 mg/L	65.3 ± 2.57
Start of flowering 100 mg/L	55.3 ± 3.65
Start of flowering 50 mg/L	57.9 ± 4.26

Table 6.2. Average number of shallow and deep pollen tubes and fertilised ovules in Chardonnay and Tempranillo vines that were shoot topped and in Cabernet Sauvignon vines that were sprayed with CCC or ethephon (± standard error).

Treatment and timing	Pollen Tubes		Fertilised Ovules
	Shallow No.	Deep No.	
Shoot topping application - Chardonnay			
Control - no shoot topping	6.56 ± 0.57	2.90 ± 0.09	1.15 ± 0.05
Before flowering (EL Stage 17)	5.40 ± 0.45	2.10 ± 0.11	1.00 ± 0.07
Start of flowering (EL Stage 19)	7.89 ± 0.34	3.50 ± 0.24	1.56 ± 0.05
Shoot topping application - Tempranillo			
Control - no shoot topping	8.10 ± 0.12	2.30 ± 0.16	0.90 ± 0.09
Before flowering (EL Stage 17)	8.05 ± 0.27	2.10 ± 0.14	0.70 ± 0.04
Start of flowering (EL Stage 19)	9.40 ± 0.27	3.40 ± 0.21	1.35 ± 0.06
CCC application			
Control - no chlormequat	4.95 ± 0.41	1.65 ± 0.32	0.80 ± 0.06
Two weeks before flowering	6.50 ± 0.33	2.10 ± 0.12	1.00 ± 0.05
One week before flowering	7.30 ± 0.21	2.55 ± 0.23	1.10 ± 0.07
Ethephon application			
Control - no ethephon	5.01 ± 0.34	1.71 ± 0.14	0.83 ± 0.09
Two weeks before flowering 100 mg/L	5.15 ± 0.23	1.65 ± 0.17	0.85 ± 0.05
Two weeks before flowering 50 mg/L	6.89 ± 0.19	1.74 ± 0.16	0.83 ± 0.04
One week before flowering 100 mg/L	4.44 ± 0.32	1.54 ± 0.15	0.76 ± 0.08
One week before flowering 50 mg/L	4.32 ± 0.21	1.42 ± 0.18	0.67 ± 0.05
Start of flowering 100 mg/L	3.89 ± 0.12	1.37 ± 0.21	0.56 ± 0.06
Start of flowering 50 mg/L	3.92 ± 0.21	1.21 ± 0.12	0.23 ± 0.06

6.4 Discussion

Reduced fruitset appears to be associated with a slow rate of pollen tube growth and ovule fertilisation and/or organic nutrients to the ovules. The finding that relatively fewer ovules were fertilised when ethephon was applied one week before flowering and at the start of flowering may indicate that ethephon (which is converted to ethylene) inhibits pollen tube growth and/or ovule fertilisation. The pollen tube is a highly polarized, rapidly tip-growing cell (Hepler et al. 2001) and one of its limitations is the control of its growth rate and direction (Parton et al. 2001).

We know that the availability and allocation of resources may contribute to low fruit-to-flower ratios (Abbott 1985; Paton and Turner 1985; Lamont and Barrett 1988). Both CCC and shoot topping have been shown to promote fruitset. It has been suggested that both practices divert organic nutrients from shoot tips to developing ovaries. Our findings support this hypothesis as the number of fertilised ovules was higher than the controls when shoot topping was applied at the start of flowering and CCC was applied one and two weeks before flowering.

A higher proportion of ovaries from vines that had been shoot topped or sprayed with CCC also displayed “healthy” cell morphology. This corresponded with a higher percentage of fruitset than the controls. The opposite was found for ovaries from vines that were sprayed with ethephon where there was a higher proportion of ovaries with “deformed” cell morphology. Fruitset and the number of pollen tubes decreased compared with the control when ethephon was applied. Interestingly, it has been shown that ethephon increased flower abscission (Weaver and Pool 1969) and this may be linked to both ovary cell morphology and pollen tube growth, thereby influencing reproductive performance.

6.5 Conclusions

In conclusion, this study has shown that cultural practices can influence pollen tube growth, ovule fertilisation and ovule cell morphology in grapevines. We have also shown that the application of ethephon before and at the start of flowering decreased fruitset in Cabernet Sauvignon. This decrease in fruitset may be a result of an increase in flower abscission. Investigations into the impact of different plant growth regulators on floral development and set may assist in crop control and avoidance of crop loss. For this reason further research is required to understand the role that both the male and female reproductive organs play in reproductive performance.

Detailed findings will be outlined in a manuscript that is currently being prepared and will be submitted to the Australian Journal of Grape and Wine Research.

7. Summary of PhD thesis by Mardi Longbottom: The reproductive biology of grapevines — factors that affect flowering and fruitset

7.1 Molybdenum experiments

In Australia young Merlot vines sometimes suffer from vegetative disorders such as slow, zigzagged growth and leaf distortion. Merlot is also particularly known as a low- and inconsistent-yielding grape variety. Previous research showed that when foliar applications of molybdenum (Mo) were applied to Merlot vines the vegetative symptoms improved. More recently, when sodium molybdate was applied to Mo-deficient Merlot, yield improved; a function of increased bunch weight brought about by bigger berries. It has also been reported that at high concentrations, molybdenum might be detrimental to yield. Experiments were conducted on own-rooted Merlot (clone D3V14) vines in commercial vineyards in the Adelaide Hills (Hills) and at McLaren Vale, South Australia.

7.1.1. Effects of molybdenum deficiency on the vegetative growth and yield of *Vitis vinifera* cv. Merlot

The aims of the current study were to: a) elucidate the mechanism by which molybdenum affects yield of Merlot; b) to monitor the effects of Mo-treatment on the balance between vine reproductive and vegetative growth; c) to monitor the residual effects of Mo-treatment on growth and yield of Merlot and; d) to determine whether high concentrations of molybdenum are detrimental to yield.

Three rates of sodium molybdate were applied to vines in springtime (control = 0g, rate 1 = 0.101g and rate 2 = 0.202g sodium molybdate per vine). Vine molybdenum status was measured prior to treatment and again at flowering time using petiole, shoot tip and inflorescence analysis. The effects on vegetative growth were monitored at veraison, during dormancy and at budburst in the seasons following Mo-treatment. At flowering time, pollen vitality, pollen tube growth and flower structure were examined. Bunch number per vine, fruitset, berry weight and berry composition were measured at harvest.

In the Hills, the controls had adequate molybdenum however, at McLaren Vale petiolar molybdenum concentration fell within the suggested deficiency range of 0.05-0.09 mg/kg in the petioles at flowering time. No visual symptoms of Mo-deficiency were observed on the experimental vines. At McLaren Vale, Mo-treatment reduced pruning weight and improved vine balance. Mo-treated vines in the Hills and at McLaren Vale were affected by delayed budburst in

the season following Mo-treatment irrespective of their Mo-status. However, no seasonal carryover of molybdenum could be detected in tissue analysis at flowering time.

Juice total soluble solids, pH and titratable acidity were not affected by Mo-treatment at McLaren Vale or in the Hills. However, juice from Mo-treated vines in the Hills had a significantly higher concentration of molybdenum than the controls. At McLaren Vale there was no significant difference in juice molybdenum concentration between treatments.

In the Hills, yield was not affected by Mo-treatment. However, Mo-treated vines at McLaren Vale had significantly higher yields (approximately double) than the Mo-deficient controls. Bunch number per vine was not affected by Mo-treatment, either in the year that treatments were applied or in the following season. However, bunches from Mo-treated vines had significantly better fruitset resulting in more berries per bunch. Berry weight was affected by Mo-treatment in one season only. Yield was not detrimentally affected on vines that received the higher rate of sodium molybdate.

In the Hills, Mo-treatment did not affect pollen numbers, pollen vitality or pollen tube growth. At McLaren Vale, where the controls were Mo-deficient, pollen vitality was not affected by Mo-treatment. However, pollen tube growth was significantly enhanced by Mo-treatment. Significantly more pollen tubes penetrated the ovules from Mo-treated vines and a higher proportion of ovaries had at least one penetrated ovule. Structural observations revealed that a significantly higher proportion of ovules from Mo-deficient vines were defective. The absence of an embryo sac in those ovules is probably the cause of pollen tube growth inhibition and subsequent poor fruitset.

The concentration of molybdenum was higher in the petioles, shoot tips and inflorescences of Merlot at MELS 25 when the vines were sprayed with sodium molybdate at MELS 12. There were strong relationships between petiolar molybdenum concentration and the concentration of molybdenum in the shoot tips and the inflorescences. The concentration of molybdenum was significantly higher in the stamens than the pistils confirming that molybdenum was differentially partitioned to the male and female parts during flower development. At McLaren Vale foliar application of sodium molybdate in spring did not affect the concentration of molybdenum in the juice at harvest. However, in the Adelaide Hills, the concentration of molybdenum in berry juice was higher in Mo-treated vines than in controls. This may have implications for growers who spray vines with sodium molybdate as a precautionary measure where the vines may not be Mo-deficient.

Shoot tip analysis eliminates potential contamination from spray application and is therefore a useful tool for monitoring molybdenum in molybdenum treated vines. There was no quantifiable carry-over of molybdenum in the vines from season to season 7; however, Mo-treated vines displayed delayed budburst in the season after treatment. Vines treated with sodium molybdate in consecutive seasons had less molybdenum in the petioles than vines treated for the first time.

Treatment of Mo-deficient Merlot vines with sodium molybdate improved bunch weight. This was a function of improved fruitset, not increased mean berry weight as previously reported. Treatment of vines with adequate levels of molybdenum did not detrimentally affect yield nor did application of two times the standard rate of sodium molybdate. Molybdenum treatment did not affect bunch number per vine nor did it affect flower number per inflorescence in the season following treatment. Treatment of Mo-deficient Merlot vines with sodium molybdate did not have a cumulative effect on yield when applied in consecutive seasons. The critical level of molybdenum for optimal fruitset on own-rooted Merlot (clone D3V 1 4) is suggested to be 0. I mg/kg in the shoot tips at modified E-L stage 25 (80% flowering).

