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Key workers:	Mr C T Biddlecombe Mr G M Saunders
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Database and Literature search for Brix enhancement in *Ribes nigrum* and similar fruit crops

Grower summary

It was felt that the UK blackcurrant industry would benefit from a detailed literature and database search on blackcurrant to investigate factors reported to affect yield and juice quality. This project will further British growers knowledge and direct future proposals that would enable targeted research to be carried out. A detailed database and literature search investigating Brix enhancement in blackcurrants and grapes, with the aim of obtaining copies of 15 to 20 relevant research papers was conducted.

Summary of the project and main conclusions

The database and literature review has produced several papers concentrating on:

- architecture of the plant and associated light interception with high levels of light resulting in higher ^{0}Brix levels and low levels of light resulting in lower ^{0}Brix

- partial defoliation where removal of a portion of photosynthetically active leaves results in increased photosynthetic rate of the remaining leaves which can increase ^{0}Brix

- thinning of fruit which has been shown to increase ^{0}Brix

- irrigation where mild water stress or adequate water supply has been shown to produce fruit with the highest ^{0}Brix

- nitrogen application where nitrogen has been shown in some cases to be a limiting factor

- potassium applications which have been shown to increase ^{0}Brix levels

- phytohormones which have been shown to affect sugar accumulation

Science section

Introduction

It was felt that the UK blackcurrant industry would benefit from a detailed literature and database search on blackcurrant to investigate factors reported to affect yield and juice quality. This project will further British growers knowledge and direct future proposals that would enable targeted research to be carried out. A detailed database and literature search investigating Brix enhancement in blackcurrants and grapes, with the aim of obtaining copies of 15 to 20 relevant research papers was conducted.

Materials and Methods

A detailed database and literature search was conducted using library and electronic facilities at:

FAST Ltd.,
Experimental Farm,
North Street,
Sheldwich,
Faversham,
Kent, ME13 0LN.

East Malling Research,
New Road,
East Malling,
Kent, ME19 6BJ.

Imperial College,
Wye Campus,
Wye,
Ashford,
Kent, TN25 5AH.

Results and Discussion

It has been found that for *Vitis vinifera* (grape) the training system affects ^{0}Brix (Cavallo, Poni and Rotundo, 2001). In all training systems the vines were planted at the same spacings but different canopy manipulation resulted in different degrees of shading and of crop architecture. Lower ^{0}Brix was associated with within-canopy shading which is of no great surprise as leaf photosynthesis is the main contributor to berry sugar levels. Light levels have also been found to correlate with ^{0}Brix for a range of other fruits. Opportunities to explore the effect of light on ^{0}Brix in *Ribes nigrum* (Blackcurrant) are possible by either use of reflective mulches or canopy manipulation by pruning to change the bush architecture.

It has also been found in grape that removal of some of the leaf material from the canopy whilst berry ripening occurs can increase ^{0}Brix in the fruit (Holzapfel and Rogiers, 2002). It is suggested that this was due to the increased photosynthetic rate of the remaining leaves. Conversely (Ezzahouani and Williams, 2003) leaf removal of basal leaves has been shown to have no effect on ^{0}Brix. The differences between the two experiments could be explained if in the second experiment older shaded leaves with less photosynthetic potential were removed.

Thinning of fruit (Ezzahouani and Williams, 2003) has been shown to increase ^{0}Brix in grape. The remaining fruit are a smaller target sink for the sugar produced in the leaves and therefore individual fruits can contain a greater quantity of sugar.

The method and quantity of irrigation has also been shown to have an effect on ^{0}Brix as well as yield in grape (Holzapfel and Rogiers, 2002; des Gachons *et al.*, 2005). In some vineyards, yield of fruit was shown to be greatest in high water application and least where low water volumes were applied and as yield increased ^{0}Brix decreased. Conversely in other vineyards a high yield was positively correlated with high ^{0}Brix indicating that irrigation management can have significant impact on fruit productivity and composition. It has been determined (Gachons *et al.*, 2005; Yakushiji *et al.*, 1998)that mild water stress produced fruit with the highest ^{0}Brix. It has been reported (Yakushiji *et al.*, 1996) that sugar accumulation in Satsuma mandarin fruit was not caused by dehydration under water stress but rather that sugars were

accumulated by active osmoregulation in response to water stress. Other reports (Esteban *et al.*, 1999) have results which suggest that the higher yields in irrigated vines did not have any adverse effect on grape must composition and hence on grape juice quality, because on the whole synthesis and accumulation processes were able to offset any dilution effects. In this case other factors such as nutrition may not have been limiting enabling the whole synthesis and accumulation processes to offset any dilution effects. Indeed it has been found (Perez-Zamora *et al.*, 2004) that water and nitrogen interactions significantly affect ^{0}Brix.

Determination of soil water can be made with Enviroscan equipment which continuously monitors soil water at a range of depths. If irrigation rates are varied, water status, yield and resultant ^{0}Brix can be monitored to give information on optimum irrigation for the crop.

Nitrogen has been shown (De Faria *et al.*, 2000) to be a significant factor in resultant ^{0}Brix in melon. Nitrogen increased ^{0}Brix up to a point where further additions of nitrogen produced no further increases in ^{0}Brix, however by then increasing the crop density, further additions of nitrogen resulted in an increase of yield at a high ^{0}Brix level. It has also been shown in melon (De Faria *et al.*, 1994) that nitrogen applications have a positive effect on ^{0}Brix and yield.

Additions of potassium have been shown to increase ^{0}Brix levels in melon, pineapple (Depaula *et al.*, 1991), strawberry and in sweet potato (George *et al.*, 2002). Potassium also increased the yield as did nitrogen but in this case nitrogen did not have an effect on ^{0}Brix.

Phytohormones such as abscisic acid (ABA), gibberellins and cytokinins have been shown to help regulate sugar metabolism and/or transport in a range of plant species. ABA has been shown (Kobashi *et al.*, 2001) to stimulate carrier mediated sugar uptake into fruit cells across the plasma membrane in peach. The paper also refers to ABA and the cytokinin indoleacetic acid (IAA) and that they have also been shown to effect sugar accumulation in apple, melon and strawberry. Gibberellic acid (GA$_3$) when applied as post-bloom treatments has been shown to increase ^{0}Brix in grape (Reynolds and de Savigny, 2004).

Mentions of chemical ripeners have also been made in the literature but no specifics have been found as to their composition and action. Although I could not obtain a copy, one potentially useful article is by G. James.

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Ecophysiology and vine performance of cv. "Aglianico" under various training systems

P. Cavallo[a], S. Poni[b,*], A. Rotundo[a]

[a]*Dipartimento di Produzione Vegetale, Università degli Studi della Basilicata, Potenza, Italy*
[b]*Istituto di Frutti-viticoltura, Università Cattolica del Sacro Cuore,
via Emilia Parmense 84, 29100 Piacenza, Italy*

Abstract

A 2-year study was conducted to evaluate ecophysiological characteristics and yield–quality performance of cv. "Aglianico" (*Vitis vinifera* L.) grapevines trained to three different trellises at the same intrarow vine spacing and bud load per meter of row length. Bilateral guyot (BG) showed the lowest vine capacity and bilateral spur-pruned cordon (BSPC), with a vertical shoot positioning had the highest total leaf area (LA) and pruning weight. Despite very comparable crop levels among trellises, quality decreased considerably in the bilateral free cordon (BFC) vines with respect to the systems with upright shoot growth. BFC vines showed significantly lower sugar concentration (°Brix), anthocyanins and phenols, and higher pH and K^+ according to a pattern frequently associated with excessive within-canopy shading. Shading was aggravated in the BFC vines by canopy rotation, which probably resulted in an increase of LA density per volume unit. Moreover, the BFC canopies had more close-to-horizontal oriented leaves and from veraison onward, placed the most functional median and apical leaves in the lower or less illuminated portion of the canopy. These factors may have combined to diminish total vine photosynthesis in BFC-trained vines. The data also pointed out that the differences among trellises could not have been predicted simply on the basis of widely accepted indicators of crop load (e.g. the yield-to-pruning weight ratio) or canopy density (e.g. leaf area-to-canopy surface area (LA/SA)). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Light interception; Gas-exchange; Shoot growth; Yield; Crop load; Leaf area index

* Corresponding author. Tel.: +39-523-599-271; fax: +39-523-599-268.
E-mail address: sponi@pc.unicatt.it (S. Poni).

1. Introduction

Labor intensive operations such as harvesting and pruning are affecting an increasing number of Italy's grapevine districts, regardless of geographical location. Training systems which allow partial or full mechanization and facilitate hand management due to their simpler structure and pruning are therefore becoming more appealing to growers (Intrieri et al., 1998). The introduction of any new trellis in a given area requires both agronomic and ecophysiological evaluations to determine its viability over time and how canopy structure interacts with the local climate. Unfortunately, most of the comparisons among different trellises performed under various Italian environments have been based on traditional growth yield–quality assessments (Lisa and Cargnello, 1991; Sottile et al., 1991; Intrieri et al., 1992; Novello, 1994), whereas the interactions of training systems with the environment (namely, light) have been either neglected or oversimplified by calculation of basic indices such as the exposed canopy surface area (SA) (Smart, 1985; Carbonneau, 1995) or the leaf area-to-canopy surface area (LA/SA) ratio (Smart, 1985). These indices are essentially based on geometrical features of the canopy and their use has been recently questioned by Mabrouk and Sinoquet (1998) and Calò et al. (1999).

A more focused approach designed to evaluate directly light interception and distribution for a given trellis can be anticipated to lead to a better interpretation of the yield–quality performance of vines, in addition to building a body of data useful to characterize each training system type regardless of the influence exerted by other factors (year, location, rootstock, etc.).

The purpose of the present study was to compare different trellises for the cv. "Aglianico" with emphasis on their agronomic performance and interactions with light availability. The trellises were the bilateral guyot (BG), the bilateral spur-pruned, vertically shoot-positioned cordon (BSPC) and the bilateral free cordon (BFC), which also differ with respect to an increasing adaptability to the mechanization of harvesting and pruning.

2. Material and methods

The trial was carried out in 1997 and 1998 in a 10-year old experimental vineyard planted with cv. "Aglianico" grafted onto 1103 P in the Melfi area (Basilicata, southern Italy). The vineyard had been planted in single rows spaced 3 m apart and trained to three different trellises: BG, BSPC and BFC (Fig. 1). The BG and BSPC canopies have a typical upright shoot growth supported by catch wires, whereas the BFC's shoots hang freely. To ensure a uniform bud load among trellises, one eight-node cane and two two-node spurs were retained on the BG vines; 7–8 short spurs were maintained on both the BSPC and BFC to yield

Bilateral Guyot

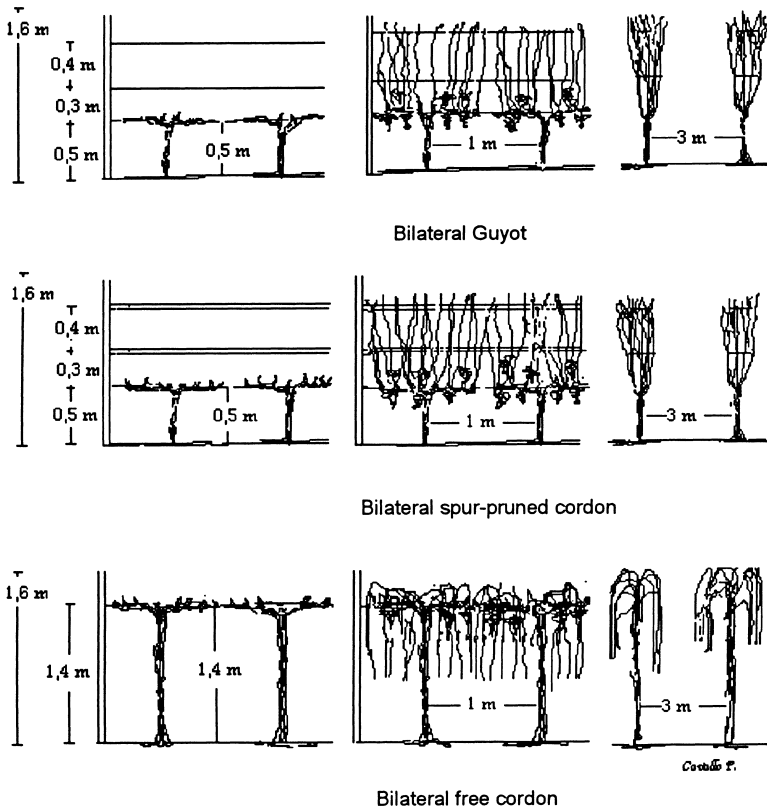Bilateral spur-pruned cordon

Bilateral free cordon

Fig. 1. Diagrams reporting trellis features, cordon heights and vine spacings of the three training systems.

approximately 20 nodes per vine per trellis. Vines did not receive supplemental irrigation during the trial, and pest control was administered on a calendar basis. Shoot thinning was done each year at the phenological stage of "separated clusters" (Baggiolini, 1952) to lower canopy density without altering the shoot number ratios among trellises.

2.1. Vegetative growth, yield and must quality

Budbreak date was estimated after Baggiolini (1952), i.e. when at least 50% of the nodes retained at pruning had crossed the "swollen bud" phase. Shoot number per vine and number of inflorescences per shoot were recorded at the "visible cluster" stage. At the same stage of growth, two shoots per vine (one for each cordon or cane) were tagged and monitored for the number of expanded leaves (both main and laterals) at varying intervals until growth cessation. For 1997 only, the lengths of each burst shoot on the spurs and canes of the test vines

were recorded at weekly intervals from the stage of "first expanded leaf" until fruit set. All shoots were trimmed at fruit set to retain a minimum number of 12–14 main leaves. Concurrently with the dates of shoot measurements, samples of main and lateral leaves representative of different positions along the shoot were taken from extra-vines on each trellis, and the LA for each leaf was measured with a LI-COR 3000 portable area meter. Combining mean LA assessment with leaf counts made it possible to estimate LA per vine on the different dates as well as the extent of LA removed by topping. The vegetative growth measurements ended with the weight of 1-year old pruning wood.

At harvest, besides recording the crop weight and cluster number of each test vine, two 100-berry samples (one per cordon or cane) were taken from the same vines, weighed and processed for sugar concentration (°Brix), titratable acidity (TA), malate, tartrate, phenols and anthocyanins. The latter two parameters were measured on berry skin disks after Iland (1988). Potassium concentration of berries was measured by atomic absorption.

2.2. Leaf gas-exchange, light interception and distribution

Assimilation (A) and transpiration (E) were measured at fruit set and veraison on eight, mature, healthy leaves per treatment, four of which were well-exposed while the other four were located in shaded parts of the canopy. Measurements were recorded at 2 h intervals from 8:00 a.m. to 4:00 p.m. with an ADC-LCA 4 portable gas-exchange system. Leaf gas-exchange records were paralleled by leaf water potential readings taken with a pressure chamber on well-lit and shaded leaves adjacent to the ones sampled for gas-exchange. Pre-dawn leaf water potentials were also measured at the same dates on eight leaves per trellis.

Total canopy light interception (TCLI) was estimated at three dates throughout the season (pre-shoot and post-shoot toppings, and veraison) on 2 m row sections of each trellis type including the cordons or canes of the test vines. The amount of light transmitted to the vineyard floor was measured under clear-sky conditions using a multiple line sensor equipped with 10 single cosine-corrected, photosynthetically active radiation (PAR) sensors (10 cm spacing) and linked to a CR10 Campbell data logger. The line sensor was moved over a level, below-canopy grid of 2 m×2 m to 10 locations per grid so as to yield a total of 100 individual light readings taken in approximately 2 min. Considering diurnal variation in light interception, the light readings were taken at three different sun angles (2 h before solar noon, solar noon and 2 h after solar noon) by moving the grid over the ground to intercept the entire ground area shaded by the canopies. Percent canopy light interception was estimated by subtracting total vine light transmission from incident radiation measured simultaneously above the canopy by an elevated, horizontal PAR sensor. An adjusted leaf area index (LAI′) was then calculated by dividing total vine LA by the mean ground area shaded by the

canopy. The light extinction coefficient (K, otherwise defined as the amount of light intercepted per unit LA) was calculated as the slope of the logarithm of light transmission vs. LAI′ after Beer's law of light attenuation throughout a canopy (Jones, 1992). Concurrently with the dates of light interception, SA was estimated after Smart (1985); LA/SA was also calculated.

Light distribution was evaluated under clear-sky conditions on the same experimental units on days adjacent to those chosen for light interception estimates. Five locations, at 40 cm spacing, were marked along the 2 m long row sections with bamboo stakes, and nine different measuring levels from cordon height were identified as follows: 20, 40, 60, 80, 100 and 120 cm above cordon, and 20 and 40 cm below cordon for BG and BSPC; 20, 40 and 60 cm above cordon, and 20, 40, 60, 80 and 100 cm below cordon for BFC. Measurement levels were adapted to the canopy geometry of the trellises featuring a top fruiting area for BFC and the reverse for BG and BSPC. Light readings were taken at each level using the same multiple sensor, which was held perpendicular to the vine row and inserted horizontally into the canopies so that its mid-point was centered along the row axis. The sensor was then quickly moved to the next location so as to complete the measurement for a single unit in less than 1 min. Light availability was given as a percent of the incident radiation measured concurrently by the elevated, horizontal PAR sensor. Data were also averaged over the "fruiting" and the "vegetative" zone of each trellis as defined in the table captions.

2.3. Statistical analysis

Data were taken in both years on three replicates per treatment represented either by single vines or 2 m row sections. Data were subjected to analysis of variance and mean separation performed by Duncan multiple range test (DMRT). Values are presented as means over the 2 years, unless a significant year×trellis interaction occurred.

3. Results and discussion

Budbreak occurred within a 2-day span over trellises, although average date of budburst was estimated on April 14 in 1997 and April 9 in 1998. The BSPC had the highest vegetative growth for total LA and pruning weight (Table 1). All vegetative parameters indicated BG as the weakest trellis, although pre-topping shoot growth was more reduced in BFC — as might be expected because of its free hanging (rather than upright) shoots. Nevertheless, BFC vine capacity offset this drawback by a higher, albeit not significantly, shoot number per vine and resulted in not being significantly different from BSPC (Table 1).

Table 1
Vegetative growth of vines trained to various trellises[a]

Trellis	Nodes (No.)/m	Shoots (No.)/m[b]	Shoot length[c] (cm)	Total leaf area (m²)/m	Lateral leaf area (m²)/m	Removed leaf area (m²)/m[d]	Pruning weight (kg)/m
BG	18.5	10	114 b	5.74 b	0.91 b	0.67 b	0.80 b
BSPC	19.5	11.5	170 a	7.40 a	1.78 a	1.02 a	1.01 a
BFC	22.5	13	104 b	7.01 a	1.69 a	0.87 ab	0.96 ab
Trellis	NS	NS	*	*	*	*	*
Trellis×year	NS	NS	NS	NS	NS	NS	NS

[a] Mean separation within columns by DMRT test. Data are given on a per meter of cordon or cane basis. NS denotes nonsignificance. *$P<0.05$.
[b] Recorded after manual shoot thinning.
[c] Maximum values recorded before topping.
[d] Removed with topping.

The variability in pre-topping shoot development along the cane (1997 data only) was higher in BG (coefficient of variation=12%) as compared to that recorded in BSPC and BFC (coefficient of variation of 6% for both trellises) for the shoots bore on spurs at different positions along cordon. Interestingly, the BFC vines reacted to shoot topping with a considerable number of laterals (Table 1). Although this response was also affected by the higher shoot number, note that the period immediately following shoot cut (July) was characterized by 18 mm of rain in 1997 and no rain in 1998 (data not reported). It appears likely that soil moisture may have limited regrowth by shifting the dependence of lateral formation onto the amount of vine reserves (i.e. more abundant in BSPC and BFC due to the presence of a permanent cordon).

Yield components varied, giving very similar crop levels per meter of row over treatments (Table 2). BSPC vines offset the lower cluster number by increased cluster weight; berry size did not differ among trellises. Given the comparable yields, the calculated crop load indices essentially reflected variations in vine capacity (Table 2). BSPC showed the highest source availability per unit of fruit mass (18.8 cm²/g), which also corresponded to the lowest yield-to-pruning weight ratio (3.5 kg/kg).

The must quality of the BFC vines differed from that of the trellises with upright shoot growth (Table 3) — significantly lower sugar concentration, anthocyanins and phenols and higher pH and K^+. This ripening pattern has often been associated with increased canopy shading (Smart, 1987; Kliewer and Smart, 1989), which in the present study does not seem attributable to a higher LA (values were similar for both BSPC and BFC) and should have actually been offset by the progressive spreading of the BFC canopies, leading to a "dilution" of LA in a larger canopy volume. A more likely explanation for

Table 2
Yield, components of yield and crop load indices recorded on vines trained to various trellises[a]

Trellis	Clusters (No.)/shoot[b]	Cluster weight (g)	Berry weight (g)	Cluster number (g)/m	Yield (kg)/m	Yield-to-pruning weight ratio	Leaf area/yield (cm^2/g)
BG	1.75 a	230 ab	2.22	18.0 a	4.13	5.8 a	13.9 b
BSPC	1.56 b	251 a	2.20	16.0 b	3.99	3.5 b	18.8 a
BFC	1.68 a	212 b	2.37	19.5 a	4.14	4.9 ab	16.7 ab
Trellis	*	*	NS	*	NS	*	*
Trellis×year	NS	NS	NS	NS	NS	NS	NS

[a] Mean separation within columns by DMRT test. Cluster number and yield given on a per meter of cordon or cane basis. NS denotes nonsignificance. *$P<0.05$.
[b] Recorded after manual shoot thinning.

altered within-canopy shading in BFC is linked to the seasonal dynamics of canopy growth and movement for the different trellises (Fig. 2). Given the cordon's rotation along the supporting wire, BFC vines began to grow asymmetrically by mid-June, i.e. before shoot topping, in both years. This effect is quite often recorded in cultivars with heavy clusters and a natural downward growth habit (Intrieri and Poni, 1997), the latter feature applying to the great majority of the grape cultivars grown in Italy (Intrieri et al., 2000). Poni et al. (1996) investigated the incremental canopy density caused by asymmetric growth in free cordon trained vines and found a doubling in the mean leaf layer number as compared to a week earlier when growth was still symmetric.

Furthermore, shifting of canopy growth towards one side of the row would also lead to overexposure of clusters located in the "empty" side of the row, which subsequently may cause poor pigmentation. A similar effect has been reported for sun-exposed berry skins of Pinot gris (Price et al., 1992) which have shown an increase in flavonol concentration, particularly a quercitin glycoside, occurring at

Table 3
Must quality of vines trained to various trellises[a]

Trellis	Soluble solids (°Brix)	TA[b] (g/l)	Tartrate (g/l)	Malate (g/l)	pH	K^+ (ppm)	Anthocyanins (mg/l)	Phenolics (mg/l)
BG	21.6 a	8.4	6.1	4.5	3.09 b	987 b	572 a	1863 a
BSPC	21.5 a	8.9	6.3	4.7	3.13 b	1009 b	483 ab	1728 ab
BFC	19.6 b	9.1	6.3	5.3	3.25 a	1290 a	426 b	1526 b
Trellis	*	NS	NS	NS	*	*	*	*
Trellis×year	NS	NS	NS	NS	NS	NS	NS	NS

[a] Mean separation within columns by DMRT test. NS denotes nonsignificance. *$P<0.05$.
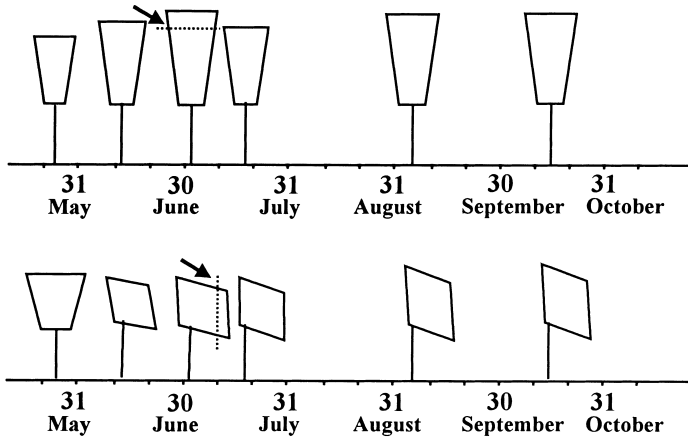[b] TA, titratable acidity.

Fig. 2. Cross-section views of the BG–BSPC canopies (top) and of the BFC canopies (bottom) drawn at different times throughout the growing season. Asymmetry of the BFC canopies is apparent even before topping (indicated by arrows and broken lines). Date of topping was averaged over years (July 2).

the expense of anthocyanins synthesis. Although aggravated by the above effects, the mean canopy density of BFC-trained vines did not significantly differ from BSPC in terms of LAI$'$ and the LA/SA ratio was actually significantly lower than the value calculated for BSPC (Table 4). According to Smart (1985), excessive within-canopy shading may occur when values of the latter index exceeds 1.5 (Smart, 1985).

Therefore, these indices proved to be unsuitable to predict and/or account for the differences in must quality highlighted by the two trellises. Additional insight into this matter is provided by looking at "local" canopy effects and "qualitative" canopy characteristics. For example, the higher values of the light extinction coefficient for the BFC vines indicate the predominance of close-to-horizontal oriented leaves, which can cause excessive light exposure of the uppermost leaf layers and prolonged diurnal shading of those located underneath.

The variations in TCLI for the different trellises reflected their canopy geometry and the vegetative flush after topping (Table 4). Pre-topping TCLI was the highest in BFC due to the canopy's widening, whereas higher TCLI for BSPC at full canopy as compared to BG relates to differences in vine vigor. Note that TCLI was not significantly affected by topping in any system. By contrast, the TCLI increment recorded at full canopy clearly matches that for lateral regrowth. The final TCLI values differed among trellises by up to 10% despite very comparable crop levels. Therefore, TCLI does not seem to be a good yield predictor in grapevine though this is at variance with the results shown for apple (Lakso, 1994). The lack of correlation between TCLI and yield in this study should also consider the fact that, due to the pruning type (short spurs retained in

Table 4
TCLI, adjusted LAI (LAI′), K, SA and LA/SA ratio calculated for vines trained to various trellises[a]

Trellis	TCLI[b] (%)			LAI′ (m²/m²)	K	SA (m²)	LA/SA (m²/m²)
	Pre-topping	Post-topping	Full canopy				
BG	33.9 b	32.2 b	43.0 b	1.86	0.63 b	2.90 a	1.95 b
BSPC	38.5 ab	35.7 b	51.0 a	1.83	0.62 b	3.20 ab	2.42 a
BFC	46.9 a	46.4 a	53.5 a	1.75	0.85 a	3.40 a	1.96 b
Trellis	*	*	*	NS	*	*	*
Trellis×year	NS	NS	NS	NS	NS	NS	NS

[a] LAI′, K, SA and LA/SA values refer to the full canopy stage. Mean separation within columns by DMRT test. TCLI, total canopy light interception, LAI′, adjusted leaf area index; K, light extinction coefficient; LA, leaf area; SA, canopy surface area. NS denotes nonsignificance. *$P<0.05$.

[b] Calculated as $100-[(I_t/I_i)100]$, where I_t is transmitted radiation and I_i is incident radiation above canopy.

all systems), bud fruitfulness (one of the main yield components) may have been more closely affected by the light availability to the basal part of the cane retained at pruning rather than by the total amount of light intercepted by the whole canopy. Furthermore, other important yield components (i.e. berry size and berry number) are affected by canopy efficiency parameters such as the amount of effective LA per unit of fruit which are not inherently considered in the calculation of TCLI.

Light availability for the vegetative zones of each system showed higher values on almost any date for trellises with upright shoot growth and the lowest ones for BFC (Table 5). Although this outcome could have largely been predicted from their reversed canopy geometry (high vegetative zones for BG and BSPC, and low for BFC), it provides further insights into explaining the unsatisfactory grape quality reached by the latter trellis. In spite of a temporary increase due to the effects of topping, the light available to the vegetative zone of BFC at full canopy (corresponding to veraison, i.e. the time point from which berry sugaring begins) was considerably lower than that of BG (−22.9%) and BSPC (−11.3%).

This implies that the median and apical leaves of BFC shoots experienced a more limiting light microclimate than the corresponding leaves of the two remaining systems. It is well demonstrated that the contribution to vine photosynthesis of leaves located towards the shoot tip after veraison is predominant as these leaves are mature but not senescing (Hunter and Visser, 1988). Conversely, BFC benefitted from higher light availability to the fruiting-renewal area, where the older basal leaves are also located (Table 5). Since the rate of leaf senescence in *Vitis vinifera* is reported to be faster than the rate recorded for other *Vitis* species (Lakso, 1993) or fruit crops (Lakso, 1994), it is

Table 5
Light availability (percent of maximum incident radiation) for the vegetative-shoot and the fruiting-shoot zones of each trellis at different times of the season[a]

Trellis	Vegetative-shoot zone[b]			Fruiting-shoot zone[c]		
	Pre-topping	Post-topping	Full canopy	Pre-topping	Post-topping	Full canopy
BG	45.4 a	58.6 a	53.0 a	26.4 b	28.9 b	21.4 b
BSPC	40.5 a	50.2 ab	40.4 a	23.3 b	27.8 b	19.3 b
BFC	30.9 b	46.4 b	29.1 b	32.0 a	35.4 a	28.7 a
Trellis	*	*	*	*	*	*
Trellis×year	NS	NS	NS	NS	NS	NS

[a] Mean separation within columns by DMRT test. NS denotes nonsignificance. *$P<0.05$.
[b] Average of readings taken at 40, 60, 80, 100 and 120 cm above the cordon in BG and BSPC, and at 60, 80 and 100 cm below the cordon in BFC.
[c] Corresponding also to the renewal shoot zone in BSPC and BFC. Average of readings taken at the cordon level, 20 cm above and below the cordon in BG and BSPC and at the cordon level, 20 cm above the cordon, 20 and 40 cm below the cordon in BFC.

unlikely that the ameliorated light availability to the basal leaves may have fully offset the decreased radiation suffered by the median and apical leaves. This effect adds to the above discussion on altered leaf angles, suggesting that overall canopy photosynthesis may have been considerably lower in BFC as compared to BF and BSPC.

Diurnal trends and maximum values of *A* and *E* measured on exposed and shaded leaves at fruit set and veraison did not differ among trellises (data not reported). Pre-dawn and total leaf water potentials were slightly more negative at fruit set in accordance with the limited rainfall registered in both years during this period. However, minimum leaf water potential recorded in well-exposed leaves (−1.35 MPa) induced no apparent stomatal closure, and therefore did not limit photosynthesis.

4. Conclusions

This 2-year trial indicated that the upright, vertically shoot positioned trellises are both viable in achieving adequate ripening in "Aglianico" grapes. The choice between BG and BSPC should primarily involve their suitability to mechanization (BG is not adaptable to mechanical pruning) and cost savings under hand management (winter pruning in BG is more laborious and time consuming). Furthermore, the higher LA registered in BSPC did not induce any further improvement in grape quality, suggesting that excessive canopy density as related to intrarow vine spacing may have occurred in this system.

BFC does not appear to be a recommendable system on the basis of this preliminary evaluation, since grape quality was lowered markedly despite crop levels similar to the other trellises and total LA similar to the BSPC vines. However, the unsatisfactory performance of the BFC could not have been predicted simply on the basis of general estimators of crop load (i.e. the crop weight per weight of cane pruning or the total leaf-to-fruit ratio) and canopy density (LA/SA and LAI′). As a matter of fact, the values of these parameters calculated for BFC were either close to optimum or non-limiting according to the reference thresholds (Smart, 1985; Mabrouk and Sinoquet, 1998). The data show that vine performance in BFC was more closely related to such factors as leaf array (*K* values suggested more horizontally oriented leaves), shoot function (the more functional leaves were located in the bottom part of the canopy during the post-veraison stage) and local effects (asymmetry of canopy growth led to leaves and clusters being either too shaded or overexposed). Accordingly, given that cordon rotation leads to an asymmetric canopy growth, it could be taken as a primary factor in causing unsatisfactory grape quality. Maintaining a more erect canopy can be envisaged as a primary goal for upgrading the efficiency of BFC-trained vines under our conditions. This could be achieved either by using coiled support wires or trimming the shoots at a quite early stage (i.e. pre-bloom) to induce a more upright growth. If coiled wires are used, another option would be to form the cordon by training two twisted canes (instead of one), so that the time needed for the cordon to stick around the wire would be shortened accordingly. The overall data also indicate the urgent need to come up with a ''physiological'' expression of crop load (i.e. the balance of carbohydrate supplies to the demand of the clusters and the vine's other organs) rather than indirect expressions such as yield-to-pruning weight.

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Nitrogen levels through fertirrigation and plant density on melon crop in a vertisol

De Faria, CMB Costa, ND Pinto, JM Brito, LTD Soares, JM

EMBRAPA, Ctr Pesquisa Agropecuaria Trop Semi Arido, Caixa Postal 23, BR-56300000 Petrolina, PE, Brazil
EMBRAPA, Ctr Pesquisa Agropecuaria Trop Semi Arido, BR-56300000 Petrolina, PE, Brazil

Abstract

This study consisted of one experiment with melon (Cucumis melo L.), carried out in a Vertisol in Juazeiro, BA, Brazil, in 1995, with the objective of evaluating the effects of nitrogen levels through fertirrigation and plant density on fruit yield and quality. The N levels were 0, 80, 130 and 180 kg/ha, combined with row spacings of 2.0 and 1.8 m and 0.20 m between plants within the row, with one or two plants/hole. The source of N was urea applied daily up to 42 days after germination, through drip irrigation. All treatments had a uniform fertilization of 120 kg/ha of P_2O_5 and 120 kg/ha of K_2O. No significant difference was caused by spacing between rows in the studied variables. Eighty kg/ha of N combined with one plant per hole gave a yield of 34.07 ton/ha, being 55,7% of fruit allocated to inside market, not significantly lower than those obtained with the highest N levels in any combination. This same N level gave fruits with 10.22 degrees Brix, significantly higher than the treatment without N and not significantly lower than the other levels. In order to get high number of good fruit for out side market it was necessary to elevate the density to two plants per hole and N level to 130 or 180 kg/ha. Mean weight of fruits increased from 1.008 to 1.705 kg with the increase in the levels of N and the decrease in plant density of two plants per hole to one plant per hole.

