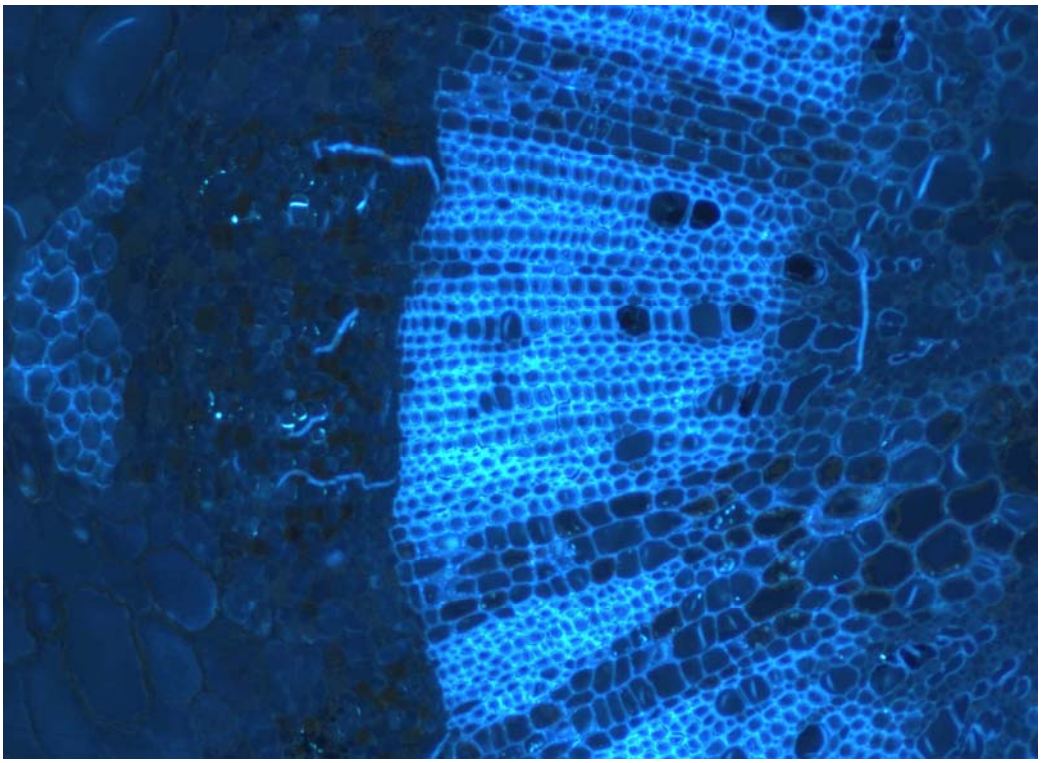
Bunchstem and berry development in Merlot and Cabernet Sauvignon and their effects on berry ripening and composition



Bunchstem vascular system in Cabernet Sauvignon at harvest

Final Report to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSU 02/03**

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Research Organisation: **National Wine & Grape Industry Centre**

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ABSTRACT

The project was conducted to investigate the relationship between bunchstem and berry development and consisted of four experiments on Cabernet Sauvignon and Merlot. Bunch size influenced not only berry and bunchstem size, but also some basic compositional parameters. The degree of reduction in flowers led to an increase in berry size and most often amino acids concentrations. An induced alteration of fruit set had implications on bunchstem and fruit composition, often altering berry amino acid and nutrient concentrations. Seasonal changes in xylem sap composition were also observed in a further investigation. The study underlines the interactive association between bunch berry number and the bunchstem structure and its vascular system influencing the final berry composition.

EXECUTIVE SUMMARY

The Australian wine industry demands consistent supply of high quality fruit for wine making, therefore grape quality and its variability are seen as a high priority. Bunch development, structure and size as well as colour development, ripening rate and asynchronous ripening are critical issues. This study was conducted to gain further understanding of physiological processes and morphological changes involved in the development of the bunchstem in relation to berry maturation and composition.

The work consisted of four field experiments and was conducted in the CSU Winery vineyard at Wagga Wagga, NSW Australia. All experiments were conducted on field grown Cabernet Sauvignon and Merlot grapevines on own roots over five consecutive growing seasons, with each experiment conducted over two seasons. The data collection included berry and bunchstem growth measurements, basic and detailed compositional determination. Further, microscopic studies were conducted on the bunchstem and the composition of the xylem and phloem was also assessed. The analysis of the nutrients was undertaken with a plasma-optical emission spectrometer (ICP), while for the amino acid analysis a high pressure liquid chromatograph (HPLC) was used. For the investigation of the vascular system of the bunchstem a microscope with fluorescence capacity was used.

The first part of the project was to investigate the dynamics of bunchstem and berry development in bunches of different sizes. The aim was to clarify the relationship between bunchstem structure and its vascular system in relation to berry development and composition. This bunchstem study (Experiment 1) was conducted on the two varieties (see above) over the first two growing seasons (2002/03 and 2003/04) of the overall project. For this purpose a number of large and small bunches in a replicated field trial were selected randomly at flowering, which were monitored until harvest. The study confirmed that the size of the bunch influenced berry number per bunch, but also a number of berry parameters differed between the two sizes. The larger bunches had generally larger berries and lower TSS levels, while they were higher in all other compositional parameters. There was a strong relationship between bunch size and bunchstem size, in both length and diameter. The vascular system size varies between positions in the rachis and laterals.

The size of the inflorescence and fruit set influence the final number of berries on a bunch. Studies have also shown that partial and full flower removal influence the bunchstem development and structure. This section of the work was designed to better understand the interaction between flower number, bunchstem development and final berry composition. The flower removal experiment was also conducted on two varieties over two growing seasons (2004/05 and 2005/06). Five treatments were implemented in a replicated field trial with five levels of flowers removed around one week before flowering (control, 25%, 50%, 75% and 100%). The berry number was proportionally reduced by the flowers removed at bloom; however the reduction was not as strong as implemented by the treatments, suggesting that the fruit set improves if more flowers are removed. The reduction in flower numbers lead to an increase in berry size and the concentrations of the amino acids proline and arginine in the berry are generally elevated. The treatments also altered basic berry composition and nutrients, but these changes were variable between varieties and years. The vascular system was

altered by the flower removal treatment, leading to disintegration of first the laterals and then the whole bunchstem system. The treatments receiving flower removal at a lower level could have impacted on the vascular system also, having partially implemented the differences in berry size and berry composition.

Fruit set can be altered by applying the plant hormone cytokinin or gibberellin to the bunch or by changing the assimilate supply to the bunch. The third experiment was designed to give a better understanding of how fruit set impacts on the bunchstem and berry development and was conducted on the two varieties and over two growing seasons (2005/06, 2006/07). Beside the treatments of bunches with gibberellic acid (GA_3) or cytokinin (BAP), a 50% flower removal treatment and a full shoot leaf removal treatment were applied. The implemented treatments did successfully change the number of berries per bunch, although the severity was not the same in both seasons. The BAP treatment increased the berries per bunch and the other three treatments lowered them, namely GA_3, flower and leaf removal. The size of the bunch implicated by the treatments on the basic berry parameters was inconsistent and there appeared to be no clear relationship between bunch size and berry size, total anthocyanins and TSS; only total phenolic was usually higher in the leaf removal treatment. The proline and arginine concentration increased during berry maturation, these were particularly pronounced for proline. The treatments altered both amino acids, but these changes were often inconsistent, probably due to seasonal variation and varietal differences. The GA_3 and leaf removal were the most consistent treatments in altering both berry amino acid and nutrient content. The GA_3 treatment lead also to shot berries in bunches, which had pedicels with a different vascular structure.

The nitrogenous compounds such as amino acids in the xylem have been shown to be relatively high around the flowering period. This experiment was designed to understand the changes occurring during the growing season, particularly in the early part of reproductive development around the flowering to fruit set period. The experiment was also conducted at the CSU vineyards on Cabernet Sauvignon and Merlot over two growing seasons as in the above experiment. The trial consisted of three replicates with four vines in two rows for both cultivars. In this trial no treatment was applied only samples were taken during the growing season for collecting xylem sap. Around flowering the amino acid concentrations in the xylem sap were highest, particularly in the bunchstem. This indicates the significance of the inflorescence or bunch as a sink at that time. High concentrations were also observed in the morning or late in the evening and could be due to the low transpiration rate at this time of the day. The variation in amino acid concentrations during the season indicates the differences in demand for these nitrogenous compounds as well as differences in N uptake during the season.

Bunchstems contain an important vascular transport system supplying the grapes with nutrients and sugar. The study has shown that there is a strong interaction between berry number, bunchstem development and a number of berry parameters. Early during berry development the number of berries influenced bunchstem development, this change appeared to impact on the final berry composition. How important the bunchstem and its vascular system on these berry parameters are needs to be further clarified. However, the management affecting berry and bunchstem development could have a large impact on grape maturation and quality.

PROJECT BACKGROUND

The development of the bunch, its structure and size as well as berry colour development, ripening rate and asynchronous ripening are critical issues in grape production. There was little information on early bunchstem development (flowering to veraison) and its impacts on berry ripening and grape composition. The fertilisation of flowers and their number reflect on bunchstem and berry development. The differences in fruit set affect the variability of berry size and number as well as the bunchstem. The structure and properties of the bunchstem influence the supply of minerals and carbohydrates to the berry through the vascular bundles (xylem, phloem) during development, but becomes particularly important during berry ripening. The rate of berry ripening and berry composition could be strongly influenced by the development of the bunchstem during the early stage of development. Berry and bunch disorders have been related to nutritional imbalance or supply of carbohydrates. Fruit set and therefore crop size is affected by sugar (inflorescence necrosis), Zn (coulure) and B (millerandage). Bunchstem necrosis is affected by Ca, Mg, N and sugar. K and N supply affects juice pH and berry colour, N affects the mixture of nitrogenous compounds important for fermentation. The effect of other nutrients on berry composition is unknown. The variety Merlot often shows poor and uneven fruit set and Cabernet Sauvignon is a variety which is prone to bunchstem necrosis. This study was aimed to contribute to the understanding of physiological processes and morphological changes involved in the development of the bunchstem in relation to berry maturation and composition.

Project code:	CSU 02/03
Project Title:	Bunchstem and berry development in Merlot and Cabernet Sauvignon and their effects on berry ripening and composition
Research Organisation:	National & Wine Grape Industry Centre (NWGIC)
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Project Site(s):	Wagga Wagga
Commencement Date:	September 2002
Completion Date:	May 2007
Project funding:	$124,271

Project aims and performance targets

- Investigate bunchstem structure and development from bloom to harvest and its effects on final fruit attributes.
- Study phloem and xylem movements to the berry and their composition and effects on berry sugar and mineral accumulation.
- Examine how berry set and bunchstem development affect final bunch size and structure and berry quality.

Outputs	**Performance Targets**
1. Information on bunchstem structure and development on two major red varieties	1. Bunchstem structure and development on the two major varieties by June 2003
2. Knowledge of nutrient and sugar accumulation into the berry	2. Sugar and mineral accumulation in grape berries of two varieties by September 2004
3. Study of phloem and xylem composition and delivery	3. Phloem and xylem composition and delivery to berries and leaves of two varieties by June 2005
4. Information on carbohydrate production on fruitfulness, berry set and bunchstem development	4. Two trials on grapevine carbohydrate production on fruitfulness, berry set and bunchstem development by September 2005
5. Results to improve berry composition presented	5. Results presented to 4 seminars by June 2005
6. Results published	6. Two publications of results in refereed journal and industry journal by September 2005

Note: The start of the project was delayed by 18 months due to difficulties in finding a suitable PhD student, therefore the completion date of performance targets was delayed.

1. The structure of the bunchstem was investigated in three experiments and two varieties; these were studying the impact of natural bunch size variation, altered flower numbers and fruit set. These related to basic measurements of the bunchstem and microscopic studies on the vascular system.

2. The sugar accumulation and the nutrient accumulation were determined in the two experiments on altered flower numbers and fruit set, both over two growing seasons and for the varieties Cabernet Sauvignon and Merlot.

3. A study was conducted to investigate the phloem and xylem composition. The delivery determination was more complex than expected and therefore the information is inconclusive and not presented. The assessment of the phloem sap has also not been finalised.

4. The impact of fruit set on bunchstem development was investigated in two trials. This study included an assessment of the vascular system by using microscopic techniques. In addition, compositional changes of the berry implement by the treatments were also determined.

5. The results were presented in several seminars as listed at the end of the report. Several posters have been presented to the industry at the NWGIC and the AWITC in Melbourne and Adelaide.

6. The results have also been published in an industry journal and refereed publications following after completion of the PhD thesis.

DETAILED PROJECT REPORT

1 General Introduction

Fruit ripening and composition are often investigated during or at the end of ripening, although at least some of the final fruit composition is influenced by berry set. The weather around flowering time and carbohydrate supply to the cluster are seen as the most dominant factors affecting berry set. Yield and berry number are affected at berry set, but the role of the bunchstem vascular system and its development on mineral and sugar supply to the grapes have not received much attention.

Carbohydrate source-sink relationships change during the growing season and the carbon assimilated will move to different sinks depending on the vine's developmental stage. The shoot tips are more important sinks during flowering than the cluster (Koblet 1969), meaning a removal of the growing tip (topping) will benefit the carbon supply to the cluster (Currle et al. 1983). Despite the competition there is a sharp increase in organic compounds flowing to the cluster indicating its increasing sink strength (Glad et al. 1992). Weather has a significant impact on the length of flowering time and fruit set, unfavourable weather conditions reducing carbon assimilation make the vine more dependent on carbohydrate reserves (Keller et al. 1995). It appears that leaves in the bunch zone are most important, particularly in its early development stage (Schultz et al. 1996). Carbohydrate production from bloom to up to three weeks after is most critical for final crop yield (Candolfi-Vasconcelos & Koblet 1990). The importance of reserve levels during this bloom-set period is not well understood (Caspari et al. 1998). Winter carbohydrate levels could be important, since cluster development and shoot growth are closely linked (Pratt & Coombe 1978). Carbohydrate reserves in the nodes are reflected in the fruitfulness the following spring (Sommer et al. 2000). Early and late bunchstem necrosis have also been shown to correlate with the weather conditions around flowering (Theiler 1985) and low supply of assimilates may induce early bunchstem necrosis (EBSN) and BSN (Keller & Koblet 1995). Poor set (coulure) and inadequately fertilised berries (millerandage) affect yield and quality at harvest (Jackson 1994). Seed number determines the time of ripening of the berries (Rapp & Klenert 1974). Fertilisation problems can be the result of B deficiency (Gärtel 1974), berries of boron-deficient vines have smaller and fewer seeds (Hardie & Aggenbach 1996). Zinc deficiency leads to a disturbed berry and bunchstem development as well as predisposition to BSN (Gärtel 1974). It appears that source-sink interactions have a significant impact on berry set affecting bunchstem and berry development.