7.2.2. Effects of mode of pollination on yield of Merlot and the interacting effects of sodium molybdate sprays

Pollination experiments were conducted on field-grown own-rooted Merlot (clone D3V14) vines in commercial vineyards in the Adelaide Hills and at McLaren Vale in 2003-04 and in 2004-05. Inflorescences were supplied with supplementary Merlot pollen (self-pollination), with pollen from another variety (cross-pollination) or they were left to pollinate naturally (open pollination). In the Hills, mode of pollination did not affect fruitset or berry weight. In 2003-04 fruitset increased significantly at McLaren Vale when inflorescences were cross-pollinated with Semillon. Applying supplementary Merlot pollen also tended to improve fruitset, however none of the treatments affected berry weight. In 2004-05 there was no significant difference between treatments. These results indicate that Merlot may be a poor producer of pollen and may suffer from self-incompatibility.

Given the significant improvements in yield gained by spring foliar applications of sodium molybdate to Mo-deficient Merlot vines, in 2005-06 a reciprocal experiment was conducted to separate the effects of Mo-treatment and mode of pollination on the male and female flower parts. The aims of this experiment were to: a) determine whether the male or female reproductive organs are more important in determining the success of fruitset of Merlot and; b) determine which remedial measure, Mo-treatment or pollination, is more effective at overcoming poor fruitset. Supplementary pollination treatments—cross-pollination (Semillon); self-pollination (Mo-deficient

pollen); self-pollination (Mo-treated pollen) and; open-pollination—were applied to Mo-treated and Mo-deficient vines.

Cross-pollinating Mo-deficient vines with Semillon significantly improved fruitset of Merlot compared to other pollination treatments on those vines, however applying molybdenum to the vines in springtime was more effective at improving fruitset. Within the Mo-treated vines the effects of supplementary pollination on fruitset were not thought to be of any practical significance.

The results of this experiment provide further evidence that Mo-deficiency affects the female flower parts more than the male reproductive organs of Merlot.

7.2 The occurrence of 'star' flowers in Australia

In 2003 faulty flowers were discovered on Canada Muscat grown in the Coombe Vineyard at the University of Adelaide's Waite Campus. The Canada Muscat flowers opened from the top in 'star' formation in contrast to normal grape flowers, which shed the calyptra from its base. Star flowers were reported in French literature in the late 1800s. They were reported to as a symptom of a 'disease' that caused 'coulure', the cure for which was vine removal. The current report is the first known report of star flowers occurring in Australia.

The entire thesis has been lodged with the GWRDC.

8. Polyamines in Molybdenum Deficient Merlot Vines

8.1 Introduction

In plants, polyamines (PAs) are believed to be involved in several physiological processes including morphogenesis, rooting, flowering and senescence (Evans and Malmberg 1989). The role that PAs play in fruit development has not been fully elucidated; however, in many fruits, PA levels and their metabolism provide evidence of their involvement in the development and ripening of fruit. Polyamines are found at all the stages of the vegetative cycle of the grapevine and particularly during berry development (Lespy Labaylette et al. 1994).

A method for categorising fruit is on the basis of their PA content in the developing fruit. The first group, which includes the apple (Biasi et al. 1988) and strawberry (Ponappa and Miller 1996), have high PA levels in the early phases of development; and as development progresses the levels gradually decrease. The second group, which includes mandarin (Nathan et al. 1984) and cherimoya (Escribano and Merodio 1994), PA levels increase during the ripening process. The changes in PA levels may reflect features of fruit growth and development in each fruit species. Grapes belong to the first group, where free, conjugated and wall-bound forms of PAs accumulate mostly at anthesis before decreasing at fruit set (Geny et al. 1997).

8.1.1 The polyamine biosynthetic pathway

The PA biosynthesis pathway is complex and is regulated at transcriptional, post-transcriptional, translational and post-translational levels (Shantz and Pegg 1999). PAs are synthesized from amino acids through decarboxylations (Figure 1). There are two potential biosynthetic pathways for synthesis polyamines in plants, and whichever predominates depends on both the species and the stage of development within a given species (Bauza et al. 1995). Martin-Tanguy (1997) suggested that PAs that are formed via ornithine decarboxylase (ODC) pathway appear to have a role in floral development, whereas PAs derived from arginine decarboxylase (ADC) are mainly involved in vegetative development.

S-adenosylmethionine (SAM) is involved in both the biosynthesis of spermidine and spermine in the PA biosynthesis pathway, and also as the source of the plant hormone ethylene as it is a common precursor for both biosynthetic pathways. Ethylene is a well-known senescence inducer, whereas PAs have antisenescence activities. Therefore, the flow of carbon atoms through SAM

could control the developmental fate of the cell, tissue or organ involved. However, the interrelationship between PAs and ethylene may vary with species, type of tissue and the experimental system used (Wang et al. 1993).

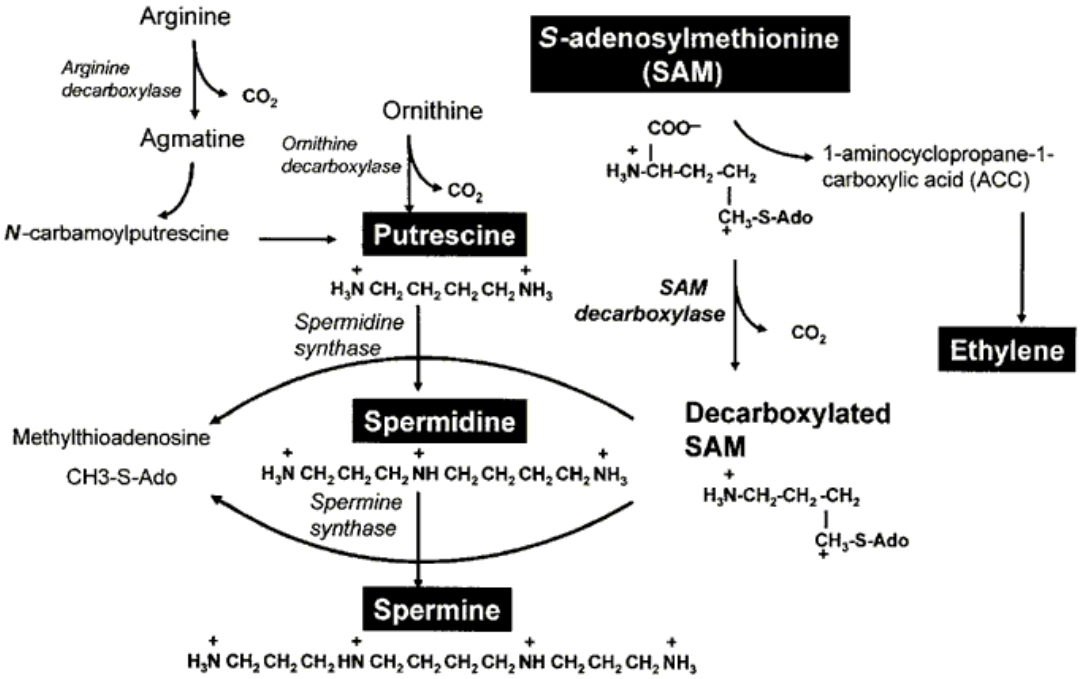


Figure 1. The Polyamine and Ethylene Biosynthesis Pathways (from Mehta et al. 2002).

8.1.2 Polyamines, Bound and Conjugated Forms

In nature, PAs often occur as free molecular bases, but they can also be associated with small molecules such as phenolic acids (conjugated forms) and with various macromolecules such as proteins (wall-bound forms). Under certain conditions these conjugates may constitute up to 90% of the total PA concentration of the cells (Galston and Kaur-Sawhney 1995).

In plants, little is known about the regulation of PA metabolism. Exogenously-applied PAs increase growth and retard softening in many fruit while inhibitors of PA synthesis treatment (D-arginine and DFMO) decrease free PAs and reduce firmness (Costa and Bagni 1983).

Upon floral initiation, conjugated PAs accumulate in shoot apices (Perdrizet and Prevost 1981). The application of exogenous PAs during fruitset resulted in an increase in fruitset and also an increase in the size of fruit which did set (Evans and Malmberg 1989). Conversely, maize mutants that lack conjugated PAs in the anthers are male sterile (Flores et al. 1989).

8.1.3 Ethylene and Polyamine Interactions

The interaction between PAs and ethylene appears to be crucial in controlling the regulation of abscission. Martin-Tanguy et al. (1993) showed that, in tobacco, inhibitors of ethylene production stimulated free and conjugated PA accumulation. PAs have also been shown to inhibit ethylene formation in several plant tissues, i.e. apple fruits, bean and tobacco leaf explants (Apelbaum et al. 1985).

8.1.4 Molybdenum

It has long been known that the rare transition element molybdenum is an essential micronutrient for plants, animals and micro-organisms (Bortels 1930). Robinson and Burne (2000) showed that Mo deficiency may be a factor associated with the "Merlot problem" of young vines and with poor fruitset of mature vines. For grapevines, a phenomenon in which the flowers set, but the resulting fruit fails to attain normal size, although seeds may occasionally form is known as *millerandage*. Ma et al. (1992) reported increased fruitset when sodium or ammonium molybdate were sprayed on vines at early flowering. They also found that the germination of pollen grains *in vitro* and pollen tube growth rate increased when Mo was added to the sucrose medium. Molybdenum uptake by plants is affected by soil, climatic and plant factors. The occurrence of Mo deficiency is often associated with acidic soils. Symptoms of Mo deficiency include symptoms associated with N deficiency; reduction and irregularities in leaf blade formation; local chlorosis and necrosis along the main vein of mature leaves as well as those described for the "Merlot problem", i.e. include stunted shoots with zigzag or distorted growth habit and small leaves.