MINERAL AND ORGANIC FERTILIZING ON THE MELON CROP IN A VERTISOL OF THE SUBMIDDLE SAO-FRANCISCO VALLEY, BRAZIL

de Faria C.M.B., Pereira J.R. and Depossideo E.L.

EMBRAPA,CTR PESQUISA AGROPECUARIA TROP SEMIARIDO,CAIXA POSTAL 23,BR-56300000 PETROLINA,PE,BRAZIL

Abstract

This work was composed of two experiments with melon (Cucumis melon L.), carried out in 1989 and 1990 in two places in a Vertisol in Juazeiro, BA, Brazil. The treatments consisted of levels of nitrogen, phosphorus, potassium, and cattle manure. The results showed that melon responded positively to nitrogen, phosphorus and potassium fertilization, but not to cattle manure in both experiments. In one experiment, it was possible to determine the economical levels for the three nutrients, which were: 74 kg/ha of N, 114 kg/ha Of P_2O_5 and 156 kg/ha of K_2O, giving an average yield of 30,425 kg/ha. The N had a positive influence on the brix and on the number of fruits. The P influenced positively the weight of fruits.

NUTRITIONAL-REQUIREMENT OF PINEAPPLE - EFFECT OF LIMING, POTASSIUM AND NITROGEN ON YIELD AND QUALITY OF FRUIT

Depaula, MB Decarvalho, VD Dasnogueira, F and Souza, LFD

CTR REG SUL MINAS GERAIS,EPAMIG,CAIXA POSTAL 176,BR-37200 LAVRAS,MG,BRAZIL

Abstract

In Minas Gerais, Brazil, pineapples are exclusively grown on leached acid soils, and a balanced nutrition is important for the growth and production of this crop. A liming - N-K trial was conducted with the aim of recommending a fertilizer for pineapple cultivation. The levels of K2O, N and lime were: K2O kg/ha (0-413-722-1031), N g/plant (0-5-10-15), lime ton/ha (0-2). The design of the trial was a factorial arrangement in split plot with three replications. Liming did not improve the production, but a beneficial effect of liming on K utilization was found. Yield significantly responded to increments of K and N. Addition of K increased the content of acid and brix in the fruit, and N was found to lower the acid content.

Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements

M.A. Esteban, M.J. Villanueva and J.R. Lissarrague

Univ Politecn Madrid, Dept Prod Vegetal Fitotecn, Escuela Tecn Super Ingenieros Agron, Madrid, Spain
Univ Politecn Madrid, Dept Prod Vegetal Fitotecn, Escuela Tecn Super Ingenieros Agron, Madrid, Spain
Univ Complutense Madrid, Fac Farm, Dept Nutr & Bromatol 2, Madrid, Spain

Abstract

The impact of water availability on the yield and must composition of Vitis vinifera L. cv. Tempranillo grapes was studied over a three-year period. Grape juice composition was compared during stages II and III of the berry growth. The object of this study was to ascertain the effect of irrigation regime on berry development and ripening, and hence on grape juice quality. Changes in berry weight, degrees Brix, glucose, fructose, titratable acidity, pH, tartaric acid, malic acid, citric acid, and mineral elements were monitored. The evolution of sugars and acids during berry growth followed patterns similar to those reported by previous investigators. Hence, water availability did not affect the accumulation patterns of the different sugars and acids. Glucose was the predominant sugar in the berries at veraison, while fructose predominated at the end of ripening, irrespective of the treatment applied.
Organic acid concentration was highest when berries were pea- sized, and it began to decrease at veraison. The rate of decrease was greater for malic acid than for tartaric acid. The concentration of tartaric acid was higher than that of malic acid from veraison on, and differences between them increased as ripening progressed. The differences between malic acid and tartaric acid were greatest in the non-irrigated treatment.
Total soluble solids, and the concentration of glucose and fructose were significantly higher in the irrigated vines than in the non-irrigated vines, mainly towards the end of ripening, except in 1992. In that year the values for the non-irrigated vines were slightly higher, though the differences were not significant on many sampling dates. The "Brix of the irrigated grapes at harvest were 2.8% to 14.9% higher than in the non- irrigated grapes. The largest increase in "Brix (67% - 124% in non-irrigated grapes and 58% -117% in the irrigated grapes) took place after veraison. Titratable acidity (TA) was significantly higher for the irrigated vines, primarily at the end of ripening. The TA of irrigated vines at harvest was 9.8% to 28.3% higher than the TA for non-irrigated vines. By expressing the data for glucose, fructose, tartaric acid,

malic acid, and citric acid in grams per berry, we observed the largest number of sampling dates with significant differences between irrigation treatments in the years 1990 and 1992. The concentrations for the mineral elements followed differing trends over the course of ripening. Potassium increased until harvest time, and calcium and magnesium decreased, but sodium did not exhibit any clear trend, rising or falling on different sampling dates. Quantitatively, the values for all the parameters studied in the non-irrigated vines tended to be equal to or greater than those in the irrigated vines, even though per-berry quantities were higher in the irrigated vines.

The results show that the effect of water deficits on the composition of the grape juice was more intense in the final year of the study, when the differences in soil water availability between treatments were greatest. The results further suggest that the higher yields in irrigated vines did not have any adverse effect on grape must composition and hence on grape juice quality, because on the whole synthesis and accumulation processes were able to offset any dilution effects.

Trellising, fruit thinning and defoliation have only small effects on the performance of 'Ruby Seedless' grape in Morocco

By A. EZZAHOUANI[1*] and L. E. WILLIAMS[2]

[1]Department of Horticulture, I.A.V. Hassan II, B.P. 6202, Rabat-Institutes, Rabat, Morocco
[2]Department of Viticulture and Enology, University of California, Davis, California 95616,USA
(e-mail: a.ezzahouani@iav.ac.ma)

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SUMMARY

The effects of trellising, thinning and defoliation on growth, yield and fruit composition were investigated in grape (*Vitis vinifera* L., 'Ruby Seedless') in Morocco over two years. Vines were grown on a double tee (DT) or a triple tee (TT) using two or three cross-arms, thinned at fruit set (29 clusters) or not thinned (35 clusters), and defoliated (removal of basal leaves at veraison up to and including the leaf opposite the first retained grape cluster) or left undefoliated. The TT trellis had greater shoot growth, leaf area, and pruning weights than the DT trellis. Twenty per cent of light reached the fruiting zone in both trellis systems. In contrast, average light in this zone ranged from 52 to 969 μmol m^{-2} s^{-1} in the defoliated vines compared with 13 to 59 μmol m^{-2} s^{-1} in the undefoliated vines. Berries on defoliated vines were up to 6 K warmer than berries on the control vines. Yield per vine was not affected by any of the treatments, with an average yield of 7.53 ± 1.85 kg per vine. Average berry weight was increased by 6% with either defoliation or fruit thinning. Total soluble solids were significantly ($P<0.05$) increased in thinned vines (17.02 ± 0.10°Brix) compared with unthinned vines (16.55 ± 0.09°Brix), whereas there was no effect of trellising and defoliation on total soluble solids. Defoliation increased fruit colour slightly in unthinned vines (absorbance values at 520 nm of 0.390 and 0.461), while thinning increased berry colour slightly in undefoliated vines (absorbance values at 520 nm of 0.390 and 0.489). Trellising, fruit thinning and defoliation have only marginal effects on the performance of grape in Morocco.

Numerous cultural practices affect vine berry development and fruit quality. Light interception by a grapevine depends on leaf area and the distribution of the leaves as affected by the shape of the plant (Smart *et al.*, 1985). Widening the trellis with the use of cross-arms, however, has at best only a small effect on total soluble solids (TSS) (Kasimatis *et al.*, 1975). The addition of a second cross-arm increases TSS without affecting berry weight or acidity (Kasimatis *et al.*, 1975). These data suggests that an increase in leaf area or light interception could improve fruit quality in grape.

Defoliation can also affect berry composition, low light from veraison to harvest reduces berry weight and TSS, and increases total acidity (Kliewer, 1971). Exposed fruit has higher concentrations of glucose and fructose (Crippen and Morrison, 1986). However, direct exposure to sun can lead to high temperatures which can reduce berry weight (Kliewer, 1971), delay sugar accumulation (Kliewer and Weaver, 1971) or slow colour development (Bergqvist *et al.*, 2001).

Cluster or berry removal can stabilize vine yields and improve quality. Cluster thinning around bloom increases the weight of remaining clusters (Bravdo *et al.*, 1984). Thinning immediately after shatter (unfertilized flowers abscising from the cluster), increase berry weight and TSS (Kaps and Cahoon, 1989).

The objective of this study was to determine the impacts of various techniques on fruit quality of 'Ruby Seedless' grapes grown in Morocco. We compared

*Author for correspondence.

double and triple tee trellises, thinned and unthinned vines, and defoliated and undefoliated vines. It was suggested that these cultural practices would affect fruit quality without affecting yield.

MATERIALS AND METHODS

Six year old 'Ruby Seedless' grapevines (*Vitis vinifera* L.) grown in a commercial vineyard near Meknes in Morocco, were used. The soil had 45% silt, 38% sand and 17% clay. Vines were planted 1.5 m apart in rows 3.0 m apart and grafted on 140 Ruggeri rootstocks. The vines were head-trained at 0.7 m and pruned to four canes of 10–12 buds each. The standard trellis used was a double tee (DT) with the lower crossarm 0.4 m wide and 0.8 m high. The second cross-arm was 0.8 m wide, and 1.2 m high. Trellis wires were attached to the ends of both cross-arms, with the fruiting canes tied to lower wires. In 1990, an extra cross-arm was added – the triple tee (TT). The additional cross-arm was 0.8 m wide, with foliage wires at both ends, and 0.4 m above the DT's higher cross-arm. Clusters were thinned to 29 clusters per vine at fruit set when the berries were 3–5 mm in diameter, whereas control unthinned vines had 35 clusters per vine. Lastly, vines were defoliated at veraison by removing all the basal leaves of the shoot up to and including the leaf opposite the cluster, or left undefoliated.

Photosynthetic photon flux density (PPFD) was measured at the soil surface and within the grapevine canopy at each level of a cross-arm for both trellis systems at solar noon on a single day in 1991 (August 2).

A LiCor line quantum sensor (model LI 190SA) was connected to a LiCor data logger (model LI-1000). Diurnal PPFD within the fruiting zone was determined with a point quantum sensor, and fruit temperature was measured using a hypodermic thermocouple. These measurements were made on a single cluster on the east and west sides of the row, for both defoliated and control vines. The light and temperature sensors were connected to a datalogger (Kane-May, model KM-1206, Cole-Parmer Inst. Co., USA).

Shoot length was measured biweekly using two shoots per vine. After harvest, four individual vines per trellis were defoliated to determine the number of leaves and leaf area per vine using a Li-Cor area meter (Model LI-3000). Pruning weights were measured when the vines were dormant. At harvest, total yield and number of clusters per vine were recorded. Samples of 100 berries per replicate were weighed and analysed for TSS using a hand-held refractometer (American Optical, Model 10430), titratable acidity (determined by titration with 0.133 N NaOH using phenolphthalein as indicator), and pH with a pH-meter (Cole-Parmer, Model PM-14). Berry colour was determined on 7 mm discs of skin taken from the apical region with 20 berries from each sample (Kliewer and Weaver, 1971). The absorbance of the extracts was read at 520 nm with a spectro-photometer (Perkin-Elmer, LAMBDA 2). Data were collected over two years.

The experimental design was a split-split plot, with trellis the main plot, and crop load and defoliation subplots (n = 4). Each main plot consisted of a single row with 32 vines and the trellis randomly assigned down the row. Crop load and defoliation treatments were randomly assigned within each trellis treatment. The data were analysed using analysis of variance and means separated using least significant difference (LSD) or Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The use of an additional cross-arm in this study stimulated vegetative growth. Shoot length measured was 10% greater and pruning weights measured during dormancy were significantly greater for the TT trellis than for the DT trellis. Mean shoot length, was

Trellis	Leaves/vine (number)	Leaf area/vine (m^2)	Leaf area/fresh fruit wt (cm^2 g^{-1})[y]
DT	787 b	14.0 b	19.0
TT	1082 a	17.3 a	22.5

[z]Mean separation in columns by Duncan's multiple range test, 5% level.
[y]Values in this column were calculated by dividing leaf area per vine by average crop weight of the DT and TT trellises, which were 7.37 and 7.70 kg per vine, respectively.

significantly ($P<0.05$) higher in the TT trellis (195.5 ± 8.7 cm) compared to the DT trellis (177.2 ± 5.7 cm) on June 25, but not before that time (Figure 1). Pruning weight was 1.17 ± 0.08 kg per vine for the TT and 1.06 ± 0.05 kg per vine for the DT trellis. Defoliation and fruit thinning had no significant ($P>0.05$) effect on pruning weights (data not presented). This is similar to that found by Kasimatis *et al.* (1975) on 'Thompson Seedless' when a second cross-arm was added to the control trellis consisting of only a single cross-arm.

Leaf area (14.0 ± 1.6 m^2 vs. 17.3 ± 0.96 m^2) and leaf number (787 ± 60 leaves per vine vs. 1082 ± 164 leaves per vine) were significantly greater for the TT trellis than with the DT trellis (Table I). Leaf area per fresh fruit weight was also 18% higher for the TT trellis. The extra cross-arm of the TT trellis in this study apparently allowed the shoots to continue an upward growth habit for longer, resulting in more leaf area per vine than when using the DT trellis. Twenty per cent of sunlight (227.4 ± 24.9 μmol m^{-2} s^{-1}) reached the fruiting zone in both trellises. Only 50% of sunlight was intercepted between the highest and the middle cross-arms of the TT trellis (Figure 2). The increased leaf area of the TT trellis may have resulted in more light being intercepted by the canopy when compared with the DT trellis, resulting in greater production of photosynthates (Williams, 1996).

Removal of leaves from around 'Ruby Seedless' clusters increased PPFD measured in the fruiting zone. The PPFD ranged from 52–969 μmol m^{-2} s^{-1} in the defoliated vines and from 13 to 59 μmol m^{-2} s^{-1} in the controls (Figure 3). Average values were 533.40 ± 315.99 μmol m^{-2} s^{-1} vs. 37.99 ± 14.81 μmol m^{-2} s^{-1}.
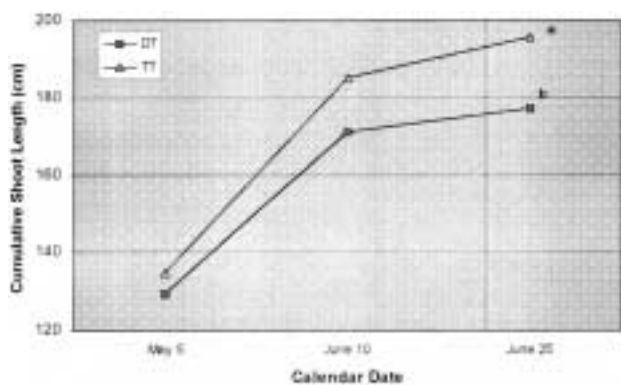

FIG. 1
The average effect measured over two growing seasons (1990 and 1991) of trellis system on cumulative shoot length of 'Ruby Seedless' grapevines. Data points followed by a different letter are significantly different at 5% level.
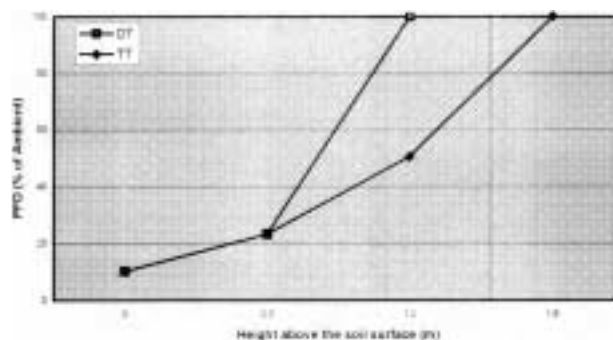

FIG. 2
The amount of light, as % of ambient photosynthetic PFD, measured at each cross-arm for the DT and TT trellis systems used in this study and at the soil surface. Measurements were made at solar noon on 2 August 1991. Each datapoint is the mean of ten measurements.
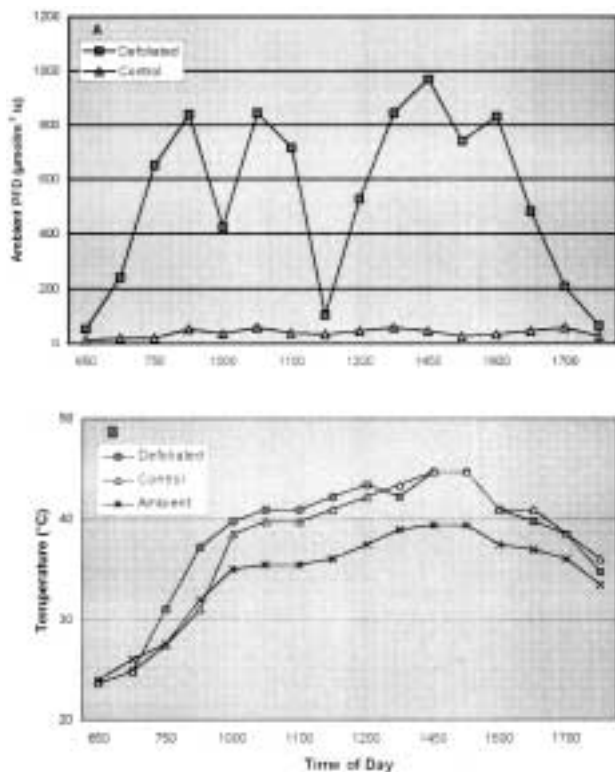
Fig. 3

The effect of leaf removal in the fruiting zone of 'Ruby Seedless' grapevines on the diurnal time course of photosynthetic PFD (A) and berry temperature (B). Measurements were made on 2 August 1991. Each value a mean of two measurements.

Average berry temperatures in the defoliated vines (38.13 ± 6.47°C) and the controls (37.35 ± 6.89°C) were higher than the air (34.44 ± 4.76°C) during most of the day. Light in the fruiting zone of the control vines was only 7% of that received by the clusters of the defoliated vines measured on a diurnal basis. Greater exposure of the fruit on the defoliated vines increased heat accumulations by approximately 2% compared with the controls. In this study berries on defoliated vines were up to 6 K warmer than berries on control vines. Other studies have found the temperatures of berries exposed to direct solar radiation can be up to 10 K warmer than those in the shade (Bergqvist et al., 2001; Kliewer and Weaver, 1971). Most studies conducted in hot climates, however, have measured a decrease in berry weight due to higher temperatures (Bergqvist et al., 2001) and direct exposure to sunlight (Crippen and Morrison, 1986; Bergqvist et al., 2001). Bergqvist et al. (2001) found that increased light exposure for fruit on the north side of east/west rows increased berry size up to a PPFD of 51 to 100 μmol m^{-2} s^{-1} when measured at midday. A greater PPFD level measured at midday on the same side of the canopy reduced berry size in that study. Therefore, the increased PPFD and heat accumulation for the defoliated treatment in this study may be responsible for the significant increase in berry weight for that treatment.

Yield was not significantly ($P>0.05$) affected by treatments, with an average yield of 7.53 ± 1.85 kg per vine (Table II). Nevertheless, the 4.5% increase in yield per vine due to the addition of a third cross-arm was close to the 6.0% increase in yield obtained for 'Thompson Seedless' grapevines in which a four-wire T trellis was compared with a two wire T trellis (Kasimatis et al., 1975). The limited effect observed in the above studies may have been due to a greater mobilization of photosynthates for vegetative growth or/and to the fact that the vines may have been over-cropped. Cluster thinning in this study resulted in a slight reduction of yield (ca. 5%) even though cluster number was reduced by 17%. However, berry weight was significantly increased by the cluster thinning treatment, as was the calculated number of berries per cluster (data not presented), resulting in some compensation of yield. Comparable results also were reported on 'Carignane' (Bravdo et al., 1984), and 'Seyval blanc' (Kaps and Cahoon, 1989) following post-bloom thinning.

TABLE II

The average effect measured over two years (1990 and 1991) of trellis system (DT – double T, TT – triple T), crop level (C – control, T – cluster thinned) and defoliation on yield (kg per vine) of 'Ruby Seedless' vines. Harvest dates were 11 and 7 September in 1990 and 1991, respectively. There were no significant differences among treatments

| | | DT | | TT | | Average effect Defoliation |
		C	T	C	T	
Defoliation	— No	7.50	6.58	7.44	7.66	**7.78**
	— Yes	8.07	7.34	7.86	7.84	**7.29**
Average effect of	**Crop**	**7.72**		**7.35**		
	Trellis	**7.37**		**7.70**		

TABLE III

The average effects of different treatments measured over a two-year period on berry weight (g/berry) of 'Ruby Seedless' grapevines. Berries were sampled on 11 September 1990 and 1 September 1991. Other information as in Table II

| | | DT | | TT | | Average effect Defoliation |
		C	T	C	T	
Defoliation	— No	2.13	2.26	2.18	2.23	**2.20**
	— Yes	2.22	2.42	2.27	2.41	**2.33**
Average effect of	**Crop**	**2.20**		**2.33**		
	Trellis	**2.26**		**2.27**		
LSD$_{0.05}$ Interactions	Trellis = n.s.[z] n.s.[z]	Crop = 0.11		Defoliation = 0.09		

[z]LSD not significant at the 5% level.

TABLE IV
The effects of the various treatments on total soluble solids of 'Ruby Seedless'. (°Brix) average across both years of the study. Other information as in Table II

		DT		TT		Average effect Defoliation
		C	T	C	T	
Defoliation	— No	16.4	16.8	16.5	16.9	**16.7**
	— Yes	16.8	17.2	16.5	17.2	**16.9**
Average effect of	**Crop**	16.6		17.0		
	Trellis	16.8		16.8		
LSD$_{0.05}$	Trellis = n.s.^{z}	Crop = 0.44		Defoliation = n.s.^{z}		
Interactions	n.s.^{z}					

TABLE V
The average effects over two years (1990–1991) of trellis system, crop level adjusted at fruit set and defoliation at veraison on the colour (absorbance at 520 nm) of 'Ruby Seedless' berries. Other information as in Table II

		DT		TT		Average effect Defoliation
		C	T	C	T	
Defoliation	— No	0.435	0.547	0.344	0.431	**0.439**
	— Yes	0.322	0.446	0.600	0.445	**0.453**
Average effect of	**Crop**	0.425		0.467		
	Trellis	0.437		0.455		
LSD$_{0.05}$	Trellis^y	Crop = n.s.^{z}		Defoliation^y		
Interactions	**Trellis × Defoliation** = 5.42					

^{y}Trellis, and defoliation LSDs not listed because of significant interaction.
^{z}LSD not significant at the 5% level.

Berry weight was significantly higher in thinned vines (2.33 ± 0.05 g) compared with unthinned vines (2.20 ± 0.03 g) and in defoliated vines (2.33 ± 0.05 g) compared with undefoliated vines (2.20 ± 0.02 g) (Table III). The significant increase in berry weight due to cluster thinning was similar to that obtained with defoliation. Even though there was no significant interaction between these two treatments, their combination resulted in the largest berries (2.42 ± 0.07 g). Trellising had no significant effect on berry weight. This is in agreement with studies conducted on 'Thompson Seedless' (Kasimatis *et al.*, 1975) (comparing a two cross-arm trellis to a single cross-arm trellis).

The TSS were significantly (*P*<0.05) increased in thinned vines (17.02 ± 0.10°Brix) compared with unthinned vines (16.55 ± 0.09°Brix) (Table IV). Trellis had no significant effect on TSS despite more leaf area per vine for the TT trellis. There was a slight increase of 0.2°Brix for the defoliated treatment when compared with the control. This corresponded to a 7.3% increase in total sugars per berry for the defoliated treatment. It was found that TSS were always less on the exposed part of clusters growing directly at the sun than berries toward the interior of the canopy for 'Seyval Blanc' grapevines (Reynolds *et al.*, 1986). This was not the case for thinning treatment in this study as there was a significant increase in TSS, in agreement with other such studies (Bravdo *et al.*, 1984). Despite no significant interactions among treatments, the combination of cluster thinning and defoliation was 0.8°Brix greater than that of the control fruit.

Defoliation significantly (*P*<0.05) decreased titratable acidity (0.45 ± 0.01 g l^{-1}) compared with the control (0.48 ± 0.01 g l^{-1}), whereas thinning and trellising had no significant effect. The average pH of the fruit was 4.05 ± 0.02 with no siginificant effects of treatments. The decrease in titratable acidity would result in the hastening of fruit ripening based on the sugar/acid ratio, (35 for control fruit, and 38 for fruit on defoliated vines). Sun-exposed fruit of 'Thompson Seedless' also were reported to have lower total acid and higher pH than shaded fruit, while TSS contents were about the same (Kliewer and Weaver, 1971). Other studies also have found that fruit exposure lowered total acidity in the fruit (Reynolds *et al.*, 1986; Smart *et al.*, 1985).

Both defoliation and thinning affected slightly berry colour. The relative berry colour as reflected by absorbance at 520 nm was unthinned/undefoliated (0.390), unthinned/defoliated (0.461), defoliated/thinned (0.456) and undefoliated/thinned (0.489) (LSD, *P* = 0.05, 0.123, Table V). Other studies demonstrated that cluster shading was negatively correlated with colour development and total anthocyanin concentration (Smart *et al.*, 1985).

CONCLUSIONS

This study demonstrated that 'Ruby Seedless' berry weight could be increased by cluster thinning at fruit-set and defoliation at versaison with even greater increases obtained by the combination of the two treatments. Fruit maturity based on soluble solids concentration and total acidity was promoted by the above treatments. The addition of another cross-arm to the control trellis had no significant effect on fruit composition and maturity, even though vegetative growth was stimulated. Taking into account the lack of an immediate effect from trellis modification and its material cost, the addition of another cross-arm would not be recommended for table grape production in this area. Cluster thinning and defoliation would be economically viable practices for increasing berry weight and enhancing fruit maturity of Ruby Seedless grapevines grown in Morocco for table grapes.

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Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L cv Sauvignon blanc in field conditions

Catherine Peyrot des Gachons,[1] Cornelis Van Leeuwen,[1,2*] Takatoshi Tominaga,[1] Jean-Pierre Soyer,[3] Jean-Pierre Gaudillère[3] and Denis Dubourdieu[1]

[1]Faculté d'Œnologie de Bordeaux, 351 Cours de la Libération, F-33405 Talence Cedex, France
[2]ENITA de Bordeaux, 1 Cours du Général de Gaulle, BP 201, F-33175 Gradignan Cedex, France
[3]INRA-ECAV, BP 81, F-33883 Villenave d'Ornon, France

Abstract: *S*-Cysteine conjugate precursors of three volatile thiols were monitored in *Vitis vinifera* L cv Sauvignon blanc grapes during fruit ripening to assess the influence of vine water and nitrogen status on the grape aroma potential in field conditions. Four dry farmed plots were studied in the Pessac-Léognan and Graves appellations (Bordeaux area) in 1998, which was a very dry vintage, and in 1999, when regular summer rainfall occurred. Soil water-holding capacity ranged from very low to high. Soil total nitrogen content was related to soil organic matter content, which was highly variable on the four plots. Vine vigour was enhanced by both high water and nitrogen status. Major compounds in grapes depended mainly on vine water status. Water deficit-stressed vines produced small berries with low sugar and low total acidity. Grape aroma potential was highest in vines under mild water deficit and moderate nitrogen supply. Severe water deficit stress seemed to limit aroma potential, as did nitrogen deficiency. Consequences for site selection and irrigation management for Sauvignon blanc are discussed.
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INTRODUCTION

Vine development and fruit composition are highly dependent on environmental conditions and particularly on vine water status and vine nitrogen status.[1] Many papers report on the influence of water deficits on vine development and yield[2–5] and on fruit composition.[4,6–10] Vine nitrogen status also greatly influences growth and yield parameters[11–13] as well as fruit ripening.[11,14–19] Most of these studies were carried out on red grape varieties. Some show a clear positive effect of water deficit on berry phenolic compound concentration and quality potential for red wine making.[4,7–10] Moderate nitrogen deficiency can also enhance phenolic compound synthesis.[10,16,18,19] As red wine quality greatly depends upon phenolic compound concentration in grapes, it seems clear that moderate environmental stress, and particularly low water and nitrogen availability, enhances quality potential in red grapes.

Very few data have been published on the influence of environmental conditions on quality potential in white grapes. Unlike in red grapes, phenolic compounds do not play a positive role in white grape quality. High phenolic content in white grape juice is responsible for unstable colour and bitterness.[20] White grape quality mainly depends upon its aroma potential. For some white wine aromas, eg terpenols, one part is directly extracted from the grapes during vinification, without transformation,[21] and another part is glycosidally bound.[22–25] However, very little of this last fraction is transformed into aroma during vinification, because glycosidase enzymes have very low activity at grape juice and wine pH.[26,27] Aromatic varieties such as Muscats produce white wines with a terpenol-dominated aroma. Their aromatic potential can easily be assessed during grape ripening either by direct gas chromatography of the free terpenols or by gas chromatography after hydrolysis of the bound terpenols.[28] The grapes of other varieties such as *Vitis vinifera* L cv Sauvignon blanc contain mainly non-volatile, bound aromas. Some of these aromas are liberated during the fermentation processes, making

the wine odorous. The evolution of the aroma potential of these grape varieties during the ripening process is much more difficult to assess.

Recently, three major aroma compounds of the wine produced from Sauvignon blanc grapes were identified: 4-mercapto-4-methylpentan-2-one (4MMP), 4-mercapto-4-methylpentan-2-ol (4MMPOH) and 3-mercaptohexan-1-ol (3MH).[29,30] Their analysis by gas chromatography coupled with mass spectrometry makes it possible to quantify aroma intensity in Sauvignon blanc wines. These aromas exist in Sauvignon blanc grapes as non-volatile *S*-cysteine conjugate precursors.[31] Peyrot des Gachons *et al*[32] developed a method for quantifying these precursors. Although this method is delicate and time-consuming, it allows the quantification of Sauvignon blanc aroma precursors directly in grape juice. Thus aroma potential in Sauvignon blanc grapes can be assessed during grape ripening.

The objective of this research was to study the influence of vine water and nitrogen status on vine development and fruit quality of Sauvignon blanc grapes in field conditions. During grape ripening, not only major compounds such as sugar and organic acids were analysed, but also precursors of three major volatile compounds of the Sauvignon blanc aroma: p-4MMP, p-4MMPOH and p-3MH. Aroma potential of Sauvignon blanc grapes varied widely in relation to vine water and nitrogen status. The possible use of these observations in site selection for Sauvignon blanc grapes and in optimising irrigation and fertilisation practices for this variety is discussed.

MATERIALS AND METHODS
Experimental plots
The most renowned white Bordeaux wines are produced in the Pessac-Léognan and Graves appellations south of the town of Bordeaux. Four plots, located in commercial vineyards in these appellations and planted with *Vitis vinifera* L cv Sauvignon blanc, were studied. Plots were medium- to high-density plantings. Vines were guyot pruned and the training system was a trellised vertical shoot positioning. Other characteristics of the plots are listed in Table 1. Plots were dry farmed and no mineral nitrogen was added during the trial.

Soils
Among these plots, two are located on Quaternary alluvium and two are located on Tertiary limestone. The soils on Quaternary alluvium are acidic and contain a high amount of gravel and sand; the soils on Tertiary limestone have a high pH and their texture is sandy clay.

The plot SG is located on a sandy/gravelly soil. Gravel content is around 40% in the topsoil (0–50 cm) and 20% in the subsoil. The subsoil is very compact and most roots are located in the layer 0–50 cm. Water-holding capacity is low (67 mm over 1 m in depth, calculated according to Rawls *et al*[33]) because of shallow rooting and soil gravel content. The pH is very low in the subsoil (4.0) but close to neutral in the topsoil owing to fertilisation practices. This plot was planted after deforestation, which explains the very high organic matter content of the topsoil (over 5%). Consequently, soil total nitrogen content is very high in spite of an organic matter C/N ratio of 26.

The plot GS is located on a gravelly/sandy soil. Although GS is developed on the same geological deposit as SG, it contains a higher amount of gravel (over 50% in the topsoil and over 60% in the subsoil). Although rooting is very deep (over 2 m), soil water-holding capacity is low because of the high amount of gravel (39 mm in the first 80 cm of the soil). Soil organic matter content is average (1.2%). The pH is low in the subsoil but close to neutral in the topsoil.

Table 1. Characteristics of studied plots SG, GS, LSB and LHB

| Characteristic	SG	GS	LSB	LHB
Soil type	Sandy/gravelly soil	Gravelly/sandy soil	Sandy clay on soft limestone bedrock	Sandy clay on hard limestone bedrock
Organic matter content (%)	5.31	1.20	0.81	1.14
Organic matter C/N ratio	26	15	12	12
Soil pH (0–50 cm)	5.7	6.4	7.7	8.0
Geological origin	Quaternary alluvium	Quaternary alluvium	Tertiary limestone (Oligocene)	Tertiary limestone (Oligocene)
Gravel content	Medium	High	Low	Low
Soil depth	Shallow	Deep	Deep	Shallow
Appellation of origin	Pessac-Léognan	Graves	Pessac-Léognan	Graves
Grape variety	Sauvignon blanc	Sauvignon blanc	Sauvignon blanc	Sauvignon blanc
Rootstock	101-14MG	3309C	41B	3309C
Vine density (vines ha^{-1})	7143	5556	8547	5556
Canopy management	Vertical shoot positioning	Vertical shoot positioning	Vertical shoot positioning	Vertical shoot positioning
Pruning system	Cane pruned (Guyot)	Cane pruned (Guyot)	Cane pruned (Guyot)	Cane pruned (Guyot)
Year of planting	1988	1985	1988	1985
Irrigation	Dry farmed	Dry farmed	Dry farmed	Dry farmed

The plot LSB (limestone soft bedrock) is located on a calcareous soil developed on a soft Tertiary limestone bedrock. The soil has a sandy clay texture. From 90 cm in depth the soil contains a significant amount of lime. The pH ranges from 7.5 to 8.0 between 0 and 90 cm in depth and is around 8.5 over 90 cm in depth. Soil water-holding capacity is high (93 mm) owing to the sandy clay texture and the deep rooting (over 140 cm). Soil organic matter and soil total nitrogen content are very low.