A well functioning bunchstem is crucial for supplying the grapes with nutrients and carbohydrates. Most work in relation to bunchstem development has been conducted in connection with the grape disorder BSN. By mid flowering (75%) the majority of the phloem and xylem bunchstem cross section is developed, a further increase in the vascular bundle system occurs until veraison when final length of the bunchstem is reached (Theiler & Coombe 1985). Gibberellic acid (GA) treatments before flowering can increase xylem development, suggesting GA deficiency produces the predisposition to BSN. Weather conditions at flowering have shown an effect on xylem development (Lang & Düring 1990) as well as on BSN (Theiler & Müller 1986). Düring and Lang (1993) noticed that xylem development in some varieties is reduced in a short distal region just below the branching point (node). They discovered a strong correlation between xylem development and susceptibility of grape varieties to BSN (Lang et al. 1994). Düring et al. (1987) also found that the xylem function is reduced and therefore the phloem becomes more important for water transport to the berry, which explains the changes in influences on the mineral composition after veraison in the berry (Düring & Oggionni 1986). Ca is imported into the berry before veraison and K mainly during berry ripening (Creasy et al. 1993, Donèche & Chardonnet 1992, Hradzina et al. 1984, Ollat & Gaudillère 1996, Possner & Kliewer 1985, Rogiers et al. 2000), the berry uptake pattern can differ between varieties (Schaller et al. 1992a, b). The disfunction of the vascular system to the berry could be affected by its early development, or its capability can impact on the rate of carbohydrate and nutrient accumulation before any disfunction appears.

Early bunch and berry development can have significant implications for fruit development during berry ripening and composition at harvest. The role of carbohydrate supply to the cluster appears to be

important during flowering, since it is competing with the shoot tip and possible storage. The development of the bunchstem happens mainly before veraison (particularly between bloom and berry set), but a well developed bunchstem will ensure good nutrient and carbohydrate supply through its vascular system.

2 General Materials and Methods

2.1. Location and conditions

Several small field experiments were conducted in the CSU Winery vineyard at Wagga Wagga, NSW Australia (35°05S 147°35E). Wagga Wagga is a hot and semi-arid area with a mean January temperature of 24ºC and average annual rainfall of 572mm. All experiments were conducted on field grown Cabernet Sauvignon and Merlot grapevines on own roots (*Vitis vinifera* L.) during 2002/03, 2003/04, 2004/05, 2005/06 and 2006/07 growing seasons (Figure 2.1). The Merlot vines were planted in 1979 and the Cabernet Sauvignon in 1999 on an eastern slope of a sandy loam (3.5 m between rows and 1.5 m between vines). The vines were drip irrigated and trained to a single bilateral cordon that was hand pruned.



Figure 2.1 Merlot grapevines in the CSU Vineyard, where all experiments were conducted.

2.2 Bunch and berry measurements

Measurements and sampling

The development of the bunchstem was followed by measuring the length and the diameter. The bunchstem length was measured from the shoot to the tip of the bunch, included the hypoclade. On the other hand, the measurement of the diameter was conducted on the hypoclade, unless this one was too short. In this case, the measurement was undertaken between the first lateral and the node (excluding the hypoclade). The developmental measurements of the bunch included also the berries and the bunchstem. The size of three tagged berries situated at the top, the middle and the bottom of the bunch was determined. The berry and bunchstem diameter development were measured using a digital calliper (Absolute Digimatic 500-196, Mitutoyo, Japan).

Bunch samples were taken with variable frequency depending on the trial for both varieties. In the bunch size study (Experiment 1) bunches were taken every two weeks, while for the flower number experiment (Experiment 2) and fruit set experiment (Experiment 3) only at key stages (flowering, fruit set, veraison, mid ripening and harvest). In experiment 1 tagged bunches used for measurements above were sampled at harvest. For the other two trials (experiments 2 & 3), due to the great number of data to collect, only tagged bunches were collected at all times.

In addition, the entire bunches were weighed, and then 25 randomly chosen berries for the bunch size study and all the berries for the other two experiments (flower removal and fruit set modification) were removed from the bunch to have the most representative sample. The berries were pulled out of the bunch, the brush remained on the bunchstem. These berries were also weighed in order to know the average weight of a berry and frozen at –20ºC until further analysis. The rest of the bunch was then stored in a classic preservative solution called FPA50.

Berry and sap analysis

The samples were homogenised at 16,000 rpm with an Ultra Turrax (T25 homogeniser, IKA-Labortechnik, Staufen, Germany) just after thawing. Total anthocyanins (colour) and total phenolics were determined by the method described by Iland et al. (2000).To reproduce the extraction conditions of the colour and anthocyanins during the fermentation, 1g of homogenate is put in 10mL of ethanol 50% for one hour, with 3 times shaking. After centrifugation, 9mL of HCl 1M are added to 1mL of liquid sub-sample. The solution is then homogenised and left for 3 hours at room temperature. The actual reading of the absorbance was performed using a spectrophotometer (UV-VIS scanning spectrophotometer, μQuant, Bio-Tek Instruments Inc, Winooski, USA) at two different wavelengths: 280nm and 520nm for total phenolic compounds and total anthocyanins, respectively.

Another part of the homogenate is put in a 10mL centrifuge tube and is centrifuged to obtain some juice for the remaining analysis. The level of sugars (TSS) in the grape juice is directly measured with a digital refractometer (Atago PR-101, Tokyo, Japan). To measure both the titratable acidity (TA) and the pH an automatic titrator (Radiometer Copenhagen TitraLab 80, TIM800 Titration Manager, ABU901 AutoBurette, SAC80 Sample Changer) associated to a computer (TimTalk program, Radiometer analytical, Lyon, France) was used. In the original method, the samples consist in 5mL of grape juice added to 15mL of distilled water. However, depending on the stage of berry development, the juice was more or less diluted.

The sub-samples of juice from the homogenate used for total anthocyanin and phenolic determination were filtered (45μm). Then the amino groups in these samples were derivatised with FMOC (Haynes et al. 1991) prior to separation and quantification by HPLC with a Hypersil C18 reverse-phase column (150 x 4.6mm ID)(Thermo 5μm ODS hypersil, Waltham, MA, USA). The column was temperature controlled at 38°C and the excitation and emission wavelengths were set at 270 and 316nm respectively on the fluorescence detector (Waters 2475, Milford, MA, USA). A 5 μL sample injection with a tertiary gradient of phosphate buffer (pH 6.5, containing 15% methanol), 15% methanol, and 90% acetonitrile was used with a flow rate of 1 ml/min, the solvent gradient was running for 40 minutes.

The mineral content was also determined from the homogenate by commercial laboratories (Environmental and Analytical Laboratories, Charles Sturt University, Wagga Wagga, NSW and Waite Analytical Services, University of Adelaide, SA). Most macro and micro plant nutrients (except N, B and Mo) were analysed after an acid digestion on an inductively coupled plasma-optical emission spectrometer (ICP-OES).

2.3 Tissue preparation and microscopy

Tissue processing

For all the microscopic studies on the vascular system a modified method of glycol methylacrylate (GMA) embedding was used (O'Brien & McCully 1981). The method consists of four main steps: preservation, dehydration, infiltration and embedding. Another step can be added if staining is required. Firstly, the samples were fixed after completion of all measurements with a preservative (FPA50, composed by 5% Formalin (40% formaldehyde), 5% Propionic acid and 90% Ethanol 50%) and remained at least 48h in the solution. The next step consisted of the dehydration of the material in alcohol. Just before starting the preparation for the microscopy, the FPA50 is replaced by a solution of ethanol 50%. The samples were then put into 70% ethanol, then in 95% ethanol and finally 3 times in pure ethanol, for 2 and a half hours in each solution.

The next step was to infiltrate the samples with GMA resin. The GMA reagent was made by mixing 93mL of liquid GMA, 7mL of liquid polyethylene glycol 400 (PEG) and 0.6 g of benzoyl perioxide. Dehydrated samples were put into a gradient of GMA (25%, 50%, 75%) mixed with ethanol, for two days in each with a renewal of the solution every day. The fixation continued in pure GMA for a week, with a renewal of the GMA solution after 2 days. The samples were finally embedded with GMA in gelatine capsules (2 days at 60°C for polymerization). The embedded samples were sectioned with an ultra thin microtome (OmU2, Reichert, Austria), using homemade glass-knives. Because of the hydrophilia of the GMA resin, we used dry sectioning techniques. For light microscopy, the sections were made to 2 to 2.5µm thick.

Image analysis

A microscope (Provis AX70, Olympus) linked to a camera and a computer has been used. Depending on the stain used, both bright field and UV fluorescence have been applied to emphasize on particular structures of the vascular system. The stain aniline blue was used, which fluorescence blue under UV light. The number and area of the bundles were measured on digital pictures with an image processing program (analySIS 5 LS Research, Image processing, Life Science, Olympus company).

3 Bunch size study (Experiment 1)

3.1 Introduction

Grapevine bunches can have different shapes, sizes and weights depending on the variety, the training and pruning system, the position on the shoot and the vigour of the vine. However, a classical bunch is always formed by a peduncle, a main axis called bunchstem or rachis (Figure 3.1). The bunchstem is more or less ramified, having a number of laterals depending on the size of the bunch. Each lateral can also be ramified and usually ends with a bunch of two to five berries. The smaller ramifications are the pedicels that link the berries with the overall bunchstem structure.

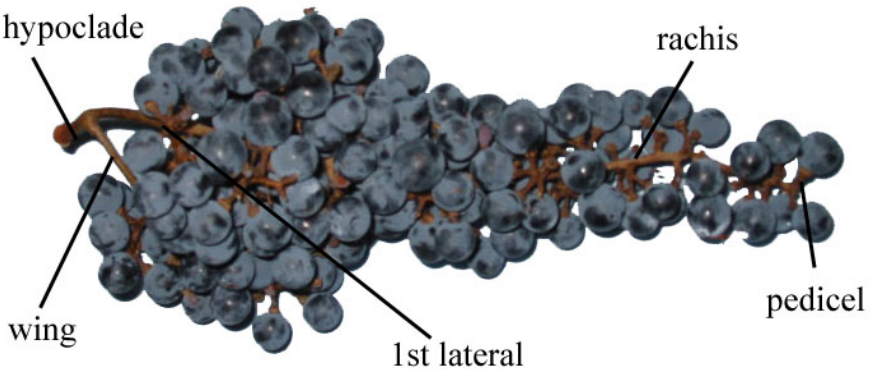


Figure 3.1 Cabernet Sauvignon bunch with the different parts of the bunchstem indicated.

The bunchstem usually represents between 3 and 8% of the total weight of a ripened bunch (Huglin & Schneider 1998; Ribéreau-Gayon et al. 1998) at harvest. Cabernet Sauvignon bunchstem represents about 8% of the total bunch weight compared to only 5% for the Merlot; this difference appears to be linked with the average berry size of each variety (Galet 2000). The growth of the rachis is influenced by flower and berry development (Theiler & Coombe 1985). The development of the rachis is divided into two phases. Firstly, there is a rapid increase in the length and a later thickening of the stem from one month before flowering to mid-bloom. After flowering 75% of the final size of the bunchstem and the vascular system are established, there are some further small changes until its final size is reached around veraison (Ribéreau-Gayon et al. 1998). During grape bunch development, the area of both vascular systems (phloem and xylem) increase simultaneously (Lang & Ryan 1994).

This part of the project was investigating the dynamics of bunchstem and berry development in bunches of different sizes. The aim was to clarify the relationship between bunchstem structure and its vascular system in relation berry development and composition.

3.2 Materials and Methods

The experiment was conducted in the CSU vineyards on two varieties Cabernet Sauvignon and Merlot over two growing seasons (see section 2 for further details). The trial consisted of four replicates with four vines in two rows for both cultivars. On each vine a large and a small bunch were selected randomly at flowering to study the development of the bunch. The development of these bunches were followed throughout the growing season. On each bunch, three of the berries (top, middle and bottom) were tagged to follow the growth of the berry size. All the tagged bunches have been collected at harvest for further analysis. The bunch development was followed throughout the growing season through measurements of bunchstem length and diameter. At harvest, basic berry quality characteristics were analysed, such as total soluble sugars content, colour, juice pH and acidity. The

size of the vascular system at harvest from different section throughout the bunchstem structure was studied using microscopic techniques in Cabernet Sauvignon and Merlot.

3.3 Results and discussion

Berry development and composition

The berry size was generally smaller in the second season of the experiment, the final size of berries coming from large or small bunches was similar in both years. However, during berry development the size of the bunches influenced berry diameter (Figure 3.2). The berry diameter changed during the berry development, most significantly from shot gun size to veraison, with smaller changes present during berry maturation.