Colin et al. (2002) showed that bunches with *millerandage* had the same PA content and composition as those at different stages of development. However, there were differences in relationships between wall-bound PAs, especially wall-bound diaminopropane (DAP), and arrested growth of berries. Berries from bunches with *millerandage* had higher wall-bound DAP content that normal berries during their development.

8.1.5 Grapes and Polyamines

Aziz et al. (2001) investigated the role that PAs play in the regulation of physiologically-induced fruitlet abscission in *Vitis vinifera* L., Pinot noir and Merlot. They found that abscission was higher in Merlot than in Pinot noir and that it was preceded by a decrease in free PA levels. Flower or fruitlet abscission is a response in plants to developmental or environmental cues and Aziz et al. (2001) suggested that PAs may play a regulatory role in flowering and initial fruitlet abscission.

8.1.6 Nitrogen

Flowering is a critical time for nitrogen supply as it is the period of development of the most intensive nitrogen uptake in grapevines (Löhnertz 1991). Coombe (1973a) was the first to demonstrate that under favourable climatic conditions fruitset in grapevines is determined primarily by the supply of organic nutrients in the inflorescences during and after anthesis and by genetic limitations. Mattoo et al. (2008) showed that transgenic tomatoes that had been transformed with a yeast SAM decarboxylase accumulated spermidine and spermine as N forms and as such had sensing and signalling responses similar to those of plants that had been treated with an exogenous addition of N.

8.2 Materials and Methods

8.2.1 Molybdenum Field Experiment

The experimental site was selected based on previous reports of Mo-deficiency occurring at a Hardy Wine Company vineyard. Two pre-flowering foliar sprays were applied to the vines, one at modified E-L stage 12 (shoots 10cm) and the second one week later. The molybdenum was applied in the form of sodium molybdate ($Na_2Mo_4.2H_2O$) at a rate of 0.101g per vine in deionised water, and delivered at a rate of 250 mL per vine. Control vines were sprayed with 250 mL deionised water per vine.

Berries were collected randomly from the experimental vines at specific flowering stages (listed in Table 8.1) and immediately frozen in liquid nitrogen.

Table 8.1. Grape flower and berry samples collected for analysis.

Date Tissue Collected	EL Stage	Description
2004-05		
03/12/2004	EL31	Berry Pea Sized
15/12/2004	EL32	Beginning Of Bunch Closure
22/12/2004	EL33	Berries Still Hard & Green
10/01/2005	EL34	Veraison
25/01/2005	EL35	Berries Beginning To Soften
07/02/2005	EL36	Berries Inter-med Brix Values
23/02/2005	EL38	Harvest Ripe
2006		
23/10/2006	EL19	Flowering Begins
02/11/2006	EL26	Flowering Ends
15/11/2006	EL27	Berry Set
30/11/2006	EL29	Berries Peppercorn Sized

8.2.2 Preparation of grape RNA and cDNA synthesis

Grape RNA was isolated essentially as described by Davies and Robinson (1996). cDNA was synthesised in 20 µl reactions from 1 µg of total RNA using the iScript cDNA synthesis kit (Bio-Rad) and the 3-end Oligo$(dT)_{20}$ primer mix according to the manufacturers instructions. cDNA reactions were diluted 10-fold before use in real time quantitative PCR analyses.

8.2.3 Quantitative real-time (qReal-Time) PCR analysis

Expression analysis of *VvACDR*, *VvACCO*, *VvELF*, *VvNitRed*, *VvODCR*, *VvSAMDC*, *VvSPDS*, *VvSPMS* genes were determined by qReal-Time PCR using an iCycler (BioRad). The expression of the genes was normalized to *VvELF*. The difference between the cycle threshold (Ct) of the target gene and the Ct of *VvELF*, $\Delta Ct = Ct_{Target} - Ct_{ELF}$, was used to obtain the normalized expression of target genes which corresponds to $2^{-\Delta Ct}$.

8.2.4 Polyamine extraction and analysis

Extraction of free, bound and pelleted polyamines was performed using a modification of the method described by Flores and Galston (1982) described in Soar et al. (2004). HPLC analysis

was on the Agilent 1100 Series HPLC machine (Agilent). Separation and quantification of the derivatized polyamines by HPLC was performed using 64% Methanol elution at 30oC with an Agilent ZORBAX Eclipse XDB-C18 column (4.6 X 150 mm, 5μM) (Agilent). Derivatized polyamines were detected using a UV-Vis detector at 254 nm.

8.3 Results

Table 8.2. Merlot bunch and berry analysis for molybdenum-treatment experimental trial for 2004-05 and 2006-07 seasons.

	# flowers/ inflorescence	# berries /bunch	# LGOs/ bunch	# Chicken berries/bunch	Fruit Set (%)	Coulure Index	Millerandage Index	Berry Weight (g)	Bunch Weight (g)
2004-05									
no Mo	351a	84a	113a	8a	24a	4.2	3.7	0.97a	83a
Mo treated	315a	121b	64b	8a	38b	3.9	3.0	1.05a	130b
2006-07									
no Mo	309a	82a	44a	4a	27a	5.9a	3.9a	0.93a	85a
Mo treated	359a	152b	64b	2a	43b	4.0b	2.9a	0.98a	156b

The flower number per inflorescence was not affected by Mo-treatment in either 2004-05 or 2006-07. However, there was a significant increase in the number of berries per bunch and hence percent fruitset with the Mo-treatment (Table 8.2).

There were no significant differences in berry weight between treatments, and there were no significant difference in the proportion of chicken berries per bunch between treatments. Chicken berries contributed less than 1% to the total berry weight per bunch for the treatment (Table 8.2).

There was a significant difference in bunch weights between the no treatment and the Mo-treatment for both seasons (Table 8.2).

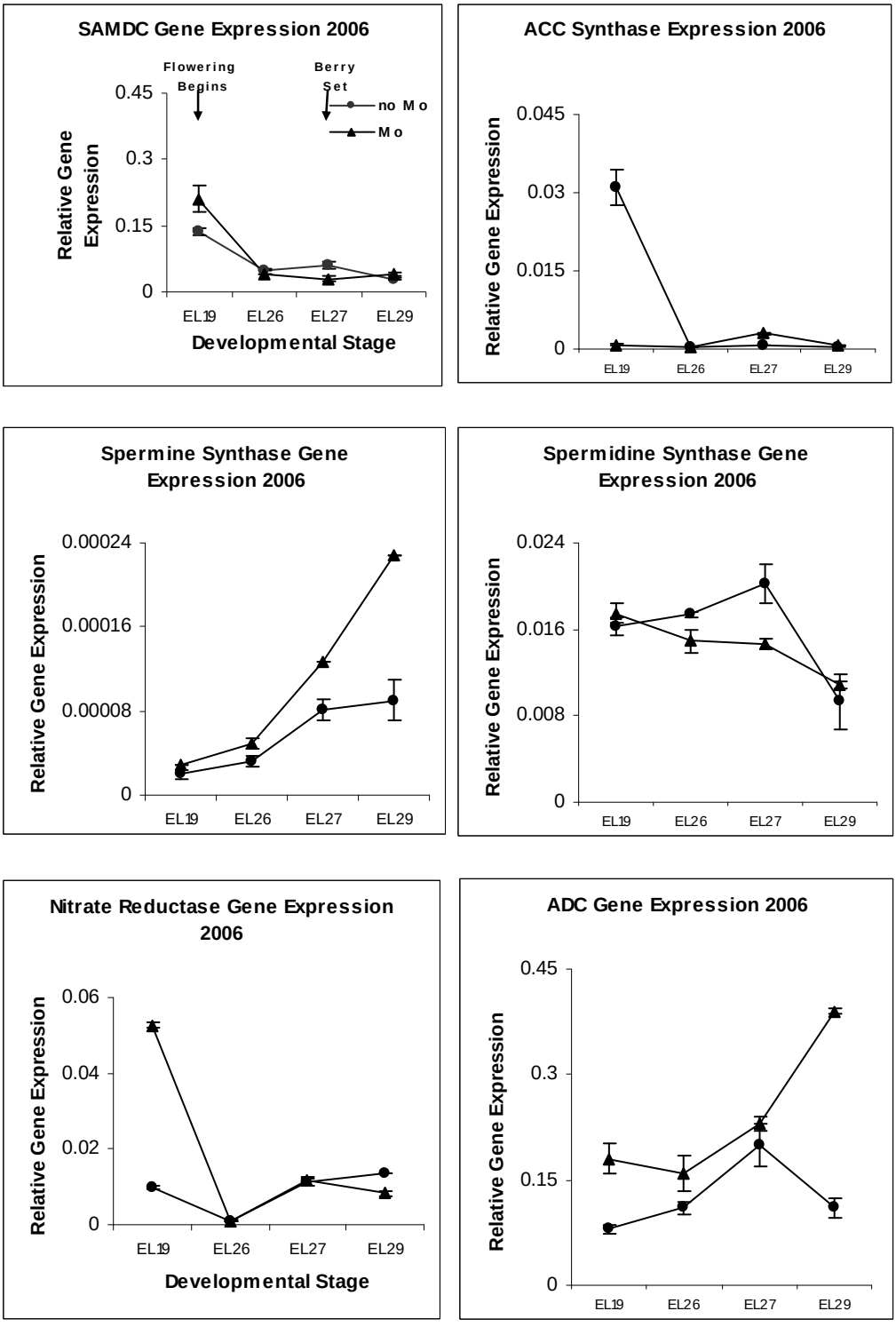


Figure 8.2. Real-Time PCR analysis of relative gene expression for selected *Vitis vinifera* L. Merlot genes in the 2004 flower and berry tissues from the molybdenum spray experiment.