The plot LHB (limestone hard bedrock) is located on a calcareous, sandy clay soil. A hard Tertiary limestone bedrock is present at 60 cm in depth. Although vine rooting is limited to 60 cm in depth, this type of bedrock can provide a significant amount of water to the vines. Using neutron moisture probes on a similar soil, Duteau[34] showed that about 50% of the water consumed by the vines in a dry vintage was supplied by the bedrock. Consequently, even in dry vintages, vines never face severe water deficit stress on this soil type. The pH of the soil is close to 8.0. Soil organic matter content is average (1.1%).

Climatic conditions

In 1998, temperatures were above average for the Bordeaux area from April to September (18.3 °C; Figs 1a and 1c). Rainfall during the growing season was low, especially in May (22 mm) and August (14 mm). This vintage can be characterised as warm and dry. The year 1999 was even warmer. The average temperature from April to September was 19.0 °C (Fig 1b). Growing season rainfall was slightly above average for the Bordeaux area, with more than 60 mm of rain in every month. No significant climatic variations among the plots were recorded during these two vintages (data not shown).
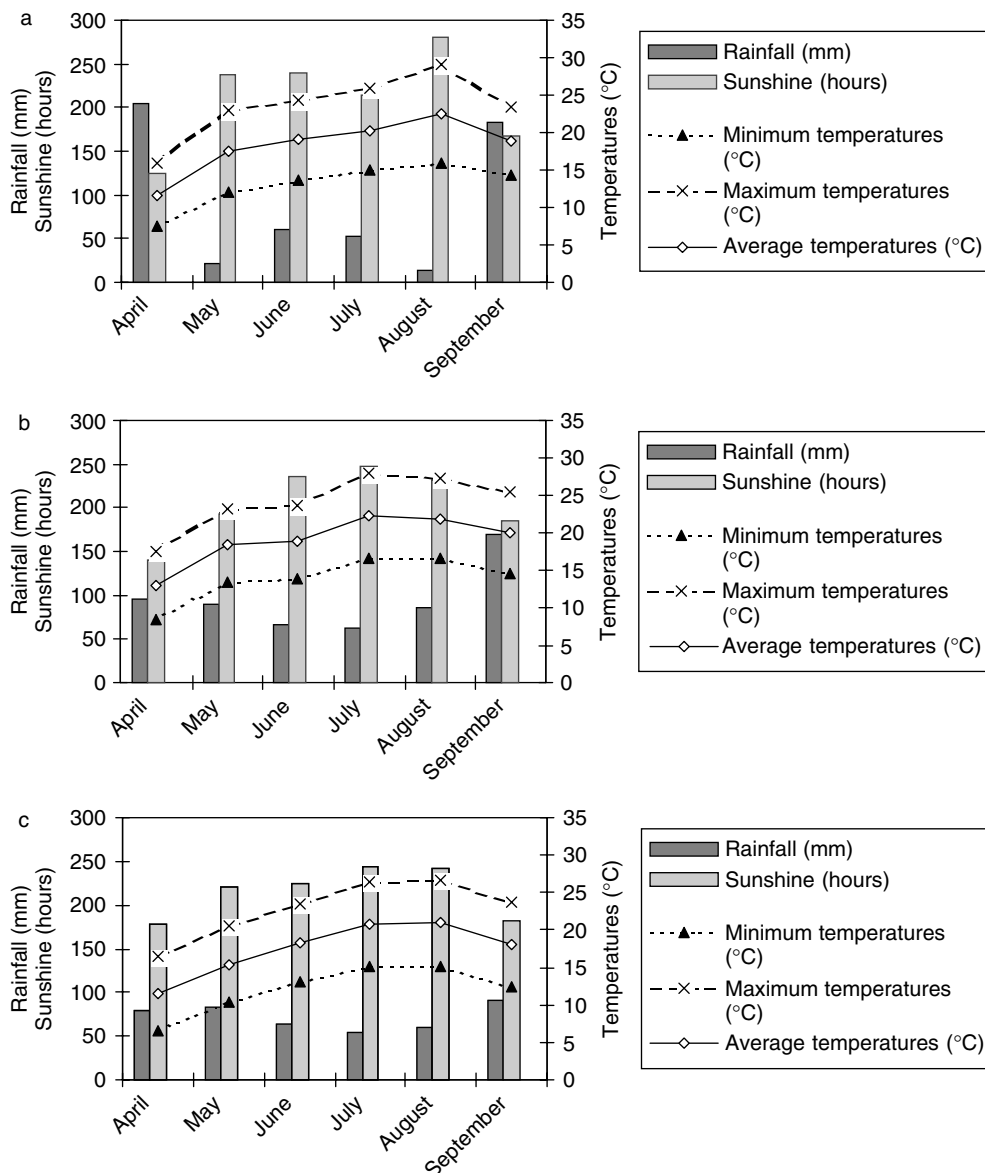
Figure 1. (a) Rainfall, sunshine hours and temperatures in 1998 in Graves and Pessac-Léognan region (data: Bordeaux Mérignac). (b) Rainfall, sunshine hours and temperatures in 1999 in Graves and Pessac-Léognan region (data: Bordeaux Mérignac). (c) Long-term means of rainfall, sunshine hours and temperatures in Graves and Pessac-Léognan region (data: Bordeaux Mérignac).

Variables measured

Vine water status
Changes in vine water status during the season were determined by five predawn leaf water potential measurements[35] carried out between the end of June and early September. Each value is the average of eight replicates. Water uptake conditions during the ripening period were also determined by measuring $^{13}C/^{12}C$ carbon isotope discrimination in grape sugars at ripeness ($\delta^{13}C$).[36,37]

Vine nitrogen status
Grape juice nitrogen content is a sensitive indicator for assessing vine summer nitrogen uptake in field conditions.[17,38] Grape juice total nitrogen and grape juice NH_4^+ contents were measured four times during grape ripening (12 replicates per plot).

Vine development and vigour
On 33 vines per plot the length of one shoot per vine was measured every 10 days until growth cessation. To prevent the cutting of these shoots by the hedging machines, they were trained horizontally on the lowest wire of the trellising system. Shoot growth rate ($cm\,day^{-1}$) was calculated for each period between two measurements. Leaf area was determined immediately after harvest by measuring leaf dry weight on 10 vines per plot according to Sepúlveda and Kliewer.[39] Pruning weight was measured in December 1998 and December 1999 on 12 plots of six vines.

Berry composition (major compounds)
Berry samples were taken at four times during grape ripening on 12 blocks per plot. Samples from each block were analysed separately and constitute replicates. Each block contained six vines. Ten berries were sampled on each vine. Berry weight was determined. Then for each block the 60 berries were manually pressed and 30 ml of grape juice was centrifuged. The following analyses were carried out on each replicate of fresh grape juice.

- Sugar content was calculated after determination of total soluble solids with a manual temperature-compensated refractometer (°Brix, RF233 model, Merck Eurolab, Fontenay sous Bois, France).
- pH was determined using an automated coupled pH meter (Cogetude, Vendôme, France).
- Titratable acidity was determined by titration with $0.05\,N$ NaOH to an end point of pH 7.0 (expressed in g tartaric acid l^{-1}).
- Organic acids were analysed using a continuous flow analytical system (Traacs 800, Bran and Luebbe, Plaisir, France) and expressed in $g\,l^{-1}$. Malic acid was determined by an enzymatic method (Bohringer, Mannheim, Germany), and tartaric acid by colorimetry after reaction with vanadic acid.
- K, Ca and Mg were determined with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Varian Vista, Mulgrave, Australia) after

1:20 (v/v) dilution with 5% HNO_3 (K expressed in $g\,l^{-1}$; Mg and Ca expressed in $mg\,l^{-1}$).
- NH_4^+ and mineral phosphorus were analysed using the Traacs 800 continuous flow analytical system according to the Berthelot method for N and the Duval method for P.
- Total nitrogen was digested with sulphuric acid/H_2O_2 according to a modified Kjeldahl method, followed by the analysis of NH_4^+ as mentioned previously.[40]

Berry composition (aroma precursors)
One lot of 25 bunches was sampled per plot. Berries were crushed under neutral gas. SO_2 was added ($50\,mg\,l^{-1}$). Skins were macerated in grape juice for 18 h at 18 °C. After maceration, skins were pressed at 0.5 MPa in a pneumatic micro-press (Bellot, Gradignan, France), SO_2 was added again ($50\,mg\,l^{-1}$) and the grape juice was filtered and stored at −20 °C until analyses. *S*-Cysteine conjugate precursors of volatile thiols were analysed following the method in Ref 32. In both vintages the concentrations of precursors of 4-mercapto-4-methylpentan-2-one (p-4MMP), 4-mercapto-4-methylpentan-2-ol (p-4MMPOH) and 3-mercaptohexan-1-ol (p-3MH) were determined at ripeness (ie harvest date of the plot, which was decided by the technical staff of the estate managing the plot). In 1998, five to seven samples were taken from the plots between veraison and harvest to show the evolution of these precursors during fruit ripening.

Data analysis
Data were analysed by means of linear regression (determination coefficient), analysis of variance and Newman–Keuls (NK) comparison of averages. The statistical analysis was carried out using Microsoft Excel and Statbox software.

RESULTS

Vine water uptake conditions in 1998 and 1999
On 9 July 1998 (Julian day 190), predawn leaf water potentials were close to zero on the four plots, showing no limitation in vine water uptake (Fig 2a). At the end of July 1998 (Julian day 208), predawn leaf water potentials were significantly more negative on SG and GS compared with LSB and LHB. Vine water deficit stress continued to increase during August on SG and GS. On 1998 August 20 (Julian day 232), water deficit stress was severe on GS (predawn leaf water potential −0.62 MPa) and very severe on SG (predawn leaf water potential −1.0 MPa). On SG the water deficit stress caused severe defoliation of the vines. Vines did not face water deficit stress on LSB and LHB. In early September, predawn leaf water potential values just showed a slight water deficit on these plots. $\delta^{13}C$ values, measured in grape sugars at ripeness, confirmed the water stress on SG and GS (Table 2). Moreover, the values indicated that water
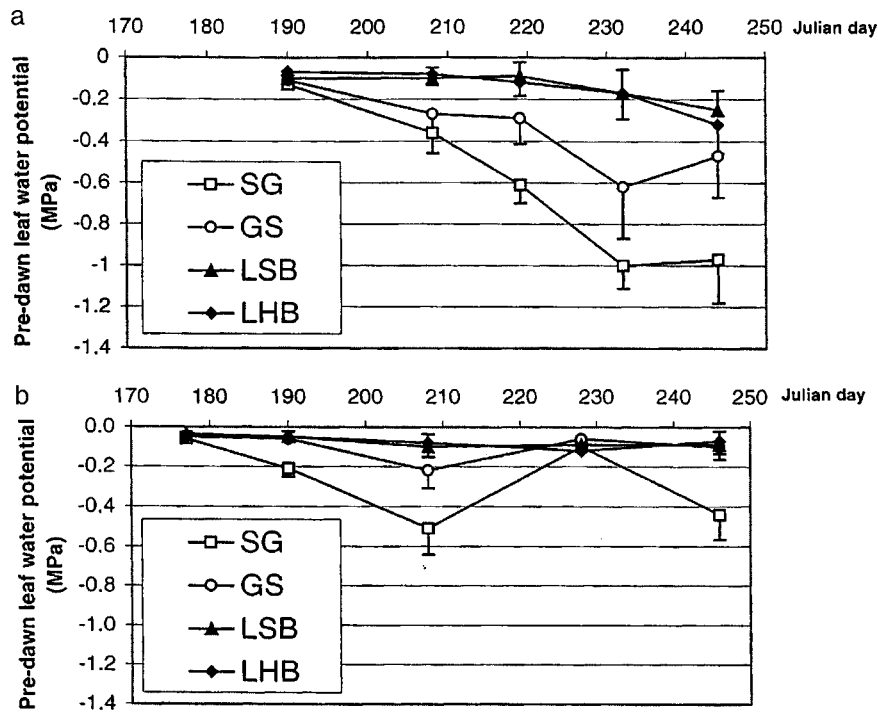
Figure 2. (a) Predawn leaf water potential values measured in 1998 (error bars indicate SD). (b) Predawn leaf water potential values measured in 1999 (error bars indicate SD).

Table 2. Carbon isotope composition of grape sugar (^{13}C/^{12}C) at harvest (δ^{13}C, p 1000)

Year	SG	GS	LSB	LHB
1998	−20.9	−22.5	−25.0	−25.9
1999	−24.5	−26.0	−26.1	−26.9

Interpretation: <−25.0, unlimited water uptake conditions during fruit ripening; −25.0 to −24.0, mild water deficit during fruit ripening; −24.0 to −22.0, moderate water stress during fruit ripening; >−22.0, severe water stress during fruit ripening. One replicate per plot for each year.

Figure 3. (a) Total nitrogen and NH$_4^+$ nitrogen in grape juice at harvest in 1998 (error bars indicate SD). (b) Total nitrogen and NH$_4^+$ nitrogen in grape juice at harvest in 1999 (error bars indicate SD).
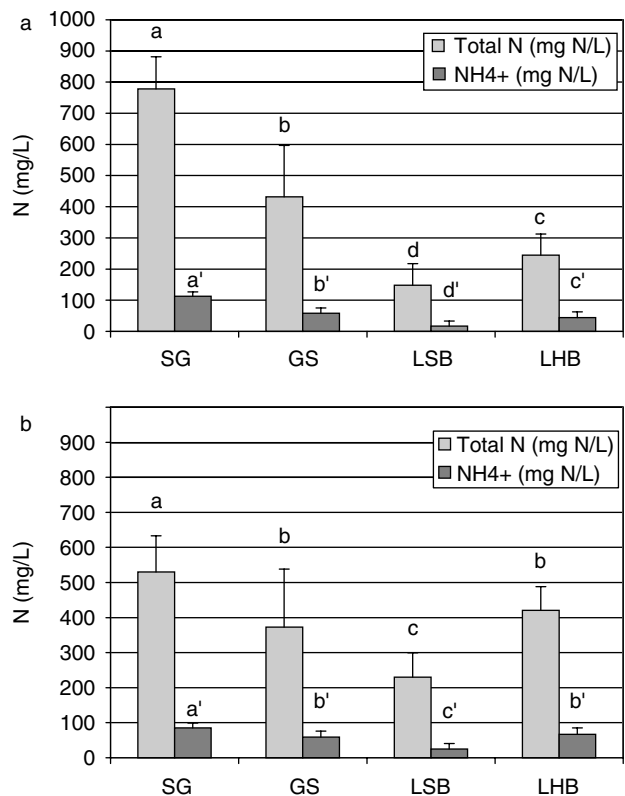
deficit during grape ripening was slightly more intense on LSB than on LHB.

In 1999, vines did not face severe water deficit stress because of higher rainfall during the season (Fig 2b). Predawn leaf water potential values did not indicate any vine water deficit on LSB and LHB. On GS a slight water deficit occurred at the end of July 1999 (predawn leaf water potential −0.22 MPa, Julian day 208), but the plants quickly recovered owing to rain in August. On SG, soil water-holding capacity is very low because of shallow vine rooting. On this plot, moderate water deficit stress occurred at the end of July, disappeared after rain in the middle of August and reappeared in early September. δ^{13}C values, measured in grape sugars at ripeness, indicated mild water deficit on SG and no water deficit on the other plots (Table 2).

Vine nitrogen status in 1998 and 1999

In 1998, grape juice nitrogen content was very high on SG, high on GS, moderate on LHB and low on LSB (Fig 3a).

In 1999, grape juice nitrogen content was lower on most plots compared with 1998 (Fig 3b). Vine nitrogen status was high again on SG, low on LSB and medium on GS and LHB. Values were not significantly different on GS and LHB for both grape juice total nitrogen and grape juice NH$_4^+$.

Vegetative development

On LHB, shoot growth rate was high and growth cessation occurred late in the season (Figs 4a and 4b). This resulted in long shoots at the end of the season (over 300 cm in 1998 and 1999, Table 3). Leaf area, pruning weight and yield were also high (Table 3). This plot, where neither vine water status nor vine nitrogen status was severely limited, can be characterised as vigorous.

On LSB, shoot growth rate was low throughout both growing seasons (Figs 4a and 4b). Although growth cessation did not occur very early, the slow growth rate resulted in short shoots at the end of the growing season in 1998 (138 cm) and in medium shoots in 1999 (246 cm, Table 3). Yield was medium in 1998 and high in 1999, while pruning weight was low in both vintages (160 g per vine in 1998 and 250 g per vine in 1999, Table 3), showing low vegetative vigour.

In 1998, shoot growth rate was high on SG at the beginning of the season but dropped dramatically when water stress occurred during July (Fig 4a). Although the shoots stopped growing earlier on this plot than on any other plot in 1998, SG produced the second longest shoots (202 cm, Table 3). Leaf area and yield were small because of the severe water stress, but pruning weight was high. In 1999, shoot growth rate varied within the season depending on vine water

status. It was high in June, decreased in July when available water became limiting and increased again in August owing to significant rainfall. Growth stopped in early September (Fig 4b). Yield was medium and pruning weight was high.

Shoot growth rate, shoot length, leaf area and pruning weight were average on GS in both vintages (Figs 4a and 4b, Table 3). Yield was low in 1999 and medium in 1998.

Pruning weight was correlated with berry NH_4^+ content ($R^2 = 0.55$; $p = 0.05$; $n = 8$) but not with vine water status. Secondary leaf area was correlated with vine water status (correlation $\delta^{13}C$–secondary leaf area: $R^2 = 0.85$; $p = 0.01$; $n = 8$), as was total leaf area (correlation $\delta^{13}C$–total leaf area: $R^2 = 0.75$; $p = 0.01$; $n = 8$). Yield and final shoot length were not correlated with vine water or vine nitrogen status.

Berry composition at ripeness (major compounds)

Harvest date was determined by the technical staff of each estate and mainly based on grape sugar/acid ratio (Table 4). Correlations were established with data collected on the four plots during two vintages ($n = 8$). Berry weight at ripeness was highly correlated with vine water status (correlation $\delta^{13}C$–berry weight: $R^2 = 0.81$; $p = 0.01$; $n = 8$). Water deficit-stressed

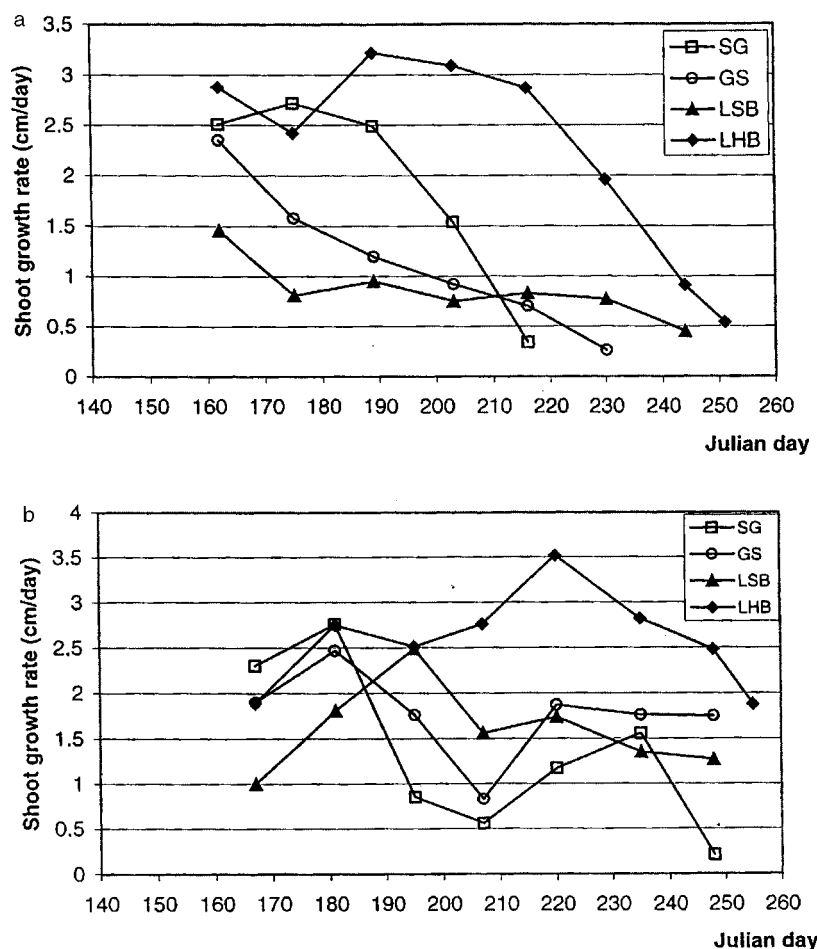**Figure 4.** (a) Shoot growth rate in 1998. (b) Shoot growth rate in 1999.

Table 3. Vine development and vine vigour parameters

1998	SG		GS		LSB		LHB	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Final shoot length (cm)	202	87	159	75	138	62	306	103
$n = 33$	b		bc		c		a	
Primary leaf area at harvest (m^2 ha^{-1})	7600	1500	6200	1900	5500	1800	8400	2100
$n = 10$	ab		ab		b		a	
Secondary leaf area at harvest (m^2 ha^{-1})	3600	1700	6300	2500	7500	2200	9100	3300
$n = 10$	b		ab		a		a	
Total leaf area at harvest (m^2 ha^{-1})	11 200	2500	12 500	5100	13 000	4000	17 500	5200
$n = 10$	b		ab		ab		a	
Pruning weight (t ha^{-1})	3.5	0.32	2.6	0.33	1.4	0.17	3.5	0.40
$n = 12$	a		b		c		a	
Yield (t ha^{-1})	8.5	2.1	12.8	3.0	12.0	3.2	19.5	3.1
$n = 12$	c		b		b		a	
1999	SG		GS		LSB		LHB	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Final shoot length (cm)	246	106	278	122	246	110	372	138
$n = 33$	b		b		b		a	
Primary leaf area at harvest (m^2 ha^{-1})	8600	1500	7100	1900	8000	1800	9800	2100
$n = 10$	ns		ns		ns		ns	
Secondary leaf area at harvest (m^2 ha^{-1})	8800	2300	11 900	3700	9200	2300	12 300	3400
$n = 10$	ns		ns		ns		ns	
Total leaf area at harvest (m^2 ha^{-1})	17 400	2700	19 000	5300	17 200	4000	22 100	5300
$n = 10$	ns		ns		ns		ns	
Pruning weight (t ha^{-1})	3.6	0.47	3.0	0.34	2.1	0.45	4.0	0.42
$n = 12$	a		b		c		a	
Yield (t ha^{-1})	11.2	2.3	8.5	2.6	15.7	5.6	18.8	2.6
$n = 12$	b		b		a		a	

Letters a, b, c indicate differences at $p = 0.05$ level (NK test); n, number of replicates; SD, standard deviation.

vines produced small berries, particularly on SG in 1998. Vine nitrogen status did not have an effect on berry size in this study. Vine water deficit stress negatively affected berry sugar content at harvest (correlation δ^{13}C–berry sugar content at ripeness: $R^2 = 0.59$; $p = 0.05$; $n = 8$). SG produced berries with very low sugar content in 1998 when vines were severely water stressed. The same plot produced berries with the highest sugar content in 1999 when water deficit was only mild. Vine nitrogen status did not affect berry sugar content at ripeness. Titratable acidity was determined by berry malic acid content ($R^2 = 0.72$; $p = 0.01$; $n = 8$) instead of by berry tartaric acid content ($R^2 = 0.22$; ns (not significant)) or berry potassium content ($R^2 = 0.02$; ns). Grape juice pH was correlated with titratable acidity ($R^2 = 0.71$; $p = 0.01$; $n = 8$) as well as with malic acid content ($R^2 = 0.71$; $p = 0.01$; $n = 8$). Titratable acidity depended on vine water status (correlation δ^{13}C–titratable acidity: $R^2 = 0.74$; $p = 0.01$; $n = 8$) but not on vine nitrogen status (correlation grape juice total nitrogen–titratable acidity: $R^2 = 0.40$; ns). The water-stressed plots SG and GS in 1998 produced berries with low titratable acidity and malic acid content and high pH. Differences in acidity among plots were much smaller in 1999 when vine water uptake conditions were similar. Water deficit-stressed

vines had a higher total nitrogen content (correlation δ^{13}C–grape juice total nitrogen: $R^2 = 0.50$; $p = 0.05$; $n = 8$).

Berry aroma potential

In 1998, p-4MMP content at ripeness was high on LHB, average on LSB and GS and low on SG (Fig 5a). In 1999, p-4MMP content was medium to high on SG and GS and low on LSB and LHB (Fig 5b). p-4MMPOH content and p-3MH content showed a similar distribution among the soils and vintages (Figs 5a and 5b). Higher levels were recorded in 1998 from vines grown in the soils on the Tertiary deposits (LSB and LHB) and in 1999 on the Quaternary alluvium (SG and GS). The concentration of p-4MMPOH was particularly high in 1999 on SG and the concentration of p-3MH peaked in 1998 on LHB. In 1999 the highest aroma potential was reached on SG and in 1998 on LSB.

Precursors of volatile thiols were analysed in 1998 on the experimental plots weekly from veraison (Figs 6a–6c) and continued until 1 or 2 weeks after harvest on 25 non-harvested vines. Concentrations of p-4MMP increased until a maximum and then decreased (Fig 6a). On SG and GS the maximum value occurred before harvest and was considerably lower compared with LSB and LHB. On LSB and

Table 4. Berry composition at harvest date (major compounds)

1998 Sampling date (calendar day) Sampling date (Julian day)	SG 14 Sept 257		GS 7 Sept 250		LSB 4 Sept 247		LHB 15 Sept 258	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Berry weight (g) $n = 12$	1.19 c	0.13	1.71 b	0.25	1.75 b	0.17	2.21 a	0.16
Sugar (g l^{-1}) $n = 12$	163 c	7	182 b	6	209 a	11	188 b	7
Titratable acidity (g tartaric acid l^{-1}) $n = 12$	5.6 b	0.47	5.0 b	0.62	7.0 a	0.45	6.8 a	0.42
pH $n = 12$	3.42 a	0.06	3.40 a	0.09	3.20 b	0.04	3.07 c	0.06
Malic acid (g l^{-1}) $n = 12$	1.34 c	0.31	1.61 b	0.38	2.41 a	0.33	2.21 a	0.25
Tartaric acid (g l^{-1}) $n = 12$	6.8 a	0.43	5.5 c	0.44	6.6 a	0.37	6.0 b	0.27
K (g l^{-1}) $n = 12$	1.489 a	0.105	1.394 b	0.075	1.525 a	0.103	1.060 c	0.095
Mg (mg l^{-1}) $n = 12$	62.3 a	3.0	54.8 b	6.0	63.1 a	10.4	45.9 c	3.4
Ca (mg l^{-1}) $n = 12$	37.8 bc	5.3	35.6 c	5.3	52.8 a	7.8	42.9 b	5.5
Mineral phosphorus (mg l^{-1}) $n = 12$	77.7 a	8.9	57.1 c	5.7	70.5 b	8.2	52.2 c	5.8

| 1999 Sampling date (calendar day) Sampling date (Julian day)	SG 2 Sept 245		GS 2 Sept 245		LSB 6 Sept 249		LHB 13 Sept 256	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Berry weight (g) $n = 12$	1.78 b	0.11	1.87 b	0.10	2.05 a	0.21	2.07 a	0.11
Sugar (g l^{-1}) $n = 12$	214 a	11	197 b	7	205 ab	14	211 a	7
Titratable acidity (g tartaric acid l^{-1}) $n = 12$	5.9 b	0.65	7.4 a	0.65	7.6 a	0.44	7.7 a	0.39
pH $n = 12$	3.31 a	0.04	3.13 c	0.03	3.23 b	0.04	3.16 c	0.04
Malic acid (g l^{-1}) $n = 12$	1.81 b	0.48	2.48 a	0.44	2.41 a	0.41	2.08 ab	0.24
Tartaric acid (g l^{-1}) $n = 12$	5.6 c	0.34	6.1 b	0.34	6.6 a	0.36	6.5 a	0.23
K (g l^{-1}) $n = 12$	1.320 b	0.060	1.361 b	0.108	1.491 a	0.077	1.95 b	0.063
Mg (mg l^{-1}) $n = 12$	56.5 a	2.9	53.9 a	6.3	49.8 a	8.6	49.5 a	3.0
Ca (mg l^{-1}) $n = 12$	20.5 c	2.3	27.0 b	2.7	37.2 a	7.1	40.6 a	5.2
Mineral phosphorus (mg l^{-1}) $n = 12$	62.5 b	4.8	66.9 ab	7.0	71.6 a	7.0	52.4 c	6.2

Letters a, b, c indicate differences at $p = 0.05$ level (NK test); n, number of replicates; SD, standard deviation.

LHB, berry p-4MMP content continued to increase respectively 1 and 2 weeks after harvest before starting to decrease. There was a considerable time span between the maximum on GS (2 September, Julian day 245) and the maximum on LHB (23 September, Julian day 266). Concentrations of p-4MMPOH increased slowly from veraison to harvest (Fig 6b). The increase was most pronounced on LHB, where it tripled during fruit ripening. Concentrations of p-3MH had a more chaotic development and, globally, remained relatively stable during fruit ripening (Fig 6c). Grape p-3MH content was highest on LHB at veraison and remained so until harvest.

DISCUSSION AND CONCLUSION
Vine water status as well as vine nitrogen status were assessed by two techniques, showing consistent results.
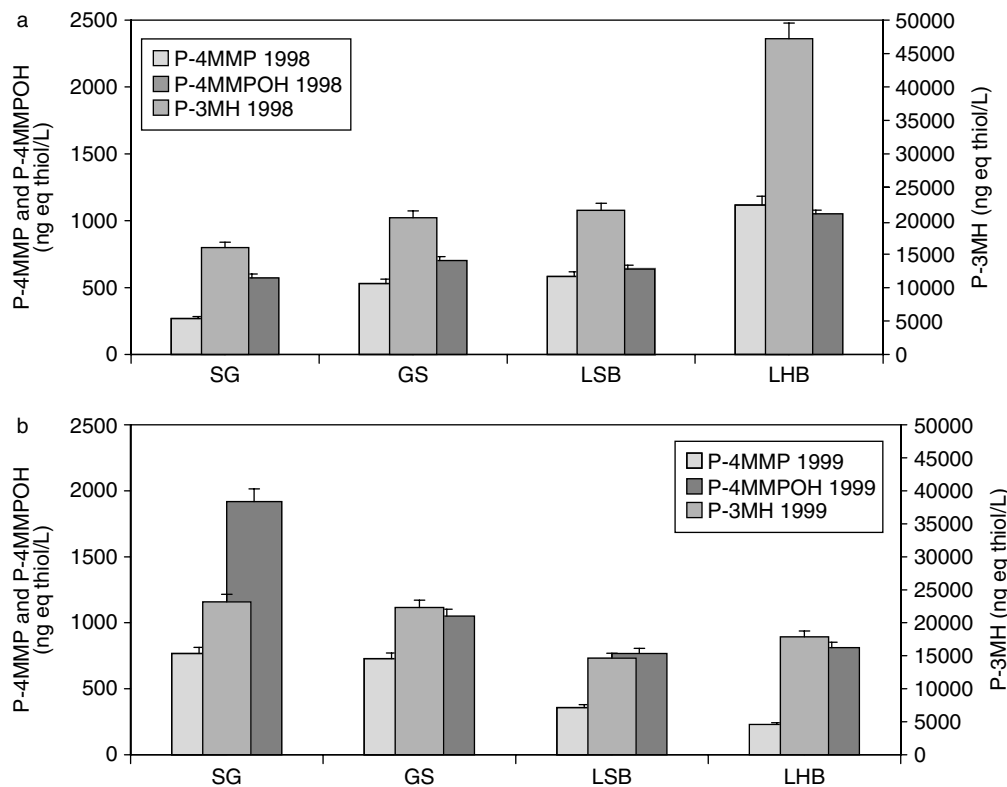
Figure 5. (a) Volatile thiol content in berries at harvest in 1998 (error bars indicate coefficient of variation). (b) Volatile thiol content in berries at harvest in 1999 (error bars indicate coefficient of variation).

δ^{13}C values were highly correlated with minimum predawn leaf water potential ($R^2 = 0.92$; $p = 0.001$; $n = 8$). Tregoat *et al*[10] showed that the combined use of these two techniques allows a precise monitoring of vine water status in field studies. Vine nitrogen status was assessed by measuring total nitrogen and NH_4^+ in grape juice according to Van Leeuwen *et al*.[17] Values of total nitrogen and NH_4^+ were highly correlated ($R^2 = 0.92$; $p = 0.001$; $n = 48$).

Vine vigour depended on both vine water status and vine nitrogen status.[18] Limiting vine nitrogen uptake resulted in low shoot growth rate during shoot development, as was the case on LSB in 1998. The effect of limiting vine water uptake was shown on SG in 1998. Shoot growth rate started at a high level but dropped as soon as vines faced water deficit. Grape juice from water deficit-stressed vines contained more nitrogen (correlation δ^{13}C–grape juice total nitrogen: $R^2 = 0.50$; $p = 0.05$; $n = 8$). It is likely that high nitrogen status increases the susceptibility of vines to water stress. High nitrogen uptake promotes shoot growth early in the season and consequently results in high leaf area. High leaf area increases vine water use and favours the emptying out of soil water reserves. When water deficit stress becomes severe, part of the leaves can fall as a reaction by the plant to protect itself against excessive water loss. This scenario clearly took place on SG in 1998. Conversely, low vine nitrogen status limits shoot growth and leaf area. Subsequently, water use by vines is diminished, as was shown by Pieri *et al*.[41]

Berry composition at harvest was more influenced by vine water status than by vine nitrogen status. Severe water deficit stress provoked low berry sugar content and low titratable acidity, because malic acid content was low. This berry composition is not favourable for producing high-quality white wines. In Bordeaux a titratable acidity of 7.5 g tartaric acid l^{-1} is considered optimum for the production of well-balanced white wines.[20] In 1998, a very dry vintage, all the experimental plot titratable acidity values were below 7.5 g tartaric acid l^{-1}. Titratable acidity was particularly low on SG and GS, which faced severe water deficit stress. In 1999, when rainfall occurred more regularly during the summer, titratable acidity was close to 7.5 g tartaric acid l^{-1} on three experimental plots (GS, LSB and LHB). Average berry sugar content was also higher in 1999. Water deficit stress reduced berry size. In this study there was no relationship between berry size and berry aroma precursor content in Sauvignon blanc.