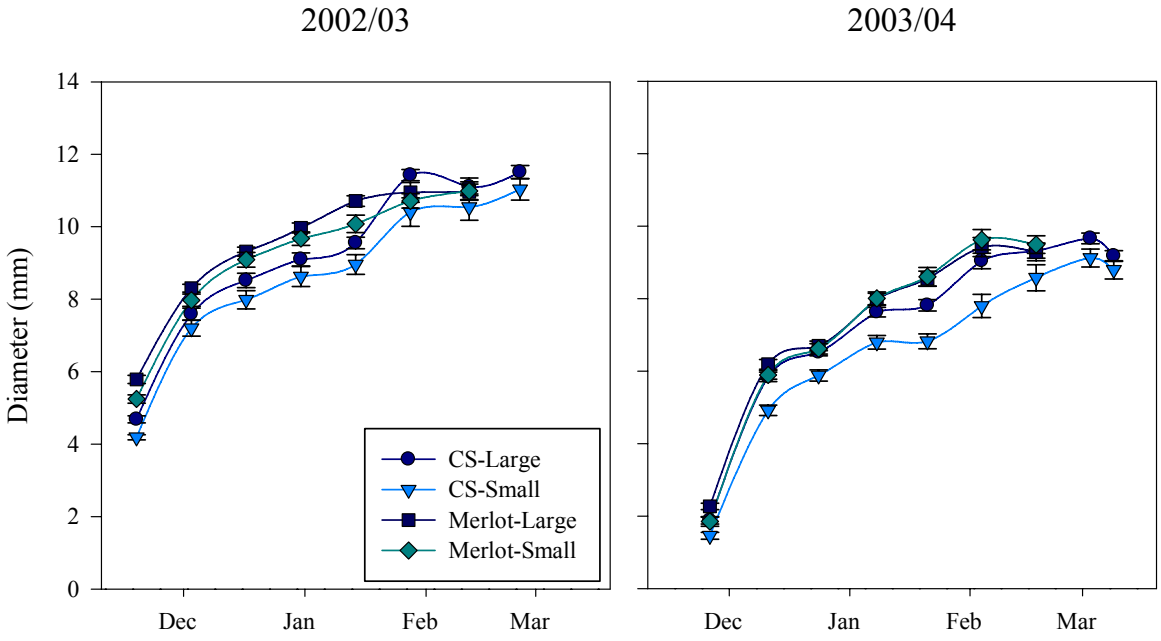


Figure 3.2 Impact of bunch size on berry diameter from two varieties and two growing seasons (standard errors of the means are shown as bars, n=4).

The final bunch weight was assessed at harvest and revealed that the difference between the large and small bunches was more distinct in the second season of the trial (Table 3.1). At harvest 2004 the large bunches weighed about double the small ones, which was mostly due to berry numbers. The berry size was also usually related to bunch size, except for Merlot in the last season. Total anthocyanins, total phenolics, and TA were higher, and pH and TSS lower in the large bunches compared to the smaller ones. Again, with the exception of Merlot in the last year for pH and TSS, this might indicate the importance of berry size on this composition parameter.

Table 3.1 Impact of bunch size on berry parameters from two varieties and two years (2003, 2004). (standard errors of the means are shown in small type, n=4)

	Cabernet Sauvignon				Merlot			
	2003		2004		2003		2004	
	Large	Small	Large	Small	Large	Small	Large	Small
Bunch wt (g)	141.0 0.255	110.2 0.465	150.7 0.161	65.7 0.113	72.6 0.095	63.4 0.121	96.3 0.099	49.0 0.080
Berries/bunch	185.4 11.20	161.3 12.95	225.9 8.60	110.3 7.04	94.4 6.39	86.7 5.56	151.8 8.04	78.2 5.14
Berry wt (g)	0.76 0.023	0.68 0.036	0.67 0.019	0.60 0.016	0.77 0.015	0.73 0.022	0.63 0.012	0.63 0.016
Anthocyanin (g FW)	0.90 0.050	0.73 0.035	1.33 0.037	1.28 0.040	0.88 0.040	0.69 0.035	0.94 0.040	0.81 0.050
Phenolics (g FW)	1.46 0.038	1.42 0.048	1.49 0.027	1.56 0.047	1.46 0.036	1.32 0.030	1.47 0.027	1.36 0.038
TSS (ºBrix)	23.1 0.331	25.8 0.505	24.1 0.332	25.4 0.616	22.4 0.305	23.9 0.365	25.3 0.330	25.0 0.320
TA (g/L)	2.66 0.042	2.82 0.058	2.70 0.050	2.85 0.088	2.91 0.076	3.21 0.134	2.71 0.057	2.90 0.036
pH	4.59 0.016	4.68 0.018	4.49 0.029	4.52 0.028	4.54 0.025	4.77 0.023	4.46 0.021	4.46 0.027

Bunchstem development and vascular system

There was a strong relationship between bunch size and bunchstem length in Merlot and Cabernet Sauvignon throughout both seasons, which was more pronounced in Merlot (Figure 3.3). Most of the growth appeared several weeks before veraison, being more pronounced and lasting longer in the larger bunches, particularly in the second growing season (2003/04). The small Merlot bunches appeared to nearly have finished their length expansion. The diameters are also distinctively different between bunch sizes, but very erratic during the berry development period, instigating the difficulties in measuring this parameter accurately. The substantial reduction in bunchstem diameter in the later part of grape maturation in Cabernet Sauvignon during the last season might be due to some internal disintegration of the bunchstem due to high temperatures and/or rain fall in the later part of grape maturation.

The rachis of the Cabernet Sauvignon below hypoclade showed the four structures, namely fibres phloem, rays and xylem (Figure 3.4). Rachis about in the middle of the bunch presented younger xylem and less cells; the bundles were more separated from each other. The first lateral had a similar structure to the main rachis with more separated bundles and less developed xylem; very similar to bunchstem structure in the middle of the bunch. Structure of the vascular system was similar for the variety Merlot, for both varieties a large bunch was selected for these pictures to allow for best detail of the sections.

3.4 Conclusions

The size of the bunch influenced berry number per bunch and a number of berry parameters: larger bunches had generally larger berries and lower TSS levels, while higher in all other compositional parameters. There was a strong relationship between bunch size and bunchstem size, in regard to length and diameter. The vascular system size varies between positions in the rachis and laterals and appeared to depend on the number of berries being supported.

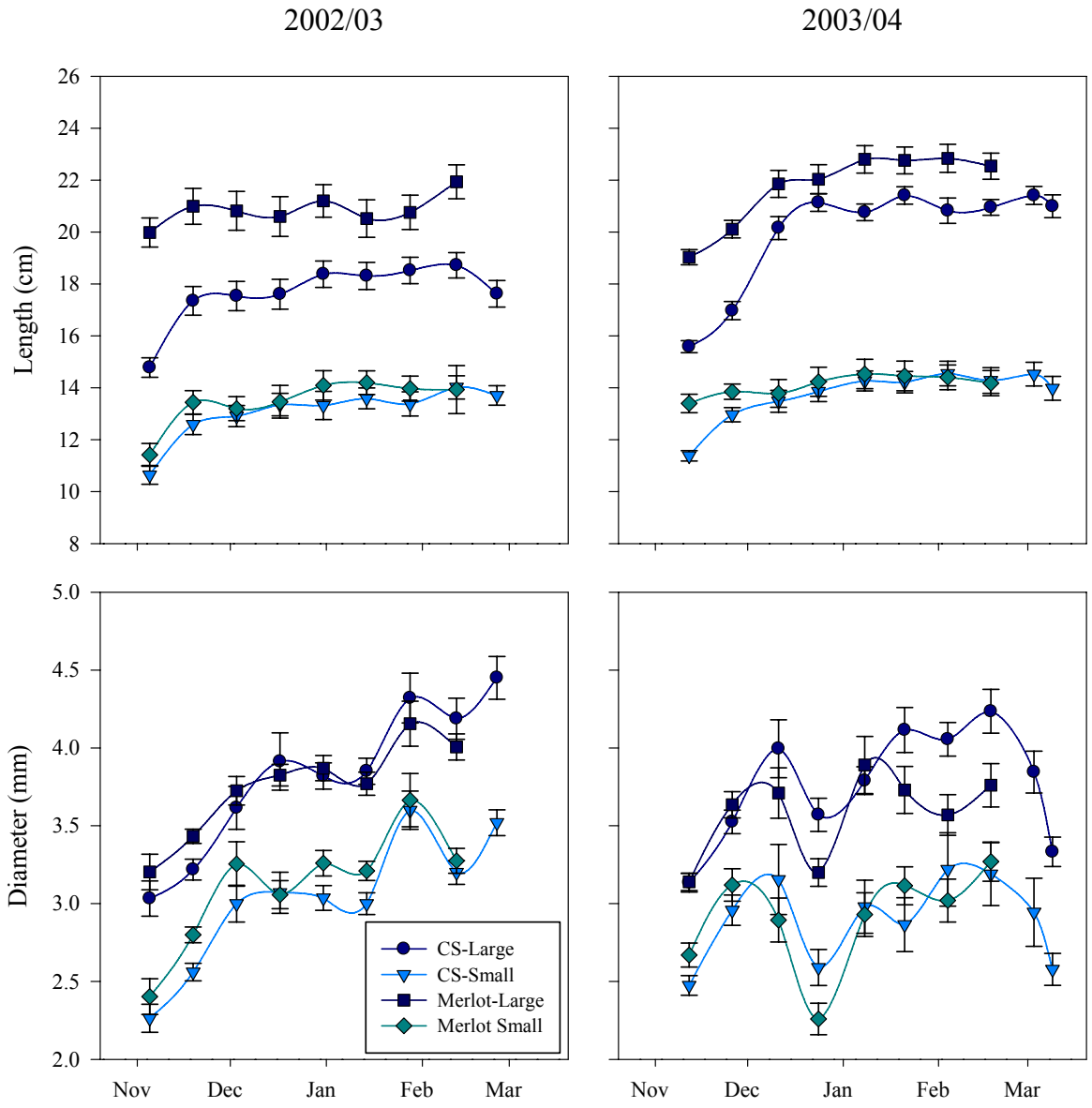


Figure 3.3 Impact of bunch size on bunchstem length (top) and diameter (bottom) from two varieties and two growing seasons (standard errors of the means are shown as bars, n=4).

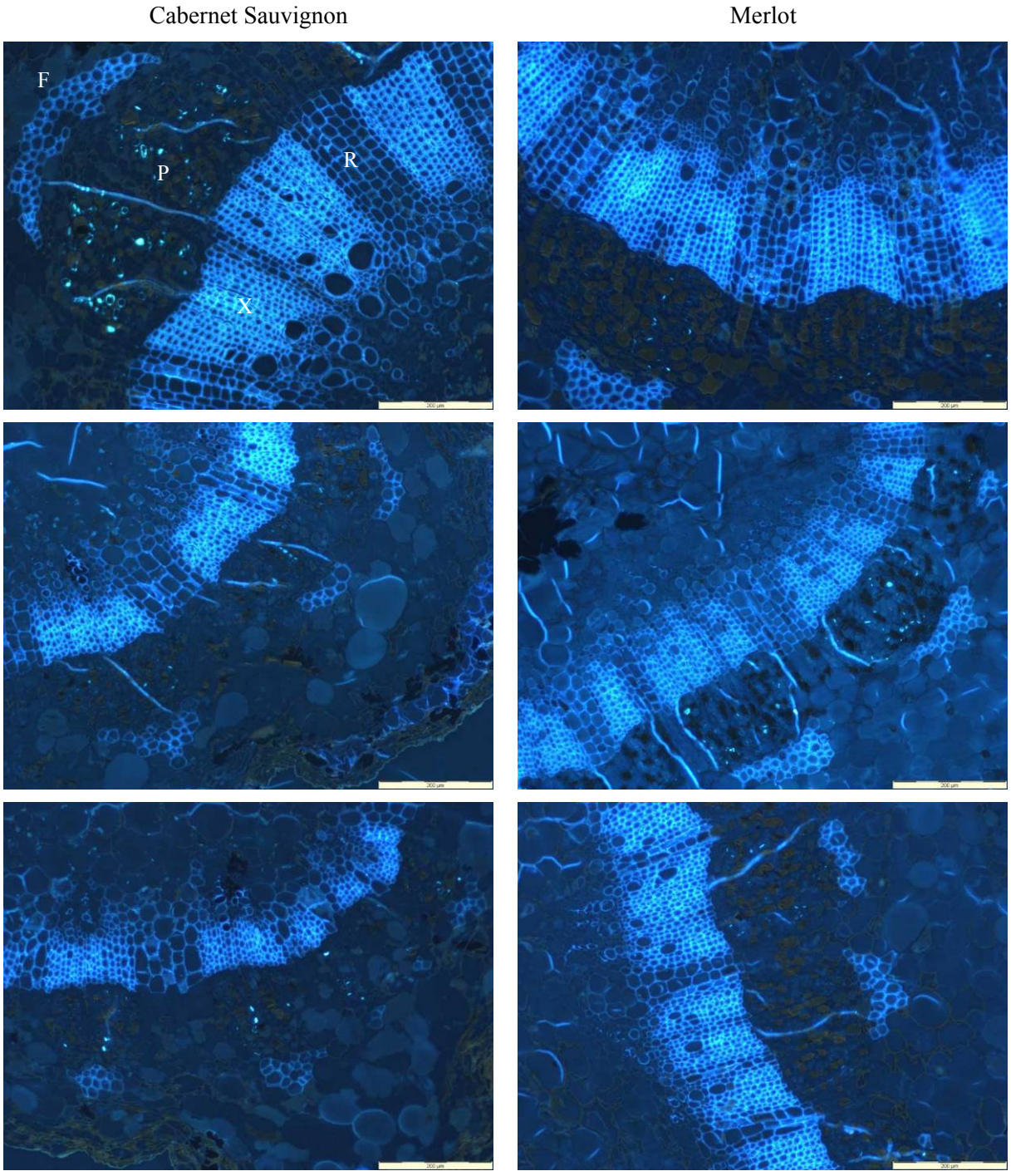


Figure 3.4 Microscopy pictures of the bunchstem structure, from top to bottom of Cabernet Sauvignon and Merlot: rachis below hypoclade, 1st lateral and mid rachis (F = fibres, P = phloem, R = rays, X = xylem).

4 Flower number removal (Experiment 2)

4.1 Introduction

The size of the inflorescence with the number of flowers is one of the factors determining final bunch size and berry number. Weather has a significant impact on the length of flowering and fruit set, therefore also impacts on the berry number of the bunch. Bad weather conditions can generate the phenomenon of coulure, which is the abortion of an abnormally high number of flowers per inflorescence. Temperature and light intensity at flowering are the most important factors for good fruit set (Ebadi et al. 1995a, b; Ebadi et al. 1996), but other factors such as mineral nutrition and plant hormones can have an influence (Srinivasan & Mullins 1981). The assimilation of carbon and the level of carbohydrate reserves can influence fruit set, which could explain the difference between varieties (Zapata et al. 2004a, b).