The most striking difference between the two treatments is the large induction of ACC synthase in the Mo deficient flowers at the beginning of flowering, suggesting the synthesis of ethylene in these flowers. This, in conjunction with the higher levels of expression of ADC in the Mo-sprayed flowers at the same time point, as well as the higher levels of spermine synthase at all the time

points collected in the 2004 season, may indicate a higher level of polyamine biosynthesis gene expression, and an alteration in the polyamine:ethylene synergy (Figure 8.2).

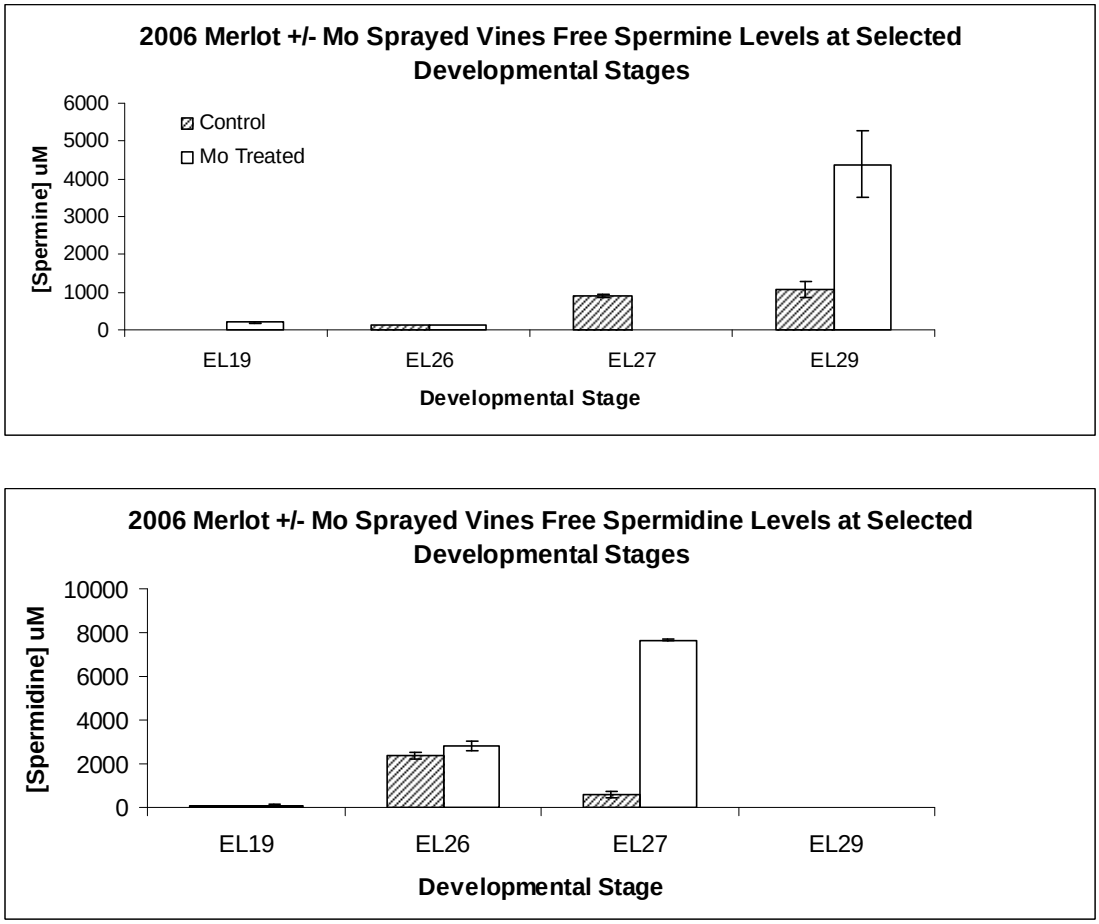


Figure 8.3. HPLC Analysis of selected free polyamine levels in the *Vitis vinifera* L. Merlot 2004 flower and berry tissues from the molybdenum spray experiment.

The molybdenum-treated vines had statistically higher levels of spermidine at two of the four flower and early berry sampling times for 2006 when compared with the molybedenum-deficient vines (at the other two time points, the levels were very low in both treatments). For spermidine, there was one time point, EL27, where the Mo-deficient vines had higher levels when compared with the Mo-treated vines; however, at the next time point, this was reversed and the Mo-treated vines had nearly fourfold higher levels (Figure 8.3).

8.4 Discussion

The increase in bunch weight on vines treated with a standard rate of molybdenum compared with the Mo-deficient controls is in agreement with the findings of Gridley (2003) and Williams et al.

(2004). In the current study, the increase in bunch weight was a result of a higher percent fruitset on bunches from Mo-treated vines. In both seasons, berries from Mo-deficient vines appeared uniform in size and development, and bunches had very few chicken berries. *Millerandage* refers to the condition where berries on bunches are arrested in development. This may include 'shot' berries (or LGOs) and chicken berries (Colin et al. 2002). In other studies (Gridley 2003; Williams et al. 2004), bunches from Mo-deficient vines had higher proportion of chicken berries than controls. This suggests that the ovaries were arrested in development at a later stage than in our study. The results here are in agreement with previous studies that reported that treatment of Mo-deficient Merlot vines with sodium molybdate can increase yield.

Longbottom and Dry (pers. comm.) showed that the application of sodium molybdate to Mo-deficient Merlot vines increased the number of penetrated ovules and frequency of ovaries with at least one penetrated ovule, as well as significantly improved pollen tube growth. This could be a significant contributor to the increase in fruitset.

The Real-Time PCR and HPLC analysis showed that the most striking differences between the two treatments were observed in the 2006 samples, which were taken from the EL 19 to EL29 stages (flowering begins to berries are 4mm in size). The 2004-05 sampling was not initiated until EL31 (berries pea-sized) and although there are some interesting results, it is the interaction between the ethylene and polyamine biosynthesis pathways in the floral developmental process that is most intriguing.

This increase in the expression of a gene in the ethylene biosynthetic pathway is a potential indicator of the mechanism which is responsible for the abscission of flowers in Mo-deficient Merlot vines, and the corresponding decrease in ACC synthase expression in the Mo sprayed vines is strong evidence that polyamine and ethylene levels are implicated in the restoration of "normal" berry development. Correspondingly, in the Mo-treated vines, the flowers sampled showed consistently higher levels of many of the polyamine biosynthesis genes when compared with the Mo-deficient control vines.

The significant induction of ACC synthase in the molybdenum-deficient flowers compared with the molybdenum-sprayed flowers indicates a potential shift in the ethylene/polyamine biosynthesis synergies. Other researchers have shown that, in tobacco, inhibitors of ethylene production stimulated free and conjugated PA accumulation (Martin-Tanguy et al. 1993). This is not surprising as it has been shown that PAs act as senescence inhibitors and ethylene as a senescence inducer and the interaction between PAs and ethylene appear to be crucial in controlling the regulation of abscission. Ruperti et al. (1998) published research in peach which showed increased

ethylene production during floral development resulted in increased fruitlet abscission. PAs have also been shown to inhibit ethylene formation in several plant tissues, i.e. apple fruits, bean and tobacco leaf explants (Apelbaum et al. 1984). This regulation may be due to metabolic competition for S-adenosylmethionine (SAM).

The application of exogenous PAs during fruit set resulted in an increase in fruit set and also an increase in the size of fruit which was set (Evans and Malmberg 1989).

The levels of free polyamines were found to be much higher in the flower and very early berry samples collected in 2006 compared with the later berry samples collected in 2004-05. This is in agreement with other researchers such as Baigorri et al. (2001) who showed that in Tempranillo the maximum level of total PAs were found at flowering in both the leaves and the flowers, as well as Geny et al. (2004) who showed that Cabernet Sauvignon berries after set had markedly decreased PA concentrations (similar to Chardonnay (Martin Tanguy et al. 1993) and Tempranillo (Baigorri et al. 2001)).

Flower or fruitlet abscission is a response in plants to developmental or environmental cues and PAs may play a regulatory role in flowering and initial fruitlet abscission (Aziz et al. 2001; Aziz 2003). Their studies investigated the role that PAs play in the regulation of physiologically-induced fruitlet abscission in *Vitis vinifera* L., Pinot noir and Merlot and showed that Merlot had low levels of PAs in its inflorescences and a high abscission rate compared with Pinot noir, which had higher PA levels and a lower rate of inflorescence abscission. They also demonstrated that the exogenous application of spermidine prior to anthesis resulted in an increase in both free and conjugated forms of both putrescine and spermidine and an inhibition in abscission. In contrast, the application of an inhibitor of the PA biosynthesis gene arginine decarboxylase caused a decrease in free PAs and an increase in floral abscission. Geny et al. (2002) demonstrated that the exogenous application of PAs increased the percentage of fertilisation in grape flowers.

9. The Presence of Extracellular Calcium Crystals in the Anthers of *Vitis vinifera.*

9.1 Introduction

To further our knowledge on the causes of poor fruit set, anther and pollen morphology was investigated by use of cryo-scanning electron microscopy. Flower samples were frozen and pollen, stamen and ovary morphology examined. While we did not see any differences in pollen, stamen and ovary morphology, we did observe the presence of calcium crystals in the stamens of the flowers.

Calcium oxalate (CaOx) is the most abundant and widespread insoluble mineral found in plants (Korth et al. 2006). It occurs as crystals of various size and morphology, and as intracellular or extracellular deposits (Franceschi and Nakata 2005). CaOx crystals are present in many higher plants (Frey-Wyssling 1935, 1981; Arnott and Pautard 1970; Franceschi and Horner 1980; Franceschi and Nakata 2005; Zindler-Frank 1987). The most common shapes of calcium oxalate crystals found in plants are druses, raphides, styloids, prisms and crystal sand (Franceschi and Horner 1980; Ilarslan et al. 1997).