4MMP is responsible for the box tree and broom aromas in Sauvignon blanc wines.[29] Its detection threshold in a model solution is very low (0.8 ng l^{-1}).[29] 4MMPOH smells of citrus zest (detection threshold in model solution 55 ng l^{-1}) and 3MH of grapefruit and passion fruit (detection threshold in model solution 60 ng l^{-1}).[30] Although the concentration of volatile thiols in wine is directly related to the concentration of their precursors,[42,43] only a small percentage of the precursors are effectively transformed into aroma during vinification. According to Peyrot des Gachons,[43] the average level of transformation is 1.4%
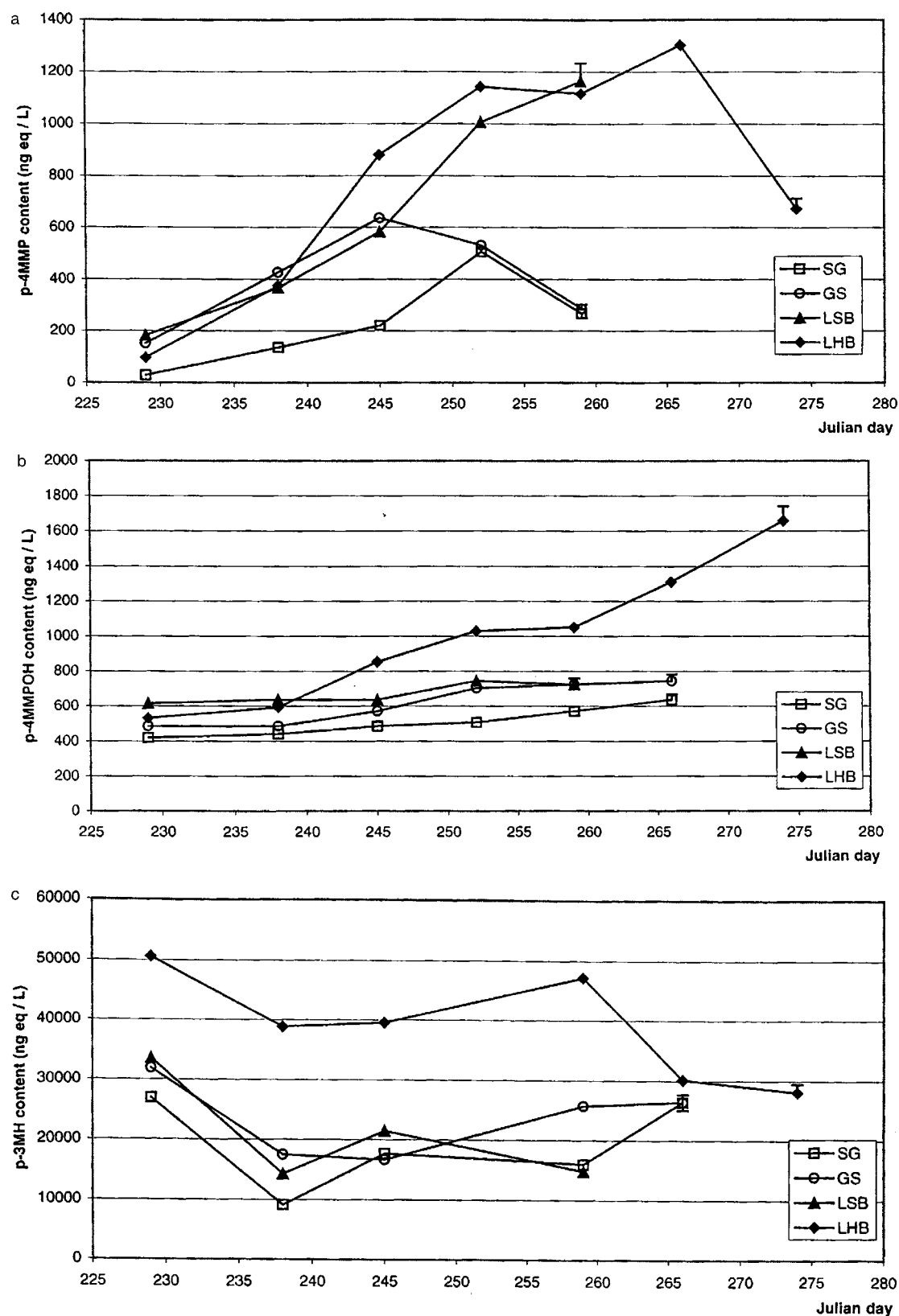
Figure 6. (a) Evolution of berry p-4MMP content during fruit ripening in 1998 (error bars indicate coefficient of variation). (b) Evolution of berry p-4MMPOH content during fruit ripening in 1998 (error bars indicate coefficient of variation). (c) Evolution of berry p-4MH content during fruit ripening in 1998 (error bars indicate coefficient of variation).

for p-4MMP, 3.0% for p-4MMPOH and 4.2% for p-3MH.

In 1998, which was a dry vintage, the highest aroma potential was achieved on the plots with the greatest water reserves (LSB and LHB). In 1999, which was a wet vintage, the highest aroma potential was achieved on the plots with the lowest water reserves. SG and GS had mild water deficit stress
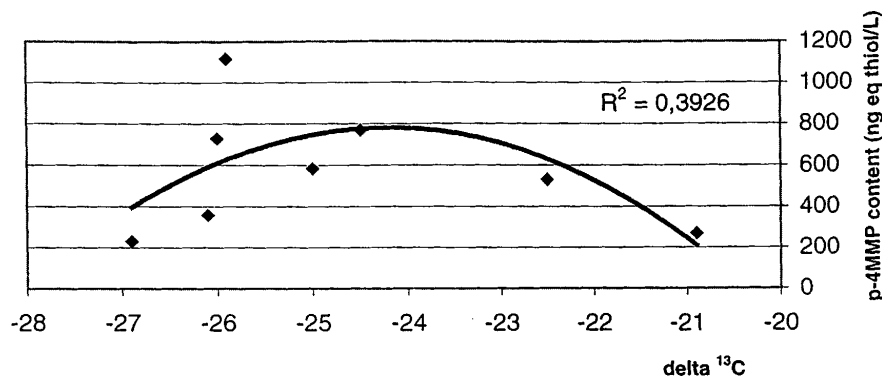
Figure 7. Correlation between berry p-4MMP content at harvest date and intensity of water deficit stress, assessed by carbon isotope composition of grape sugar.

in 1999. These results seem to indicate that severe water deficit stress limits aroma potential in Sauvignon blanc grapes but that mild water deficit might enhance it. When the four soils and two vintages are plotted together, berry p-4MMP content is highest when vines face mild water stress ($-26 < \delta^{13}C < -24$, Fig 7). This is consistent with the observation that white Bordeaux wines generally lack aroma expression in dry vintages.[20] Low vine nitrogen status on LSB might explain the lower concentrations of volatile thiol precursors on this plot in 1998 compared with LHB, while vine water status was similar. Despite a tendency of depressed aroma potential when vine nitrogen status is low, no correlation can be established with the available data between vine nitrogen status and grape aroma potential, because the effect of vine water status interferes with the effect of vine nitrogen status. For example, on SG in 1998, vine nitrogen status is high but aroma potential remains low because of severe water stress. Although these results need to be confirmed by experiments in controlled conditions, they seem to indicate that the highest aroma potential is built up in Sauvignon blanc grapes when vines face mild water deficit stress and when nitrogen status is non-limiting.

Considering perception threshold values of volatile thiols and the percentage of transformation of precursors into odorous thiol, p-4MMP and p-3MH have a higher contribution to the aroma potential of the Sauvignon blanc grapes in this study than p-4MMPOH.[43] Because p-3MH content remained relatively stable from veraison to harvest, variations in aroma potential of Sauvignon blanc grapes during fruit ripening depended mainly on the evolution of p-4MMP content. In 1998, grapes were picked after the maximum p-4MMP content on SG and GS and before the maximum on LSB and LHB. Ideally, grapes should be picked when p-4MMP content is the highest. However, it was not possible to pick grapes earlier on SG and GS because of low sugar content. When p-4MMP content was maximum, sugar content was only $160\,g\,l^{-1}$ on SG (9 September, Julian day 252) and $174\,g\,l^{-1}$ on GS (2 September, Julian day 245). Conversely, grapes could have been picked a few days

later on LSB and LHB, but not very much so, because otherwise titratable acidity would have been too low.

The results of this research can be used in site selection for non-irrigated Sauvignon blanc vineyards. For maximum aroma expression in Sauvignon blanc grapes, water deficit stress should not be severe and nitrogen status should not be limiting. Shallow and gravelly soils are better suited for high potential red grape production, while deeper soils are better adapted for Sauvignon blanc. However, soils for Sauvignon blanc should not provide an excessive amount of water and nitrogen to the vines. Mild water deficit could possibly have a positive effect on aroma potential in Sauvignon blanc grapes. Excessive nitrogen can enhance susceptibility to *Botrytis*.[20] In irrigated vines, Sauvignon blanc should be watered to achieve and maintain a mild deficit level. Nitrogen deficiency should be avoided, as well as excessively high vine nitrogen levels. The same recommendations can be given for a number of other white grape varieties of *Vitis vinifera* L (Gewürztraminer, Petit Manseng, Gros Manseng, Sémillon), because volatile thiols also make up part of their aroma.[44]

Grape sugar and titratable acidity level monitoring is universally used to determine harvest date. In red grapes the analysis of anthocyanin and tannin can provide interesting further information. The assessment of the aroma potential in white grapes could also be helpful and give interesting information to growers.

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RIPENING GRAPES TO SPECIFICATION: IDENTIFYING MANAGEABLE FACTORS DETERMINING GRAPE COMPOSITION & QUALITY THROUGH CARBOHYDRATE SINK-SOURCE RELATIONSHIPS

Final Report to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **DAN 98/1**

Principal Investigators: **Dr Bruno Holzapfel & Dr Suzy Rogiers**

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

Date: **27 September 2002**

Grape and Wine Research and Development Corporation

Final Report, September 2002

Project Code   DAN 98/1

Project Title   RIPENING GRAPES TO SPECIFICATION: IDENTIFYING MANAGEABLE FACTORS DETERMINING GRAPE COMPOSITION & QUALITY THROUGH CARBOHYDRATE SINK-SOURCE RELATIONSHIPS

Contents

ABSTRACT

A regional survey and field trial were used to investigate the impact of delayed ripening on berry composition in Shiraz. The survey examined sugar and colour accumulation trends across vineyards of the Riverina over two seasons. TSS, colour and yields varied significantly among vineyards and between years as did sugar and colour accumulation during berry maturation. Irrigation type and temperature were two factors that accounted for some of this variability. The field trial was used to investigate vine source-sink relationships and these were altered by reducing the source (leaves) or sinks (fruit, trunk, roots) by crop removal and girdling. There was an impact of these treatments on ripening and colour development during the first half of maturation, however by harvest the treatment effects were no longer significant and there were no differences in final berry composition.

EXECUTIVE SUMMARY

The objective of this project was to investigate the impact of delayed ripening on berry composition. A 2-year survey was conducted across ten Shiraz vineyards in the Riverina to gain knowledge of the extent of variability in berry ripening rates and vine productivity (yield, vigour). An average ripening vineyard was chosen to conduct a trial where vine source (leaves, wood reserves) and sink (fruit, wood storage) sizes were altered with the aim to change berry ripening rates.

The rates and extent of berry sugar and colour development are integral factors contributing to berry quality. The survey used to study these factors spanned across the 1998/99 and 1999/2000 ripening seasons of the Riverina. Berry ripening rate data was accumulated along with vineyard management information. Information on factors influencing the decision to harvest was also collected. Each of the vineyards had 10 sampling plots from which 100 berries were collected from veraison to harvest in weekly intervals, yield and pruning weights were also determined. Half of the berry sample was juiced for TSS, pH and TA determination and the other half was used for berry colour determination.

In the first season the harvest date was mainly dictated by berry ripeness levels and the schedule of the processing winery. In the second year, it was dictated by the weather and the winery. Generally higher yields lead to lower berry TSS and colour, however these correlations were not very strong and even less pronounced in the second season. The relationship between colour and TSS was much stronger. The findings indicate that high yielding vineyards can mature grapes properly and can have good berry colour. Low yielding vineyards do not necessarily produce good mature grapes or intense berry colour. Therefore, the potential exists to improve yield, sugar and colour levels simultaneously within a particular vineyard. The data also suggest that the optimum grape quantity/quality relationship differs between vineyards.

The survey indicated that management practises across the vineyards were diverse. In particular, there was large variation in soil management including fertilisation regime, cover crop selection, irrigation method (drip, furrow, flood) and amounts of water applied. The last two factors had a significant affect on vineyard performance. Five vineyards were distinctly different in the amount of water applied and the type of irrigation system in place. By plotting yield per vine in the second season against the pruning weights of the first season, it was observed that higher yields correlated with higher pruning weights. A vineyard with low furrow irrigation was at the lower end of the productivity scale, whereas a high furrow irrigated vineyard was at the higher end. Other vineyards lay in between. Therefore, in this population of vineyards, high water application rates lead to vigorous vegetative growth in the first season. This resulted in high yields in the following season. Perhaps there were ample reserves to draw from in these vigorous vineyards.

Another group of vineyards behaved differently however. In this population, yield varied widely even though the pruning weights of the previous season were very similar. The variation in yield was due to bunch numbers per vine, whereas berries per bunch and berry weight played a minor role. The higher number of bunches per vine (or metre of cordon) was due to higher shoot numbers per vine and more bunches per shoot. These results suggest a significant shift in vine balance towards grape production is

possible without a need for vigorous canopies. The high yielding vineyards also had berries with high colour levels indicating that the yield/quality trade off is not always necessary.

The survey also brought out how different vineyards can be in the amount of variability they contain. Two vineyards differed strongly in the amount of variation there was within that particular vineyard. Yield in vineyard I ranged between 9 and 14kg/vine and pruning weight ranged from 1.0 to 1.8kg/vine. The variability in vineyard II was much larger. Yield ranged from 6 to 22kg/vine and pruning weight ranged from 1.0 to 2.6kg/vine. The higher yielding plots of the vineyard I did not result in lower berry colour levels, and brix levels were only slightly lowered. This again indicates that good composition and high yields can be achieved simultaneously. An EM survey indicated little within-vineyard variation in both vineyards, but an aerial image conducted before harvest 2002 revealed that the vines in vineyard II had large canopy size differences. The within-vineyard variation was therefore most likely due to poor vineyard establishment rather than soil differences.

The survey was conducted over two seasons and weather was more extreme in one season than the other. January 1999 was very hot with 7 days where the daily maximum was in excess of 40ºC and no daily maximums dipping below 30ºC. The mean daily temperature was higher in January of 1999 by 5.6ºC than January of 2000. In the early ripening period, sugar and colour per berry increased at a faster rate in 2000 as compared to 1999. These data indicate that the extreme temperatures of the 1999 season resulted in delayed ripening and this culminated in significantly lower colour and sugar levels at harvest. Berry size was also much lower in the 1999 harvest as compared to the 2000 harvest. Lack of rain during late ripening of 1999 resulted in increased rates of weight loss and this resulted in smaller berries.

The second part of the project consisted of a trial to study the source-sink relationships in vines and their impact on grape maturation and berry composition. Vineyard management and environmental conditions can influence sugar accumulation and colour development. Leaf photosynthesis is the main contributor to berry sugar levels; a small contribution can come from stored reserves or berry photosynthesis. The vine stores reserves in wood which can also become a competing sink during grape maturation. Source-sink relationships are regulated by assimilate supply and demand and these alter during the growing season. Leaves are a source of assimilates, (except in early development) wood can be source or a sink, while the fruit are basically only a sink. Trunk girdling, leaf and fruit removal change the source-sink relationships in a vine.

Five treatments were applied in early January 2001 (veraison): Control (C), Leaf removal (LR), Bunch removal (BR), trunk Girdling (G), Girdling and Bunch removal (G+BR). Berry composition, yield and pruning weight data were collected. The C and G treatments produced the highest yields while the BR treatment resulted in half the yield of the control. The pruning weights were highest in the two girdling treatments (G, G+BR) with the lowest weights recorded in the LR and BR treatment. The LR treatment had the highest photosynthesis rate. The two girdling treatments had the lowest rates (G, G+BR)and the control and BR were in between. As in this study, the removal of part of the source (leaf) often enhances the photosynthetic rate of the remaining leaves while sink (bunch) removal often lowers leaf photosynthesis. Shoot growth in spring was reduced by the girdling treatments, while bunch removal increased shoot growth slightly compared to the control. Girdling effectively blocked assimilate movement and reduced their production while the removal of bunches most likely left more assimilate available for shoot growth.

The largest berry weights were seen in the LR treatment. As in the control, these berries consistently accumulated weight until nearly harvest. The berries of the G+BR treatments had a weight maximum at mid ripening, and as a result they were the smallest at harvest. Berry sugar accumulation in early ripening was slowed down by the LR treatment and increased by BR+G treatment. This relationship reversed during late ripening, and at harvest berry sugar was highest for the LR treatment. Glucose and fructose concentrations in the juice at harvest were similar to the TSS data of the different treatments. Juice pH tended to increase and TA to decrease in all treatments during ripening. At harvest the control treatment had the highest pH and the lowest TA, while the LR treatment produced the lowest pH and highest TA. Early in berry maturation the concentration of malic acid was much higher than that of tartaric acid. As time progressed the concentration of malic acid continued to decrease with

very little difference between treatments. At harvest, berries of the G+BR treatment had the highest tartaric acid levels while the BR treatment berries had the lowest.

Berry colour increased significantly during the first two weeks of January with large differences between treatments. Berries from the combined G+BR treatment had nearly double the colour on a fresh weight basis of the LR treatment. At harvest, however, the control berries had the highest colour. This is because colour continued to accumulate right until harvest while in the G, BR, and G+BR treatments the colour maximum was reached two weeks before harvest. The mono-glycosylated anthocyanins also showed a strong increase in the first four weeks of berry maturation (mid ripening). Malvidine 3-glucoside and all the other anthocyanins were higher in the G+BR treatment compared to the other treatments. At harvest the three major anthocyanins (malvidin 3-glucoside, malvidin 3-acethylglucoside, malvidin 3-p-coumarylglucoside) were highest in the control and lowest in the combined treatment (G+BR). These three anthocyanins contributed to about 80% of the total anthocyanins while cyanidin 3-glucoside was the constituent with the lowest concentration.

The results of this project have provided information on the variability in ripening rates that can exist within a growing region and also within a vineyard. It has provided insights on the impact of vineyard management on vine productivity and grape quality. The results also indicate that berry ripening can be affected by climatic conditions. Furthermore, changes in source or sink size can alter the development of colour and sugar within the berry.

PROJECT OVERVIEW

Project code: DAN 98/1

Project Title: RIPENING GRAPES TO SPECIFICATION: IDENTIFYING MANAGEABLE FACTORS DETERMINING GRAPE COMPOSITION & QUALITY THROUGH CARBOHYDRATE SINK-SOURCE RELATIONSHIPS

Research Organisation: National & Wine Grape Industry Centre

Project supervisors:

Dr Bruno Holzapfel   National Wine & Grape Industry Centre, NSW Ag
Dr Suzy Rogiers   National Wine & Grape Industry Centre, NSW Ag

Research staff:

Dr Chris Steel   National Wine & Grape Industry Centre, CSU
Ms Kerry DeGaris   Wine Grapes Marketing Board
Ms Gioia Small   National Wine & Grape Industry Centre, NSW Ag
Mr David Foster   National Wine & Grape Industry Centre, NSW Ag

Project Site(s): Riverina

Commencement Date: September 1998

Completion Date: August 2001 (extended to June 2002)

Project funding: Funds provided by GWRDC
1998/99 $25,000
1999/00 $32,385
2000/01 $30,318
2001/02 $5,000

Additional funds provided by NSW Agriculture's R&D Initiative Account
1998/99 $15,000
1999/00 $15,000
2000/01 $15,000

Project Objectives/Outcomes:

Objectives:

- Determine range in sugar accumulation rates (slow, normal, fast) across vineyards.
- Determine factors affecting harvest date.
- Investigate which vineyard management factors affect sugar accumulation rates.

- Clarify the relationships between carbohydrate production, canopy size, crop load and berry susceptibility to delayed ripening.
- Investigate the impact of berry ripening and sugar accumulation rates on major berry components (anthocyanins, phenolics, sugars, organic acids) so as to improve the ability to relate ripening behaviour to grape quality.
- Further understand the relationship between vine productivity and grape quality and its implications for vineyard management.

Outcomes:

- General aspects influencing sugar accumulation and colour identified.

- Directions for further work on sugar accumulation and colour development determined.

- Possibilities to minimise slow interrupted berry sugar accumulation in vineyards indicated.

- Insights into the relationship between carbohydrate production, crop load and berry maturation revealed.

- Better understanding on the impact of grape maturation on major grape composition parameters.

- Further knowledge of factors impending on the relationship between yield and quality will significantly improve the ability to produce grapes to specification.

Outputs and Performance Targets

Outputs	Performance Target
1999/2000 (year 1)	
1. Information on internal (crop load, canopy) and external (vineyard management, climate) factors affecting berry ripening	1. Survey of fifteen vineyards in the MIA by July 2000
2. Growers knowledgeable of the outcomes of survey	2. Presentation of results at two seminars by September 2000
2000/2001 (year 2)	
3. Information on the effect of grape ripening on berry composition, particularly colour	3. Trial over two seasons with treatments which alter the source-sink relationship by July 2001
4. Seminars and further publication	4. Two seminars presented and one publication in journal by September 2001

The output and performance targets 1 and 2 refer to the survey (see section 2.1), the information from the vineyards was collected and the growers and industry informed (see section 7). Due to a number of reasons, only 10 vineyards appear in the result section (see explanation 2.1.2). Output and performance target 3 refers to the field trial (see section 2.2). A trial was conducted in the 1999/2000 season but results are not reported here since they were similar to the 2000/2001 season and only three treatments were applied due to growers' concerns. The output and performance target 4 refers to both parts of the project (see section 7). Please note that output and performance targets were not required for the 1998/99 year and therefore not present.

DETAILED PROJECT REPORT

1 GENERAL INTRODUCTION

Grape sugar accumulation, phenolic development and flavour production determine grape quality. They are processes critical to grape variety selection, vineyard location, fruit pricing, viticultural strategies, harvesting date, wine processing and wine market value. The industry needs to specify that quality using measurable parameters. However, factors that affect sugar accumulation are not well defined; factors that affect phenolic and flavour components are even less understood; sugar accumulation does not consistently relate to phenolic and flavour.

Environmental conditions can slow down or interrupt sugar accumulation in the later phase of berry ripening. This may delay harvesting, and it may reduce grape quality and vine carbohydrate reserves. Its occurrence needs to be investigated. In disrupting the normal ripening of the berry, the process also provides a valuable further means of studying the mechanisms of sugar accumulation; of studying the comparative role of storage reserves and photosynthetic ability on ripening and carbohydrate metabolism; and studying the link between sugar accumulation, phenolic development and flavour production.

This work was conducted from 1998 to 2001 and had two aspects. First a survey was conducted over 2 years of 15 vineyards in the Riverina to investigate the rate of ripening in Shiraz and potential connections between management and internal relationships (vegetative/generative growth) in these vineyards. The second part consisted of a field trial in which the source-sink relationships of the vine were altered so as to influence berry maturation rates.

2 RESEARCH INVESTIGATIONS

2.1 Productivity of Shiraz vines under irrigation

2.1.1 Introduction

Berry sugar, colour and flavour are composition parameters determining quality of wine grapes. There is some existing literature addressing how climate and vineyard management affects these parameters. For instance, high yields and vigour often reduce grape quality (Jackson & Lombard 1993) by delaying maturation (Bravdo et al. 1985, Lombard & Jackson 1993). The effects of these factors on grape quality may be the result of altered carbohydrate source-sink relationships.

Carbohydrate source-sink relationships are complex and alter during the growth season. Initial growth of the shoot depends on carbohydrate reserves in the permanent structure (Williams 1996). The leaves reach about half of their final size before they begin to export carbohydrates (Koblet 1977). Before anthesis, translocation of photosynthates from the grapevine leaves is towards the shoot tips (Hunter & Visser 1988a). After berry set the fruit becomes a very large sink (Hunter & Visser 1988b), and after harvest most of the recently assimilated photosyntate is translocated to the permanent structure of the vines (Williams 1996). Thus, carbohydrate concentrations in the wood are highest in winter (Sommer & Clingeleffer 1995). These results indicate that the amount of carbon fixed by the vine and partitioned to the trunk and cordons varies through the growing season. Vine age, vineyard establishment, and genotype also influence partitioning (Mullins et al. 1992). This work will examine the influence of that partitioning on ripening behaviour.

The carbohydrates in fruit originate to a small extent from stored carbohydrates (Korkas et al. 1996, Mansfeld & Howell 1981) and from berry photosynthesis (Di Macro et al. 1977), but mainly from leaf photosynthesis (Koblet 1977, Düring & Alleweldt 1980, Glad et al. 1992a, b). The rate of photosynthesis of grapevine leaves is affected by light intensity (Kliewer 1980, Koblet 1984),

temperature and relative humidity (Sepúlveda & Kliewer 1986), leaf age (Intirieri et al. 1992, Koblet 1984), water supply (Downton et al. 1987, Ruhl & Alleweldt 1990), nutrition (Williams & Smith 1985, Skinner & Matthews 1990, Korkas et al. 1994a, b), pruning (Kriedemann et al. 1975, Hofäcker 1978), crop load and seasonal pattern (Kriedemann 1977). The accumulation of sugars in the berry begins suddenly after veraison (Coombe 1989). Sucrose is transported from the leaves into the berry via phloem and is hydrolysed by invertase to glucose and fructose (Mullins et al. 1992).

High temperature can cause changes in the distribution pattern of carbohydrates (Sepúlveda et al. 1986) despite reasonable photosynthetic activity (Mullins et al. 1992). During heat stress the movement of assimilates was predominantly towards the shoot tip, as opposed to trunk, root and clusters in control vines (Sepúlveda et al. 1986). The delay in sugar accumulation accompanying high temperatures can often result in unwanted juice composition (Kliewer & Lieder 1970).

As in sugar accumulation, grape colour is influenced by a myriad of factors. Climatic conditions (Pirie & Mullins 1977) training system (Reynolds et al. 1994, Smart et al. 1985), water supply (McCarthy et al. 1995, McCarthy 1997, Nadal & Arola 1995) and nitrogen (Delas 1993, Jackson & Lombard 1993, Treeby et al. 1997) influence colour of the grape. A strong correlation between concentrations of anthocyanins and sugar in the skin has been found (González-SanJose & Diez 1992, Kataoka et al. 1983, Matushima et al. 1989, Pirie & Mullins 1977, 1980) perhaps because sugars are the initial precursors of anthocyanins (Haazdina et al. 1984) but also likely is that anthocyanin synthesis is triggered by developing sugar concentrations, or that both are independently triggered at veraison. The anthocyanins increase during ripening (Fernandez-Lopez et al. 1992), although a maximum concentration is reached before harvest (Pirie & Mullins 1977, Ribéreau-Gayon 1971, Somers 1976).

Carbohydrates seem to play a significant role in colour/flavour development and berry composition. This may arise through colour and flavour depending upon sugar accumulation, or they may be independent processes. Previous work has not used slow, interrupted sugar accumulation to examine these possibilities.

The use of different practises by grape growers indicates that potential sources and sinks will differ between vineyards. Further knowledge about the distribution and management of carbohydrates, particularly in the later phase of fruit ripening will be important for the different climatic wine regions.

2.1.2 Materials and methods

2.1.2.1 Background of the vineyards and climatic conditions

The study was conducted on own rooted Shiraz in the Murrumbidgee irrigation area (MIA) in NSW. A number of vineyards were selected by utilising results from the Wine Grapes Marketing Board (WGMB) from the 1997/98 season. This survey identified the variability of berry colour in the MIA and directed these investigations to vineyards that showed clear differences in this grape parameter.

The selected study sites have been set up on 15 properties in Griffith, Yenda and Leeton. Five vineyards were omitted for different reasons, the most common one was the yield determination could not be obtained. In each vineyard 10 five vine replicates were distributed randomly in an area of 10 rows by 50 vines. The area in the vineyard was chosen at a certain distance from the irrigation water outlet (usually 25 vines into the row).

2.1.2.2 Measurements of berry parameters

Each replicate in every vineyard was sampled weekly from early January (veraison) to harvest for two seasons (1998/99 and 1999/2000), covering the whole berry ripening period. Two lots of berries (50 each) were taken in these vineyards until the vineyards were harvested. One sample was used for determining berry weight and measuring TSS with a digital bench refractometer (PR-101 ATAGO, Tokyo, Japan), the other sample was frozen for colour determination. Shortly before or at the commercial harvest yield was determined by weighing grapes from each plot in these vineyards. At the last sampling date in every vineyard the juice pH and TA (g/L) was determined by using an automatic titrator (TitraLab 80, Radiometer Copenhagen, Lyon, France). Berry colour and total

phenolics were determined by the method described by Williams et al. (1995) using a spectrophotometer (UV-2101PC, Shimadzu, Kyoto, Japan).

2.1.2.3 Vine productivity and vineyard information

The yields and pruning weights were determined from the vineyards by weighing the grapes and prunings from every five-vine plot. The growers or managers were asked to fill out a questionnaire with vineyard details, management and reason(s) for their harvest decision. Additional soil information was obtained from each vineyard taking soil samples at 30 and 60cm for the determination of soil structure by using a soil kit (LaMotte, Extech Equipment Pty. Ltd, Boronia, Vic 3155, Australia).

2.1.2.4 Data analysis and weather data

The data were compiled and manipulated in the data base Excel 97 and further analysed using the graphic program Sigma plot 2001 (version 7.0). Weather data was obtained from CSIRO Land and Water at Griffith.

2.1.3 Results and discussion

2.1.3.1 Management information and vineyard performance

The vineyards were mainly on relatively light soils (sandy loam, sandy clay loam, sandy clay). The Shiraz vines were around 10 years or younger, only S3 was significantly older (Table 1.1). The planting spacing of most vineyards was 1.8 x 3.6m and vines were trained on one or two wires. The clones were PT23, ESA, BURC30 or unknown. The management of vineyards (Table 1.2) varied in regard to irrigation (system, amount) and nitrogen application (amount, timing). In the first season the harvest decision was mainly dictated by berry ripeness and the winery schedule, while in the second year it was the weather and the winery. Rainfall in the region reached 89mm over the harvest period with the majority falling during the Shiraz harvest. Generally the harvest date was similar in both years, except for vineyards S3 and S15.

Table 1.1 Location and vineyard characteristics of the Riverina Survey.

Site	Area	Soil Type*	Clone	Age of Vines	Irrigation	Trellis wires	Spacing (m) vine x row	Cover crop	Pruning	Harvest method
S3	Yoogali	Scl/Sc	?	38	Furrow	2	1.8 x 3.6	no	Hand	Hand
S5	Yoogali	Scl/Scl	?	7	Furrow	1	1.8 x 3.6	no	Hand	Mech.
S6	Hanwood	Scl/Sscl	PT23	9	Flood	1	1.5 x 3.6	yes	Mech.	Mech.
S7	Hanwood	Cl/Scl	ESA	5	Furrow	1	1.8 x 3.6	no	Hand	Mech.
S9	Hanwood	Scl/Sc	ESA	10	Furrow	2	1.8 x 3.6	no	Mech.	Mech.
S10	Stanbridge	Scl-Sl/	ESA	11	Furrow	1	2.4 x 3.6	no	Mech.	Mech.
S11	Leeton	Sl/Scl	PT23	7	Drip	1	1.8 x 3.3	yes	Mech.	Mech.
S12	Wamoon	Sl/L	PT23	5	Furrow	1	1.8 x 3.3	no	Hand	Mech.
S13	Leeton	Sl/Sc	BURC30	6	Drip	1	1.8 x 3.6	yes	Mech.	Mech.
S15	Widgelli	Sl/Scl	?	4	Furrow	2	1.8 x 3.6	no	Hand	Mech.

* 30/60cm soil sample, Sc = Sandy clay, Scl = Sandy clay loam, Sl Sandy loam, Cl = Clay loam, Sscl = Sandy sandy clay loam

There were significant differences in berry size, Brix, pH, TA and colour between the 10 properties and between the two seasons (Table 1.3). In the first season (1998/99) the veraison date, the time to reach a particular TSS level during berry ripening and the harvest date differed between vineyards. The harvest period stretched over one month and the grower decisions to take the crop off were not solely based on berry sugar levels. Berry TSS levels measured varied from 20.8 to 24.7º Brix across the vineyards, with two vineyards containing grapes that did not ripen properly. Berry colour differed

considerably between vineyards (0.44 to 0.99mg/g FW) as did berry weight (0.83g to 1.37g). The smaller berries generally had the best colour. Juice pH ranged from 3.57 to 4.24 and TA from 2.68 to 4.89g/L. The highest value for TA was in vineyard S15, which also had the lowest brix level. The yield varied three fold (12.4t/ha to 27.6t/ha), but the variation in pruning weight was less pronounced (1.4 to 3.2t/ha).

Table 1.2 Water and N application rates, harvest date and reason for harvesting as part of two-year Riverina survey

Site	1998/1999				1999/2000			
	ML/ha	Kg N/Ha	Harvest date	Decision*	ML/ha	Kg N/Ha	Harvest date	Decision
S3	6.0	55.2	26-Feb	O/B	?	0	8-Mar	Wi/We
S5	?	21.0	7-Mar	B	?	67	14-Mar	Wi
S6	8.5	127.5	16-Mar	Wi/B	6.0	0	20-Mar	We
S7	6.0	103.5	8-Mar	Wi/B	?	23	12-Mar	Wi
S9	?	32.2	5-Mar	Wi	?	0	6-Mar	B
S10	?	36.0	17-Mar	?	2.0	22	14-Mar	We
S11	2.5	68.0	18-Mar	Wi/B/We/Pi	1.5	51	22-Mar	We
S12	?	211.3	25-Mar	O	7.5	135	22-Mar	?
S13	1.0	18.7	5-Mar	Wi	?	34	3-Mar	?
S15	5.0	130.0	8-Mar	O/We	?	130	20-Mar	Wi

* B = Brix, O = own, Pi = pickers, Wi = winery, We = weather

Table 1.3 Riverina Shiraz vineyard productivity and berry composition during the 1998/99 season (standard errors of the means are shown in small type).