During fruit set the carbohydrate supply from main leaves affects the number of berries per bunch and the bud fertility (Candolfi-Vasconcelos & Koblet 1990). For the final crop yield, carbohydrate production from bloom to three weeks after is thus the most critical period, because it affects the final berry number and size. Theiler (1974) showed a necrosis of the rachis on the parts (lateral or tips) fully de-berried at flowering on *Vitis vinifera* cv. Rote Gutedel. If a single flower remains on the rachis, it stays green and turgescent. The rachis also remains entire and alive when the berry removal is applied after fruit set. Theiler and Coombe (1985) studied the development of the rachis in the variety Roter Gutedel and the counter-effect of some plant hormones treatments. Dipping the bare peduncle in either NAA or gibberellic acid (GA) gave the same result as leaving a berry, meaning that the peduncle remains green and turgescent. The other hormone treatments delayed the peduncle abscission, and sometimes the decay.

The size of the inflorescence and fruit set influences the final number of berries on a bunch, studies have also shown that partial and full flower removal influence the bunchstem development and structure. This work was designed to better understand the interaction between flower number, bunchstem development and final berry composition.

4.2 Materials and Methods

The experiment was conducted at the CSU vineyards in two varieties Cabernet Sauvignon and Merlot over two growing seasons (2004/05 and 2005/06). Further details are described in section 2 in this report. The trial consisted of four replicates with five vines in two rows for both cultivars. On each plant, five treatments have been applied to five bunches located at the same level of the canopy and were randomly marked. The treatments are linked with a percentage of flowers removed (control, 25%, 50%, 75% and 100%) and were conducted around one week before flowering. Since it was critical to apply all the treatments in a single day on the same cultivar, bunches of flowers (secondary branching) have been cut off close to the peduncle; closer to the tip of the rachis single flowers were removed.

Bunches of each treatment were collected at key times during the ripening period, veraison, mid-ripening and harvest. One vine per replicate is associated with one of the key stages. For the three stages basic berry parameters were determined and the berries were analysed for amino acid concentrations and nutrient levels (see section 2 for further details). The berries per bunch were also determined for each of these stages, but only the harvest date is presented. The vascular structures were assessed under the microscope for some of the major stages and for a number of treatments, but here it will only be reported on the control and 100% flower removal.

4.3 Results and discussion

Berry number and composition

The number of berries most commonly correlated with the flower removal treatments, Cabernet Sauvignon showed higher berry number in the last season, this was also present in some instances in Merlot. This indicates that the inflorescences might have been bigger in that season or the fruit set conditions were better, at least for the variety Cabernet Sauvignon. This means the berry number is lower if more flowers were removed before bloom, following the treatment pattern (Figure 4.1). The removal of flowers does not correlate to a strong reduction in berry numbers as implemented by the treatments before bloom, suggesting that the fruit set improves if more flowers are removed. There are a few exceptions in the last year regarding the relationship between flower number and berry number, for instance at veraison in Cabernet Sauvignon and mid ripening in Merlot, where the 25% flower removal treatment had a similar or higher berry number than the control.

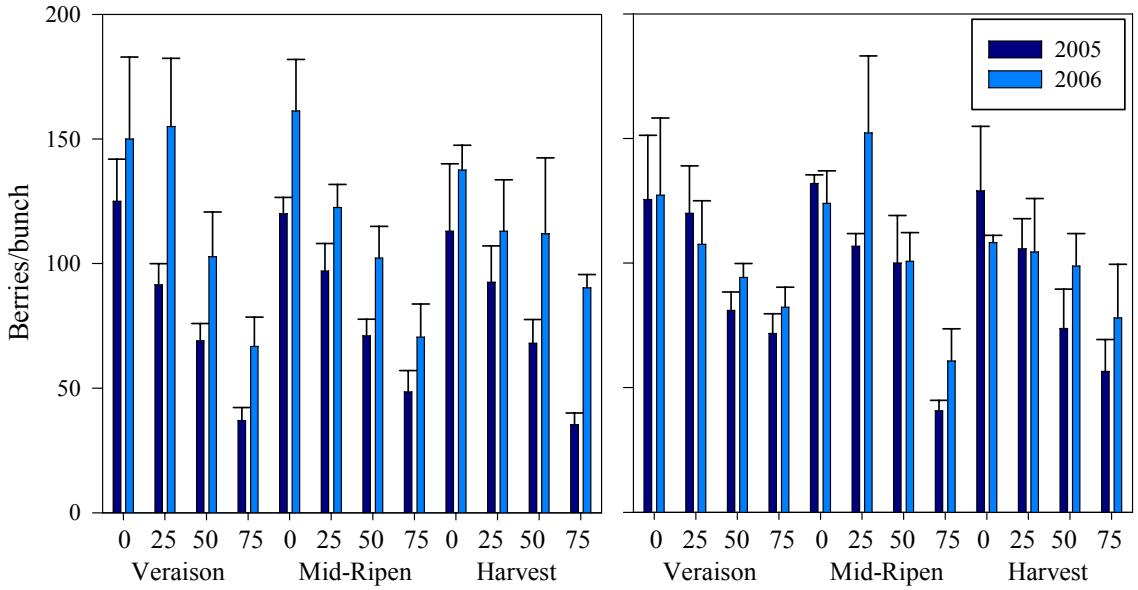


Figure 4.1 The impact of flower removal on berry number in Cabernet Sauvignon (left) and Merlot (right) with the numbers on the X axis indicating the severity of the flower removal in percent; (standard errors of the means are shown as bars, n=4).

Berry weight approximately doubled in both years in Cabernet Sauvignon, this increase from veraison to harvest was more pronounced in Merlot (Table 4.1). Total anthocyanins and TSS also increased during grape maturation, while the phenolic contained declined. Berry size was higher in Cabernet Sauvignon than in Merlot at harvest, particularly in the last season. In Cabernet Sauvignon berry size in both years was lowest in the control, while in Merlot this was reached with 25% flower removal, generally the berry size increased with the severity of flower removal. This suggested more resources allocated to the lower number of berries, allowing for increased growth. Total anthocyanins and phenolics were higher in Cabernet Sauvignon than Merlot at harvest in both seasons, this difference was more pronounced at harvest 2006. Berry colour and phenolics in Cabernet Sauvignon was highest in 75% flower removal at harvest 2005 and in the following season in the control treatment. In Merlot this was the opposite between years, here the highest total anthocyanins were determined in the control in 2005 and in the 75% flower removal in 2006. Berry maturity was similar between varieties at harvest 2005, while in the last season Cabernet Sauvignon was harvested with higher TSS levels. In

the first season Cabernet Sauvignon control berries and Merlot of the 25% flower removal treatment showed the highest TSS. In the last season the differences between treatments was relatively low in both varieties. The results show that flower number impact on berry size and some basic composition parameters, but the impact differs between varieties and years.

Table 4.1 Influence of flower removal treatments on berry composition in Cabernet Sauvignon and Merlot (standard errors of the means are shown in small type, n=4).

		2005				2006			
	Flower removal	**Berry wt. (g)**	**Anthocyanin (g FW)**	**Phenolics (g FW)**	**TSS (ºBrix)**	**Berry wt. (g)**	**Anthocyanin (g FW)**	**Phenolics (g FW)**	**TSS (ºBrix)**
					Cabernet Sauvigon				
Veraison	0	0.49 0.04	0.02 0.00	1.74 0.13	6.63 0.45	0.35 0.03	0.08 0.02	2.52 0.26	7.78 0.96
	25	0.50 0.01	0.02 0.01	1.61 0.20	6.65 1.05	0.39 0.03	0.10 0.03	2.32 0.28	8.25 1.11
	50	0.50 0.03	0.01 0.01	1.60 0.11	5.08 0.09	0.38 0.02	0.12 0.03	2.24 0.14	8.58 0.84
	75	0.50 0.03	0.01 0.00	1.58 0.08	5.48 0.53	0.39 0.02	0.08 0.01	2.23 0.11	7.65 0.48
Mid ripen	0	0.89 0.02	0.67 0.10	1.04 0.06	18.30 0.31	0.76 0.07	0.59 0.03	0.90 0.02	17.23 0.60
	25	0.77 0.08	0.48 0.14	1.16 0.09	15.33 1.53	0.81 0.06	0.74 0.04	0.92 0.02	17.48 0.41
	50	0.82 0.05	0.65 0.13	1.07 0.08	17.00 0.79	0.79 0.02	0.67 0.12	0.91 0.07	18.23 1.15
	75	0.79 0.09	0.47 0.09	1.13 0.17	15.45 1.01	0.84 0.06	0.65 0.05	0.89 0.04	17.50 0.78
Harvest	0	0.99 0.05	1.06 0.04	1.03 0.05	24.10 0.31	0.82 0.04	0.96 0.08	0.95 0.07	24.15 0.60
	25	1.07 0.05	0.90 0.29	0.85 0.27	23.70 0.14	0.76 0.10	0.94 0.08	1.03 0.09	25.63 1.50
	50	1.01 0.07	1.06 0.06	1.05 0.06	23.80 0.31	0.88 0.04	0.94 0.05	1.00 0.04	23.58 0.52
	75	1.02 0.02	1.21 0.16	1.11 0.10	24.03 0.33	1.00 0.02	0.87 0.03	0.92 0.03	22.48 0.59
					Merlot				
Veraison	0	0.65 0.07	0.02 0.01	1.34 0.11	8.48 0.98	0.40 0.05	0.01 0.00	1.96 0.10	6.25 0.12
	25	0.54 0.03	0.02 0.01	1.63 0.14	7.10 1.21	0.37 0.01	0.01 0.00	1.77 0.15	6.15 0.20
	50	0.62 0.04	0.02 0.00	1.32 0.04	8.13 0.46	0.42 0.02	0.02 0.02	1.79 0.11	6.28 0.26
	75	0.65 0.02	0.01 0.00	1.27 0.19	7.23 0.74	0.42 0.04	0.01 0.00	1.84 0.09	6.35 0.41
Mid ripen	0	0.96 0.08	0.42 0.04	1.12 0.08	15.88 0.42	0.85 0.06	0.46 0.02	0.98 0.04	19.73 0.21
	25	0.95 0.07	0.35 0.13	0.97 0.05	15.05 1.18	0.68 0.07	0.38 0.06	1.23 0.12	15.90 1.28
	50	0.96 0.05	0.39 0.07	0.99 0.03	15.70 0.78	0.79 0.04	0.39 0.04	1.01 0.05	16.63 0.41
	75	1.02 0.05	0.23 0.04	0.92 0.10	14.23 0.74	0.71 0.08	0.39 0.06	1.09 0.07	16.45 0.73
Harvest	0	1.06 0.03	1.28 0.11	1.23 0.09	22.43 0.58	0.83 0.05	0.69 0.10	1.00 0.07	20.53 1.63
	25	1.13 0.11	1.23 0.19	1.19 0.08	22.25 0.51	0.74 0.08	0.69 0.10	0.98 0.05	20.90 0.53
	50	1.23 0.05	1.04 0.10	1.07 0.04	22.45 0.37	0.81 0.02	0.67 0.09	0.95 0.02	20.65 0.95
	75	1.25 0.03	1.20 0.08	1.16 0.02	22.35 0.39	0.92 0.04	0.74 0.14	0.96 0.06	21.38 0.70

The amino acid concentrations were determined at the beginning, in the middle and at the end of berry maturation to investigate the impact of flower removal on this group of compounds. Proline usually increased from veraison to harvest, arginine peaks at mid ripening in Cabernet Sauvignon and Merlot at harvest (Figure 4.2). Concentrations of arginine were highest at harvest 2005, while proline was highest at harvest 2006. Differences between seasons and varieties are often seen as well as the pattern of concentrations observed over the berry ripening period. The two major amino acids varied between varieties, proline was highest in Cabernet Sauvignon and arginine in Merlot. In Cabernet Sauvignon both amino acids are increased with the amount of flowers removed before bloom, except for the 75% removal treatment in 2005. Merlot shows the same pattern for the 2005 harvest, but in 2006 a slight decline can be observed if more flowers are removed at bloom. Overall the amino acid concentrations are in the mid range compared to levels reported by others, the number of berries on the bunch altered the level of these two major amino acids in the berry.

A number of macro nutrients (Ca, Mg, K and P) in the berry have been assessed at the same times as

the amino acids. The nutrients showed a similar accumulation pattern between varieties in the first season, but not in the last season (2005/06). The accumulation pattern also varied between years (Table 4.2). In the first growing season the nutrient Ca was lowest at harvest and highest at veraison, while in 2006 this did not apply for Cabernet Sauvignon, where the highest level was observed at mid ripening. Mg was lowest at mid ripening in the first season, while in the last season at harvest. K usually increased during berry ripening and was higher in Merlot grapes. P increased also during ripening in both seasons, but this accumulation was less pronounced in the last season in both varieties. In Cabernet Sauvignon the treatment with 50% flowers removed at bloom had the highest concentrations of Ca, Mg and P, while the nutrient K is highest in the 25% treatment. In the 2005/06 season, it was the control with the highest K and the 25% flower removal treatment. In Merlot the treatment with 75% flowers removed at bloom has the highest Ca, Mg and P, while the control has the highest K in both seasons. In the last season Ca and P have the highest level, while Mg is not changed. The impact of flower removal appears to be more pronounced in Cabernet Sauvignon than in Merlot, at least for the nutrients Ca, Mg and P.

Bunchstem vascular system

Removing flowers will surely affect the development of the inflorescence, and then the bunch in its whole, bunchstem and berries. Decay of berry-less parts of the bunchstem has been confirmed with the present study: about one week after applying the treatment (around flowering time), the drying process started, followed by a simple fall of the dry part (about one month after flowering). This was after the bunchstem diameter and lengths declined soon after the treatment implementation (data not presented). Symptoms were similar to the ones of bunchstem necrosis (BSN). However, BSN only occurs where the berries are present and appears around veraison; while the decay in this study appears earlier in the season and on the de-flowered parts of the bunch. The cause has thus to be different, even if the result looks the same. Some bunches of the most severe treatments (-75% and -50%), more obvious or earlier on Merlot than on Cabernet, with most remaining berries on one side have curved bunchstem. This can be linked with a differential growth of the rachis depending on the presence or absence of berries. The microscopic pictures of Cabernet Sauvignon bunches at fruit set (Figure 4.2) revealed that the removal of 100% flower induces a disorganisation of the vascular system first in the lateral, then in the main axis.