While CaOx crystals are found commonly in long-living organs such as roots, stems and leaves, they are also present in floral organs such as stamens (Buss and Lersten 1972, Horner and Wagner 1980, 1992; Tilton and Horner 1980). Extracellular CaOx crystals mixed with pollen has been observed in other plant genera such as *Arum* (Hegelmaier 1871), *Calla* (Hegelmaier 1871), *Pinellia* (Hegelmaier 1871) and *Zantedeschia* (Hegelmaier 1871). CaOx crystals have been observed in tissue within anthers of higher plants (Schmid 1976; Horner and Wagner 1980; Meric and Dane 2004).

The physiological function of extracellular CaOx crystals still remains unknown. There is some evidence to support the hypothesis that CaOx can act in defence against chewing insects (Binns 1980; Ward et al. 1997; White 1997; Ruiz et al. 2002). Another hypothesis is that CaOx precipitation serves to sequester excess calcium and thereby remove it from active metabolism (Webb et al. 1995). CaOx has also been shown to bind toxic oxalate (Borchert 1984), play a role in salt stress and homeostasis (Hurkman and Taraka 1996) and the detoxification of heavy metals (Nakata 2003).

CaOx crystals have been reported in Vitaceae by some researchers (Metcalfe and Chalk 1950; Webb and Arnott 1982; Cody and Horner 1983; Cody and Cody 1987, Webb et al. 1995; Hardie et al. 1996; Arnott and Webb 2000; DeBolt et al. 2004, Collins et al. 2006). In *Vitis*, calcium oxalate crystals have been found to have raphide-like morphology (Webb et al. 1995). To date the presence of CaOx crystals in the anthers of grapevine flowers has not been reported. This study describes the appearance of CaOx crystals in the anthers of *Vitis vinifera*.

9.2 Materials and Methods

Plant material in this study was collected from several *Vitis vinifera* varieties in a vineyard located at the Waite Research Precinct, South Australia during November/December 2005 and 2006. Inflorescences were collected from the following varieties: Cabernet Sauvignon, Cabernet Franc, Riesling, Shiraz, Sauvignon Blanc, Merlot, Pinot Noir, Chardonnay, Zinfandel, Tempranillo, Emperor, and Blush Seedless. Ten inflorescences from vines for each different variety were randomly selected for analysis from each of the varieties selected for observation. Freshly opened flowers, whose anthers were both dehisced and not dehisced, were excised on an agar plate, and the anthers were then removed from the flowers. Specimens from flowers that had not opened were also collected for comparison.

For the observation and X-ray analysis in a Field Emission Scanning Electron Microscope (FE-SEM) (Philips, XL30), the pistil and anthers were excised from the flower and glued to the aluminium stub. Samples were dipped in liquid nitrogen and later coated in platinum. The aluminium stub was mounted on the cooling stage and the specimen was observed and analysed by a FE-SEM with an EDS detector (Adelaide Microscopy, The University of Adelaide).

9.3 Results

Crystals were observed in all anthers observed under the microscope. They varied in morphology and different types of crystals were observed next to each other. Crystal structures were not observed on other flower parts in any of the flowers collected for this study.

Using X-ray analysis we were able to confirm that the crystals were calcium oxalate (Figure 9.1). A number of different crystal types were observed and are shown below (Figure 9.2). The crystal types include raphides, druses, styloids, prisms and crystal sand. Not all were observed in each of the inflorescences from the different varieties used in this study. Table 9.1 illustrates crystal abundance, whether the crystals were present in the anthers and/or the ovary, at what stage of development the samples were taken and the type of crystals observed.

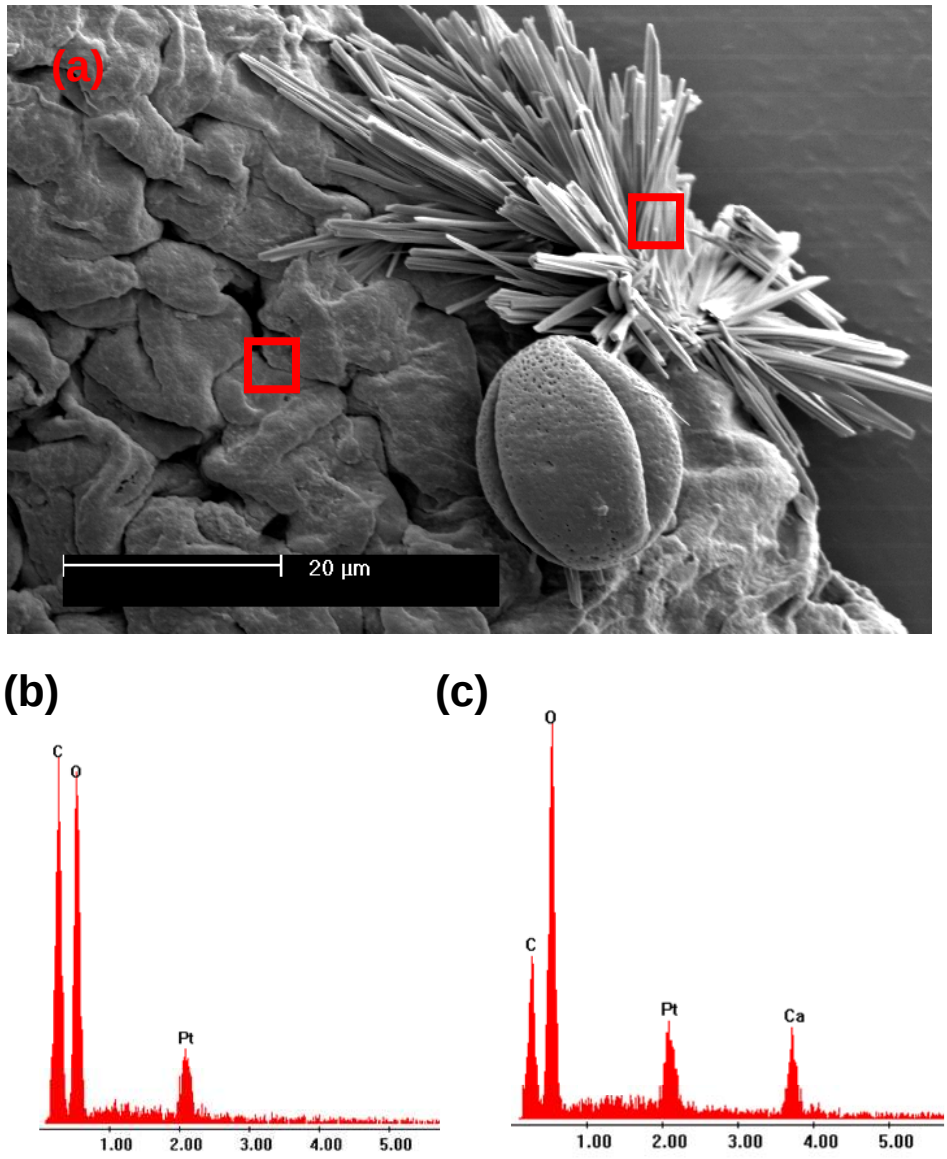


Figure 9.1. Electron micrographs of *V. vinifera* taken by FE-SEM and X-ray analysis from pollen grains and crystals. (a) Many crystals were observed around pollen grains. Pollen grains were spheroid in shape. The square areas were X-ray microanalysed. (b) Stigma surface. The emission of C-Kα, O-Kα and Pt-Kα (platinum was used to coat the sample) were detected. (c) Crystals. Intense emission of Ca-Kα were detected as well as C-Kα and O-Kα.