Site	Brix	TA g/L	pH	Berry wt. g	colour mg/Fw	Yield T/ha	Pruning wt. T/ha
S3	24.7	3.57	3.57	1.29	0.99	14.8	3.0
	0.42	0.10	0.10	0.03	0.05	1.21	0.18
S5	22.4	3.13	3.97	1.37	0.64	17.8	1.7
	0.14	0.08	0.01	0.03	0.02	0.45	0.09
S6	23.7	2.89	4.24	1.30	0.66	27.6	2.7
	0.32	0.08	0.03	0.05	0.05	1.91	0.13
S7	22.6	3.14	4.01	1.24	0.61	19.1	2.0
	0.25	0.10	0.03	0.03	0.03	0.66	0.11
S9	22.8	2.80	4.15	1.02	0.80	25.3	2.3
	0.20	0.06	0.02	0.02	0.03	2.79	0.22
S10	24.6	2.79	4.22	0.83	0.79	12.4	1.4
	0.27	0.07	0.04	0.04	0.04	1.32	0.15
S11	23.5	3.13	4.19	1.27	0.49	27.3	3.2
	0.46	0.16	0.04	0.03	0.06	1.48	0.17
S12	24.4	4.02	4.08	1.03	0.92	23.1	2.4
	0.31	0.28	0.03	0.03	0.03	1.35	0.11
S13	23.7	2.68	4.19	1.01	0.75	16.7	2.0
	0.18	0.11	0.04	0.03	0.03	0.80	0.11
S15	20.8	4.89	3.71	1.32	0.44	18.8	2.7
	0.25	0.11	0.02	0.03	0.06	0.66	0.11

Near the end of the second ripening season (1999/2000) many vineyard managers left the grapes on the vines to increase brix levels. This often resulted in Botrytis entering and making the harvest decision one based on minimising disease spread rather than by brix. The brix levels were generally

lower then in the year before (Table 1.4), ranging from 18.7 to 24º. This may be because in a number of vineyards brix levels did increase further after a certain point in the ripening period. For example S6 reached its peak brix in mid February but was not harvested for another 4 weeks. Colour levels were generally higher in most vineyards in the second season. Of all the vineyards the highest reading was 1.18mg/g FW and the lowest was 0.48mg/g FW. Berry size was not a factor leading to the more intense colours because berries were larger (0.94 to 1.42g) than the previous year. The TA levels were similar to the previous year from 2.60 to 4.69 and the juice pH tends to be higher in the second year ranging from 3.90 to 4.30. Yields were more variable then in the first year with the range being between 8.7-27.6t/ha. S5 and S10 were particularly low yielding vineyards. The yield of a particular vineyard went either up or down compared to the previous year. Pruning weights were much higher in the second year, ranging from 2.6 to 4.4t/ha.

Table 1.4 Riverina Shiraz vineyard productivity and berry composition during the 1998/99 season (standard errors of the means are shown in small type).

Site	Brix	TA g/L	pH	Berry wt. g	colour mg/Fw	Yield T/ha	Pruning wt. T/ha
S3	22.28	3.77	3.99	1.11	1.01	20.91	2.81
	0.09	0.04	0.01	0.02	0.03	0.89	0.15
S5	21.71	3.74	4.08	1.42	0.76	12.52	2.62
	0.12	0.07	0.01	0.04	0.02	0.45	0.11
S6	21.11	3.80	3.91	1.44	0.95	20.73	3.53
	0.16	0.19	0.03	0.07	0.05	1.48	0.20
S7	23.43	3.80	3.90	1.08	1.00	22.00	2.95
	0.18	0.06	0.02	0.04	0.03	1.22	0.14
S9	23.68	3.90	4.16	1.26	1.07	18.94	3.07
	0.10	0.08	0.02	0.03	0.03	2.18	0.32
S10	22.35	2.60	4.15	1.22	1.02	8.74	2.31
	0.28	0.10	0.03	0.07	0.04	1.04	0.22
S11	21.33	3.78	4.00	1.50	0.86	27.58	3.32
	0.19	0.07	0.02	0.06	0.04	0.89	0.11
S12	21.43	3.57	4.02	1.12	1.18	27.62	3.71
	0.11	0.05	0.01	0.02	0.03	1.34	0.14
S13	23.97	3.24	4.30	0.94	1.10	18.91	2.66
	0.28	0.06	0.02	0.03	0.03	1.19	0.11
S15	18.74	4.69	3.89	1.41	0.48	20.31	4.40
	0.27	0.13	0.01	0.03	0.03	1.22	0.15

Generally higher yields lead to lower berry TSS and colour, however these correlations were not very strong and even less pronounced in the second season. The relationship between colour and TSS was much stronger; as has been found by others (Pirie & Mullins 1980). These findings indicate that high yielding vineyards can mature grapes properly and can have good berry colour, and that low yielding vineyards do not necessarily produce good mature grapes or high berry colour. In fact vineyards yielding in the medium range had some of highest berry colour levels in the first season, and in the second year one of the highest yielding vineyards (S12) had the highest colour. This indicates that the potential exists to improve vineyard yield, sugar and colour levels simultaneously and that the yield/quality trade-off is not always necessary. These data also indicate that the optimum grape quantity and quality relationship differs between vineyards. It has been suggested in the past that an equilibrium of crop load versus vegetative development is important for quality fruit (Eynard & Gay 1992). Each vineyard must find this equilibrium point so that both yield and quality can be maximised. Additional information presented in the following sections on vineyard management, vine balance and vineyard environment will provide some insight into reasons for the variability in berry colour and sugar levels as well as yield.

2.1.3.2 Yield components and berry quality under contrasting vineyard management

Management practises across the vineyards were diverse. In particular, there was large variation in soil management including fertilisation regime, cover crop selection, irrigation method (drip, furrow,

flood) and amounts of water applied. The last two factors had a significant effect on vineyard performance. Five vineyards were distinctly different in the amount of water applied and the type of irrigation system in place. By plotting yield per vine in the second season against the pruning weights of the first season, it was observed that higher yields correlated with higher pruning weights (Figure 2.1). A vineyard with low furrow irrigation was at the lower end of the productivity scale, whereas a high furrow irrigated vineyard was at the higher end. Other vineyards lay in between. Therefore, in this population of vineyards, high water application rates lead to vigorous vegetative growth in the first season. This resulted in high yields in the following season. Perhaps there were ample reserves to draw from in these vigorous vineyards. A similar relationship was evident by plotting pruning weight of the first season against sugar per vine of the second season (Figure 2.2). High water application lead to vigorous vegetative growth and this also resulted in high sugar levels per vine.
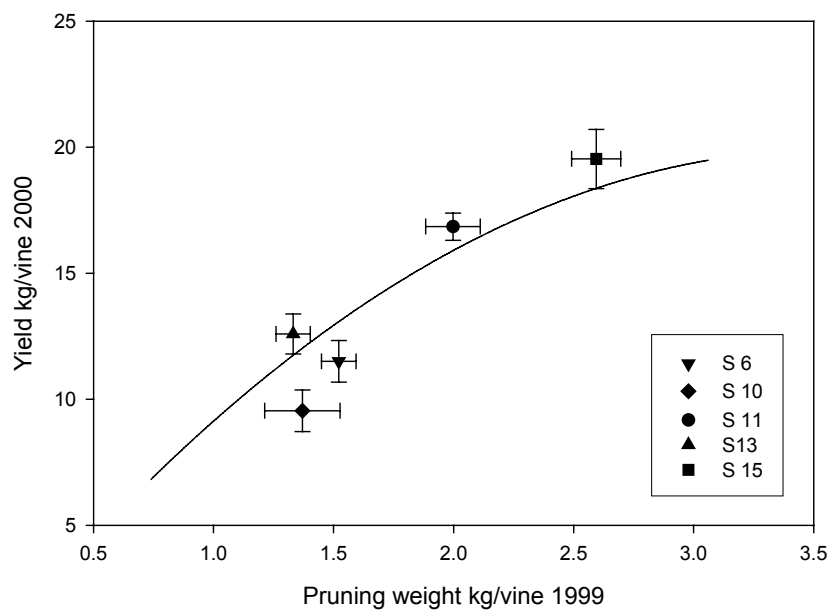
Figure 2.1 Yield in season 2 as a function of pruning weight of the prior season for vineyards differing in irrigation strategies.

The relationship between yield and total soluble solids (measured in brix) was as has been reported numerous times in the literature. Yield was highest in the high water application (furrow) vineyards and low in the conservative drip and meagre furrow irrigated vineyards. As yield increased Brix levels decreased (Figure 2.3). Berry colour (measured as total anthocyanins) was similarly affected by yield (Figure 2.4). There was a strong relationship between brix and colour as described by a number of researchers.

Other berry parameters such as berry size, TA, and pH were also affected by irrigation (data not presented). The results indicate that irrigation management and system can have significant impact on vine productivity, its internal relationships and on berry composition in a hot irrigated wine region.
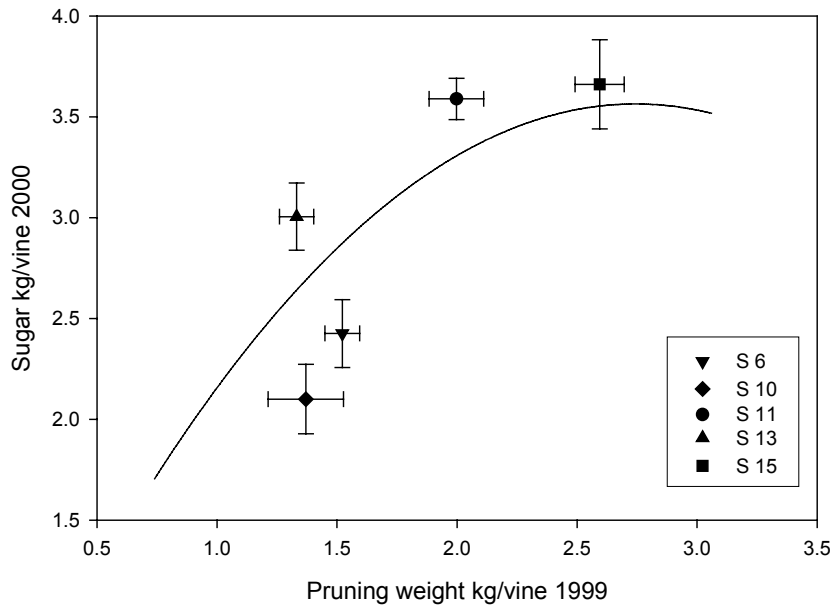
Figure 2.2 Sugar per vine in season 2 as a function of pruning weight of the prior season for vineyards differing in irrigation strategies.
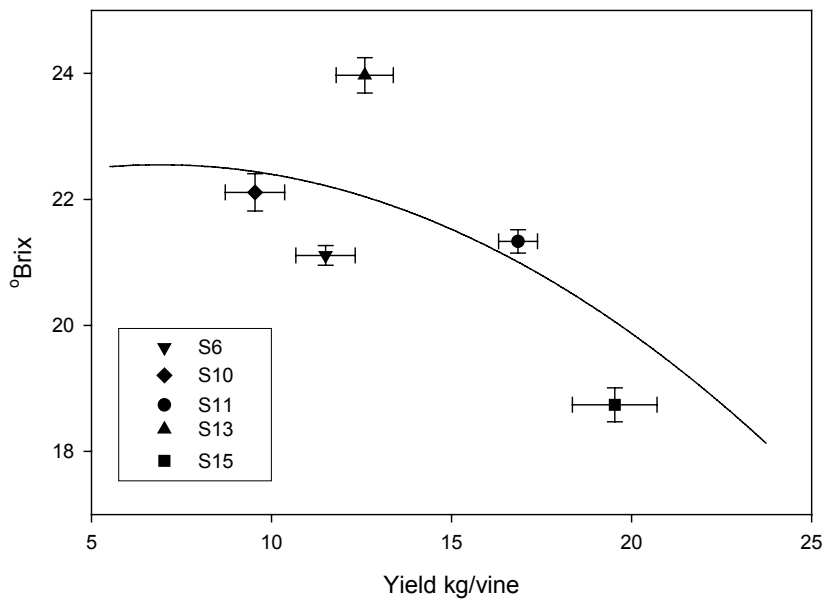
Figure 2.3 Total soluble solids (TSS) versus yield at harvest in the second season (1999/2000).
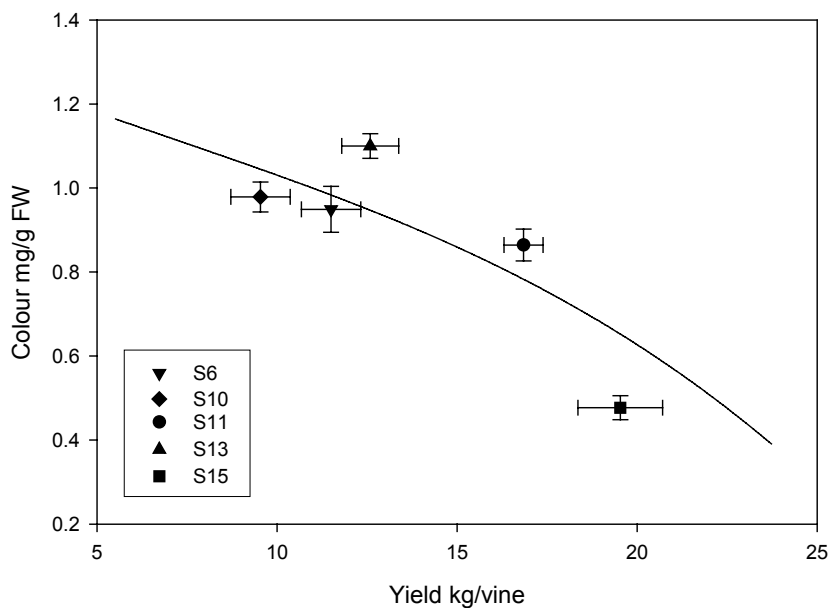
Figure 2.4 Berry colour versus yield at harvest in the second season (1999/2000).

2.1.3.3 Sources of variation in yield components under either flood or furrow irrigation

Another group of vineyards behaved differently however (Figure 3.1). In this population, yield varied widely even though the pruning weights of the previous season were very similar. The variation in yield was due to bunch numbers per vine, whereas berries per bunch and berry weight played a minor role (Table 3.1). The higher number of bunches per vine (or metre of cordon) was due to higher shoot numbers per vine and more bunches per shoot. The highest shoot number appears to be in the medium yielding vineyards (S7, S 9, S6), which also have less growth in spring. These results suggest a significant shift in vine balance towards grape production is possible without a need for vigorous canopies. The high yielding vineyards also had berries with high colour levels indicating that the yield/quality trade off is not always necessary.
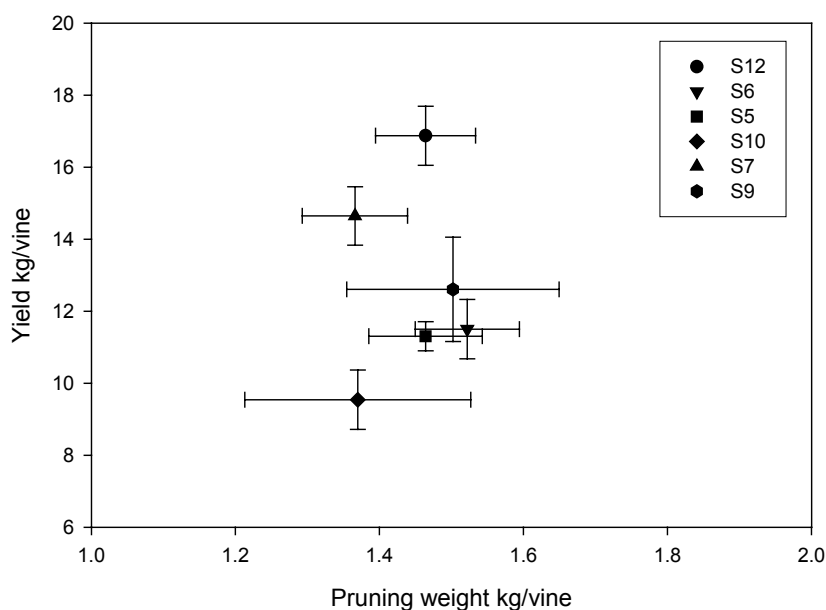
Figure 3.1 Yield in the second season (1999/2000) versus pruning weight from the first season (1998/99).

Table 3.1 Yield components, shoot numbers, pruning weight and production ratio in six Shiraz vineyards (standard errors of the means are shown in small type).

Site	Bunches/ m	Berries/ bunch	Bunch wt. g	Berry wt. g	Yield kg/m	Shoots/ m	Bunch/ Shoot	[1)]Pruning wt. kg/m	[2)]Production Ratio
S12	90.43	103.12	116.29	1.12	9.25	70.94	1.37	1.31	7.04
	8.61	14.70	17.87	0.02	0.45	4.51	0.19	0.06	0.15
S7	97.64	81.86	87.35	1.08	8.03	78.71	1.29	1.12	7.23
	8.18	7.94	8.25	0.04	0.44	6.03	0.13	0.06	0.39
S9	76.80	83.59	106.27	1.26	6.91	88.67	1.00	1.24	5.54
	12.70	10.89	14.69	0.03	0.80	7.62	0.28	0.12	0.27
S6	73.92	74.95	108.90	1.44	7.57	73.30	1.04	1.50	4.99
	6.54	6.44	12.40	0.07	0.54	6.39	0.10	0.07	0.24
S5	56.30	81.34	117.59	1.42	6.20	53.52	1.06	0.91	7.00
	3.80	6.95	12.62	0.04	0.22	2.92	0.06	0.05	0.39
S10	36.94	83.98	105.47	1.22	3.47	54.92	0.71	0.76	4.87
	3.99	11.41	16.78	0.07	0.41	5.66	0.07	0.08	0.58

[1)]pruning weight from year 1 (1998/99); [2)]yield fom year 2 (1999/2000)/pruning weight from year 1 (1998/99)

Yield did not have a clear affect on berry colour (Figure 3.2), although the vineyard with the highest berry colour had also the highest yield. This vineyard had also the second lowest brix levels. The medium yielding vineyards S7 and S9 had the highest brix levels. Berry TA ranged from 2.6 to 3.9g/L where vineyard S10 was by far the lowest with 2.6g/L. Juice pH was relatively high in all vineyards (3.9 and 4.16) (data not presented).
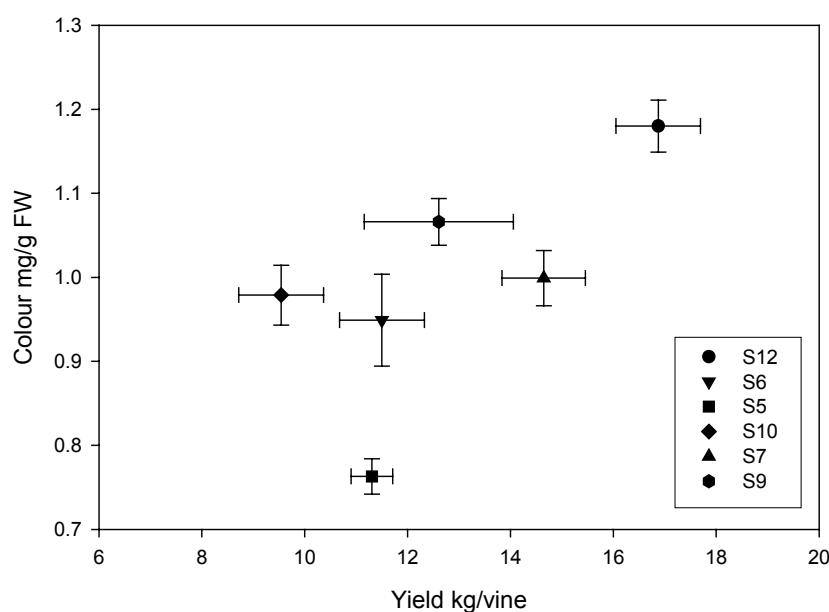
Figure 3.2 Berry colour versus yield at harvest in the second season (1999/2000).

These investigations suggest a significant shift in vine balance towards grape production is possible without necessarily impacting on grape quality. Vineyard management towards optimum bunches per shoot and shoots per vine is crucial for sustainable grape production.

2.1.3.4 Vineyards with low and high variation

Vineyards can differ strongly in the amount of variation there is within that particular vineyard. Yield in vineyard S5 ranged between 9 and 14kg/vine and pruning weight ranged from 1.0 to 1.8kg/vine (Figure 4.1). The variability in vineyard S9 was much larger. Yield ranged from 6 to 22kg/vine and pruning weight ranged from 1.0 to 2.6kg/vine. The higher yielding plots of the vineyard S5 did not result in lower berry colour levels, and brix levels were only slightly lowered (Figures 4.2 and 4.3). This again indicates that good composition and high yields can be achieved simultaneously. The variability in the brix and colour relationship was slightly less in vineyard S5. Despite the large differences in yield and pruning weight in vineyard S9, the variation in berry colour and brix was quite similar to vineyard S5 (Figure 4.4).
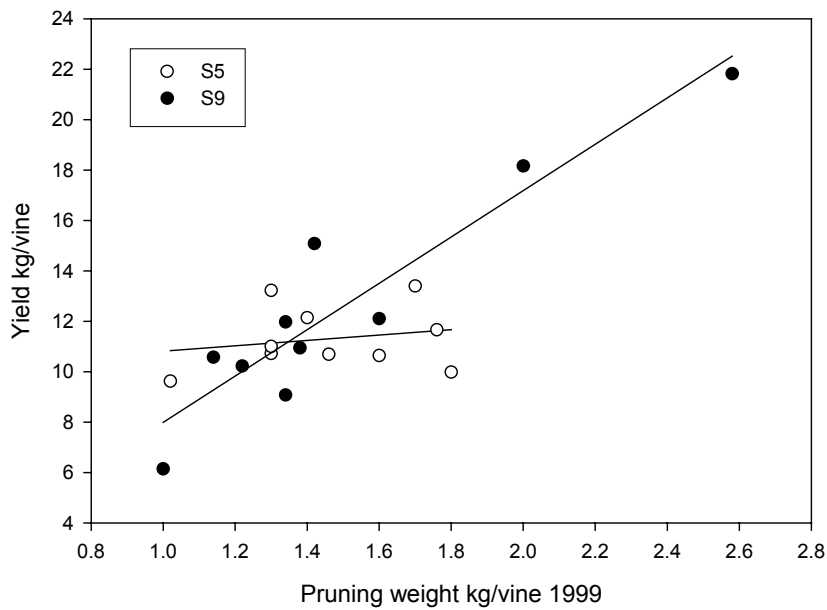
Figure 4.1 Yield in the second season (1999/2000) versus pruning weight from the first season (1998/99) in a low and high variable vineyard.
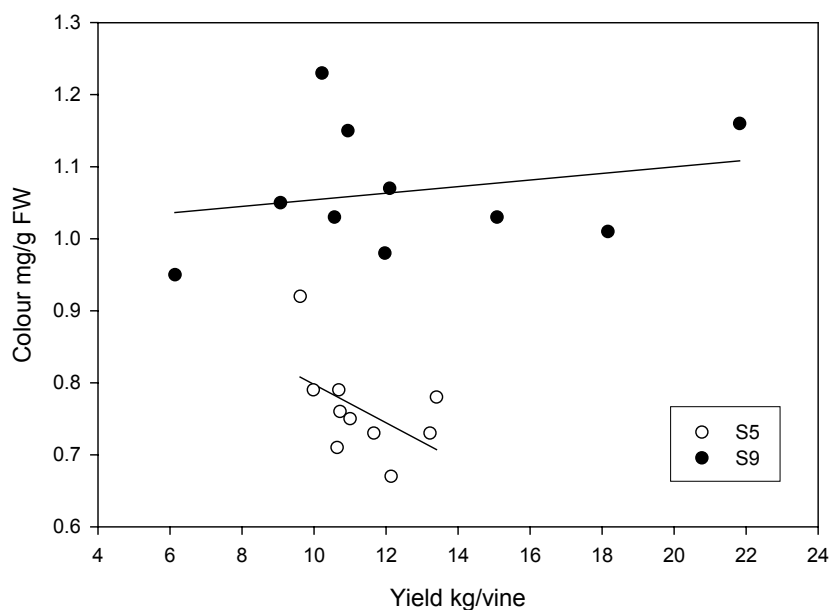
Figure 4.2 Berry colour versus yield at harvest in the second season (1999/2000).

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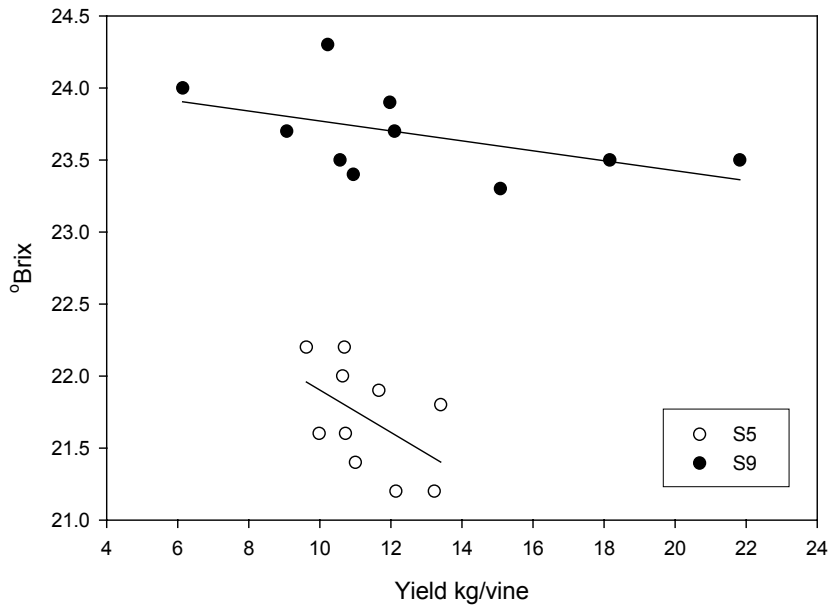
Figure 4.3 Total soluble solids (TSS) versus yield at harvest in the second season (1999/2000).
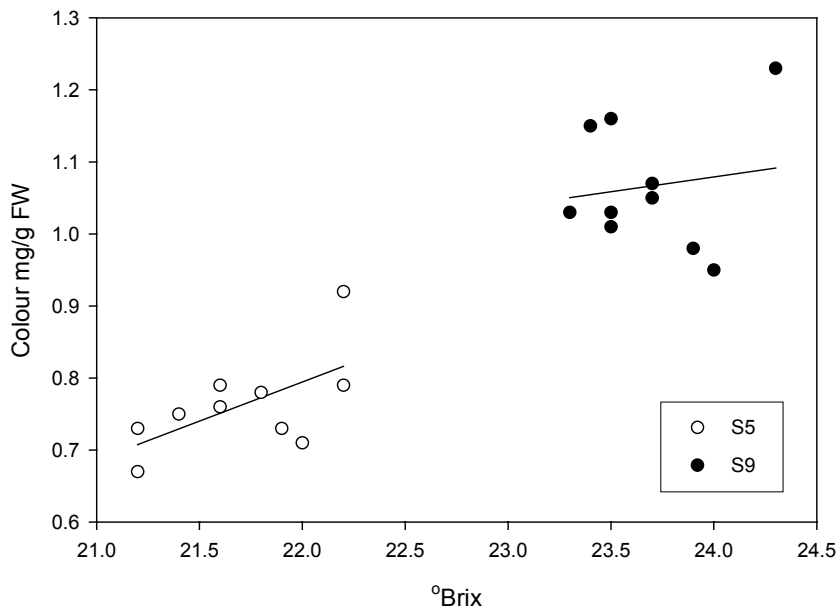
Figure 4.4 Berry colour versus brix at harvest in the second season (1999/2000).

An EM survey showed a similar degree of variation between the two vineyards and could not explain the large variation of vineyard S9 (Figure 4.5, left). The aerial image conducted before harvest 2002 revealed that the vines in S5 were very even, were vineyard S9 showed the large differences observed in yield and pruning weights were imminent (Figure 4.5, right). The variation was therefore most likely due to poor vineyard establishment rather than soil differences.

Figure 4.5 Aerial image of a low (S5) and high variable vineyard (S9) in 2002; false colour image (left) and EM survey (right).

SITE S5
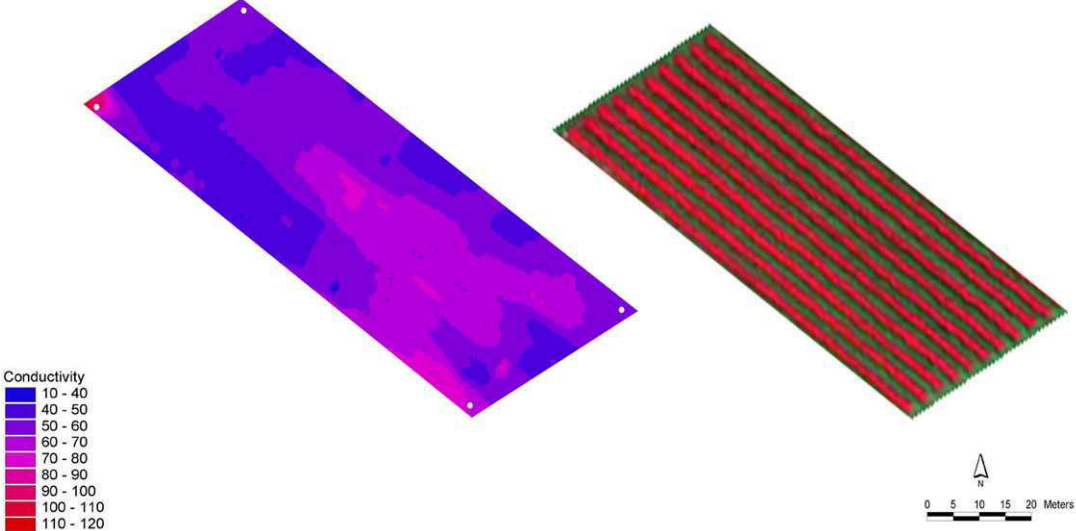
SITE S9
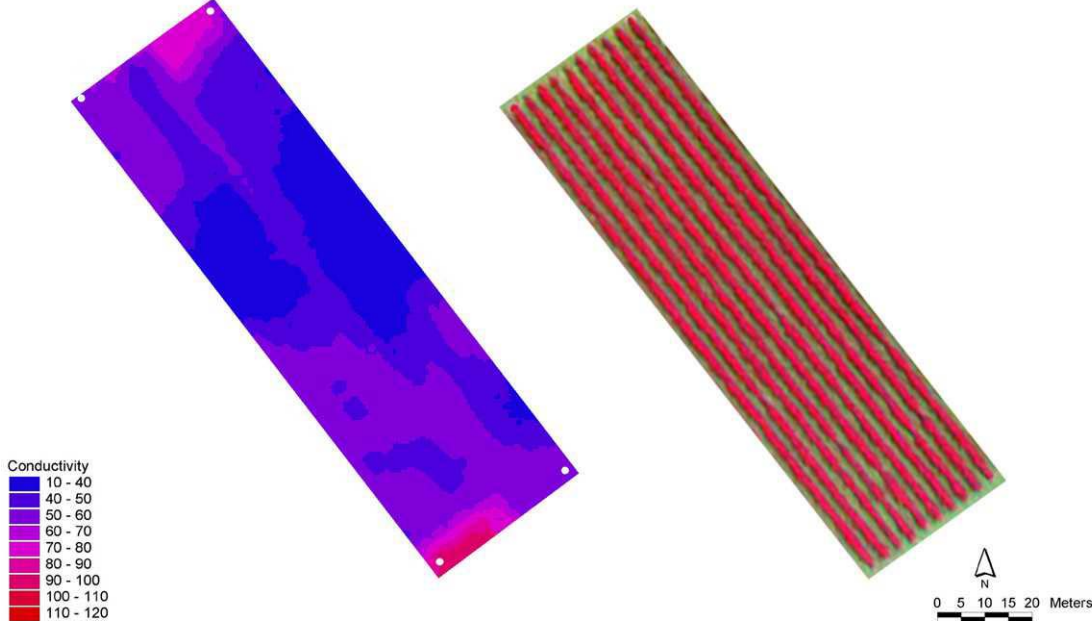
2.1.3.5 Vineyard environment and berry ripening

Climatic conditions during berry ripening varied between seasons. January 1999 was very hot with 7 days where the daily maximum was in excess of 40ºC and no daily maximums dipping below 30ºC (Figure 5.1). The mean daily temperature was higher in January of 1999 by 5.6ºC than January of 2000. This resulted in 100 more cumulative heat units by the end of the ripening period in 1999 than 2000. During January of 1999 there were 4 significant rain events, but in January 2000 there was only one (Figure 5.1). During the remainder of the ripening period very little rain fell in 1999 until the very end of ripening, but there were 4 large rain events in 2000.
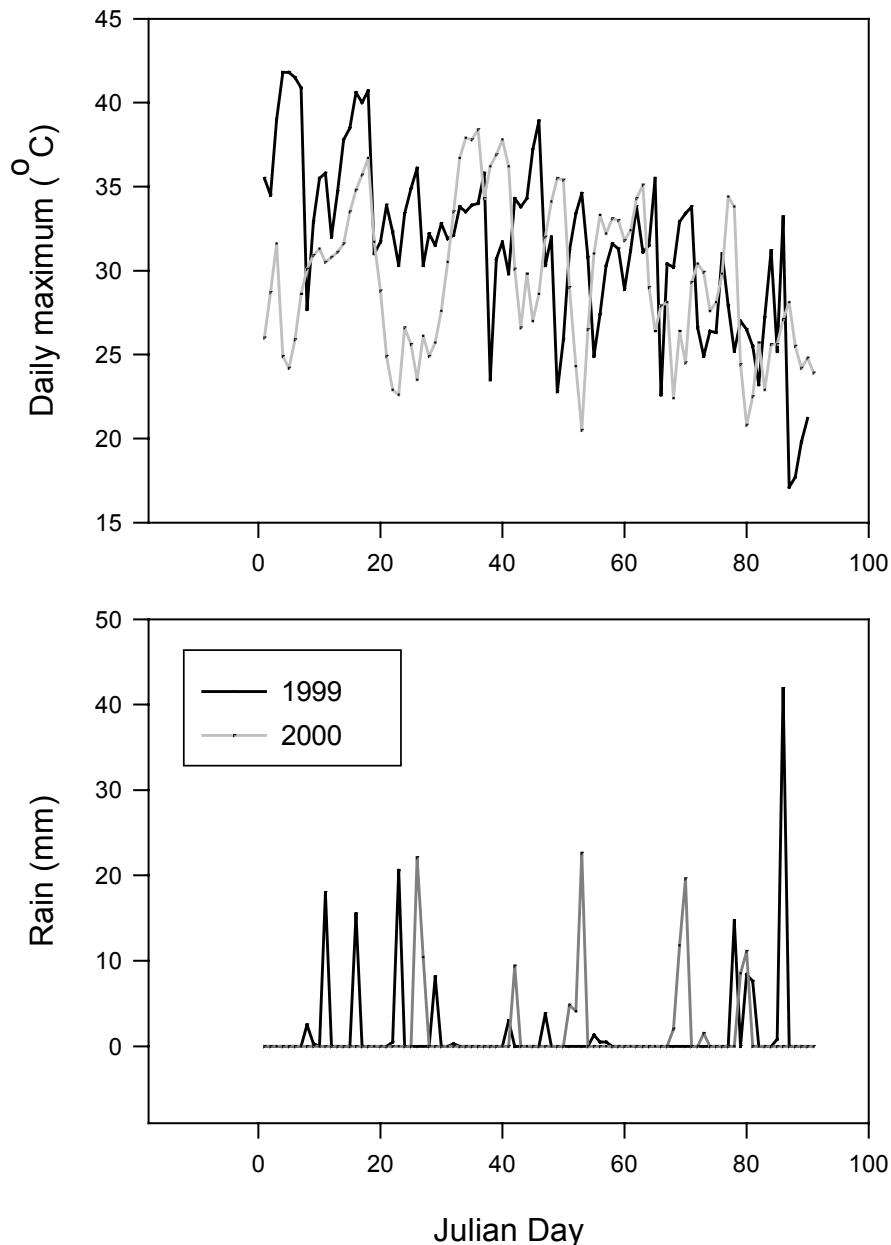
Figure 5.1 Daily maximum temperature and rain during the 1999 and 2000 seasons.