4.4 Conclusions

The berry number was proportionally reduced by the flowers removed at bloom, however the reduction was not as strong as implemented by the treatments, suggesting that the fruit set improves if more flowers are removed. The reduction in flower numbers lead to an increase in berry size and the concentrations of the amino acids proline and arginine in the berry were generally elevated. The treatments also altered basic berry composition and nutrients, but these changes were variable between varieties and years. The vascular system was altered by the flower removal treatment, leading to disintegration of first the laterals and then the whole bunchstem system. The treatments receiving flower removal at a lower level could have impacted on the vascular system also and have partially implemented the differences in berry size and berry composition.

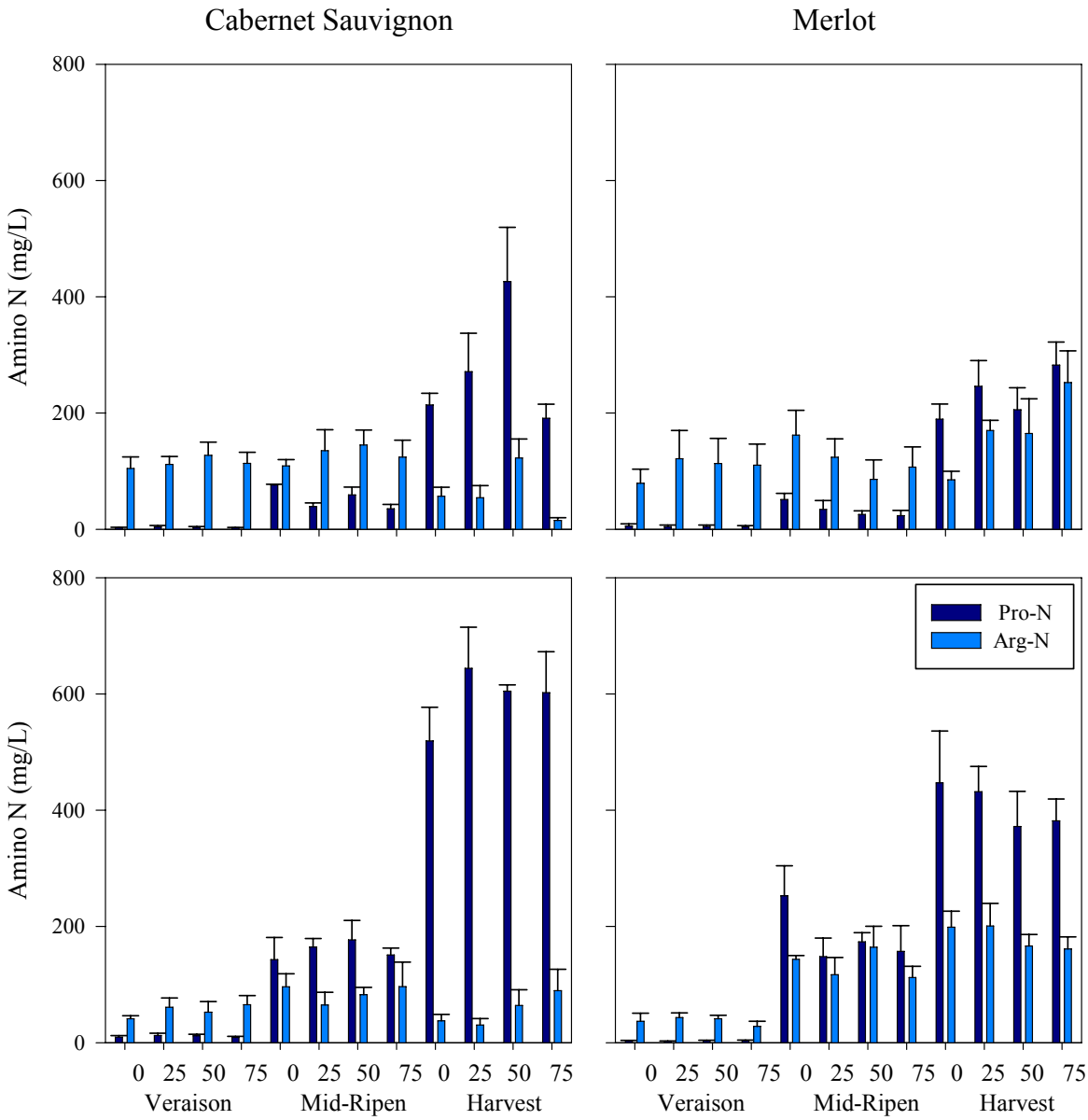


Figure 4.2 Amino acid concentrations at veraison, mid ripening and harvest in Cabernet Sauvignon and Merlot with the numbers on the X axis indicating the severity of the flower removal in percent, season 2004/05 (top) and season 2005/06 (bottom), (standard errors of the means are shown as bars, n=4).

Table 4.2 Influence of flower removal treatments on berry nutrient concentrations in Cabernet Sauvignon and Merlot. (standard errors of the means are shown in small type, n=4)

		2005				2006			
	Flower Removal	Ca mg/kg	Mg mg/kg	K mg/kg	P mg/kg	Ca mg/kg	Mg mg/kg	K mg/kg	P mg/kg
				Cabernet Sauvignon					
Veraison	0	289 25.8	119 3.2	1346 26.4	217 6.2	435 40.3	181 8.4	2675 118.1	345 17.1
	25	303 24.5	129 4.1	1289 29.8	217 9.2	433 42.1	172 16.8	2325 110.9	303 14.9
	50	393 13.8	149 6.4	1318 47.7	233 0.4	483 36.8	186 13.4	2500 129.1	330 7.1
	75	289 18.3	135 4.4	1398 59.5	253 8.3	535 25.3	192 12.4	2475 47.9	328 21.4
Mid ripen	0	217 26.2	114 7.4	2068 270.3	216 15.9	615 80.5	174 9.0	2400 191.5	325 24.0
	25	224 42.1	125 10.6	2232 233.0	226 29.4	708 67.3	195 10.2	2425 209.7	360 39.2
	50	214 23.0	111 2.2	1522 86.7	216 5.3	588 58.9	177 12.4	2400 108.0	343 8.5
	75	177 8.8	106 3.4	2426 219.0	223 7.3	660 50.5	187 5.6	2325 103.1	368 11.1
Harvest	0	227 9.2	126 1.9	2250 62.1	301 8.9	243 8.5	132 2.5	3025 25.0	333 8.5
	25	268 43.8	132 7.9	2413 252.7	291 26.9	510 28.7	164 5.9	2500 150.0	335 15.5
	50	301 64.3	148 15.9	2304 176.1	299 51.3	385 33.0	142 8.1	2400 81.6	308 16.8
	75	200 6.3	124 5.9	2403 199.4	279 36.0	428 15.5	154 1.5	2500 165.2	325 8.5
				Merlot					
Veraison	0	382 25.9	124 7.1	1469 52.7	250 27.7	615 80.5	174 9.0	2400 191.5	325 24.0
	25	365 22.7	127 9.9	1581 114.1	272 30.9	708 67.3	195 10.2	2425 209.7	360 39.2
	50	385 35.5	126 5.5	1379 23.1	266 11.4	588 58.9	177 12.4	2400 108.0	343 8.5
	75	370 32.3	123 7.1	1456 46.2	270 13.8	660 50.5	187 5.6	2325 103.1	368 11.1
Mid ripen	0	274 24.6	104 4.1	1810 240.2	243 16.5	398 28.7	144 2.8	3175 62.9	388 32.5
	25	237 20.8	105 6.1	1910 247.3	229 5.7	480 39.2	154 7.7	3100 173.2	388 24.6
	50	349 40.1	121 4.3	2026 173.3	229 19.5	453 20.2	137 6.1	3025 103.1	348 21.4
	75	217 13.9	78 25.3	2214 145.7	228 17.4	545 48.0	154 9.5	3000 81.6	360 14.7
Harvest	0	278 64.3	117 10.4	2134 202.2	333 50.2	465 37.7	146 2.8	3225 85.4	385 35.9
	25	264 18.2	115 7.1	1876 147.8	282 19.4	440 19.6	145 4.4	3100 108.0	378 38.6
	50	302 36.5	116 10.3	1861 93.2	327 56.9	440 28.6	145 3.3	3000 91.3	360 31.9
	75	340 61.4	128 17.9	2093 170.1	356 73.2	418 17.5	146 4.1	3125 170.2	378 39.7

Main rachis

First lateral

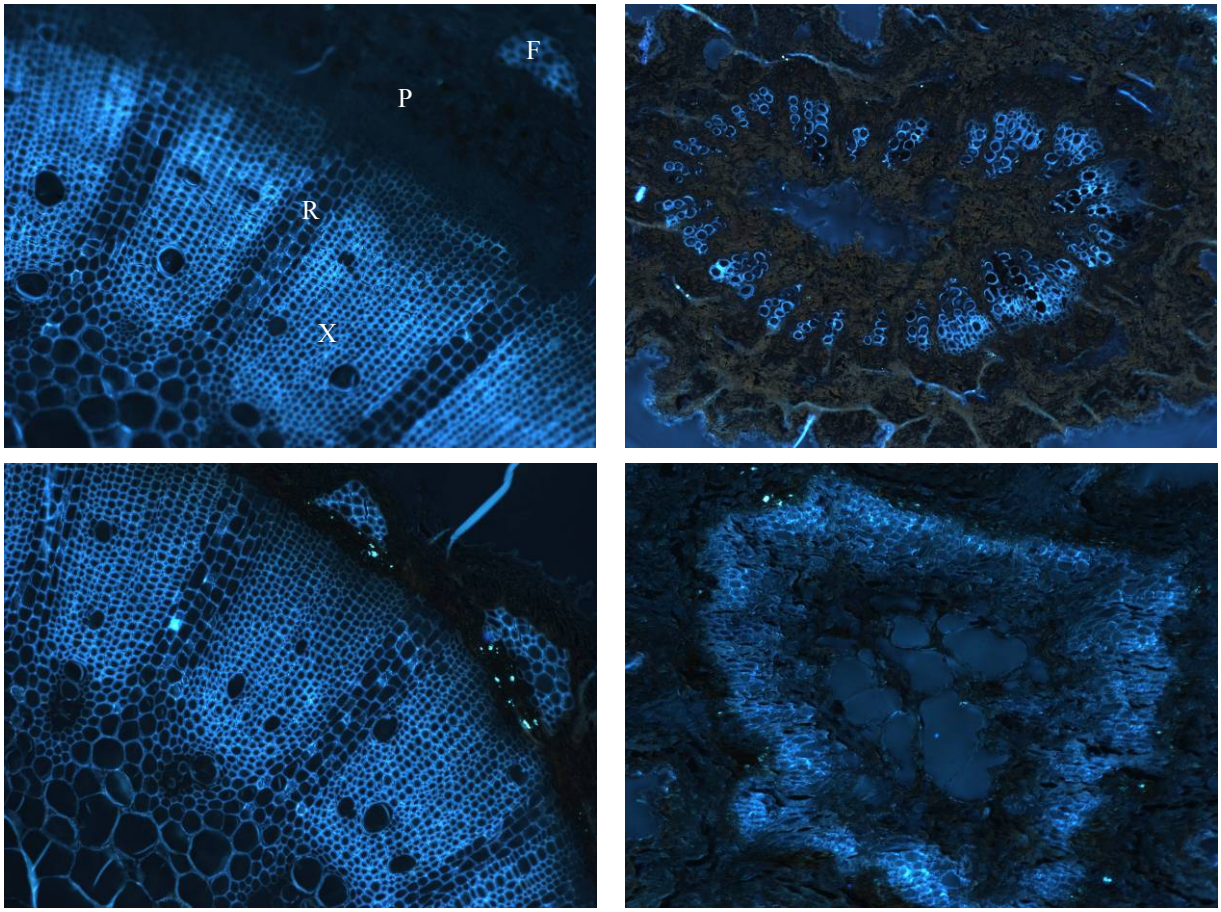


Figure 4.3 Microscopy pictures of the bunchstem structure at fruit set of the control (top) and 100% flower (bottom) removal treatment in Cabernet Sauvignon (F = fibres, P = phloem, R = rays, X = xylem.

5 Fruit set alteration (Experiment 3)

5.1 Introduction

The external and internal factors influencing fruit set have been described in the previous section. This relates to climatic conditions around the flowering and fruit set period as well as carbohydrate production and partitioning. The alteration of carbon supply to the inflorescence and the application of plant hormones can influence the final number of berries on a bunch.

The climatic conditions also have a significant impact on the length of flowering time and fruit set period. Unfavourable weather conditions during the bloom to fruit set period reduce carbon assimilation and make the vine more dependent on carbohydrate reserves (Keller et al. 1995). Leaves in the bunch zone seem to be most important in supplying bunches with assimilates (Schultz et al. 1996). The shoot tips are more important sinks during flowering than the inflorescences (Koblet 1969), meaning a removal of the growing tip will benefit the carbon supply to the inflorescences (Currle et al. 1983) and will influence the fruit set. Carbohydrate production from bloom to up to three weeks after is most critical for final crop yield (Candolfi-Vasconcelos & Koblet 1990).

Cytokinins are the hormones implied in cell elongation (Davies 2004). Their role in flower development is through the increased sink capacity of the inflorescence compared to vegetative organs (Huglin & Schneider 1998). Srinivasan and Mullins (1981) have produced inflorescences from tendrils by applying 6-benzylaminopurine (BAP). Sprays of cytokinins have been tested to stimulate the growth of inflorescences. Depending on the concentration of the solution, the results have been contradictory. Srinivasan and Mullins (1976) have shown that low concentration stimulates inflorescence lengthening and appearance of flowers; while at higher concentration the final number of berries per bunch has not been increased (Weaver et al. 1966). The commonly used synthetic cytokinin N-(2-chloro-pyridyl)-N'-phenylurea (CPPU) in table grape vineyards can increase fruit set and decrease the number of seeds per berry (Hayata et al. 1995).

Gibberellins (GA) have been used to reduce fruit set and compactness of some cultivars of table grapes (Huglin & Schneider 1998), and thus the incidence of bunch rot. GA_3 is also involved in the inhibition of flower initiation (Boss et al. 2003), but this response is cultivar-dependant. The results obtained after spraying exogenous gibberellins solutions have thus been contradictory. Khursid et al. (1992) have shown that GA_3 increases both the number of flowers per inflorescence and its length. On the other hand a decrease in fruit set, berry number and weight, and yield have been implemented by applying this plant hormone. GA_3 also seems to have a carry-over effect on both bud fertility and flower number per bunch to the following season (Dokoozlian et al. 2001, Weyand & Schultz 2005).