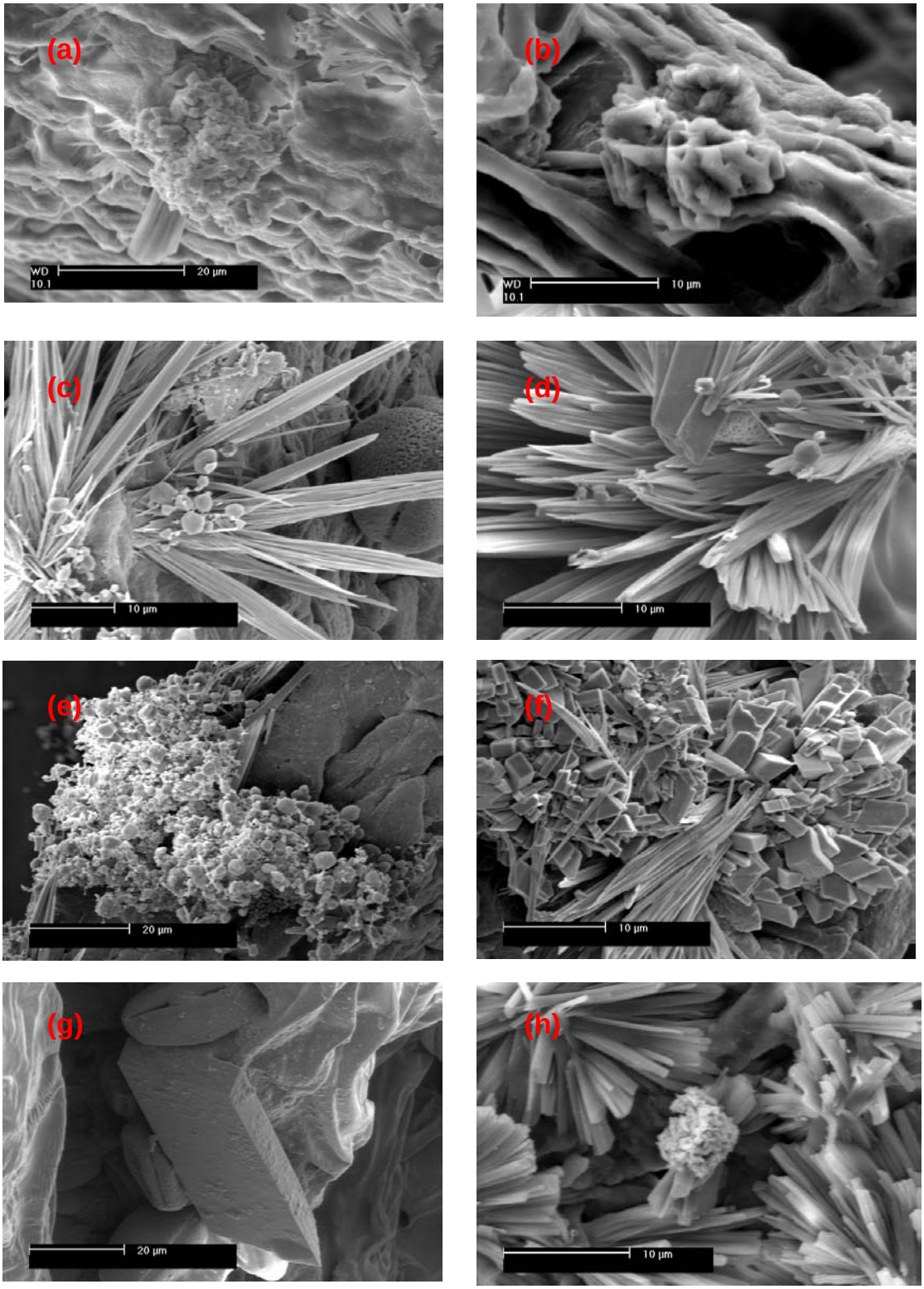


Figure 9.2. Scanning electron micrographs of calcium crystal types identified on the anthers of *Vitis vinifera* (a-h). (a,b) druse crystals, (c,d) raphide, styloid and crystal sand, (e) crystal sand, (f) prism and raphide crystals, (g) prism, and (h) druse and raphide crystals.

Table 9.1. Crystal abundance, localisation, stage of development and the type of crystal form/s observed for each of the varieties investigated in this study.

		Presence or absence		Stage of development		
Variety	**Crystal abundance**	**Anthers**	**Ovary**	**Before flowering**	**During flowering**	**Crystal forms observed**
Sauvignon Blanc	+	+	-	-	+	raphide, druse
Chardonnay	++	+	-	-	+	prism, druse, raphide, styloid
Pinot Noir	++	+	-	-	+	prism, druse, raphide, styloid
Zinfandel	+++	+	-	+	+	raphide, druse, styloid
Tempranillo	+	+	-	-	+	raphide, druse, styloid
Cabernet Sauvignon	+++	+	-	+	+	raphide, druse, styloid, crystal sand
Cabernet Franc	+++	+	-	+	+	raphide, druse, styloid, crystal sand
Riesling	+	+	-	-	+	druse, crystal sand
Merlot	+++	+	-	+	+	raphide, druse, styloid, crystal sand
Shiraz	++	+	-	-	+	raphide, druse, styloid, crystal sand
Emperor	+	+	-	-	+	druse
Blush Seedless	+++	+	-	+	+	raphide, druse, crystal sand

9.4 Discussion

This is the first time that calcium crystals have been reported in grapevine anthers. Several different crystal types were observed as well as differences in the relative abundance of these crystals. Horner and Wagner (1980) suggested that the different crystal forms observed in anther development could be due to the influence of microchemical gradients and varying ion concentrations that exist in the connective tissue. It has been suggested that typically cells that produce crystals are usually young cells that are in the process of differentiation (Horner and Wagner 1992). While it is not known what causes the occurrence of crystals it appears that this process is not random (Horner and Wagner 1992) and that crystal formation may be under genetic control (Ilarslan et al. 2001). It has been shown that environmental and chemical conditions such as temperature, pressure, pH and ion concentrations may affect crystal growth, location and properties (Francheschi and Horner 1980).

X-ray analysis on single crystal structures showed a prominence of calcium and oxygen. This strongly suggests that all the crystal forms observed in this study were composed of calcium oxalate.

We observed that when the anther was dehisced and pollen grains released, the CaOx crystals adhered to pollen grains. It has been suggested that crystals formed in the presence of pollen may play a defensive role against pollinators or in pollen liberation (D'Arcy et al. 1996). Another hypothesis is that the crystals dissolve into aqueous drops and supply the pollen with the calcium ion needed for germination and pollen tube growth (Iwano et al. 2004).

The abundance of CaOx crystals varied between varieties. One explanation for this is a study performed by Francheschi (1989): they suggested that when calcium was in excess so too were the levels of calcium oxalate. It was also suggested that calcium crystals could be mobilised when calcium was in demand. This means that calcium would not just be a part of the precipitation process but would also involve cellular specialisation. Another explanation is that the presence of CaOx crystals in the anther may act as a means for removing excess oxalic acid from the plant system (Iwano et al. 2004).

9.5 Conclusions

We know in other plants that calcium crystals in the anthers can act as a defence mechanism to protect the pollen grains and/or may also play a role in calcium supply at a critical stage in development. We have very little knowledge on the role of calcium in floral development. Further studies are needed to investigate the effects of different cultural practices and the environment on the status of calcium and CaOx crystals and how these impact on reproductive development and especially on pollination and germination processes.

A manuscript outlining these findings is an advanced state of preparation and will be submitted to the Journal of Plant and Cell Physiology as this will be the first publication of the presence of the crystals in grapevine anthers.

A summarised report of the cellular responses shown above will be prepared for an industry audience (for example- Australian Viticulture).

10. Conclusions

When this project was originally conceived in consultation with the GWDRC—and as a result of an industry workshop—it was envisaged, by all parties, that this would be a pilot project that would investigate many aspects of the flowering and fruitset process. For this reason, this project has been wide-ranging in its approach, from phenological and climatic studies in the field, to anatomical studies of pollination and fertilisation, and studies of gene expression during flowering. It was predicted that this approach would not only improve our understanding of the flowering and fruitset process under Australian conditions but, just as importantly, would also lead to the development of further projects as 'spin-offs' so that research on flowering and fruitset would continue well into future. Although our understanding has been improved, it remains to be seen if flowering and fruitset research will be supported from hereon.

To our knowledge, this is the first study in which the reproductive performance of a large cross-section of winegrape varieties has been quantified over four seasons and across a wide range of climatic regions. It is one of few studies where fruitset has been calculated using flower number per inflorescence/bunch and not simply inferred from counts of berry number. It is clear that variation in flower number from season to season may be significant, particularly for Cabernet Sauvignon. The high variability of total berry number for some varieties (e.g. Merlot, Chardonnay, Cabernet Sauvignon) may be as much a consequence of variation in flower number as variation in fruitset. Furthermore, the relationship between berry number and fruitset may not be sufficiently robust to justify the use of berry number as an 'index' of fruitset. Certain varieties have a reputation for "poor fruit-set" that has been inferred from relatively low berry number per bunch, e.g. Tempranillo, Sauvignon Blanc, Pinot Noir and Chardonnay (in some locations). However, we have shown that, compared with other varieties, all of these varieties tend to have moderate set—and higher than that for Cabernet Sauvignon and Merlot which tend to have consistently low set, but moderate to high flower number. The value of the new indices CI and MI has been more than satisfactorily demonstrated in this study. Rather than description of grapevine reproductive performance in a subjective and imprecise manner, these indices provide a means by which performance may be quantified; and furthermore, the individual expressions of coulure and millerandage may be separated. There appear to be significant differences between the varieties in this study with regard to expression of both CI and MI (Table 3.2).

From the point of view of our research, it was unfortunate that, for the regions and the four seasons studied, there was not a repetition of the climatic events that occurred during the spring of 2000 that were associated with the poor fruitset of that season. In that particular season, lower than average temperature conditions occurred in most SA regions during the flowering and fruitset

period. For most variety and site combinations in our study, there was no obvious seasonal effect on fruitset. Even for those cases where there did appear to be a seasonal effect, e.g. Adelaide Hills Chardonnay shoot topping experiment (Chapter 5), the % fruitset of the relatively poor season was higher than the nominal threshold, i.e. 35%, recommended for 'low' fruitset in Table 3.2. This is, of course, one of the challenges of any investigation of the relationship between climate and plant performance when field experiments are employed. For this reason, it is clear that further work in this field should employ controlled environments to complement any field-based studies.

For the reasons outlined in the previous paragraph, we are not able to conclude that the *first aim of this project, i.e. to determine the climatic conditions that are beneficial or detrimental to flower development and fruitset, and the frequency of occurrence in Australian regions*, has been completely met, to our satisfaction, at this stage. Preliminary analysis of the temperature data collected during the four seasons has indicated that, for most varieties, there was not an apparent relationship between the temperature regime during the flowering/set period and the reproductive performance at a particular site. For this reason, much more detailed analysis of our data set will be necessary in order to attempt to explain, for some varieties, why certain sites consistently had lower set than others, irrespective of season. In a few cases, there does appear to have been a seasonal effect—but for some of these, temperature difference is not the explanation. In retrospect, this first project aim was probably overly ambitious because it was reliant on the occurrence of significant climatic differences between seasons (which did not eventuate).

Nevertheless, we have acquired much new information on varietal reproductive performance during this project. The results from this regional experiment (and other experiments in this project) have shown that the incidence of poor fruitset has probably been overestimated for certain varieties and seasons in Australian regions in the past. Also, it is possible that the impact of seasonal temperature on flowering and fruitset may have been over-estimated in some cases. Therefore, we strongly recommend that reproductive performance of the main winegrape varieties should be quantified in each region in every season by utilising the methodology described in this report. This will potentially enable some reduction of the incorrect assumptions about seasonal effects on fruitset that are currently widespread. This particular task could be readily achieved by regional organisations through the use of monitored sites in their regions.