Berry fresh weight increased, peaked and then sometimes declined during ripening (Figure 5.2). Season did not have a consistent affect within a vineyard (ie in some vineyards maximum and final berry size were larger in 1999 while in other vineyards they were larger in 2000). Maximum berry

weight ranged from 1.00 (vineyard 10, cautious flood irrigation) to 1.54 g (vineyard 5, furrow irrigation) in 1999 and 1.06 (vineyard 13, cautious drip irrigation) to 1.65 (vineyard 11, excessive drip irrigation) in 2000. The timing of the maximum ranged from 34 to 41 Julian days in 1999 and 26 to 61 Julian days in 2000. It is uncertain what causes this variation in the timing of the volume maximum but it may be related to variation in flowering. Final berry weight ranged from 1.01 to 1.37 in 1999 and 0.99 to 1.50g in 2000 and was dependent on when the crop was harvested. Berry dry weight increased until 55 Julian days in 2000 and then plateaud at 0.33 g.

In 2000, once weight loss did set in, the downward trend was not always consistent, and there was weight gain again in vineyards S3, S7, S9, S10, and S13 at 47 and/or 54 Julian days in 2000. This may have been due to rain events that occurred shortly before the sampling occasion. This gain in weight, after the downward trend had set in was not evident in the 1999 season except for vineyard 12 and this may be because there were no rain events above 4 mm during this period in this year. Vineyard 12 was the only vineyard that had not been harvested before 78 Julian days when 31 mm fell over 4 days. Weight had increased by 0.10 g the subsequent sampling date (84 Julian days). A sudden drop in weight at the very end of the season, such as in vineyard S10 in 2000 was attributed to bee damage.
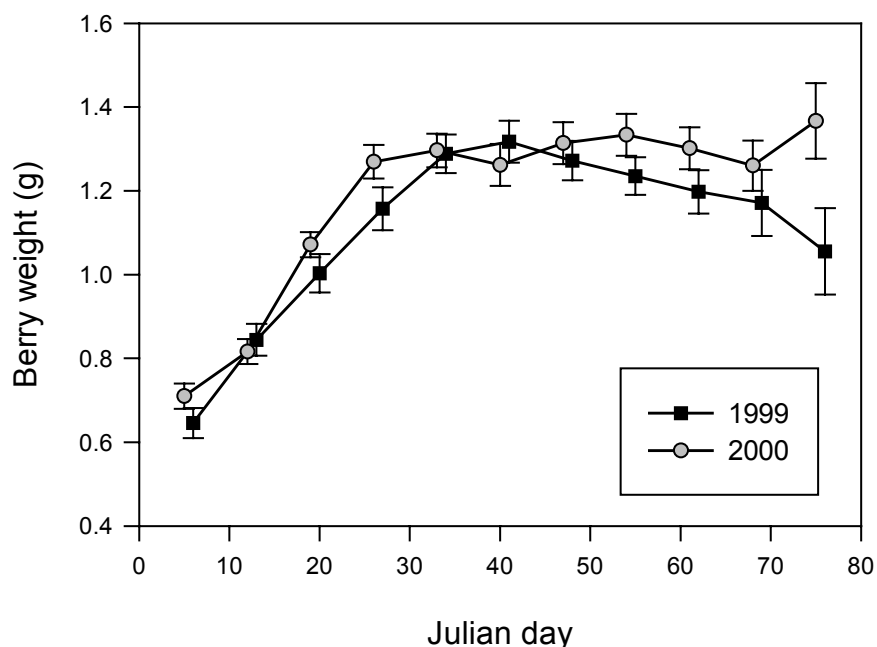
Figure 5.2 Berry weight averaged for all vineyards during 1999 and 2000.

At the start of sampling in early January, Brix ranged between 5 and 7 across the twelve vineyards in both seasons (Figure 5.3). Final Brix was dependent on when the berries were harvested and ranged from 20 to 25 in 1999 and 18 to 24 in 2000. Though accumulation of Brix was faster in early 2000, final Brix was higher by 1° at 70 Julian days in 1999. Sugar levels in the berry at harvest ranged from 200 to 300 mg berry^{-1} in 1999 and from 220 to 320 mg berry^{-1} in 2000. On average, sugar levels were equal at 70 Julian days between the two seasons.

In the early ripening period, sugar per berry increased at a faster rate in 2000 as compared to 1999 (Figure 5.4). This rapid rate of increase in 2000 was lessened after 30 Julian days.

Very high temperatures (above 35ºC) during the early ripening period (up to February 1, ca 250 mg sugar berry^{-1}) had an effect on sugar accumulation rates. However, in the 25 to 35ºC maximum range temperature did not have an effect on sugar accumulation rates during the early ripening period. Similarly, during the middle and late ripening phases (beyond February 1, or 250 mg sugar berry^{-1})

maximum temperatures in the 25 to 35ºC range did not have an effect on sugar accumulation rates. Other possibly intrinsic factors must be contributing to the steady decline in sugar accumulation rates through the mid- and late ripening phases.
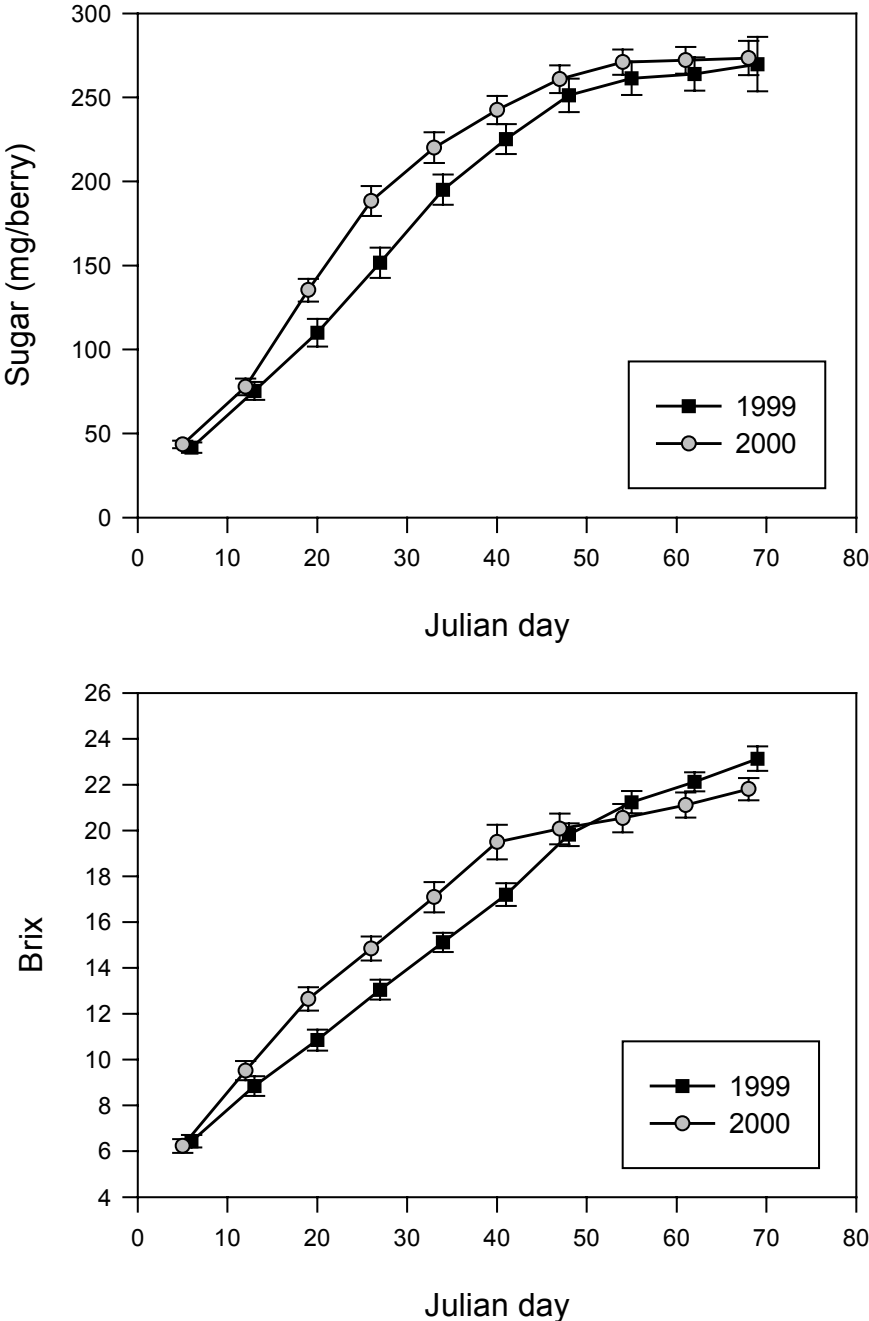

Figure 5.3 Sugar and Brix of berries averaged across all vineyards during 1999 and 2000.

The rate of sugar accumulation dropped to less than 0 beyond 60 Julian days in 2000. It is not until this point that the increase in brix was solely due to concentration of existing sugars rather than a combination of sugar concentration upon shrinkage and sugar import from the vine. In 1999, the sugar accumulation rate did not reach negative values. Therefore, the increase in brix subsequent to the weight maximum was not solely due to a concentrating effect.

Sugar accumulation rates ranged from 31 to 48 mg berry[-1] week[-1] prior to the weight maximum in 1999. Subsequent to the weight maximum the rates ranged from 4 to 26 mg berry[-1] week[-1]. Therefore the rates were much higher in the first part of the ripening season. Sugar accumulation and brix increase rates after the weight maximum were not correlated to sugar accumulation and brix increase rates prior to the weight maximum. Sugar accumulation varied between vineyards in both seasons (Figure 5.5). In the first season vineyard S3 had about double sugar accumulation than vineyard S8 until mid ripening. Another example from the second season, is the high accumulation rates before mid ripening of vineyard S5 and sugar loss close to harvest compared to vineyard S7 not having this pronounced high and negative accumulation rates.

In both 1999 and 2000, berry colour concentration increased and plateaud, and total content per berry increased, plateaud and then declined (Figure 5.6). The decline is not apparent on a fresh weight basis because at this stage of ripening the berries are shrinking and the anthocyanins are concentrating. Final colour on a per berry and fresh weight basis was higher in 2000 compared to 1999 for all vineyards. Colour at 70 Julian days ranged from 0.4 to 1.0 and averaged at 0.6 in 1999, and ranged from 0.5 to 1.2 and averaged at 0.9 mg g fwt[-1] in 2000. The higher values of the 2000 harvest may be due to the faster accumulation rates during the early ripening period of 2000.
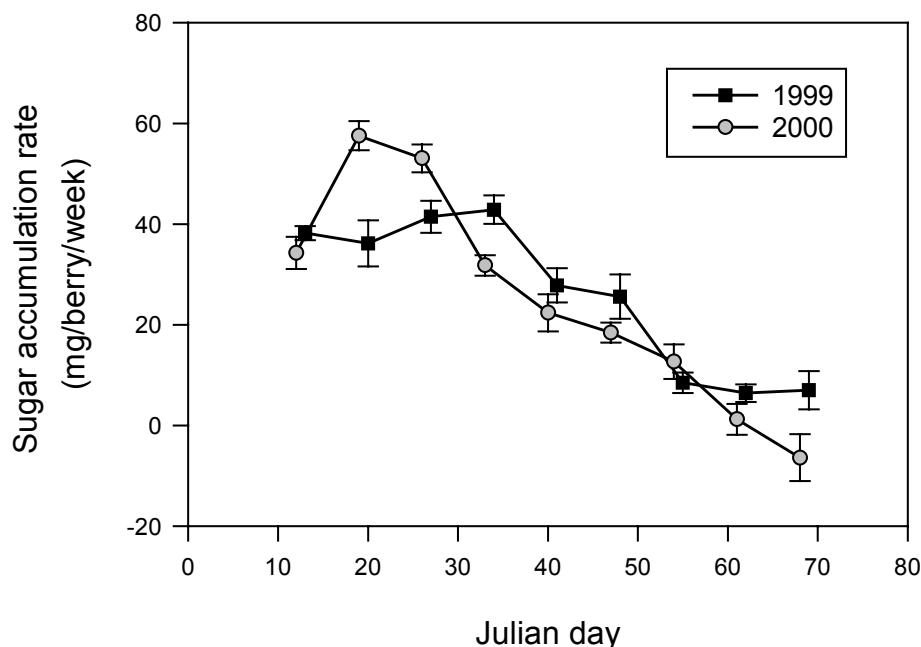

Figure 5.4 Sugar accumulation rates during 1999 and 2000.

Anthocyanin accumulation rates during the earlier phase of ripening were season dependent and temperatures in excess of 34 °C were inhibitory. Radiation was not a limiting factor in either season.

The colour maximum intensities differed by 35% upon the onset of anthocyanin degradation. Berry sugar levels at the onset of degradation, however, were similar between the two seasons, as was the calendar date (Figure 5.7). When we examined the relationship between berry sugar concentration (Brix) and colour concentration (on a fresh weight basis) during late ripening two different patterns emerged. In late ripening of 1999, the colour concentration remained stable while the Brix continued to increase. In 2000, the colour concentration remained fairly stable but the Brix did not continue to increase to the same extent as in 1999. Berry shrinkage during late ripening was more severe in 1999 and this would contribute to the more extensive Brix increase in 1999. Due to this shrinkage the anthocyanin concentrations remained stable even though anthocyanin degradation on a per berry basis occurred.
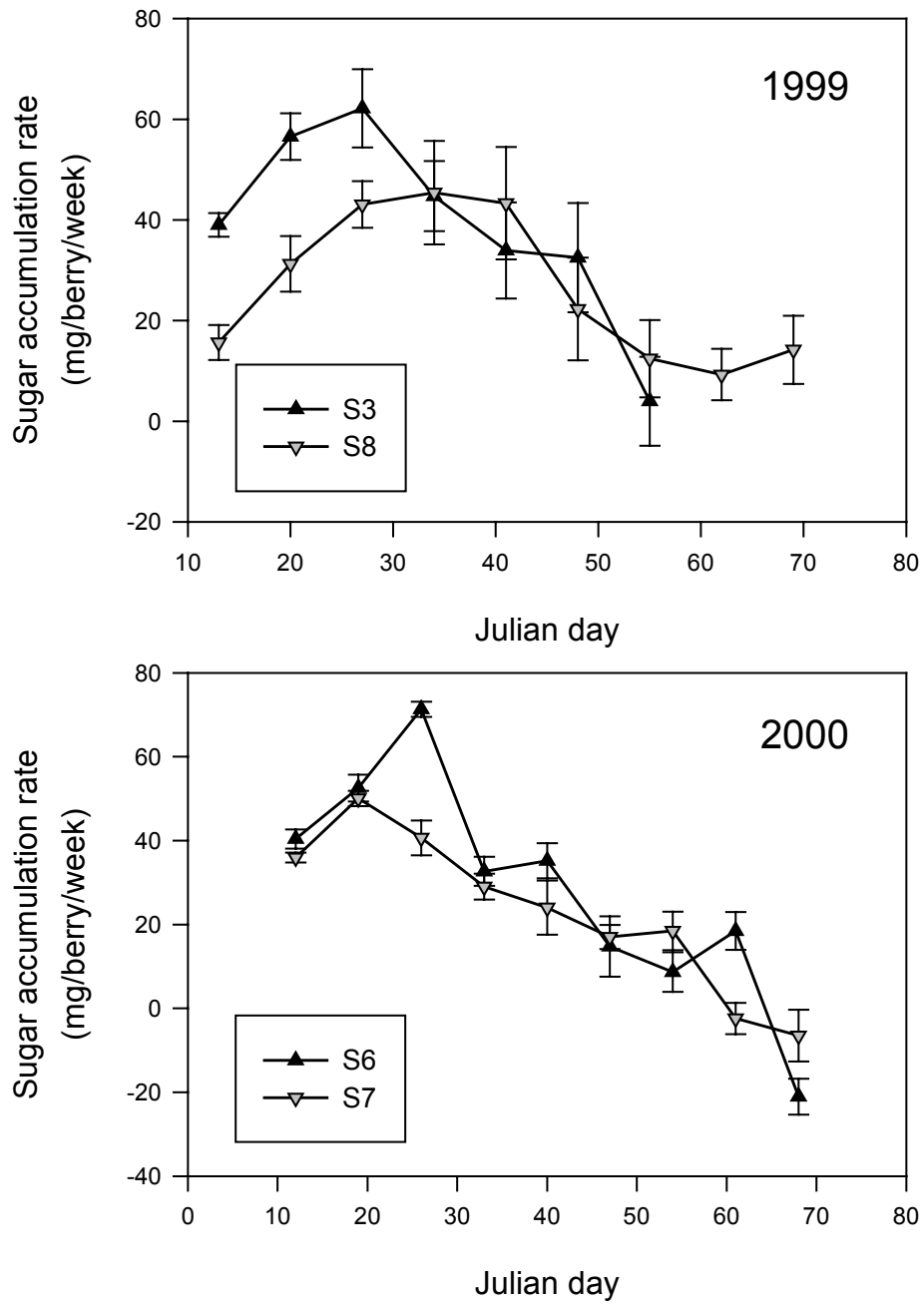
Figure 5.5 Two vineyard extremes in sugar accumulation rates during 1999 and 2000.
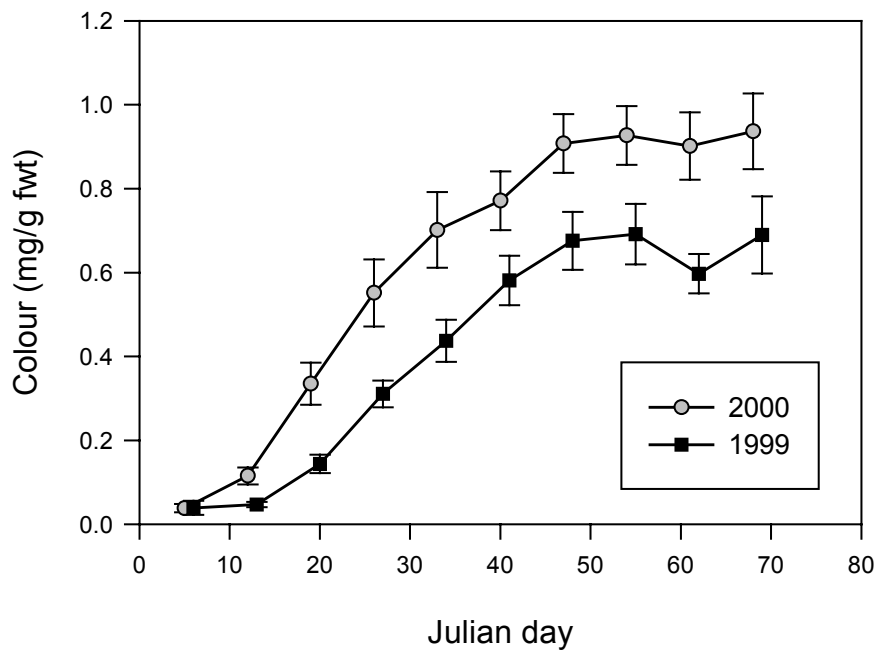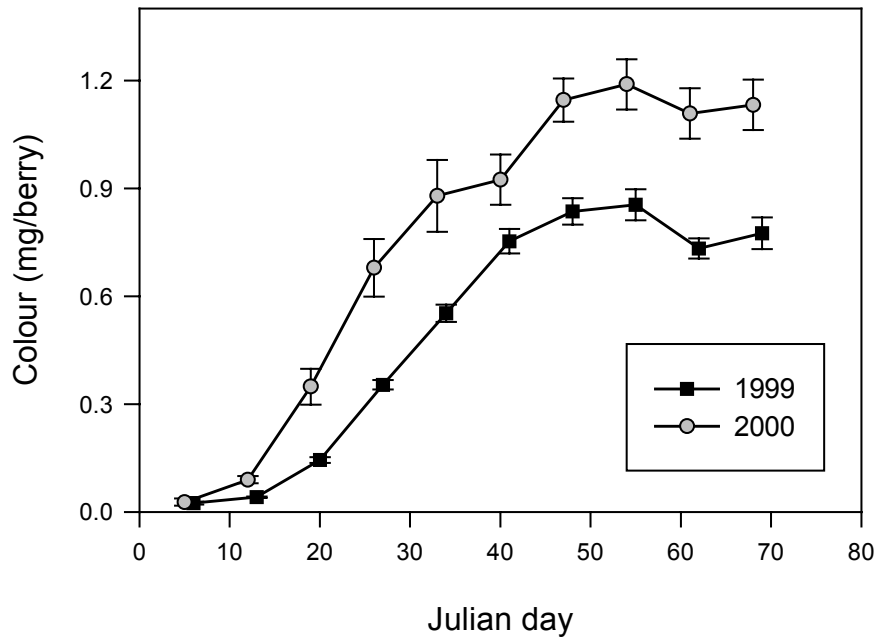
Figure 5.6 Berry colour development during 1999 and 2000 ripening period.
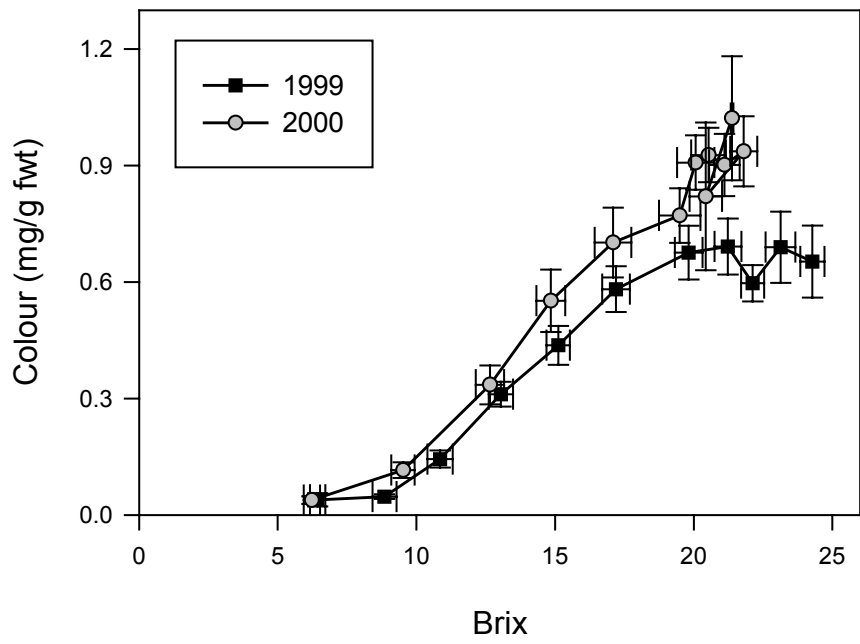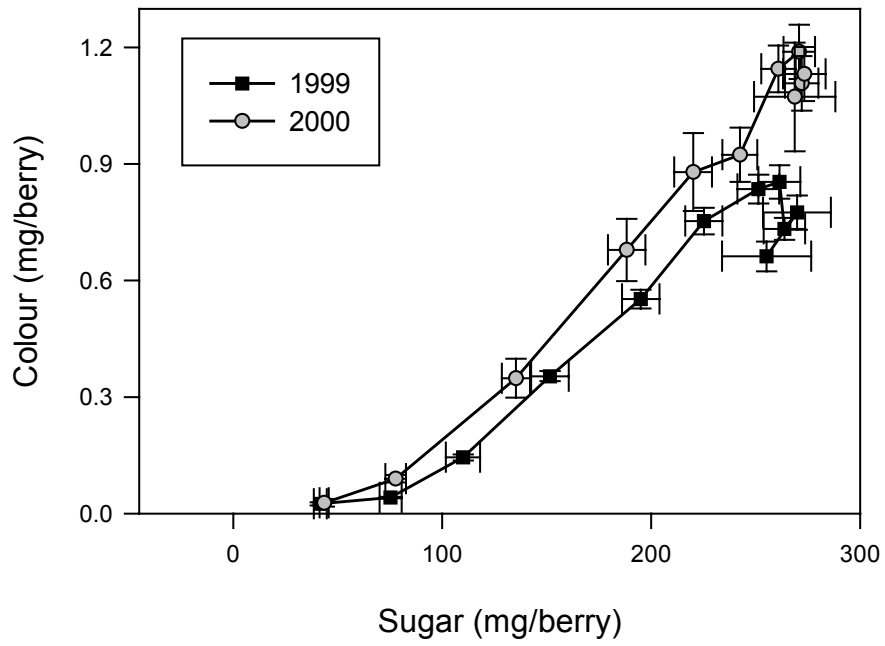
Figure 5.7 Colour formation as a function of sugar levels in berries during two ripening periods.

2.2 Source-sink relationships in Shiraz vines

2.2.1 Introduction

Traditionally sugar level (Brix/Baume) has been the only quality parameter measured by wineries to determine when grapes are ripe. Colour in Shiraz berries may act as an indicator of potential wine colour and quality (Henschke et al. 1990). Vineyard management and environmental conditions can influence both sugar accumulation and colour development which may be the result of altered source-sink relationships within a vine. Production practices used to maximise grape quality parameters or yield can have a significant effect on the source-sink relationship of the grapevine (Williams 1996)

Source-sink relationships are regulated by assimilate supply and demand and will alter during the growing season. Fruits often are the largest sinks but this will depend on the development stage of the vine (Sepúlveda et al. 1986). Sucrose produced via leaf photosynthesis is translocated from leaves to fruits where it is then broken down to glucose and fructose (Sepúlveda & Kliewer 1986). The environmental conditions that can influence leaf photosynthesis include light intensity (Smart 1991), temperature (Morrison 1990), relative humidity (Sepúlveda & Kliewer 1986) and leaf age (Intireri et al. 1992). Water Supply (Ruhl & Alleweldt 1990), nutrition (Keller & Koblet 1995) and pruning (Kriedemann et al. 1975) are management practices that can be manipulated by growers to indirectly affect crop load or yield (Jackson & Lombard, 1993).

The ripening phase of a grape berry begins at veraison (approximately 8 weeks after flowering) when sugars start to accumulate (Coombe 1989). At this time fruit becomes soft and in red grapes a change in colour occurs due to anthocyanin accumulation in the skin (Boss et al. 1996). During this period the main sink is the fruit cluster, while main shoots and laterals constitute relatively weak sinks (Sepúlveda et al. 1986). Anthocyanins and sugar accumulation continue to increase during ripening (Fernandez-Lopez et al. 1992) and there has been shown to be a strong correlation between the two (Gonzàlèz-San Jose & Diez 1992).

Leaf removal around bunches has been demonstrated to increase berry sugar levels (Jackson & Lombard 1993) as it opens up the canopy which increases the exposure of clusters to sunlight (Smart, 1991). Leaf removal around the bunch zone has also been demonstrated to increase anthocyanins and phenols in Cabernet Sauvignon if done between four weeks after capfall and veraison, later removal has little effect (Jackson & Lombard 1993). Smart (1991) suggests leaf removal should occur anytime between fruit set and 2-4 weeks before veraison and that enough leaves should be retained so 60% of the fruit is visible.

Bunch thinning provides a way of setting conditions which ensure an adequate supply of sugar to the berry so that sugar accumulation and secondary metabolite production are appropriate (Iland et al. 1995a). Bunch thinning trials undertaken in Waikerie on Shiraz where vines were thinned to one bunch per shoot at veraison concluded an increase in concentration of sugars by 4% and an increase in the glucose-glycosyl (G-G) reading by 15% (Iland et al. 1995b). The improvement in the concentration of berry G-G could be associated with a higher leaf area to fruit ratio. A trial undertaken on Pinot Noir (Iland et al. 1995a) indicated thinning increased the amount of colour per berry, the treatment using one bunch per shoot providing the maximum colour, but with a yield loss of 50%. It was demonstrated that there is an optimum leaf area to fruit ratio that plateaus once it reaches 30 cm^2/g, in response to colouration of fruit. The success of bunch thinning will depend on the initial yield, the degree to which bunch thinning occurs and the photosynthetic effectiveness of the leaves supplying the berries with carbohydrates. If leaves are shaded or stressed then the removal of bunches may not improve fruit composition (Iland et al. 1995a).

Rojas-Lara & Morrision (1989) demonstrated that leaf shading and cluster shading have different effects on grape berry development. They showed leaf shading delayed and reduced the rate of berry growth and sugar accumulation, while cluster shading had little effect on these processes. Cluster shading significantly affected anthocyanin accumulation. Morrison & Noble (1990) demonstrated that the effect of leaf and cluster shading on fruit composition is probably due to a combination of the direct effects of temperature. Temperature has been shown to be particularly responsible for colour

development, with the optimum being between 17-26ºC (Jackson et al. 1993). Photosynthetic apparatus is not damaged by high temperatures and neither is the translocation of photosynthate but they can delay sugar accumulation by changing the distribution pattern of carbohydrates with less moving into the berries and more moving towards the shoot tips (Sepúlveda et al. 1986). Sudden heat stress can cause large decreases in anthocyanin content in grape berries (Crippen & Morrison 1986)

Canopy management is a way growers can manipulate light penetration and temperature to encourage greater colour and sugar accumulation in red grapes. Topping (leaf and shoot tip removal) undertaken on Shiraz during cluster elongation through to veraison produced elongated bunches, increased yield and brix (Jackson & Lombard 1993). Shoot topping, a traditional management technique used by growers to avoid over hanging shoots has been suggested by Poni & Intrieri (1996) as a way to promote laterals that come to be mature around veraison as the younger leaves of the laterals are photosynthetically more active than the main leaves. The belief being this may enhance fruit quality by transporting carbohydrates to the ripening grapes.

Trunk girdling (the removal of a strip of phloem) has been used in table grapes as a way to increase berry size and is usually done after flowering (Roper & Williams 1989). Wine grapes are girdled at veraison to hasten maturity by earlier colour maturation and/or sugar ripeness (Jensen et al. 1981). Girdling has been shown to reduce the net photosynthetic rate and stomatal conductance in grapevine leaves (Roper & Williams 1989, Hofäcker 1978). Further work has also found that girdling can cause an increase in carbohydrates above the girdle (Roper & Williams 1989).

Knowledge on the distribution of carbohydrates in the grapevine is limited, but what is known indicates they are crucial for berry sugar accumulation. Sugar content is often correlated with berry colour, the rate of ripening effects this, and other berry quality parameters. This project will investigate the distribution of carbohydrates and how this can affect two key berry quality attributes, sugar and colour. A number of management techniques will be studied that alter the source sink relationship in a vine. This may provide growers with answers on how to manipulate their vines to produce better quality Shiraz grapes.

2.2.2 Material and methods

2.2.2.1 Experimental set up and treatments

The trial was conducted during the 2000/01 growing season on a commercial vineyard planted in 1990 to *Vitis vinifera* cv. Shiraz. The vineyard was located in Hanwood, south of Griffith, NSW. The site was chosen for its consistent sugar and colour accumulation patterns exhibited in previous work undertaken by Holzapfel et al. (1999). The traditional management factors included flood irrigation, with an average application rate of 5 ML/ha. The vines were mechanically hedged, with hand clean up undertaken in August. The vines have been mechanically harvested for a number of years.

Five treatments were applied on January 3 (2001) coinciding with veraison as follows:

1. Control (C), no treatments applied.

2. Leaf removal (LR): each cane having every second leaf removed (50% leaves) to represent the removal of a source of assimilate.

3. Bunch removal (BR), every second bunch was removed (50% bunches) to represent the removal of sinks that would normally uptake assimilate.

4. Girdling (G), the removal of a 50mm strip of phloem 25cm below the branching of the two cordons, followed by maintenance of the girdle on January 11, February 1 and 15. This treatment represented the removal of a source and sink.