These findings suggest that fruit set can be altered by applying the plant hormone cytokinin or gibberellin to the bunch or by changing the assimilate supply to the bunch. This experiment was designed to give a better understanding how fruit set impacts on the bunchstem and berry development from bloom to harvest.

5.2 Materials and Methods

The study was conducted at the CSU vineyards in the varieties Cabernet Sauvignon and Merlot over two growing seasons (2005/06, 2006/07). Further details are described in section 2 in this report. The trial consisted of four replicates with four vines in two rows for both cultivars. On each vine, five treatments have been implemented on bunches of five shoots, which were randomly marked. The treatments are as follows: control, 50% flower removal per bunch (as explained in 4.2), all leaves removed on the shoot (except the tip and the last 4 unfold leaves), bunch dipping in gibberellic acid (GA_3) solution at 75ppm, bunch dipping in a cytokinin (6-benzylaminopurine, BAP) solution at 250ppm. All the treatments were applied in a single day on the same cultivar.

The treated bunches are collected at key times: flowering, fruit set, véraison, mid-ripening and harvest. One vine per repetition is associated with one of the key stages up to mid-ripening. At harvest, all the

remaining tagged bunches are picked. In this section, the same three key stages as the previous chapter were also investigated for bunch development, and berry development and composition. The berry composition was investigated at both basic and detailed levels (see section 2 for further details). The vascular system structures were also investigated under the microscope at harvest for the two hormones and the leaf removal treatments (since control and flower removal being similar to experiment 2), the GA3 treatment response is partly presented in this report.

5.3 Results and discussion

Berry development and composition

The treatments are meant to impact on fruit set and ultimately on the berry number per bunch to investigate changes in berry composition and bunchstem structure. The response to the treatments to berry number at harvest was variable, except for the 50% flower removal treatment. The berry numbers were always lower in both years and varieties (Figure 5.1). The cytokinin BAP was aimed to increase fruit set, but the effect on berry number was only present in the second season, similarly the shoot leaf removal treatment reduced berry number per bunch only in that year. The GA_3 treatment was more effective in reducing berry numbers in the 2005/06 season, only a relatively small reduction was observed in the second season of the experiment. Severity of the impact of the hormone treatment on the fruit set might be influenced by slight differences in the timing of the treatment. The seasonal differences for the leaf removal treatment could be due to a variation in reserve levels of the shoot, by influencing fruit set. Overall the selected treatments altered the berry number per bunch as intended to investigate the impact on the berries and the bunchstem.

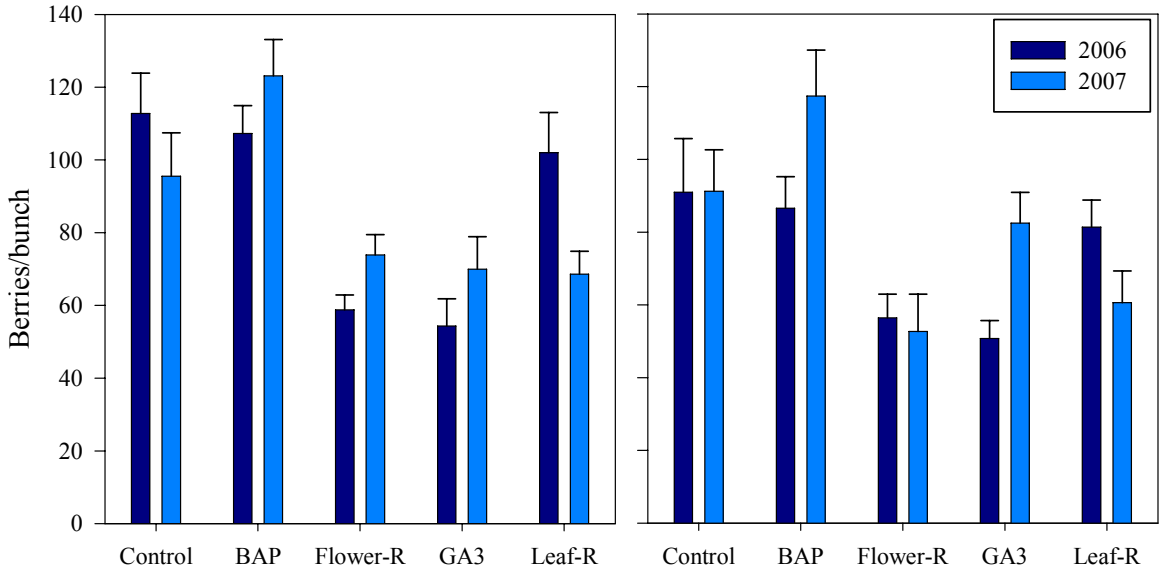


Figure 5.1 The impact of fruit set and flower removal treatments on berry number in Cabernet Sauvignon (left) and Merlot (right); (standard errors of the means are shown as bars, n=4).

The berry weight was highest at mid ripening in Cabernet Sauvignon in the 2005/06 season, due to damage by bees around harvest. Generally the increase during berry maturation in berry weight was higher in Merlot than in Cabernet Sauvignon, double and 1/3 respectively. The size of the bunch implicated by the treatments on the basic berry parameters was inconsistent and there appeared not to be such a clear relationship between bunch size and berry size as observed in the flower removal

experiment reported in section 4.3. Similarly anthocyanins and TSS were not consistently altered by the treatment, while total phenolic was usually higher in the leaf removal treatment. The results indicate that there is a variable response to the treatments and the consequential size of the bunch between varieties and years, which might have to do partially with different treatment response on the size of the bunch.

Table 5.1 Influence of fruit set and flower removal treatments on berry composition in Cabernet Sauvignon and Merlot (standard errors of the means are shown in small type, n=4).

		2006				2007			
		Berry wt. (g)	**Anthocyanin (g FW)**	**Phenolics (g FW)**	**TSS (ºBrix)**	**Berry wt. (g)**	**Anthocyanin (g FW)**	**Phenolics (g FW)**	**TSS (ºBrix)**
					Cabernet Sauvigon				
Veraison	Control	0.38 0.03	0.12 0.04	2.05 0.08	9.00 0.97	0.46 0.02	0.40 0.05	1.57 0.06	14.58 0.54
	BAP	0.31 0.01	0.08 0.02	2.28 0.12	7.95 0.46	0.42 0.03	0.30 0.08	1.61 0.07	13.70 0.87
	Flower-R	0.35 0.01	0.04 0.01	2.20 0.10	7.30 0.28	0.49 0.02	0.33 0.05	1.58 0.05	13.63 0.38
	GA3	0.37 0.01	0.08 0.02	1.71 0.05	8.03 0.38	0.53 0.02	0.44 0.07	1.63 0.08	14.63 0.63
	Leaf-R	0.37 0.01	0.20 0.05	1.92 0.10	9.05 0.68	0.46 0.04	0.47 0.02	1.61 0.05	14.30 0.52
Mid ripen	Control	0.71 0.03	0.97 0.05	1.32 0.08	19.50 0.52	0.61 0.01	1.18 0.17	1.48 0.10	19.20 0.79
	BAP	0.77 0.03	0.62 0.02	0.99 0.02	19.40 0.39	0.60 0.03	1.31 0.12	1.56 0.04	19.60 0.94
	Flower-R	0.82 0.01	0.79 0.07	1.03 0.04	18.08 0.43	0.63 0.02	1.42 0.09	1.56 0.07	19.80 0.37
	GA3	0.81 0.02	0.82 0.09	1.05 0.05	17.73 0.40	0.65 0.03	1.22 0.04	1.40 0.03	18.75 0.67
	Leaf-R	0.70 0.02	0.81 0.03	1.13 0.02	18.50 0.17	0.51 0.01	1.17 0.12	1.55 0.10	17.63 0.90
Harvest	Control	0.65 0.02	1.02 0.04	1.22 0.04	25.50 0.52	0.63 0.02	1.45 0.09	1.47 0.08	23.95 0.53
	BAP	0.66 0.03	1.11 0.05	1.29 0.04	24.89 0.61	0.66 0.02	1.58 0.09	1.52 0.06	25.04 0.27
	Flower-R	0.78 0.03	1.15 0.05	1.28 0.05	24.82 0.65	0.65 0.02	1.58 0.05	1.59 0.04	24.14 0.98
	GA3	0.73 0.04	1.05 0.03	1.15 0.03	24.11 0.50	0.70 0.03	1.47 0.05	1.44 0.06	24.41 0.34
	Leaf-R	0.69 0.03	1.08 0.04	1.26 0.03	25.00 0.42	0.58 0.02	1.73 0.08	1.82 0.07	25.21 0.29
					Merlot				
Veraison	Control	0.44 0.03	0.01 0.01	2.10 0.08	7.40 0.49	0.31 0.02	0.02 0.00	2.29 0.13	5.98 0.09
	BAP	0.44 0.04	0.01 0.00	2.02 0.11	6.75 0.35	0.33 0.04	0.04 0.02	2.27 0.17	6.88 0.55
	Flower-R	0.40 0.06	0.00 0.00	1.91 0.08	6.43 0.13	0.30 0.04	0.03 0.01	2.25 0.21	6.15 0.23
	GA3	0.39 0.05	0.01 0.00	1.28 0.16	6.98 0.34	0.32 0.02	0.04 0.01	2.24 0.17	6.43 0.60
	Leaf-R	0.36 0.05	0.02 0.01	2.09 0.17	7.30 0.37	0.29 0.03	0.02 0.01	2.11 0.07	5.93 0.27
Mid ripen	Control	0.79 0.05	0.45 0.12	1.13 0.06	18.90 0.89	0.53 0.05	0.90 0.12	1.45 0.09	19.63 0.67
	BAP	1.09 0.27	0.52 0.15	0.98 0.14	18.50 0.63	0.50 0.02	0.94 0.03	1.60 0.02	19.98 0.22
	Flower-R	0.68 0.05	0.46 0.10	1.01 0.04	18.78 1.43	0.55 0.01	0.77 0.09	1.30 0.08	19.23 0.14
	GA3	0.81 0.06	0.61 0.06	0.84 0.05	19.28 0.69	0.54 0.03	0.79 0.08	1.35 0.03	19.25 0.71
	Leaf-R	0.64 0.05	0.55 0.09	1.13 0.10	19.00 0.90	0.44 0.02	0.92 0.14	1.48 0.10	19.50 0.51
Harvest	Control	0.94 0.04	0.63 0.04	0.97 0.04	21.38 0.46	0.60 0.02	0.97 0.05	1.10 0.04	21.97 0.30
	BAP	0.91 0.03	0.61 0.02	0.94 0.03	20.75 0.28	0.61 0.02	0.89 0.05	1.07 0.03	22.28 0.21
	Flower-R	0.91 0.05	0.65 0.04	1.01 0.05	20.43 0.44	0.61 0.02	0.87 0.04	0.96 0.05	21.59 0.30
	GA3	0.83 0.04	0.61 0.04	0.79 0.03	20.54 0.42	0.58 0.02	0.89 0.05	1.08 0.04	22.10 0.21
	Leaf-R	0.84 0.04	0.75 0.05	1.08 0.04	21.81 0.46	0.53 0.02	0.94 0.03	1.13 0.03	21.13 0.30

In the first season proline and arginine appears to be higher in Merlot than in Cabernet Sauvignon, at least at veraison and mid-ripening. The harvest date could not be properly assessed due to the bee damage as mentioned earlier. The two varieties responded similarly in the 2006/07 growing session, with considerable increase in concentration from veraison to mid ripening. Followed by a small increase in arginine and a strong increase in the proline content of the berry until harvest. Arginine levels were highest in the GA_3 treatment and lowest in the leaf removal treatment at harvest 2006 in Merlot and 2007 in Cabernet Sauvignon. The proline levels were also the lowest in the leaf removal treatment and were highest in the GA_3 treatment in the last season in Cabernet Sauvignon. The

increases of amino proline N in berries during grape maturation have been observed by others, a decline of arginine N was not observed in this experiment. The treatments altered the berry proline and arginine N, but these changes were often inconsistent, probably due to seasonal variation and varietal differences.

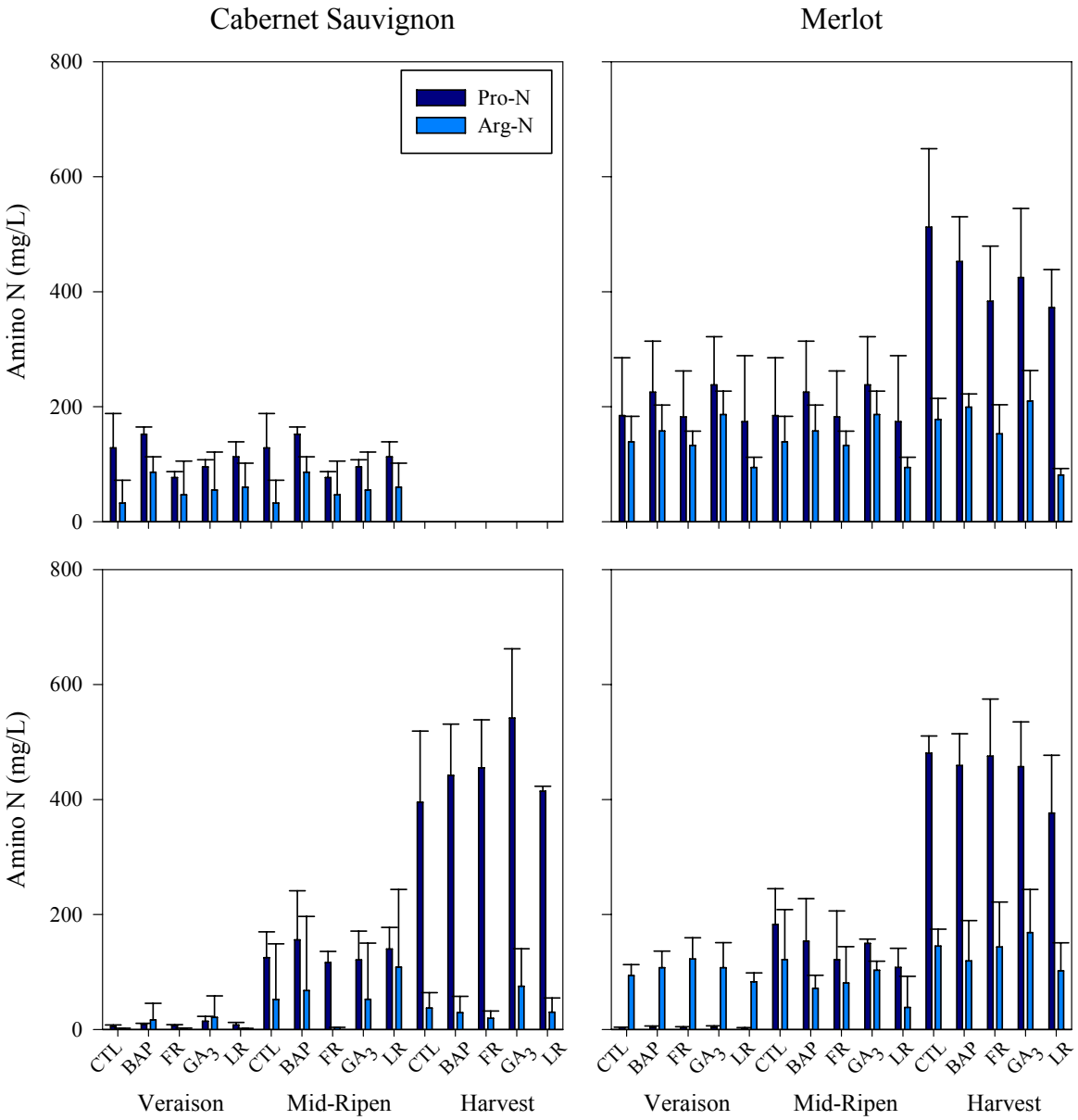


Figure 5.2 Amino acid concentrations at veraison, mid ripening and harvest in Cabernet Sauvignon and Merlot, influenced by treatments altering fruit set (BAP, GA3 and LR = leaf removal) and the flower removal (FR) treatment (season 2005/06 top and season 2006/07 bottom)(standard errors of the means are shown as bars, n=4).