The second aim of this project was to determine a means by which flowering time may be accurately predicted. This aim has been met and we have shown that this can be achieved either by the use of a degree-day model or by the use of an indicator variety such as Ganzin Glory. From a grower's standpoint, it is the latter which is likely to be the most cost-effective method. Ganzin Glory (or Canada Muscat which we have found during the course of this project to be an equally-

early flowering variety) can either be planted as an own-rooted vine or grafted. Because the indicator vines will flower up to 2 weeks earlier than Chardonnnay, this is ideal for the optimal timing of the CCC foliar spray to improve fruitset which should be applied 7 to 10 days before flowering (Chapter 5).

The third aim of this project was to investigate the effectiveness of cultural practices and to determine those most suitable for improving fruitset in seasons of poor set. This study investigated the effectiveness of shoot topping and CCC application on fruitset and other yield components with Cabernet Sauvignon, Chardonnay and Tempranillo. Treatments were applied before and during the flowering period. Fruitset and yield per vine increased in response to treatment, especially when shoot topping was applied from the start of flowering. All varieties at both locations responded to shoot topping to some degree even though none of the seasons could be classified as problematic for fruitset. Fruitset and thus yield can be improved by cultural practices in all seasons; but the magnitude of the response will be greatest in those seasons when fruitset is limited by climatic conditions or other factors. All varieties at both locations responded positively to shoot topping to some degree. Therefore, we recommend that shoot topping be applied every season on these varieties because it is likely to be cost-beneficial. Shoot topping can be mechanised, does not require chemical input and the optimal timing is easily determined. In practice, a CCC foliar spray applied 7 to 10 days prior to flowering (E-L 17 to 18) would be good insurance for Cabernet Sauvignon grown in the Adelaide Hills and in other regions where poor fruitset is a common occurrence. CCC is registered for use on Cabernet Sauvignon in Australia. These recommendations have enabled us to satisfy *the fourth aim of this project, i.e. to produce recommendations on practices to improve set.*

The results described in Chapters 6 and 7 have shown that cultural practices such as Mo foliar spray (in the case of Merlot only), CCC foliar spray or shoot topping can exert an effect on fruitset via an influence on pollen tube growth, ovule fertilisation or ovule cell morphology. For example, in the case of Mo on Merlot, pollen tube growth was significantly enhanced by Mo-treatment on Mo-deficient vines. Also, significantly more pollen tubes penetrated the ovules from Mo-treated vines and a higher proportion of ovaries had at least one penetrated ovule. Structural observations revealed that a significantly higher proportion of ovules from Mo-deficient vines were defective—the absence of an embryo sac in those ovules is probably the cause of pollen tube growth inhibition and subsequent poor fruitset. The application of ethephon before or during early flowering resulted in decreased fruitset due to increased flower abscission. This suggests that endogenous levels of ethylene may play some role in the increased incidence of flower abscission in some seasons. Therefore, it is recommended that further research on the role of endogenous growth regulators in flower abscission should be conducted, particularly with those varieties such as Cabernet

Sauvignon, Merlot, Shiraz, Tempranillo and Sauvignon Blanc which have naturally high CI values in some regions. The results of this project have enabled us to satisfy *the fifth aim of this project, i.e. to develop an understanding of the mechanisms that induce or prevent the development of abscission zones leading the shedding of grape flowers/ovaries/berries.*

Although the PhD study by Mardi Longbottom was not included as part of this project when it was originally conceived, the provision of funding from GWRDC to assist with the operation of her study lead to its incorporation within the project. This has been of great benefit to the project with significant value-adding. It is clear that application of Mo foliar spray will only be of benefit to fruitset and yield when vines are deficient in the first instance. Therefore, before application, tissue analysis should be conducted in order to verify this deficiency. Furthermore, we recommend that shoot tips should be used for this purpose in preference to the standard petioles. The critical level of molybdenum for optimal fruitset on own-rooted Merlot is suggested to be 0. I mg/kg in the shoot tips at E-L stage 25 (80% flowering). Treatment of Mo-deficient Merlot vines with sodium molybdate improved bunch weight. This was a function of improved fruitset, not increased mean berry weight as previously reported. Treatment of vines with adequate levels of molybdenum did not detrimentally affect yield nor did application of two times the standard rate of sodium molybdate. Mo treatment did not affect bunch number per vine nor did it affect flower number per inflorescence in the season following treatment. Treatment of Mo-deficient Merlot vines with sodium molybdate did not have a cumulative effect on yield when applied in consecutive seasons. The results from pollination experiments indicate that Merlot may be a poor producer of pollen and may suffer from self-incompatibility.

The final aim of the project was to develop an understanding of the role of polyamines, ABA and amino acids in the flowering process and their relationship with environmental factors and cultural practices. The results of Chapter 7 have provided a first step on the road to our understanding of the role of polyamines and ethylene in particular in the flowering and fruitset process. Real-Time PCR and HPLC analysis showed an interaction between the ethylene and polyamine biosynthesis pathways in the floral developmental process. This increase in the expression of a gene in the ethylene biosynthetic pathway is a potential indicator of the mechanism which is responsible for the abscission of flowers in Mo-deficient Merlot vines. Also, the corresponding decrease in ACC synthase expression in the Mo sprayed vines is strong evidence that polyamine and ethylene levels are implicated in the restoration of normal berry development. Correspondingly, in the Mo-treated vines, the flowers sampled showed consistently higher levels of many of the polyamine biosynthesis genes when compared with the Mo-deficient control vines. The significant induction of ACC synthase in the Mo-deficient flowers compared with the Molybdenum-sprayed flowers indicates a potential shift in the ethylene/polyamine biosynthesis

synergies. Flower or fruitlet abscission is a response in plants to developmental or environmental cues and PAs may play a regulatory role in flowering and initial fruitlet abscission. This unique observation of the induction of ethylene biosynthesis gene expression in the Mo-deficient vines compared with the induction of the polyamine biosynthesis genes in the Mo-treated vines may indicate a new and novel field of reproductive control in grapevines.

Using the primers developed in the Merlot Mo experiments, we recommend that the expression of the polyamine and ethylene biosynthesis genes should be investigated. We currently have tissue available from the 2007-08 season from an experiment in a commercial vineyard in the Adelaide Hills which has been routinely sprayed with Molybdenum. Flower tissues were harvested post spraying to investigate the short-term gene expression profile after Mo application. In addition, other cultural practices (shoot topping, CCC and Ethrel application) were also applied on both the Mo-treated and control vines to further expand our understanding of the gene expression profiles induced by these treatments.

Chapter 9 describes the first report of calcium crystals in grapevine anthers. These crystals may act as a defence mechanism to protect the pollen grains and they may also play a role in calcium supply. Because there is little knowledge on the role of calcium in floral development, further research is required.

The research to date on the effect of limited water availability on yield has focused almost exclusively on berry development. During the course of this project, we observed a strong interaction between pruning regime and water stress which resulted in 100% inflorescence necrosis soon after flowering in the spring of 2006. Therefore, it is our contention that it is vitally important to determine the effect of water stress on all yield components, and particularly during early inflorescence development. If inflorescence development is detrimentally affected in any way at an early stage, then the fate of the eventual yield has been determined. If water supply is limited, then the ability to inform growers as to the potential effect of the application of limited water will not only be desirable but also critical to the long term outlook for those regions.

In 2007/08 we took advantage of an opportunity to collaborate with Drs Chris Soar and Victor Sadras to collect samples from their SARDI (GWRDC-funded) project which is investigating the effect of elevated temperatures at key growth stages through the growing season. We collected samples from the controls and the treatment which increased temperatures by approximately 3ºC for two weeks from the start of flowering. Our preliminary findings were surprising and showed a decrease in both fruitset and yield in response to elevated temperature. The decrease in fruit set was associated with a significant increase in CI, ie. increased flower abscission. This is a

significant finding because it shows that slightly elevated temperature conditions, in the absence of water stress, may detrimentally affect fruitset and yield. Therefore, we strongly recommend that further work on the impact of both higher temperatures and water deficit during the flowering period on fruitset and yield is essential in the context of climate change.

11. Key Recommendations

1. Terminology: We strongly recommend that the terms " hen and chicken", " chicken berry" and "shot berry" be no longer used by Australian growers because they are imprecise and currently used incorrectly. In their place we recommend that berries should be described as either "seeded" or "seedless" and that the term "live green ovary" (or LGO) be used as appropriate. Also, the terms "coulure" and "millerandage" should be used to describe reproductive performance. We accept that there may be some objection to the use of foreign language terms; however, with time, they will become accepted in the same way as "veraison" and many other examples.

2. Regional assessment of reproductive performance: The only valid method to quantify reproductive performance, i.e % fruitset, coulure index and millerandage index, is to measure both flower number per inflorescence and berry number per bunch. Too often, the former is ignored and just the latter is used as an indicator of fruitset. As we have shown, low flower number may be the main cause of low berry number per bunch, not poor fruitset. Because measurement of flower number is a time-consuming process, we recommend that each region should fund the collection of both flower number per inflorescence and berry number per bunch data from a range of standard varieties every season so that the determination of reproductive performance indices may be done every season. This would potentially reduce the incorrect assumptions about the causes of low berry number per bunch in certain seasons that are widespread in the industry.

3. Shoot topping: We recommend that shoot topping be applied every season at those locations and for those varieties for which poor fruitset is a common occurrence, e.g. Cabernet Sauvignon, Merlot, Pinot Noir, Chardonnay. In terms of the timing of the operation, depending on variety and location, any time between the start of flowering (E-L 19) and end of flowering (E-L 26) is likely to achieve a positive response. In reality, the time between these two stages is relatively short (e.g. 4 to 8 days in most seasons) and for commercial application it may be necessary to span these two stages in a large vineyard operation. It can be mechanised, does not require chemical input and the optimal timing is easily determined. All vineyards with a VSP trellis generally shoot top as a normal practice for canopy control around this time anyway; therefore, a slight adjustment to the timing will minimise the operating cost of the procedure. Because of the magnitude of the likely yield response, this procedure is likely to be very cost-beneficial.