5. Girdling and Bunch removal (G+BR) a combination of treatments 3 & 4 and represents the removal of more sinks from the vine.

The trial site was designed to suit a 5 x 5 Latin Square. Each replicate consisted of 2 panels of four vines each (eight vines per treatment, per row). The six central vines were used for all measurements and sampling, but some measurements were restricted to the central vines.

2.2.2.2 Field measurements to determine vine productivity

Yield was determined at harvest (February 23, 2001) from the six vines from each replicate and weights recorded. Pruning weights were determined by weighing the combed prunings from each replicate after leaf fall (June 25, 2001) to calculate the pruning weights for each treatment on a per vine basis. Bud counts and budburst was undertaken in September 2001, to determine viability of vines and timing of budburst. Shoot length measurements were conducted on a weekly basis from budburst and measured the same-tagged shoot (number) on each vine until mid-November (flowering) when shoot length slowed.

Photosynthesis measurements were undertaken using a CO_2 gas analyser (LCA–4, Analytical Developmental Company Ltd., Hoddesdon, England). Measurements were undertaken on December 28, January 10, 17, 31, February 7, 15 and 21. These days provided clear, cloud free periods to take readings on two leaves per replicate (sixth fully enfolded leaf from the end of the shoot tip). The central vine from each replicate was used for this purpose. The central vine of each replicate was chosen to provide and accurate representation of the plot.

2.2.2.3 Basic and specific berry measurements and analysis

Samples of 100 berries were taken from different parts of various bunches per replicate on a weekly basis from veraison (January 3) until harvest. Samples were picked during the time period of 10-11 am. They were then transported to the laboratory in an esky with an internal temperature of between 1-4ºC. Within 24 hours of berries being sampled, the berries were separated into 50 berry lots. The first 50 were weighed to determine average berry weight. These were then processed in a Juicer that is able to separate the seeds and skins from the juice. The measurement of Brix was then taken using a digital refractometer (PR-101 ATAGO, Tokyo, Japan). The remainder of the juice was then frozen for further analysis on pH and TA at a later date. The other 50-berry sample was frozen at -80ºC for later analysis of sugars, anthocyanins, organic acids and phenolics.

Berry colour and total phenolics were determined by the method described by Williams et al. (1995) using a spectrophotometer (UV-2101PC Shimadzu, Kyoto, Japan). The juice samples used for pH and TA determination were defrosted in a water bath (40ºC) to enable for adequate redissolving of tartaric and malic acid crystals. The samples were then centrifuged to separate the juice from other sediment. PH was measured with a glass electrode, which was part of the automatic titrator (TitraLab 80, Radiometer Copenhagen, Lyon, France). TA was measured by titration with a base to an end point of pH 8.2 (20ºC), and the results were expressed in g/L tartaric acid.

2.2.3.4 Determination of detailed berry components

Organic acid and sugar analysis was conducted using a HPLC technique developed by Frayne (1986). The determination was undertaken on frozen homogenate (-80°C) from an initial 50 berry sample which were homogenised using an Ultra-Turrax. Enough homogenate was placed in a 10ml test tube to three quarters fill it. The test tube was then placed in a centrifuge and spun at 3000rpm for 10 minutes. 500ul was removed from the tube and placed in a 2ml eppendorf tube. PVP and activated charcoal was added to drop out any remaining anthocyanins remaining in the sample. The samples were then vortexed to mix the samples and allowed to sit for a minimum of half an hour. The samples were then placed in an eppendorf centrifuge for a period of 2 minutes at 14000rpm to settle out the PVP and charcoal. Four external standard solutions were prepared in water. Each one contained varying concentrations of glucose (32.5-150g/L), fructose (32.5-150g/L), malic (2-10mg/L) and tartaric acids (1-4g/L).

HPLC analysis was conducted using a HPLC (Alliance 2690, Waters, Milford, MA, USA) equipped with a photodiode array detector (PDA 996 Waters) and a differential refractometer detector (DR 410, Waters) in series after the PDA detector. Using a 4 x 3mm REZEX-RDM guard column (Phenomenex, Torrance, CA, USA) and two 300mm x 7.8mm Aminex HPX-87H organic acid analysis cation exchange columns (BIORAD, Hercules, CA, USA) connected in series. The columns were maintained at 60°C. the mobile phase was 0.065% H_3PO_4 using distilled water obtained from a Millipore Milli-Q water purification system. The flow rate was 0.7ml/min and an injection volume of 10ul. Tartaric and malic acids were detected by UV, glucose and fructose detected by RI. The peaks were identified

using external calibration based on peak area. The components were identified by a comparison of their retention times with those of the standards.

The anthocyanin analysis was undertaken on frozen homogenate (-80°C) from an initial 50 berry sample, which were homogenised using an Ultra-Turrax. Once homogenised a sub sample is taken and placed in a screw top centrifuge tube (approx 1.5g). The mixture is then centrifuged at 12000g for ten minutes. The supernatant is poured off and the remaining pellet is weighed. For every gram of pellet 2mls of 50% methanol is added. The pellet is resuspended and mixed. The sample is then placed on an eppendorf thermomixer for 1 hour to extract anthocyanins. The samples are then placed back on the centrifuge for 10 minutes at 12000g. Once completed the supernatant is collected and injected into the HPLC. An external standard solution of malvidin-3-glucoside (Extrasythese, Lyon, France) was prepared in methanol. The concentrations ranged from 0.4mg/ml to 0.1mg/ml. 5μL of prepared sample was injected via a Goldpak C18 column 4.6mm x 25cm into a Waters HPLC system equipped with a photodiode array detector (PDA). Mobile phase A was phosphoric acid (1%) and phase B acetonitrile (100%), each run took 55 minutes. The individual anthocyanins were determined by comparing their peaks against a known standard (malvidin-3-monoglucoside) and by comparing their retention times and elution order with previous studies of grape anthocyanins (Wulf & Nagel 1979).

2.2.3.5 Statistical and data analysis

The results were manipulated in Excel 97, further analysed using the graphic program Sigma plot 2001 (version 7.0). The statistical analysis was conducted in Genstat release 6.1 Rothhamsted Experimental Station. An analysis of variance was applied to the results of the determinations to test for significant differences between the five experimental treatments on each of the sampling dates. For locating the significant differences, least significant difference (LSD) was used (p=0.05).

2.2.3 Results and discussion

2.2.3.1 Vine response to altered in source and sink relationship

Yield and pruning weights are presented in Table 1.1. Under the conditions presented the control treatment and girdling produced the highest yields with the bunch removal treatments having half the yield of the control. This was expected since approximately half of the crop was removed at veraison. The yield of the leaf removal treatment was also lowered. The pruning weights are highest in the two girdling treatments with the lowest weights recorded in the LR and BR treatment, but not significantly different from the control. This indicates that more photo assimilates would have remained above the girdle under the G and G+BR treatments.

The Leaf removal treatment had the highest photosynthesis rate, the two girdling treatments (G, G+BR), the control and BR were in between and similar. It appeared that girdling and bunch removal had an additive effect on lowering the photosynthesis. The removal of part of the source (leaf) makes the remaining leaves more effective and less sinks lower leaf photosynthesis. Kriedemann and Lenz (1972) suggest that the reduction in photosynthesis is probably due to the build up of assimilate due to the elimination of roots as an effective sink.

The shoot growth in spring was strongly reduced by the girdling treatments in the previous year during berry ripening, and bunch removal increased shoot growth to some extent compared to the control. The effect of the girdling could be due to lowered reserves in the trunk and roots or poorly reconnected phloem.

Table 1.1 Yield and pruning weights affected by leaf removal, bunch removal and girdling.

Treatment	Yield (kg/vine)	Pruning weight (kg/vine)
Control	6.16 a	1.54 bc
Leaf Removal (LR)	5.19 b	1.44 c
Bunch Removal (BR)	3.52 c	1.46 c
Girdling (G)	6.44 a	1.88 a
Bunch Removal + Girdling (BR + G)	3.13 c	1.81 ab

significant differences between means within columns are indicated by different letters (p=0.05)
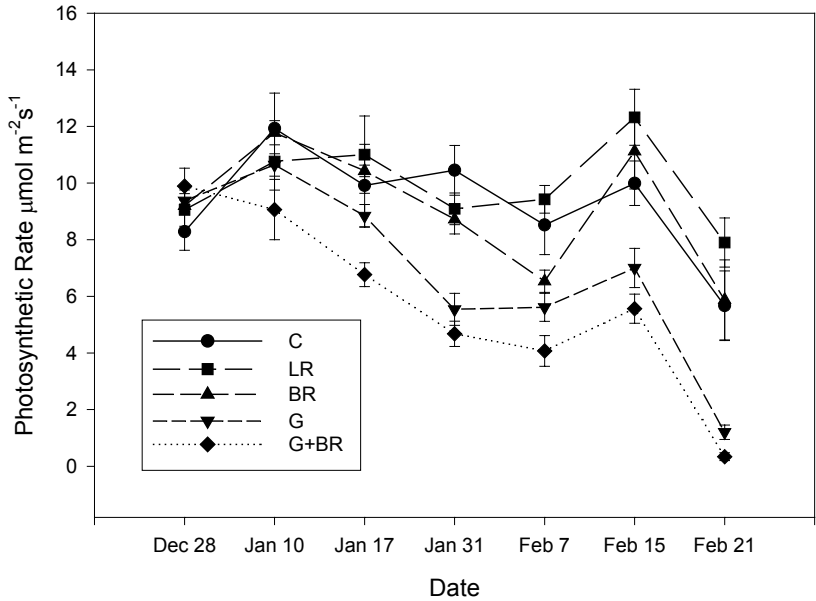
Figure 1.1 Photosynthesis during berry ripening 2001.
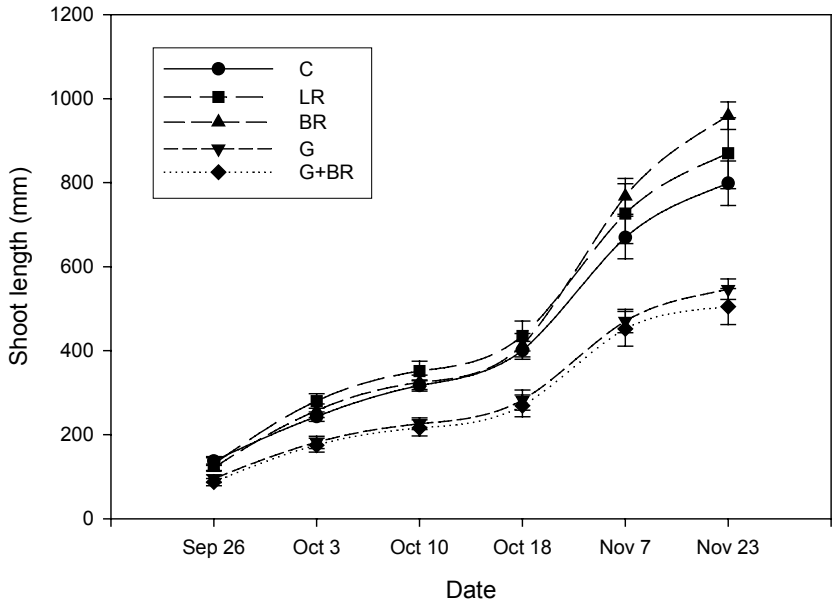
Figure 1.2 Shoot growth in early spring 2001.

2.2.3.2 Berry sugar accumulation and berry acids

Berry weights increased rapidly in all treatments until date 4 (24 January), the G+BR treatment berry weight maximum was reached at date 5 (31 January). This treatment has also the lowest berry weight at harvest (Figure 2.1). The largest berry weights were seen in the leaf removal treatment, which continued its consistent accumulation of weight up until the second to last sampling date, similarly to the control. The other two treatments, namely bunch removal and girdling were intermittently in their final berry weight and timing of maximum berry weight at date 6 (7 February).

Figure 2.1 Evolution of berry weight during grape maturation effected by several treatments in Shiraz.
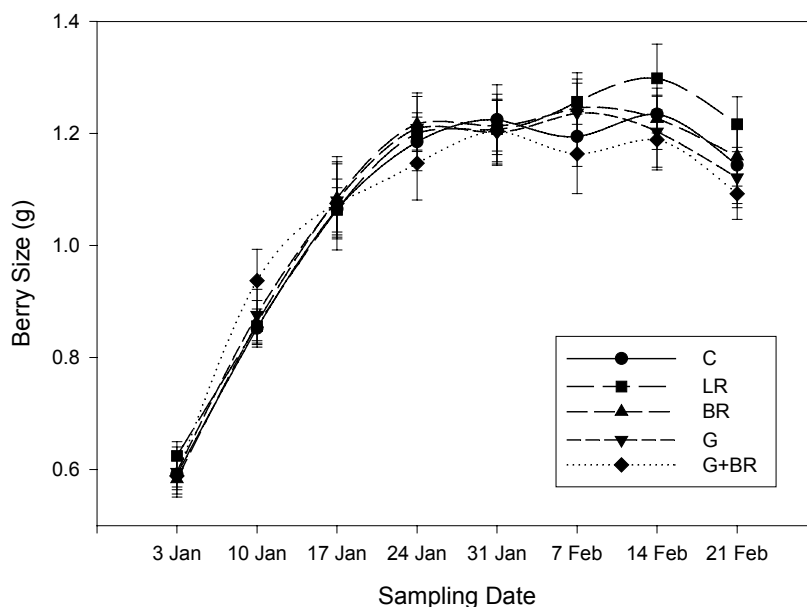

The onset of rapid sugar accumulation occurred in all treatments immediately after veraison, with a steady increase in all treatments except LR. This treatment, as seen in Figure 2.2, had a reduced rate of sugar accumulation for a period of three weeks before reaching similar levels as the control. At harvest the differences are not significant, but the girdling treatment had the highest Brix level at harvest with 24.3° and the BR having the lowest of 23.8°. The berry sugar accumulation is slowed down by the LR treatment and increased by BR + G treatment, but at harvest time the final sugar in the berry showed the reverse, where LR has the highest amount of sugar per berry with 296.5mg (Figure 2.3). This is basically due to the differences in berry size at harvest, but also due the differences in photosynthesis described earlier. Glucose and fructose concentration in the juice was similar to the brix levels, at mid ripening LR treatment had the lowest levels and G and G+BR the highest, but at harvest the these treatments had the lowest concentrations in the juice (Table 2.1).

Table 2.1 Glucose and fructose concentrations in g/L in Shiraz juice during berry ripening effected by leaf removal, bunch removal and girdling.

| | Jan-10 | | Jan-24 | | Feb-07 | | Feb-21 | |
	Glucose	Fructose	Glucose	Fructose	Glucose	Fructose	Glucose	Fructose
Control	70.9	62.0	108.6 b	104.6 cd	132.1 a	127.1	147.4 a	144.1 a
LR	66.4	47.0	104.3 b	99.8 d	128.0 a	121.3	144.1 a	141.6 a
BR	70.1	61.9	115.1 a	109.9 bc	123.5 a	118.5	147.1 a	141.5 a
G	67.3	60.1	118.8 a	114.5 ab	129.5 a	123.5	137.4 b	135.8 b
G+BR	62.7	54.5	119.7 a	117.1 a	106.9 b	103.8	123.0 c	129.3 c

signifcant differences between means within columns are indicated by different letters (p=0.05)
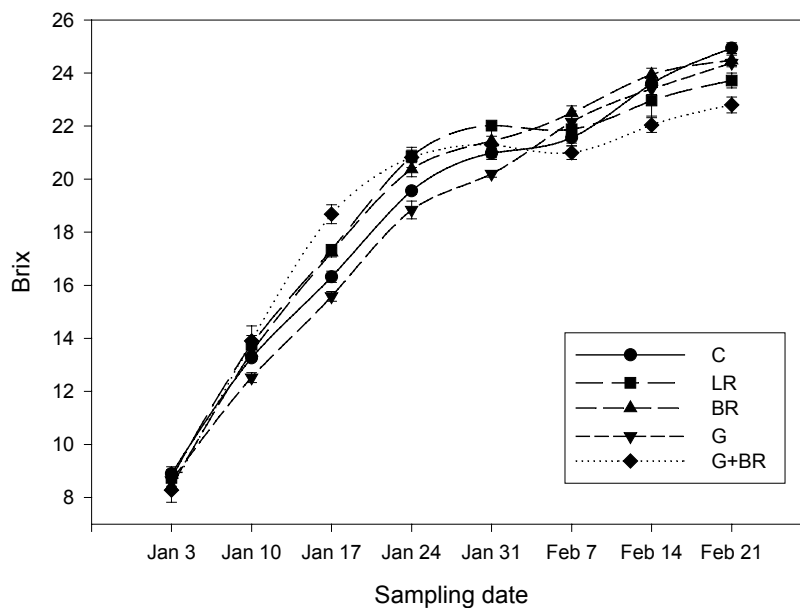
Figure 2.2 Total soluble solids (TSS) in ripening Shiraz under several treatments.
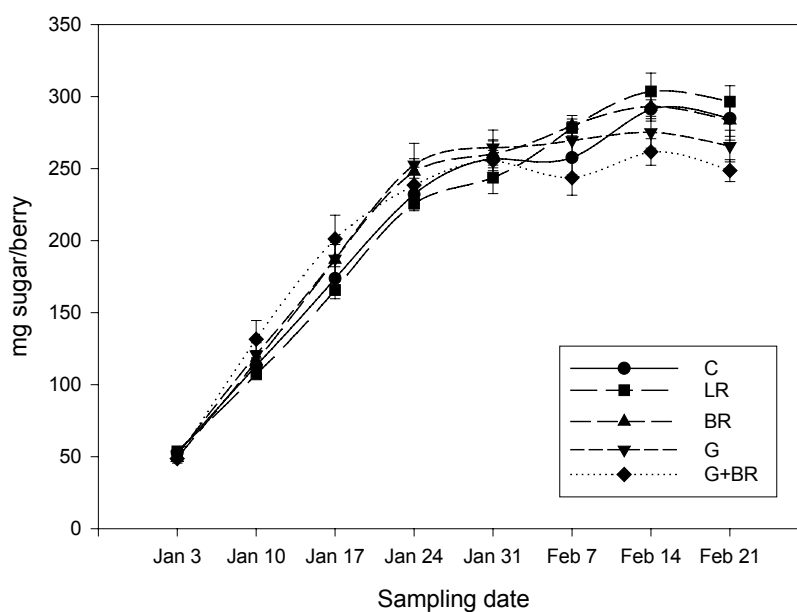
Figure 2.3 Berry sugar accumulation during maturation in Shiraz affected by several treatments.

Throughout the period of maturation of the Shiraz studied in all treatments there tended to be an increase in pH and decrease in TA (Table 2.2). At harvest the control treatment had the highest pH of 4.08 and the lowest TA of 4.9 g/L, while the LR treatment produced the lowest pH of 3.95 and highest TA of 6.4g/L. Early in berry maturation the concentration of malic acid (7.87-10.92 g/L) was much higher than that of tartaric acid (3.56-4.64g/L) (Table 2.3). As time progressed the concentration of malic acid continued to decrease with very little difference between treatments. Malic acid was not significantly affected by any of the treatments but varied between 1.46 and 2.34g/L. The G+BR showed the highest with 5.71g/L tartaric acid levels at harvest and BR the lowest with 4.97g/l. Tartaric acid reaches its lowest point around date 4 (January 24) and TA and then begins to increase again, this increase could be explained to changes in berry size.

Table 2.2 TA in concentrations (g/L) and ph in Shiraz juice during berry ripening affected by leaf removal, bunch removal and girdling.

| | Jan-10 | | Jan-24 | | Feb-07 | | Feb-21 | |
	TA	pH	TA	pH	TA	pH	TA	pH
Control	11.63	2.90	6.39	3.38 b	4.54	3.70	4.90	4.08 a
LR	11.28	2.87	6.00	3.30 c	4.45	3.66	6.40	3.95 b
BR	8.44	2.88	5.68	3.34 bc	4.55	3.76	5.55	4.03 ab
G	9.60	3.30	5.04	3.42 b	3.96	3.73	6.15	3.97 b
G+BR	9.52	2.86	6.91	3.53 a	4.30	3.73	6.10	4.03 ab

significant differences between means within columns are indicated by different letters (p=0.05)

Table 2.3 Tartaric and malic acid concentrations in g/L in Shiraz juice during berry ripening affected by leaf removal, bunch removal and girdling.

| | Jan-10 | | Jan-24 | | Feb-07 | | Feb-21 | |
	Tartaric A	Malic A	Tartaric A	Malic A	Tartaric A	Malic A	Tartaric A	Malic A
Control	4.38 ab	8.46	3.53 a	4.49	4.45	3.21	5.45 a	2.09
LR	4.64 a	10.92	3.30 b	4.36	4.34	3.06	5.19 b	2.22
BR	3.69 bc	7.87	3.11 c	4.48	3.99	3.20	4.97 b	2.30
G	3.56 c	8.71	3.25 bc	4.31	4.49	3.04	5.37 ab	1.46
G+BR	3.60 c	10.76	3.43 ab	4.27	4.16	2.66	5.71 a	2.34

significant differences between means within columns are indicated by different letters (p=0.05)

2.2.3.3 Berry colour and anthocyanins

Berry colour increases significantly in the first two weeks, where large differences between treatments are present (Figure 3.1). The combined treatment G+BR has nearly double the colour compared to the LR treatment. At harvest all treatments are between 1.16 and 1.29 mg/g FW and the control tend to be higher than the other treatments. In the treatments which were girdled, bunches removed or both reached the maximum colour two weeks before harvest. This was surprising, since berry weight decreased in these treatments as well.
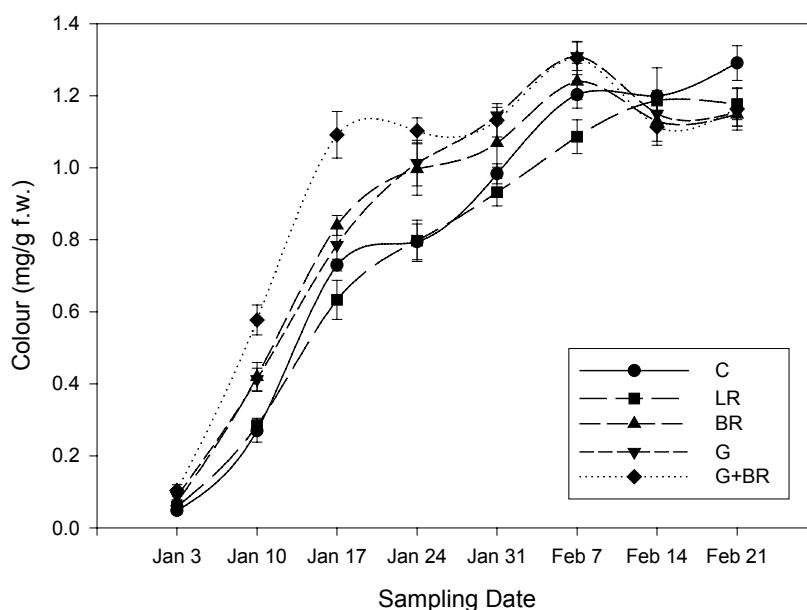
Figure 3.1 Berry colour development during berry ripening under different treatments.

The single anthocyanins showed also the strong increase in the first four weeks of the berry ripening, with similar effects of the treatments at mid ripening (24 January) as observed with the total colour. malvidine 3-glucoside and all the other anthocyanins are higher in the G+BR treatment compared to the others (Table 3.1). At harvest the three major anthocyanins (malvidin 3-glucoside, malvidin 3-acethylglucoside, malvidin 3-p-coumarylglucoside) are highest in the control and lowest in the combined treatment (G+BR). These three anthocyanins make about 80% of the total anthocyanins, cyanidin 3-glucoside contributed the least to the total berry anthocyanins (Figure 3.2).

It appears that the vines were in balance and some of the treatments had a negative effect on final berry colour, although berry colour was higher at mid ripening in the combined treatment. This suggests that the colour maximum was altered by these treatments, this difference was particularly high between the LR and the G+BR treatments two weeks after veraison.

Table 3.1 Anthocyanins (mg/g FW) in Shiraz grapes at mid ripening and harvest effected by leaf removal, bunch removal and girdling (Mv = Malvidin 3-glucoside, Mv-ac = Malvidin 3-acethylglucoside, Mv-cou = Malvidin 3-p-coumarylglucoside, Pn = Peonidin 3-glucoside, Pt = Petunidin 3-glucoside, Pn-cou = Peonidin 3-p-coumarylglucoside, Dp = Delphinidin 3-glucoside, Cy = Cyanidin 3-glucoside).

Date		Mv	Mv-ac	Mv-cou	Pn	Pt	Pn-cou	Dp	Cy
Jan-24	Control	0.234	0.111b	0.099	0.046c	0.018	0.010b	0.02b	0.014b
	LR	0.212	0.090b	0.082	0.052c	0.018	0.010b	0.026b	0.018b
	BR	0.188	0.098b	0.084	0.092b	0.016	0.010b	0.026b	0.014b
	G	0.248	0.114b	0.079	0.066bc	0.040	0.020a	0.024b	0.018b
	G+BR	0.390	0.172a	0.132	0.164a	0.018	0.014b	0.042a	0.034a
Feb-21	Control	0.304a	0.170a	0.171	0.066	0.018	0.018	0.022	0.012
	LR	0.250ab	0.132ab	0.138	0.076	0.022	0.018	0.018	0.014
	BR	0.278a	0.136a	0.124	0.060	0.018	0.014	0.020	0.014
	G	0.284a	0.152a	0.121	0.072	0.024	0.014	0.020	0.014
	G+BR	0.198b	0.102b	0.098	0.076	0.014	0.020	0.016	0.016

significant differences between means within columns are indicated by different letters (p=0.05)
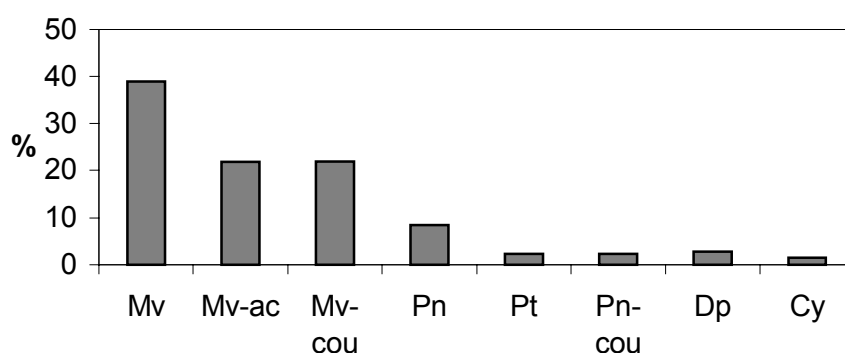
Figure 3.2 Distribution of eight anthocyanins at harvest in Shiraz berries.

3 GENERAL DISCUSSION AND FUTURE WORK

This work provides an indication of the wide range in yields and berry composition that can exist within a region (Riverina). Sugar accumulation rates varied significantly between vineyards and years, and this resulted in a one-month Shiraz harvest period in the 1998/99 season and a three-week harvest period in the 1999/2000 season. High yields were correlated with lower brix and colour in one group of vineyards (see section 2.1.2), however the opposite was apparent in another group of vineyards where high yields and good composition were achieved simultaneously (see section 2.1.3). One factor separating the behaviour of these two groups was pruning weight of the previous season. The first group of vineyards had increasing yield with pruning weight while the second group had a wide range of yield despite similar pruning weights. This suggests that vineyards can be managed to improve berry composition by optimising the balance between yield and shoot growth. This balance will be different for each vineyard. The strongest impact on vine productivity and berry quality was irrigation and canopy management.

The source reduction treatment resulted in an increase the photosynthetic rate of remaining leaves. The removal of the sinks reduced leaf photosynthesis, and this resulted in similar final berry sugar levels as the control. The exclusion of the roots and trunk as sinks also lead to higher pruning weights in these vines. The two girdling treatments significantly reduced berry glucose and fructose levels at harvest. This could be due to the diversion of assimilates to shoots rather than to berries, or it may be due to reduced photosynthesis. Altering vine source sink-relationships in the field trial however did not impact significantly on final berry ripeness, including total colour. There was an effect of source reduction (leaves) and sink reduction (bunches, trunk + roots) on these parameters only until about mid ripening. This indicates that in the particular vineyard where the trial was conducted there were plenty of leaves to ripen the fruit fully. A higher yielding vineyard, with more vigorous growth may have responded differently. One might also consider that the slowing of ripening rates at the end of maturation may not be due to a source-sink imbalance but rather a dysfunctional vascular system.

Further work is required to understand sink-source relationships in vines. Irrigation management appears to be critical and is an important factor that must be investigated in conjunction with source-sink dynamics. Understanding a vineyard's optimum balance between fruit and vegetative production is crucial to achieving optimum yield and composition simultaneously.

4 SUMMARY

This project consisted of a survey and a field trial. The survey examined differences in sugar accumulation and colour development between vineyards in the Riverina (MIA) over two seasons. Vineyard management and productivity information was also collected. TSS, colour and yield varied significantly among vineyards and years, as did sugar and colour accumulation during berry maturation. The relationship between colour, yield and TSS differed between vineyards and was influenced by irrigation management and canopy size.

The field trial was conducted to study vine source-sink relationships by reducing the source (leaves) or sinks (fruit, trunk, roots) through crop removal or girdling. The impact of these treatments on ripening and colour development was strong during the first half of berry maturation, but were no longer significant during late ripening and thus at harvest. The vines internal balance was likely readjusted to these altered source and sink sizes so that any impact these treatments might have had on final berry composition was eliminated.

5 ACKNOWLEDGMENT

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7 OUTPUTS

Publications, workshops, presentations

Referred Scientific Publications

HOLZAPFEL, B., ROGIERS, S., DEGARIS, K. and SMALL, G. (2002): Yield components and berry quality under contrasting vineyard management. Aust. J. Grape & Wine Res. (in preparation)

HOLZAPFEL, B., ROGIERS, S., DEGARIS, K. and SMALL, G. (2002): Sources of variation in yield components under either flood or furrow irrigation. Aust. J. Grape & Wine Res. (in preparation)

ROGIERS, S., HOLZAPFEL, B., DEGARIS, K. and SMALL, G. (2002): Vineyard environment and berry ripening. Aust. J. Grape & Wine Res. (in preparation)

Conference & Symposium Presentation

HOLZAPFEL, B., ROGIERS, S., DEGARIS, K. and SMALL, G. (2000): Abstract Poster: Identifying factors effecting grape berry ripening and berry colour development. 5th ISCCV&O Melbourne, Australia

DEGARIS, K., HOLZAPFEL, B., STEEL, C. and ROGIERS, S. (2001): Summary Poster: Source Sink relationships within Shiraz grapevines. Proc. 11th Australian Wine Ind. Tech. Conf. Adelaide, Australia

Extension and Industry Publications

HOLZAPFEL, B., ROGIERS, S., DEGARIS, K. and SMALL, G. (1999): Ripening grapes to specification: effect of yield on colour development of Shiraz grapes in the Riverina. The Australian Grapegrower & Winemaker 428, 24-28

DEGARIS, K., HOLZAPFEL, B., ROGIERS, S. and SMALL, G. (2000): Ripening grapes to specification: the second year. The Australian Grapegrower & Winemaker 443, 24-28

Other Presentations (workshop presentations, seminars, guest lectures)

HOLZAPFEL, B. and DEGARIS, K. (1999): Ripening grapes to specification: Identifying manageable factors determining grape composition & quality through carbohydrate sink-source relationships. Seminars presented on the 9 June (NWGIC seminar, Wagga Wagga), 31 August (WGMB seminar, Griffith) and 4 November (NWGIC opening, Wagga Wagga)

HOLZAPFEL, B. (2000): Ripening grapes to specification: Identifying manageable factors determining grape composition & quality through carbohydrate sink-source relationships. (2nd Season), WGMB seminar, Griffith, 28 August

DEGARIS, K., (2000): Source/Sink relationships in Shiraz grapevines. NWGIC Seminar Series, 2nd Session, Wagga Wagga, 23 August

DEGARIS, K., (2000): Shiraz colour development and sugar accumulation in the Riverina. WGMB seminar, Griffith, 28 August

ROGIERS, S. and HOLZAPFEL, B. (2000): Grape berry ripening and shrivel in Shiraz. NWGIC Seminar Series, 2nd Session, Wagga Wagga, 2 November

SMALL, G. (2001): Yield versus quality: the facts. Hunter Valley 23 May, Mudgee 26 May, Murimum 2 July, Griffith 12 December

Some of the findings have also been used in the 'Research to Practise Quality Workshops' and in lectures at the CSU in form of case studies and crop forecasting lecture by Gioia Small.

8 ATTACHMENTS

HOLZAPFEL, B., ROGIERS, S., DEGARIS, K. and SMALL, G. (1999): Ripening grapes to specification: effect of yield on colour development of Shiraz grapes in the Riverina. The Australian Grapegrower & Winemaker 428, 24-28

DEGARIS, K., HOLZAPFEL, B., ROGIERS, S. and SMALL, G. (2000): Ripening grapes to specification: the second year. The Australian Grapegrower & Winemaker 443, 24-28

HOLZAPFEL, B., ROGIERS, S., DEGARIS, K. and SMALL, G. (2000): Abstract Poster: Identifying factors effecting grape berry ripening and berry colour development. 5th ISCCV&O Melbourne, Australia

DEGARIS, K., HOLZAPFEL, B., STEEL, C. and ROGIERS, S. (2001): Summary Poster: Source Sink relationships within Shiraz grapevines. Proc. 11th Australian Wine Ind. Tech. Conf. Adelaide, Australia

Effect of abscisic acid (ABA) on sugar accumulation in the flesh tissue of peach fruit at the start of the maturation stage

Kenji Kobashi[1], Sumuko Sugaya[2,*], Hiroshi Gemma[2] & Shuichi Iwahori[2]
[1]*Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan;*
[2]*Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan*
(*author for correspondence, e-mail: ssumiko@sakura.cc.tsukuba.ac.jp)

Abstract

Experiments were conducted on ^{14}C-sorbitol, fructose, and glucose uptake into flesh discs, and sorbitol efflux from the discs, with and without ABA application to examine the effect of abscisic acid (ABA) on sugar accumulation in peach fruit flesh at the start of the maturation stage in relation to membrane transport. Total uptake of ^{14}C-sorbitol, fructose, and glucose into flesh discs was effectively promoted by ABA at a concentration of 10^{-5} *M*. PCMBS (*p*-chloromercuribenzensulfonic acid)-sensitive uptake, which was considered as carrier-mediated uptake, of sorbitol into the discs was clearly stimulated by ABA at 10^{-5} *M*, compared with glucose and fructose uptake. Sorbitol efflux from the discs across the tonoplast was restricted by ABA at 10^{-5} *M*. ABA application to developing fruit increased sugar accumulation in the fruit. Estimated ABA concentration in this fruit was approximately 10^{-5} *M*. These results indicate that sugar accumulation in peach fruit flesh is stimulated by ABA at a concentration of 10^{-5} *M* both *in vitro* and *in vivo*. ABA stimulates uptake of sugars, especially sorbitol, into the flesh by enhancing carrier-mediated transport possibly across both tonoplast and plasma membrane.