The changes in nutrient concentrations were similar between years but different between the two varieties (Table 5.2). For instance, K accumulated in Cabernet Sauvignon until harvest, while in Merlot only minor changes took place from mid ripening to harvest. The concentrations of P also changed only minimal during grape maturation. Commonly Mg and Ca were lowest at mid ripening and highest at veraison. The impact of the treatments was variable between years and varieties,

although K is usually highest in the leaf removal treatment at harvest and veraison. This treatment effect is also partially reflected in the Mg and P berry concentration at harvest. The berries of bunches treated with GA_3 often have low K levels. As observed for the amino acid levels the GA_3 and leaf removal treatments appear to have altered mineral concentration of the berries, which might be due to altered allocation patterns implemented by these treatments.

Table 5.2 Influence of fruit set and flower removal treatments on berry nutrient concentrations in Cabernet Sauvignon and Merlot (standard errors of the means are shown in small type, n=4).

		2006								2007							
		Ca mg/kg		Mg mg/kg		K mg/kg		P mg/kg		Ca mg/kg		Mg mg/kg		K mg/kg		P mg/kg	
Cabernet Sauvignon																	
Veraison	Control	450	75.6	188	22.1	2850	119.0	353	19.3	355	29.0	153	6.7	2550	64.5	313	8.5
	BAP	410	32.4	181	6.5	2750	144.3	330	10.8	335	28.7	143	7.7	2275	75.0	283	14.9
	Flower-R	527	75.1	207	9.8	2733	76.4	373	37.9	450	28.6	171	7.8	2375	110.9	305	6.5
	GA3	440	48.1	187	8.3	2675	217.5	300	8.2	350	29.4	165	6.1	2230	174.8	260	8.2
	Leaf-R	305	48.6	152	13.1	2875	62.9	298	8.5	318	37.9	156	13.8	2775	94.6	295	9.6
Mid ripen	Control	212	11.7	127	7.3	2975	217.5	303	17.0	313	22.9	135	3.1	2425	75.0	280	4.1
	BAP	255	19.4	128	3.0	2950	132.3	295	10.4	265	13.2	136	1.9	2600	81.6	295	2.9
	Flower-R	268	21.7	132	4.5	2725	110.9	293	4.8	303	16.5	142	5.8	2475	75.0	285	5.0
	GA3	254	24.6	126	6.2	2625	125.0	285	13.2	345	24.0	136	7.7	2450	119.0	273	12.5
	Leaf-R	207	10.5	125	4.6	3175	47.9	295	2.9	303	31.2	137	8.6	2700	141.4	283	13.8
Harvest	Control	358	10.3	154	4.4	3325	110.9	378	11.8	393	20.2	163	6.6	2825	62.9	350	13.5
	BAP	338	2.5	160	4.0	3500	81.6	375	8.7	363	11.1	156	2.3	2875	25.0	338	8.5
	Flower-R	360	12.2	160	5.8	3075	85.4	365	14.4	440	20.4	169	3.7	2875	75.0	355	5.0
	GA3	378	56.0	153	4.1	3050	170.8	338	12.5	383	17.5	152	3.5	2700	40.8	315	11.9
	Leaf-R	315	61.6	150	9.1	3225	314.6	350	22.7	428	18.9	183	4.1	3375	47.9	385	8.7
Merlot																	
Veraison	Control	595	61.2	174	10.2	2450	86.6	390	27.4	805	123.5	214	29.5	2800	147.2	480	75.8
	BAP	640	51.8	188	8.8	2650	86.6	405	33.0	630	92.6	189	22.2	2650	202.1	430	43.4
	Flower-R	723	82.5	182	12.3	2367	28.9	390	26.5	810	65.4	205	20.7	2850	253.3	493	72.7
	GA3	557	60.5	157	10.8	2400	86.6	317	41.9	710	35.4	209	11.8	2675	110.9	458	37.3
	Leaf-R	518	128.5	171	25.8	2925	137.7	383	57.9	813	133.8	243	26.0	2900	150.0	483	63.7
Mid ripen	Control	383	40.7	140	4.4	3025	149.3	390	31.6	485	45.9	171	5.4	3025	149.3	418	19.7
	BAP	443	79.1	142	9.5	3367	76.4	377	20.2	415	38.6	157	1.9	2925	62.9	398	32.0
	Flower-R	445	36.6	141	4.9	3050	28.9	350	12.9	463	30.4	161	6.4	3025	75.0	415	19.4
	GA3	355	41.7	131	9.2	2650	28.9	318	19.3	500	21.6	162	6.0	2850	86.6	388	13.8
	Leaf-R	385	37.5	143	5.5	3100	158.1	380	31.9	420	21.8	158	5.3	3033	160.7	380	21.8
Harvest	Control	423	96.5	140	31.6	2975	666.5	360	82.3	540	33.4	163	2.4	2975	118.1	405	18.5
	BAP	420	94.8	140	31.4	2875	645.2	365	86.4	578	66.3	166	9.7	3000	0.0	415	36.6
	Flower-R	388	95.5	139	31.1	2900	652.3	350	80.1	580	39.2	173	7.8	2900	141.4	405	26.0
	GA3	393	92.7	145	33.7	3000	768.9	348	81.1	543	15.5	167	3.8	2875	143.6	405	29.6
	Leaf-R	398	97.0	150	34.2	3225	730.9	380	88.4	535	22.2	171	4.4	3050	28.9	368	18.9

Bunch and bunchstem development

Bunches treated with BAP showed more compactness with shorter and thicker pedicels, while the berry size appeared to be more heterogeneous. However, measurements of the length of the bunchstem revealed that the leaf removal treatment had the shorter bunchstems, particularly in the 2005/06 season (data not presented). The leaf removal treatment applied also before bloom led to brown pedicels and tip of rachis in Cabernet Sauvignon as well as berries appearing darker. The pedicels of normal berries and shot berries implemented by the GA_3 treatment differed in their internal structure (Figure 5.4). The

vascular bundles in the pedicels of shot berries were much less developed compared to the pedicels of normal berries.

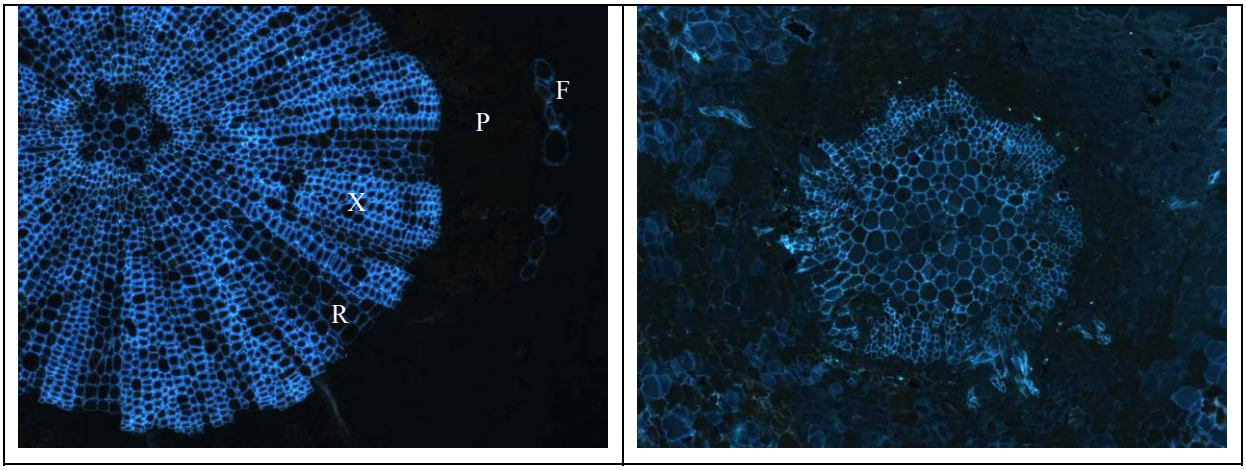


Figure 5.4 The alteration implemented by the GA_3 treatment on the vascular structure of the pedicels from normal berry (right) and shot (left) at harvest in Merlot (F = fibres, P = phloem, R = rays, X = xylem).

5.4 Conclusion

The implemented treatments have successfully changed the number of berries per bunch as intended, although the severity was not the same in both seasons the trial was conducted. The BAP treatment increased the berries per bunch and the other three treatments lowered them, namely GA_3, flower and leaf removal. The size of the bunch implicated by the treatments on the basic berry parameters was inconsistent and there appeared to be no clear relationship between bunch size and berry size, total anthocyanins and TSS, only total phenolic was usually higher in the leaf removal treatment. The proline and arginine concentration increased during berry maturation. These were particularly pronounced for proline. The treatments altered both amino acids, but these changes were often inconsistent, probably due to seasonal variation and varietal differences. The GA_3 and leaf removal were the most consistent treatment in altering both berry amino acid and nutrient content. The GA_3 treatment lead also to shot berries in bunches, which had pedicels with a different vascular structure.

6 Sap collection study (Experiment 4)

6.1 Introduction

Xylem has been often studied for its composition of nitrogenous compounds and cations in relation to the effect of this composition on the uptake of soil nutrients. Xylem sap is mostly composed from water, but contains nitrogenous compounds such as amino acids, nitrate and hormones. In addition, several cations are found in the xylem sap such as K, Ca and Mg (Peuke 2000).

N fertilisation increases the concentration of nitrogenous compounds in the xylem exudates of grapevine (Roubelakis-Angelakis & Kliewer 1979). The amounts and the proportion of nitrate N and glutamine N in the xylem depends on the level of N supply. The nitrate portion of the total N is higher under high N supply (Alleweldt & Merkt 1992). Nevertheless glutamine is the most important N transport form for the vine (Anderson & Brodbeck 1991). Around bud break, the diurnal changes in the concentrations of organic N in the xylem for grapevines were due to glutamine. The amino-N varies around bud-break and concentrations increase with higher root temperature (Anderson & Brodbeck 1989). There are also variations during the growing season, in Sylvaner the content is highest shortly before flowering and decline from then until harvest (Alleweldt & Merkt 1992). Similarly in the phloem, there is a sharp increase in organic compounds including amino acids at flowering to the inflorescences indicating the increasing sink strength of these organs (Glad et al. 1992).

The nitrogenous compounds such as amino acids in the xylem (and phloem) have been shown to be relatively high around the flowering period. This experiment was designed to understand the changes occurring during the growing season, particularly in the early stages of reproductive development around the flowering to fruit set period.

6.2 Materials and Methods

The experiment was conducted at the CSU vineyards on two varieties Cabernet Sauvignon and Merlot, over two growing seasons as was the previous experiment (2005/06 and 2006/07). Further details of the vineyard are described in section 2 in this report. The trial consisted of three replicates with four vines in two rows for both cultivars. In this trial no treatment was applied. Only samples were taken during the growing season for collecting xylem (phloem sap was also collected, but the analysis of the samples is still in progress and therefore not presented in this report).

In each of the two years of the study 20 petioles and 10 bunches were collected at dawn for both cultivars from a week before flowering to harvest. In the period until fruit set samples were taken twice a week, and then every two weeks until harvest for the xylem analysis. Immediately after their collection, the samples were brought back to the laboratory and the xylem was extracted by centrifugation at 2000 rpm for 20 minutes at 4°C (Nikolic & Römheld 2002). The xylem sap extracts were placed to -20°C until further chemical analysis and the amino acids concentration was determined by using an HPLC (see section 2.2 for further details).

6.3 Results and discussion

The xylem sap extracted from the bunchstems and petioles during season contained several amino acids, with glutamine (Gln) and arginine (Arg) presented in the following figures. Other amino acids were also present, such as glutamic acid and aspartic acid. There was a considerable difference between the years, variety and tissue from which the xylem was extracted (Figure 6.2). Gln concentrations were usually much higher in xylem sap from both bunchstems and petioles, except in Merlot in the 2005/06 season at harvest. Cabernet Sauvignon had a much higher concentration of Gln in the bunchstem and petiole xylem sap in the 2006/07 season, while Merlot showed similar levels in both years. The higher amino acid concentrations around flowering underline the importance of the inflorescence or bunch as a sink at that time.