4. CCC application: In practice, a CCC foliar spray applied 7 to 10 days prior to flowering (E-L 17 to 18) would be good insurance for Cabernet Sauvignon grown in the Adelaide

Hills and in other regions where poor fruitset is a common occurrence. CCC is registered for use on Cabernet Sauvignon in Australia.

5. A cheap aid to the timing of CCC application: The determination of "7 to 10 days before flowering" is not a simple task for many growers. Although this can be achieved either by the use of a degree-day model, we recommend the use of an indicator variety such as Ganzin Glory or Canada Muscat. These can be either planted as an own-rooted vine or grafted to existing vines. Only one of two such vines is necessary for a large vineyard block. The Ganzin Glory has the advantage that it does not produce fruit that will be picked by a machine harvester. Because the indicator vines will flower up to 2 weeks earlier than the earliest standard winegrape varieties in commercial use, this is an ideal tool for the optimal timing of the CCC foliar spray.

6. Tissue analysis to determine Mo deficiency in Merlot: It is clear that application of Mo foliar spray will only be of benefit to fruitset and yield when vines are deficient in the first instance. Therefore, before application, tissue analysis should be conducted in order to verify this deficiency. Furthermore, we recommend that shoot tips should be used for this purpose in preference to the standard petioles. The critical level of molybdenum for optimal fruitset on own-rooted Merlot is 0.1 mg/kg in the shoot tips at E-L stage 25 (80% flowering).

7. Treatment of Mo-deficient Merlot vines with sodium molybdate to increase fruitset and yield: The applications of a single foliar spray of sodium molybdate at 300g/ha concentration at E-L stage 12 is recommended.

12. Future Research

- Investigation of the relationship between climatic variables particularly temperature and flowering/fruitset should be conducted in controlled environments so as to complement any field-based studies.
- More detailed analysis of our data set will be necessary in order to attempt to explain, for some varieties, why certain sites consistently had lower set than others, irrespective of season.
- Reproductive performance of the main winegrape varieties should be quantified in each region in every season by utilising the methodology described in this report. This will potentially enable some reduction of the incorrect assumptions about seasonal effects on fruitset that are currently widespread. This particular task could be readily achieved by regional organisations through the use of monitored sites in their regions.
- Investigate the effect of a small temperature increases and/or water stress during the flowering period on eventual fruitset and vegetative growth. How will climate change affect this and what management strategies can be applied?
- Further research on the role of endogenous growth regulators in flower abscission should be conducted, particularly with those varieties such as Cabernet Sauvignon, Merlot, Shiraz, Tempranillo and Sauvignon Blanc which have naturally high CI values in some regions.
- Determine the long-term effects of Mo treatment after multiple seasons on vine and soil health. Determine the fate of Mo when vines with adequate levels of Mo are treated with sodium molybdate sprays.
- Investigate the underlying cause of Mo deficiency in Merlot and the mechanism by which Mo affects embryo sac development.
- Investigate the relationship between polyamines and ethylene during the flowering period and the interaction with environmental stress and/or cultural practices. Investigate the induction of the polyamine biosynthesis genes in the Mo treated vines—this may indicate a new and novel field of reproductive control in grapevines. Can “normal" fruit set be achieved in the Mo deficient vines by application of exogenous polyamines?
- Investigate the role of calcium in flower development of grapevines.

Appendix 1: Communication

Results and information from this project were presented in industry meetings, conferences and workshops and are shown below.

Industry and scientific presentations, meetings and workshops

September 2004	Nepenthe Viticulture, Meeting, Charleston, SA
	Flowering and fruit set meeting, Southcorp Coonawarra offices, Meeting, Coonawarra, SA
	Hardy Wine Company, Meeting, Reynella, SA
	McLaren Vale Growers Association, Grower meeting, McLaren Vale, SA
November 2004	'Molybdenum deficiency in *Vitis vinifera*' workshop, University of Adelaide, Waite campus, Adelaide, SA
	"The effects of molybdenum deficiency on flowering and yield of Merlot", Barossa Valley Viticultural Technical group, SA
July 2005	Adelaide Hills Grapegrowers Association, Lenswood Research Centre, Lenswood, SA
	'Flowering and fruit set' workshop, University of Adelaide, Waite Campus, Adelaide, SA
	'Transforming flowers to fruit' seminar, Mildura Arts Centre, Mildura, VIC
February 2006	6th International Cool Climate Symposium for Viticulture and Oenology, Christchurch, New Zealand.
August 2006	Cabernet Sauvignon Pollination experiments in the Limestone Coast, Limestone Coast Wine Industry Council Technical Seminar.
	Paper presentation entitled "Role of molybdenum and polyamines in grapevine berry development and ripening" at the 8th International Congress of Plant Molecular Biology, Adelaide Convention Centre, Adelaide, SA.
September 2006	Adelaide Hills Grapegrowers Association, Lenswood Research Centre, Lenswood, SA
July 2007	'Flowering and fruit set' workshop, 13th Australian Wine Industry Technical Conference, Adelaide, SA

Key Industry and Scientific Paper Presentations

Longbottom, M. Molybdenum and fruitset of Merlot, Australian Society of Viticulture and Oenology Seminar 'Transforming flowers to fruit', July 2005.

Collins, C., Masi, E., Dry, P. and Kaiser B. Role of molybdenum and polyamines in grapevine berry development and ripening. 8th International Congress of Plant Molecular Biology paper presentation, conference proceedings. 20th to 25th August 2006, Adelaide, Australia. p 61.

Dry, P.R. Measurement and terminology—getting it right. Workshop on flowering and fruitset, 13th Australian Wine Industry Technical Conference, Adelaide, July 2007.

Longbottom, M. Advances in the understanding of the relationship between Mo and Merlot. Workshop on flowering and fruitset, 13th Australian Wine Industry Technical Conference, Adelaide, July 2007.

Collins, C. How does climate influence flowering and fruitset. Workshop on flowering and fruitset, 13th Australian Wine Industry Technical Conference, Adelaide, July 2007.

Poster Presentations
Longbottom, M. L., Dry, P. R. and Sedgley, M. (2004). The occurrence of "star" flowers in grapevines: observations in the 2003/4 season. 12th Australian Wine Industry Technical Conference, Melbourne, Australia.

Longbottom, M. L., Dry, P. R. and Sedgley, M. (2004). Effects of molybdenum on fruit set and yield of *Vitis vinifera* cv. Merlot. 12th Australian Wine Industry Technical Conference, Melbourne, Australia.

Collins, C. and Dry, P.R. (2006). Potential management of flowering and fruitset in cool climate vineyards.6th International Cool Climate Symposium for Viticulture and Oenology, 6-10th February, Christchurch, New Zealand.

Longbottom, M. L., Dry, P. R. and Sedgley, M. (2006) Effects of molybdenum on fruit set and yield of *Vitis vinifera* cv. Merlot. International Cool Climate Symposium, Christchurch, New Zealand

Collins, C. and Dry, P.R. (2007). Understanding flowering and fruit set of grapevines, The 13th Australian Wine Industry Technical Conference. 28th July to 2nd August, Adelaide, Australia.

Publications

Peer reviewed publications
Longbottom, M. L., Dry, P. R. and Sedgley, M. (2004). A research note on the occurrence of 'star' flowers in grapevines: Observations during the 2003-2004 growing season. Australian Journal of Grape and Wine Research **10**, 199-202.

Longbottom, M. L., Dry, P. R. and Sedgley, M. (2008). Observations on the morphology and development of star flowers of *Vitis vinifera* L. cvs Chardonnay and Shiraz. Australian Journal of Grape and Wine Research (in press).

Collins, C. and Dry, P.R. (2008). Response of fruitset and other yield components to shoot topping and CCC application. Australian Journal of Grape and Wine Research (accepted).

Longbottom, M. L., Dry, P. R. and Sedgley, M. (2008). Effects of sodium molybdate foliar sprays on molybdenum concentration in the vegetative and reproductive structures and on yield components of *Vitis vinifera* cv. Merlot. Submitted to the Australian Journal of Grape and Wine Research.

Industry Journal Publications

Longbottom, M. L., Dry, P. R. and Sedgley, M. (2004) Foliar application of molybdenum pre-flowering: effects on yield of Merlot. The Australian and New Zealand Grapegrower & Winemaker 491:36-39.

Collins, C. and Dry, P. R. (2006) Manipulating fruitset in grapevines. The Australian and New Zealand Grapegrower and Winemaker, Annual Technical Issue, No. 509a, 38-40.

Bartsch, T., Collins, C. and Dry, P.R. (2007) Shoot trimming effects on Pinot Noir. The Australian and New Zealand Grapegrower and Winemaker, No. 517, 22-25.

Collins, C. and Dry, P.R. (2008) Understanding and manipulating fruitset in the vineyard. ASVO Australian Grapegrower, July 2008.

Longbottom, M. L., Dry, P. R. and Sedgley, M. (2008) A review of the processes and terminology used to describe grape flowering, berry development, fruitset and fruitset disorders. The Australian and New Zealand Grapegrower & Winemaker Annual Technical Issue.

Publications in advanced state of preparation

Collins, C., Gilliham, M., Ford, C and Dry, P.R. (in preparation). Calcium oxalate crystals in the anthers of Vitaceae. Journal of Cell and Plant Physiology

Collins, C. and Dry, P.R. (in preparation). The effect of plant growth regulators on pollen tube growth and ovule development in grapevines. Australian Journal of Grape and Wine Research

Wheeler, S., Dry, P.R., Kaiser, B.K., Ford, C.F. and Collins, C. (in preparation). Changes in polyamine content and expression in molybdenum deficient grapevines (*Vitis vinifera* L.). Journal of Plant Physiology.

Collins, C. and Dry, P.R. (in preparation). Grapevine phenology and prediction of flowering time. Australian Journal of Grape and Wine Research.

Appendix 2: Intellectual Property

There was no patentable intellectual property arising from this project.

Appendix 3: References

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Appendix 6: Budget Reconciliation