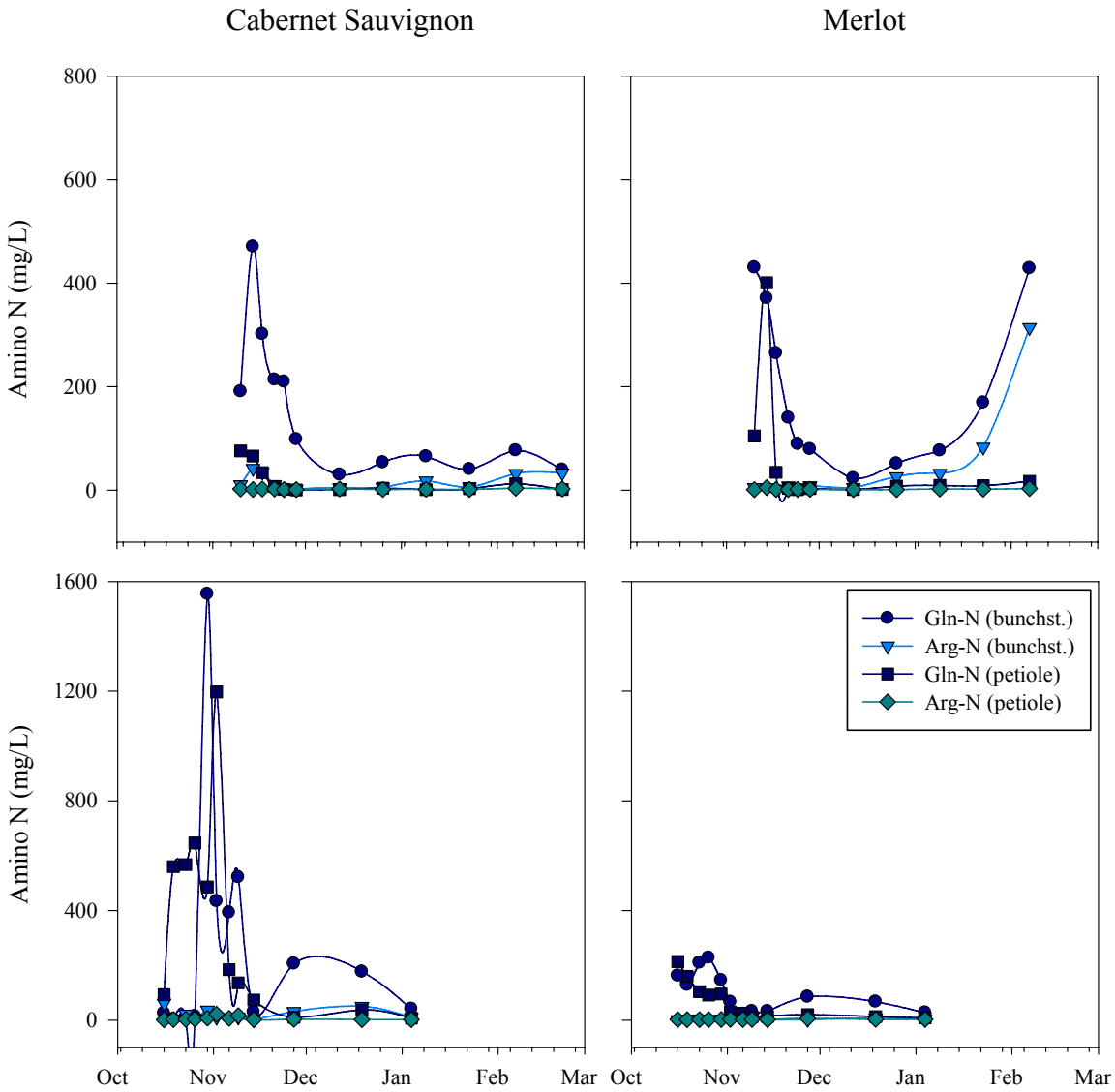


Figure 6.2 Seasonal changes of amino acids in the xylem sap of petioles and bunchstems in Merlot and Cabernet Sauvignon for season 2005/06 (top) and 2006/07 (bottom).

Strong variation was also observed during the day between the two varieties, the time of collection was conducted during the season (pre or post-veraison) and the two tissues (bunchstem, petiole). The concentrations of glutamin (Gln) were much higher in the bunchstems than in the petioles. As observed in the concentration during the season, glutamine was much higher at three weeks before veraison than after veraison or Merlot at the same time, particularly early in the morning. At pre-veraison the xylem sap Gln concentration was lowest at 10am, similar to Merlot at this stage. After veraison bunchstem xylem sap Gln levels in Cabernet Sauvignon were also lowest at 10am, but highest at 10pm. In Merlot the maximum concentration of Gln was early in the morning and lowest at 6pm. The high concentrations in the morning or late in the evening are most likely due to the low transpiration rate at this time of the day.

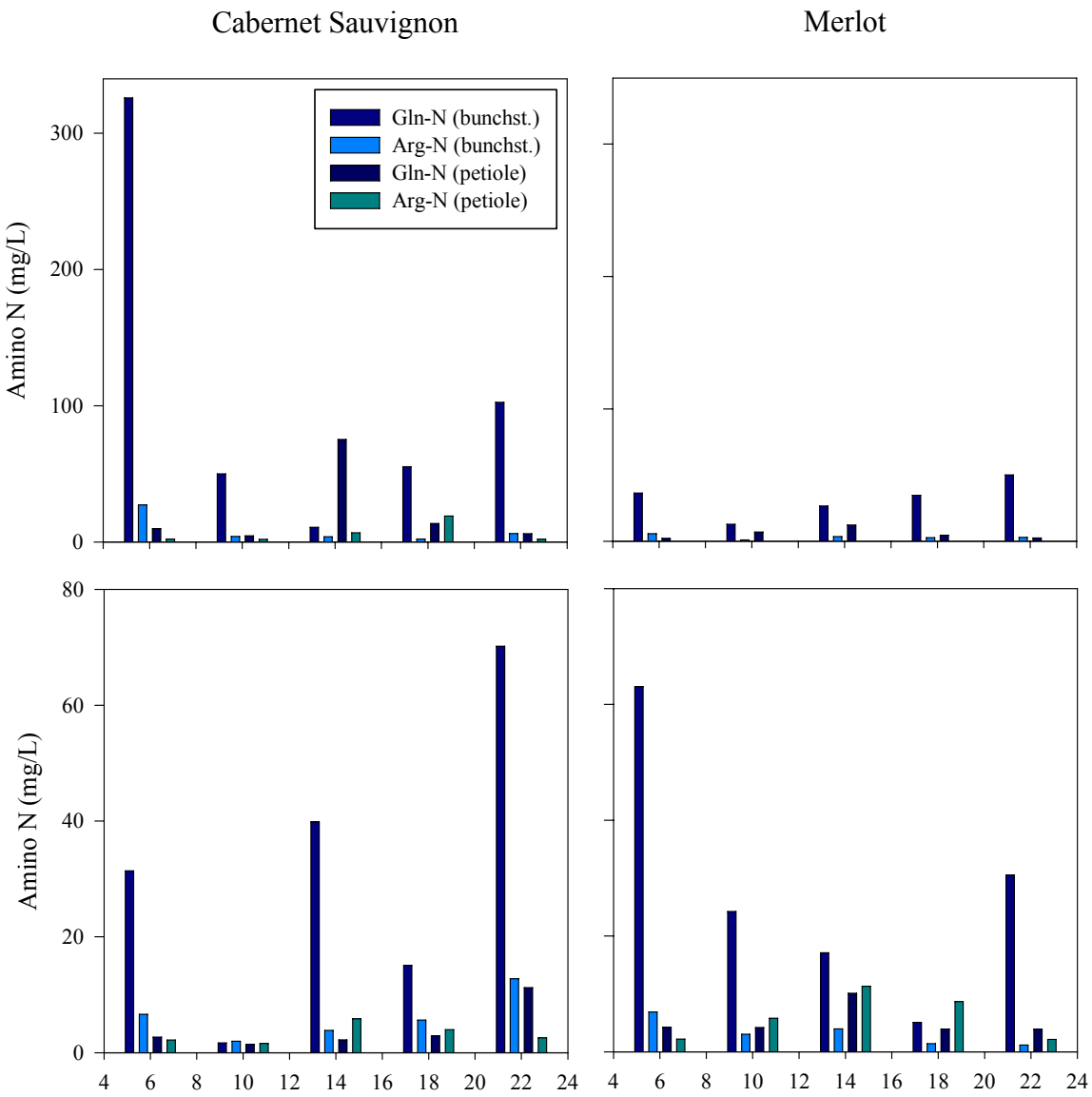


Figure 6.2 Diurnal curves of amino acids in the xylem sap of petioles and bunchstems three weeks before (top) and three weeks after veraison (bottom) in Merlot and Cabernet Sauvignon.

6.4 Conclusions

The variation in amino acid concentrations from shortly before bloom to harvest indicates the differences in demand for these nitrogenous compounds as well as differences in N uptake during the season. The results also point out the difficulty of variation and sufficient sample collection to determine the concentrations of amino acids in xylem of petioles and bunchstem tissues. Therefore it is very difficult to conclude on the delivery rate of the amino acids supplied via the bunchstem xylem to the berry.

7 Recommendations

The study has revealed that there is a strong interaction between berry number of a bunch, bunchstem development and a number of berry parameters. During early berry development the number of berries influenced the bunchstem development. This change appeared to impact on the final berry composition. Although the analysis of collected information on bunchstem vascular structure has not been finalised, further clarification is required on the importance of bunchstem and its vascular system on these berry parameters. This would include more detailed investigations on the berry composition of different parts of the bunch in relation to the size of the vascular system. The dynamics of xylem (and phloem) composition during a season and during the day would require further work, since it would be influenced by the vineyard nutrient and water supply. The gained knowledge is also a good basis for work on physiological disorders of grape bunches such as bunchstem necrosis, which was only touched on briefly during the project. The impact of vineyard management, particularly irrigation, on development and the function of the bunchstem should also be addressed in future work. Information on bunch and berry development should be presented together with the development of the bunchstem. A better understanding of the growth dynamics of the bunchstem could lead to the development of measures influencing the frame structure for the berries. These could impact on the compactness of the bunch, with implications for berry microclimatic conditions. Despite the importance of the bunchstem vascular transport system in supplying the grapes with nutrients and sugar, knowledge about this part of the bunch is still limited, particularly how it can be affected by vineyard management.

8 Acknowledgments

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10 Outputs

Refereed Scientific Publications

Gourieroux A., Rogiers S., Gray J. & Holzapfel B. (2008): The bunchstem vascular structure during berry development in bunches of different sizes in Merlot and Cabernet Sauvignon. (in preparation)

Gourieroux A., Rogiers S., Gray J. & Holzapfel B. (2008): The impact of flower removal on bunchstem development and berry amino acids in Merlot and Cabernet Sauvignon. (in preparation)

Gourieroux A., Rogiers S., Gray J. & Holzapfel B. (2008): Effect of pre-bloom treatments on fruit-set and bunchstem development in Merlot and Cabernet Sauvignon. (in preparation)

Conference & Symposium Presentation

Holzapfel, B., Gray J. & Rogiers, S. (2003): Bunchstem development, bunch size and berry characteristics in Merlot and Cabernet Sauvignon. Poster presentation. NWGIC Annual Meeting, Wagga Wagga, June 19

Gourieroux A., Holzapfel B., Gray J. & Rogiers S. (2004): Bunchstem and berry development in Merlot and Cabernet Sauvignon and their effects on berry composition. Poster presentation. 12th AWITC, Melbourne, July 25-28

Gourieroux A., Holzapfel B., Gray J. & Rogiers S. (2006): Effect of pre-bloom treatments on bunchstem development and berry composition in Merlot and Cabernet Sauvignon. NWGIC Symposium, Wagga Wagga, June 22

Gourieroux A. (2007): Effect of pre-bloom treatments on bunchstem development and berry composition in Merlot and Cabernet Sauvignon. NWGIC Annual Symposium, Wagga Wagga, June 21

Gourieroux A., Holzapfel B., Gray J. & Rogiers S. (2007): Bunchstem development and berry composition in Merlot and Cabernet Sauvignon. Poster presentation. 13th AWITC, Adelaide, July 29 – August 1

Extension and Industry Publications

Gourieroux A., Holzapfel, B., Gray J. & Rogiers S. (2004): Bunchstem and berry development in Merlot and Cabernet Sauvignon. The Australian and NZ Grapegrower and Winemaker, 490, 34-37

Holzapfel, B. (2004): Bunchstem and berry development in Merlot and Cabernet Sauvignon. WWAI Update 2004, 61-62

Holzapfel, B. (2006): Bunchstem and berry development in Merlot and Cabernet Sauvignon. WWAI Update 2006, 52-53

Gourieroux A., Rogiers S., Gray J. & Holzapfel B. (2007): The bunchstem vascular structure during berry development and the impact of flower removal in Merlot and Cabernet Sauvignon. The Australian and NZ Grapegrower and Winemaker (in preparation)

Other Presentations (workshop presentations, seminars, guest lectures)

Holzapfel, B. (2003): Bunchstem and berry development in Cabernet Sauvignon and Merlot. Riverlink Workshop on Cabernet Sauvignon, Merbein, July 29, 15-16

Gourieroux A. (2004): Bunchstem and berry development in Merlot and Cabernet Sauvignon and their effects on berry ripening and composition. NWGIC Seminar, Wagga Wagga, March 29

Gourieroux A. (2004): Bunchstem and berry development in Merlot and Cabernet Sauvignon and their effects on berry ripening and composition. CSU PhD Workshop Day, July 21

Gourieroux A. (2005): Effect of flower removal on bunchstem development in Merlot and Cabernet Sauvignon. CSU PhD Workshop Day, July 20

Gourieroux A. (2005): Source-sink relationships and Carbon – Nitrogen balance in grapevine fruiting cuttings. NWGIC, Wagga Wagga, June 1 (results of DEA, at INRA Bordeaux)

Gourieroux A. (2006): Effect of pre-bloom treatments on bunchstem development and berry composition in Merlot and Cabernet Sauvignon. CSU PhD Workshop Day, July 19

Gourieroux A. (2006): Effect of plant hormones on berry and bunchstem development. Vineyard Field Day, Griffith, September 7

Gourieroux A. (2007): Bunchstem development in Merlot & Cabernet Sauvignon. Seminar Limestone Coast, Wynns Coonawarra Estate, Coonawarra, August 3