Abbreviations: ABA – (+)-2-cis, 4-trans-abscisic acid; CCCP – carbonylcyanide *m*-chlorophenylhydrazone; DAFB – days after full bloom; GC – gas chromatography; HPLC – high performance liquid chromatography; PCMBS – *p*-chloromercuribenzensulfonic acid; PVPP – insoluble polyvinylpolypyrrolidone; TSS – total soluble solid content

1. Introduction

Sugar accumulation is an important factor in determining the quality of peach fruit [24]. Peach fruits accumulate sugars rapidly during the relatively short maturation stage [13, 16]. Sorbitol is the main translocated sugar in peach, which is then converted to glucose and fructose in the fruit. During the maturation stage, sucrose is synthesized from glucose and fructose, accumulating to become the dominant fruit sugar [13, 16]. The mechanisms controlling sugar accumulation in peach fruit are, however, still not clear.

It is known that sugar accumulation in many fruits is affected by plant hormones, such as indoleacetic acid (IAA) and abscisic acid (ABA) [2, 18, 19, 20, 21, 26]. We showed previously that total sugar content in peach fruit, accompanied by changes in sorbitol metabolism, increased with an exogenous ABA application to the developing fruit [13]. Little is known, however, about the effect of ABA on sugar accumulation in peach fruit flesh at the level of cell or tissue physiology as related to membrane transport.

It is considered that membrane transport may play an important role in sugar transport in plants. This is especially so for apoplastic loading and unloading, uptake into sink cells from the apoplast, and uptake into the vacuole from the cytoplasm [5, 10, 22]. Deposition of sugars in the vacuole in flesh cells occurs during fruit maturation [27]. Uptake of sugar

into flesh cells is thought to occur via an apoplastic pathway rather than via a symplastic pathway in grape and tomato fruits especially during the maturation stage [22]. Membrane transport across the plasma membrane as well as across the tonoplast may be responsible for regulating sugar accumulation in the flesh cells of peach fruit. Yamaki and Asakura [26] indicated that sorbitol uptake into the protoplast and into the vacuole isolated from apple fruit flesh was stimulated by IAA and ABA, respectively. From these results, it is possible that membrane transport across the plasma membrane and across the tonoplast in fruit flesh are regulated differently by plant hormones.

Measurements of ^{14}C-sugar uptake into, and sugar efflux from, flesh discs are convenient methods to indirectly investigate membrane transport regulation of sugars in flesh cells and vacuoles [18, 19, 20, 21]. Using the former method, uptake rates into flesh cells can be determined by incubation in a labelled sugar solution. Furthermore, behavior of carrier-mediated uptake can be inferred by using p-chloromercuribenzensulfonic acid (PCMBS), which is a membrane impermeable inhibitor of carrier proteins [3, 7, 9]. By the latter method, sugar efflux velocity across the plasma membrane and across the tonoplast can be determined separately, and these values not only show membrane permeability, but also reflect the sugar uptake across the membranes which may suppressively affect the sugar efflux [20]. Furthermore, in both methods, the effect of ABA on membrane transport can be investigated by adding ABA to the incubation media.

The purpose of this study is to examine the involvement of membrane transport in the ABA-induced sugar accumulation in peach fruit. For this purpose, the effects of ABA on ^{14}C-sorbitol, fructose, and glucose uptake into and sorbitol efflux from fruit flesh discs were investigated. In addition, ABA application to developing peach fruit was conducted, and the ABA concentration in fruit flesh after application was compared with that affecting ^{14}C-sugar uptake into flesh discs.

2. Materials and methods

2.1. *Plant material*

Fruit on a twelve-year-old peach [*Prunus persica* (L.) Batsch cv. Hakuho] tree at the Agricultural and Forestry Research Center, University of Tsukuba were used for each experiment.

2.2. *[14]C-sugar uptake*

Experiments on ^{14}C-sorbitol, fructose, and glucose uptake were conducted following the method of Ofosu-Anim et al. [20, 21]. Fruits of similar size were harvested in the early morning 90∼93 days after full bloom (DAFB), and immediately used for flesh disc preparation.

Cylinders (12 mm in diameter) were removed from the fruit mesocarp with a cork borer and flesh discs 1.6 mm thick were prepared from the midsections of the cylinders with a razor blade. Thirteen to fourteen discs, ca 1.7 g fresh weight, were pooled from seven fruits, and preincubated for 30 min in 10 ml of medium (20 mM MES-NaOH Buffer, pH 6.0, containing 10 mM each of sorbitol, glucose, and fructose, 2 mM CaCl$_2$, 200 mM mannitol) in an incubator at 25 °C with constant shaking. After preincubation the medium was replaced with the same medium except for inclusion of ^{14}C-sorbitol, fructose, and glucose (3.1 KBq $\cdot$ ml^{-1} in the medium, original specific activity; 10.7 or 11.4 MBq $\cdot \mu$mol^{-1}, Amersham Pharmacia Biotech, UK) and incubated for 1 h in the same manner as the preincubation. At the end of the uptake period, the discs were washed with cold incubation medium without radiolabel for 5 min to remove the labelled compounds on the surface and in the apoplast. The washing was repeated three times. Sugars were extracted from discs with 5 ml of 80% ethanol at 80 °C for 30 min, and radioactivity in 1-ml aliquots was determined with a scintillation counter (LS 5000TA, Beckman Instruments Inc. CA) after adjusting to the original volume.

For studying the effect of ABA on the uptake of sugars, S-ABA [(+)-2-cis, 4-trans-abscisic acid, provided by Toray Co., Tokyo] at different concentrations (10^{-4} M, 10^{-5} M, or 10^{-6} M) was added to the media. ABA was added to both the preincubation and the incubation media, because total uptake was enhanced by adding ABA to both media (data not shown). PCMBS (p-chloromercuribenzensulfonic-acid)-sensitive uptake was obtained by subtracting PCMBS-insensitive uptake, which was observed in the presence of PCMBS, from total uptake in the absence of PCMBS. One mM PCMBS was only added to the incubation medium, because uptake rate was not different either by adding the reagent to both the media or only to the incubation medium (data not shown). These experiments were replicated four times.

In addition, the percentage of intact cells (those not broken during incubation) to total cells in flesh discs

was determined by measuring the amount of phenolic compounds remaining in the discs after incubation [27]. A mean of 96.5% of the total cells in the disc were not broken during incubation (data not shown).

2.3. *Sorbitol efflux*

Measurements of sorbitol efflux were carried out using a compartmental analysis method, following the method of Ofosu-Anim and Yamaki [18, 19] and Yamaki and Ino [27]. Fruit were harvested at 90 DAFB for experiments with ABA, and at 97 DAFB for those with inhibitors of carrier-mediated transport. Flesh discs were prepared in the same manner as the ^{14}C-sugar uptake experiments described above, except discs were 3 mm in thickness. Forty discs, weighing about 10 g, were placed in a mesh container and quickly dipped in 30 ml of 2 mM CaCl$_2$ at 0 °C to remove surface sugars released from cut and damaged cells. The discs were then incubated in 100 ml of the same medium and aerated at 0 °C for 2 h. Aliquots of 0.5 ml were withdrawn at the end of each interval for assay. The amount of sorbitol in each aliquot was determined by gas chromatography (GC; GC-9AM, Shimadzu, Tokyo, Japan) after removal of proteins by precipitating with Ba(OH)$_2$ and ZnSO$_4$, and trimethylsilylation [13]. At the end of the 2-h incubation period, the discs were ground and sorbitol remaining in the discs was extracted twice with 50 ml of 80% ethanol for 24 h at room temperature, then amounts were determined by GC. Velocity constants for sorbitol across the plasma membrane and tonoplast were calculated from the time course reduction in the amount of sorbitol in the discs during incubation [27]. ABA (10^{-5} M) or inhibitors of carrier-mediated transport [1 mM PCMBS or 0.1 mM carbonylcyanide m-chlorophenylhydrazone (CCCP)] were added to the medium to determine the effect of ABA and the inhibitors on efflux of sorbitol from the discs. These experiments were replicated three times. In addition, percentage of intact cells in the discs after incubation was determined by the method described above, a mean of 87.7% of the total cells in the disc were not broken during incubation (data not shown). This value is similar to that of Yamaki and Ino [27].

2.4. *ABA application to developing fruit*

Five hundred mg·liter^{-1} (1.89 mM) ABA, which enhances sugar accumulation in peach fruit [13], was sprayed over the whole surface of developing peach

fruit at 93 DAFB. Six fruit were collected at intervals of 7 to 12 days from 61 DAFB to 116 DAFB. Fruits were weighed, and total soluble solid content (TSS) was measured for each fruit with a hand refractometer (N1, Atago Co., Tokyo, Japan). The six fruits were then separated into three groups of two fruits, and the flesh was finely diced and well mixed within each group. Dry weight of the diced flesh was weighed after lyophilization, and the lyophilized sample was used to determine ABA content. Mean ABA content was calculated from the three replicates. Moisture content in fruit flesh was calculated from fresh weight and dry weight of diced flesh.

ABA content was determined by the method of Uthaibutra and Gemma [23]. In this method, the lyophilized sample (0.5 g) was ground and extracted twice with 50 ml of cold 80% ethanol containing 0.5 g insoluble polyvinylpolypyrrolidone (PVPP). The extract was evaporated in vacuo at 40 °C to water phase, adjusted to pH 2.5 with 0.1 N HCl and extracted three times with 20 ml of 100% ethylacetate. The ethylacetate phase was evaporated to dryness and the remaining residue was dissolved in 1 ml of distilled water including 0.05 ml of 100% methanol. The extract was then purified by passing it through a Seppak C18 cartridge (WAT051910; Waters Co., Milford, Mass.) and eluting with 1 ml of 60% methanol. The eluted sample was centrifuged (3000 × g, 2 min) after adding 0.03 g PVPP. The supernatant was filtered through a nitrocellulose membrane filter (pore size 0.45 μm, A045A013A; ADVANTEC Co., Tokyo, Japan) and injected into a high performance liquid chromatograph (HPLC; 8000 series; Toso Co., Tokyo, Japan) equipped with a super ODS column (Super ODS, Toso Co.) and UV detector (254 nm). The conditions of HPLC analysis followed that of Uthaibutra and Gemma [23]. ABA concentration per flesh moisture, as well as ABA content per fresh weight was determined.

3. Results

3.1. ^{14}C-sugar uptake

Sorbitol uptake by flesh discs was almost linear over 60 min (Figure 1A). The effects of pH on PCMBS-insensitive and PCMBS-sensitive uptake of sorbitol into flesh discs are shown in Figure 1B. Total and PCMBS-sensitive sorbitol uptake showed an optimum at pH 6.0. PCMBS-insensitive uptake was lower below
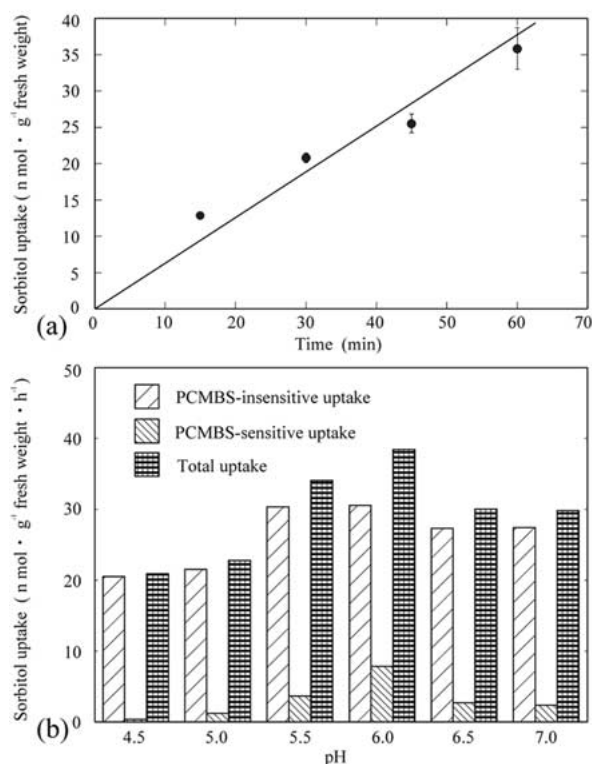
Figure 1. Time-course curve (A) and pH-dependence (B) of sorbitol uptake into peach fruit tissue discs at the start of the fruit maturation stage. Each point represents a mean of three replications ± standard error (A). Each bar represents a mean of two replications (B).

Table 1. Velocity constant for sorbitol efflux across the plasma membrane and the tonoplast in peach fruit flesh tissue as affected by incubation with 10^{-5} M ABA, measured at the start of the fruit maturation stage. Values are means of three replications ± standard error

	Tonoplast ($\mu g \cdot min^{-1}$)	Plasma membrane ($\mu g \cdot min^{-1}$)
Control	1.92 ± 0.36	6.28 ± 2.11
10^{-5} M ABA	1.09 ± 0.03	5.90 ± 1.43

M. For each sugar, ABA at 10^{-5} M showed the most effective stimulation of the total and PCMBS-sensitive uptake by flesh discs, whereas an effective concentration of ABA stimulating PCMBS-insensitive uptake was 10^{-4} M for sorbitol and glucose, and was 10^{-5} M for fructose (Figure 2). PCMBS-sensitive uptake and PCMBS-insensitive uptake of sorbitol in the presence of ABA at 10^{-5} M and 10^{-4} M, respectively, were much more clearly enhanced, as compared with the uptakes of the other sugars in this study.

3.2. *Sorbitol efflux*

Efflux curves of sorbitol from flesh discs were constructed from the log values of the amount of sorbitol that remained in the discs at the end of each incubation period (Figure 3). The final linear part of the curve was equated with efflux from the vacuole [24]. Subtracting the vacuole component from the amount of sorbitol in the discs at each time interval gave an efflux curve representing loss from compartments other than the vacuole, the final phase representing efflux from the cytoplasm [24]. Velocity constants of sorbitol efflux across the tonoplast and plasma membrane were calculated from the slopes of the efflux curves for vacuolar and cytoplasmic content (Table 1). The value across the plasma membrane was higher than that across the tonoplast for both discs incubated with and without ABA at 10^{-5} M. Velocity constant across the tonoplast decreased with ABA, although that across the plasma membrane was not affected by ABA. Velocity constants for discs incubated with or without inhibitors of carrier-mediated transport are shown in Table 2. Velocity constant across the tonoplast tended to increase with the inhibitors, especially CCCP, although it varied widely particularly in the presence of the inhibitors. No pronounced tendency was observed in the velocity constant across the plasma membrane in discs incubated with and without the inhibitors.
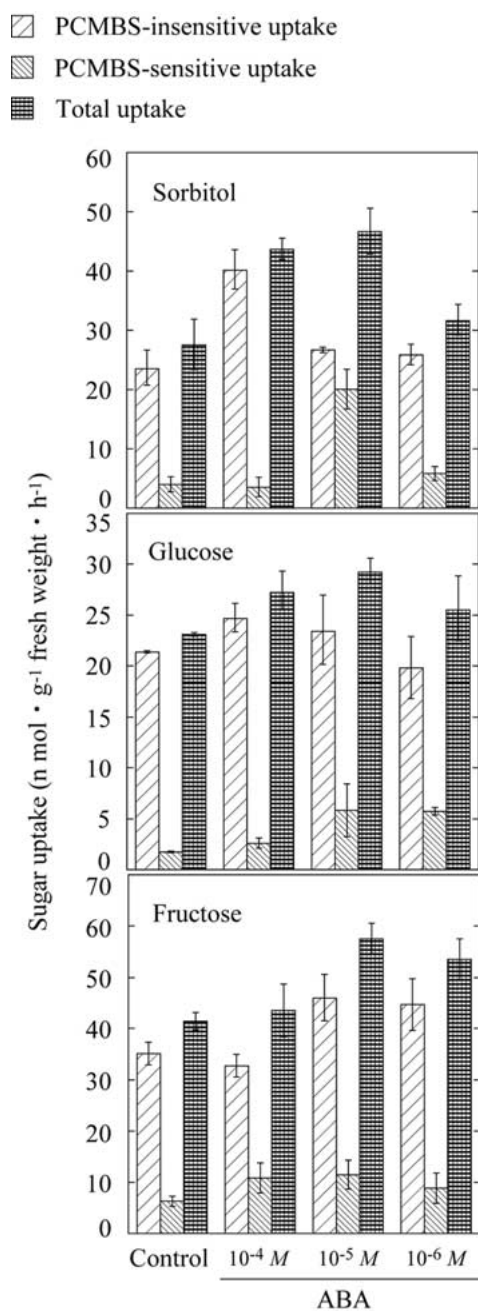
pH 5.5, while it was almost constant between pH 5.5 and 7.0.

ABA at each concentration tended to stimulate the total uptake of sorbitol, glucose, and fructose by flesh discs (Figure 2). As for sorbitol, PCMBS-sensitive uptake was considerably enhanced by ABA at 10^{-5} M, but PCMBS-insensitive uptake was enhanced by ABA at 10^{-4} M. Total sorbitol uptake, therefore, increased in the presence of ABA at 10^{-4} M and 10^{-5} M, but not at 10^{-6} M. Total uptake of glucose tended to be lower than that of sorbitol and fructose in both the control and ABA-treated discs. PCMBS-sensitive uptake of glucose was enhanced to some extent by ABA at 10^{-5} M and 10^{-6} M, and the PCMBS-insensitive uptake was slightly stimulated by ABA only at 10^{-4} M. Total glucose uptake somewhat increased in the presence of ABA at 10^{-4} M and 10^{-5} M, but not at 10^{-6} M. With respect to fructose, PCMBS-sensitive uptake was slightly enhanced by ABA at 10^{-4} M and 10^{-5} M, although PCMBS-insensitive uptake was enhanced by ABA at 10^{-5} M and 10^{-6} M. Total fructose uptake increased in the presence of ABA at 10^{-5} M and 10^{-6}

Figure 2. Effect of ABA on sugar uptake into peach fruit tissue discs at the start of the fruit maturation stage. Each bar represents a mean of three replications ± standard error.
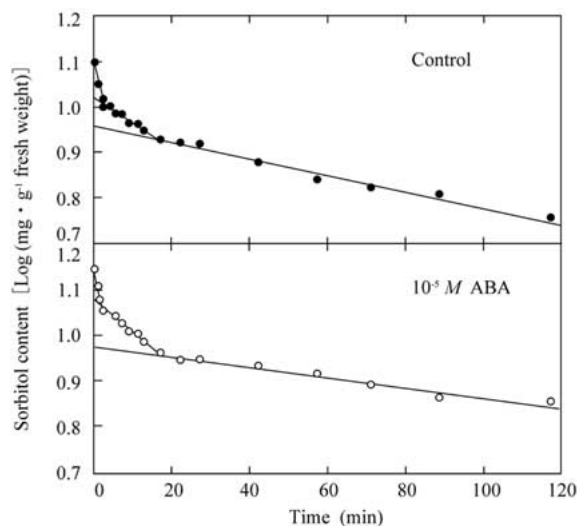

Figure 3. Time course reduction in sorbitol content in peach flesh discs during incubation with 2 mM CaCl$_2$ (control; upper) or 2 mM CaCl$_2$ + 10^{-5} M ABA (10^{-5} M ABA; lower), measured at the start of the fruit maturation stage. Each plot shows the data from three replications.

Table 2. Velocity constant for sorbitol efflux across the plasma membrane and the tonoplast in peach fruit flesh tissue as affected by incubation with inhibitors of active transport, measured at the start of the fruit maturation stage. Values are means of three replications ± standard error

	Tonoplast (μg·min^{-1})	Plasma membrane (μg·min^{-1})
Control	2.80 ± 0.53	20.60 ± 6.71
PCMBS[z]	5.72 ± 2.02	23.84 ± 9.42
CCCP[y]	8.22 ± 6.63	14.32 ± 4.14

[z]p-chloromercuribenzensulfonic acid.
[y]carbonylcyanide *m*-chlorophenylhydrazone.

3.3. *ABA application to developing fruit*

Changes in fruit weight, TSS and ABA content in fruit flesh are shown in Figure 4. An application of 1.89 mM ABA to developing fruit at 93 DAFB markedly increased fruit weight and TSS in the fruit flesh, while the enhancement in fruit weight (104 DAFB) preceded that in TSS (116 DAFB). ABA content per fresh weight of fruit flesh was considerably increased at 104 DAFB by the ABA application, although the content in the ABA-applied fruit was overtaken by that in the control at 116 DAFB. ABA concentration calculated from ABA content per fresh weight and moisture content in fruit flesh at 104 DAFB is shown in Table 3. The ABA concentration in the ABA-applied
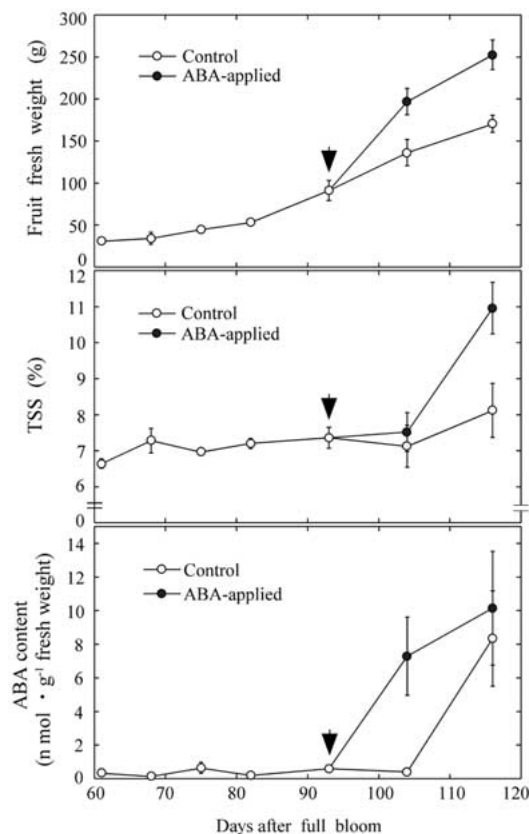
Figure 4. Effects of an exogenous ABA application on growth, total soluble solid content (TSS), and ABA content in developing peach fruit. Arrows show date of the ABA application. Each point represents a mean of six replications for fruit weight and TSS, and a mean of three replications for ABA content, ± standard error.

fruit was $8.88 \pm 2.87 \times 10^{-6}$ M, a value similar to that which most effectively stimulated total and PCMBS-sensitive uptake of ^{14}C-sugars into flesh discs and more than 17 fold of that in the control. Moisture content in fruit flesh was not different between the ABA-applied fruit and the control at 116 DAFB (data not shown), as well as at 104 DAFB (Table 3).

4. Discussion

From the ^{14}C-sugar uptake experiments, it is indicated that the total and PCMBS-sensitive uptake of sorbitol, fructose, and glucose into flesh discs were effectively promoted by ABA at 10^{-5} M (Figure 2). Enhancement

Table 3. ABA concentration in peach fruit flesh at DAFB 104 (11 days after an exogenous ABA application), calculated from ABA content and moisture content in fruit flesh. Values are means of three replications ± standard error

	Moisture content (%)	ABA concentration in fruit flesh (M)
Control	82.72 ± 0.74	$0.50 \pm 0.04 \times 10^{-6}$
ABA-treated	82.36 ± 0.67	$8.88 \pm 2.87 \times 10^{-6}$

of sorbitol PCMBS-sensitive uptake was much higher than that of glucose and fructose. PCMBS-insensitive uptake of sorbitol and glucose was enhanced in the presence of ABA at 10^{-4} M, although that of fructose was enhanced by ABA at 10^{-5} M and 10^{-6} M. The stimulatory effect of ABA on PCMBS-insensitive uptake was most clearly observed in sorbitol. These results indicate that PCMBS-sensitive and PCMBS-insensitive uptake of sorbitol, fructose, and glucose into peach flesh discs are stimulated by ABA at different concentrations, and the enhancement of both PCMBS-sensitive and PCMBS-insensitive uptake by ABA was the most significant for sorbitol. It was reported, however, that in flesh discs of strawberry and melon fruits ABA at 10^{-4} M, 10^{-5} M, and 10^{-6} M did not stimulate PCMBS-sensitive uptake of sucrose, glucose, and fructose, but ABA at 10^{-5} M stimulated PCMBS-insensitive uptake of each sugar [18, 19].

It is important to consider how and where PCMBS-sensitive and PCMBS-insensitive uptake is occurring in the flesh tissue. PCMBS-sensitive uptake is considered as carrier-mediated uptake, since PCMBS is an inhibitor of sugar carrier protein [7]. Carrier-mediated transport consists of two types of transport, i.e. active carrier-mediated transport, which is energy-dependent and H^+-coupled transport, and facilitated diffusion, which is sugar concentration gradient-dependent. But in this experimental system it can not be determined which of these two types of carrier-mediated uptake is corresponding to the PCMBS-sensitive uptake. It is known that PCMBS can not penetrate into cells across the plasma membrane [3, 9]. Thus, in experiments using tissue discs in a medium with PCMBS, such as the present study, it is likely that the carrier-mediated (PCMBS-sensitive) and PCMBS-insensitive uptake of sugars occur only across the plasma membrane. Therefore these results suggest that ABA stimulates carrier-mediated (PCMBS-sensitive) sugar uptake into flesh

cells across the plasma membrane. On the other hand, exogenously applied ABA acts not only on the plasma membrane but also on the tonoplast in tissue discs, because ABA has high membrane permeability in acid conditions (below pH 6.5) [6]. Therefore, it is possible that in this study the PCMBS-insensitive sugar uptake across the plasma membrane was enhanced indirectly through the stimulatory effect of ABA on active carrier-mediated uptake across the tonoplast [20]. Active carrier-mediated sugar uptake into vacuoles may play a role for facilitating PCMBS-insensitive sugar uptake into the cells, which may facilitate diffusion, by keeping a concentration of an imported sugar in cytoplasm relatively lower [5, 10]. By facilitated diffusion, a sugar moves down a sugar concentration gradient across a membrane through a carrier protein. PCMBS-insensitive sugar uptake is thought to be a low-affinity carrier-mediated uptake system, which is different from the PCMBS-sensitive carrier-mediated uptake system, rather than simple diffusional uptake [7, 15]. Thus the PCMBS-insensitive uptake may be considered as facilitated diffusional uptake across the plasma membrane regulated by active carrier-mediated uptake across the tonoplast. Sugar metabolism in the flesh cells might also affect the PCMBS-insensitive uptake of a sugar into the cells, because sugar metabolism can maintain a lower concentration of an imported sugar in the cytoplasm [5, 10].

From these considerations, it may be suggested that in this study carrier-mediated uptake of sugars across the plasma membrane was stimulated by ABA at 10^{-5} M, and PCMBS-insensitive uptake of sorbitol and glucose across the plasma membrane was stimulated indirectly by ABA at 10^{-4} M through the stimulation of the active carrier-mediated uptake across the tonoplast. On the other hand, Ofosu-Anim et al. [18, 19] suggested that ABA acted on carrier-mediated uptake across the tonoplast rather than across the plasma membrane in flesh tissue of strawberry and melon. Yamaki and Asakura [26] indicated that ABA stimulated active uptake of sorbitol into vacuoles isolated from apple fruit flesh, which could support our suggestion, but not into the protoplast. A mechanism of enhancement of sugar accumulation by ABA in peach fruit flesh may be different from those in fruit flesh of strawberry, melon, and apple, although further studies are required on sugar uptake into the vacuole and protoplast isolated from peach flesh. In this study, it is also suggested that an optimum ABA concentration stimulating carrier-mediated sugar uptake may be

different between the plasma membrane and the tonoplast in peach fruit as mentioned above. The difference might be caused by dilution or degradation of the applied ABA in the cytoplasm, since ABA concentration in the cytoplasm will directly affect sugar uptake across the tonoplast. A different sensitivity of sugar uptake system to ABA between across the plasma membrane and across the tonoplast might be also responsible for this difference.

In this study, stimulatory effects of ABA on sugar uptake were most prominently observed for sorbitol. Since sorbitol is the main translocated sugar in peach, it is considered that the stimulation of sorbitol uptake into flesh cells and vacuoles is mainly responsible for enhancement of sugar accumulation in peach fruit by ABA. Carrier-mediated and PCMBS-insensitive uptake of glucose and fructose into flesh discs were, however, observed both in the presence and the absence of ABA at rates which were not so different from that of sorbitol. These results suggest that uptake of these reducing sugars, which are considered to be converted from sorbitol in peach fruit [13, 14, 16], into flesh cells and vacuoles may also contribute to sugar accumulation in the peach fruit.

In the experiments on sorbitol efflux from flesh discs, the velocity constants across the tonoplast were lower than that across the plasma membrane (Table 1), which is consistent with the idea that the tonoplast is the most resistant barrier to sugar release [18, 19, 27]. In this study, sorbitol efflux from flesh discs across the tonoplast decreased with ABA at 10^{-5} M, but that across the plasma membrane was not affected by ABA. It is considered that the restriction of sugar efflux from flesh discs may not only merely show the membrane permeability, but may also reflect the sugar uptake across the membranes [20]. With this idea and the results of the ^{14}C-sugar uptake experiments in this study, it is possible that restriction of sorbitol efflux from peach flesh discs across the tonoplast in the presence of ABA reflected the enhancement of sorbitol uptake across the tonoplast into the vacuole by ABA. These results are not in agreement with Ofosu-Anim and Yamaki [18, 19] who indicated that efflux of sucrose, glucose, and fructose from flesh discs of strawberry and melon fruits were restricted by ABA at 10^{-5} M but the restriction site was the plasma membrane rather than the tonoplast. This disagreement may be caused by the differences in mechanism of stimulatory effect of ABA on sugar accumulation between peach fruit and that of strawberry and melon fruit.

In this study, restriction of sorbitol efflux by ABA at 10^{-5} M occurred in the tonoplast, whereas the stimulation of ^{14}C-sorbitol uptake by ABA at the same concentration occurred in the plasma membrane. A reason for the difference is not clear, but it may be that the effective concentration of ABA stimulating sugar transport is different in the two experimental systems due to a difference in some conditions of the incubations such as osmolarity and temperature of the media. It is probable, therefore, that the restriction of sorbitol efflux across the tonoplast by ABA at 10^{-5} M is related to the stimulation of PCMBS-insensitive uptake of ^{14}C-sorbitol by ABA at 10^{-4} M, which was suggested in this study to be regulated by the uptake across the tonoplast.

Sorbitol efflux from flesh discs across the tonoplast tended to increase with two different inhibitors of carrier-mediated transport, especially with CCCP (Table 2). Although, data are widely variant possibly due to the relatively late stages of the fruit used for this experiment (97 DAFB). The differences in the value of the controls for the two experiments (Tables 1 and 2) may be caused by a difference in fruit stage used for these experiments. CCCP is a highly membrane permeable metabolic uncoupler that indirectly inhibits H$^+$-coupled carrier-mediated transport by reducing the electrochemical potential of H$^+$ across the membrane [4, 12, 18, 19]. Thus, these results imply the presence of active carrier-mediated uptake of sorbitol across the tonoplast, which is consistent with the idea discussed from the results of ^{14}C-sugar uptake in this study. However it is unclear why the inhibitors did not stimulatively affect the sorbitol efflux across the plasma membrane whereas the results of ^{14}C-sugar uptake suggested the presence of carrier-mediated uptake of sorbitol also across the plasma membrane. This contradictory result might be caused by the difference in the conditions of the two experimental systems or a difference in the carrier-mediated uptake system between across the plasma membrane and across the tonoplast.

ABA application to developing peach fruit at 93 DAFB significantly increased fruit weight and TSS in fruit flesh (Figure 4). ABA content per fresh weight in fruit flesh was considerably increased by ABA application at 104 DAFB (Figure 4), simultaneously with the increase in fruit weight. On the same date, TSS in ABA-applied fruit did not increase. It is considered, however, that sugar accumulation in fruit was actually stimulated by ABA at 104 DAFB, because fruit growth increased in the ABA application

without any changes in TSS and moisture content in fruit flesh on the same date (Table 3). This means that the total sugar content of the fruit increased. ABA concentration in flesh of ABA-applied fruit at 104 DAFB was $8.88 \pm 2.87 \times 10^{-6}$ M (Table 3), which is fairly close to 10^{-5} M, the ABA concentration which effectively stimulated ^{14}C-sugars uptake into flesh discs. In contrast, ABA concentration in flesh of the control fruit on the same date was $0.50 \pm 0.04 \times 10^{-6}$ M, much lower than 10^{-6} M, the ABA concentration which failed to show any stimulatory effect on total uptake of ^{14}C-sorbitol and glucose into flesh discs. These results indicated that an optimum ABA concentration stimulating sugar uptake into flesh tissue obtained by *in vitro* experiments was similar to the concentration in flesh tissue which enhances sugar accumulation in developing fruit *in vivo*.

Activities of carrier-mediated sugar transporters are determined by the activities of the sugar carrier protein, H$^+$-ATPase or H$^+$-inorganic pyrophosphatase (H$^+$-PPase); the latter affects H$^+$-coupled active carrier-mediated transports (proton-sugar symport) by producing an electrochemical potential of H$^+$ across the membrane [4]. Little is known, however, about the proton-sugar symport in fruit tissues. Yamaki and Asakura [25] indicated that sorbitol uptake into the protoplast isolated from apple fruit flesh occurred via a proton-sorbitol symport and the optimum pH was 6.0. The optimum pH is coincident with that of the carrier-mediated uptake of sorbitol by flesh discs in this study (Figure 1B), implying the existence of a proton-sorbitol symport in the plasma membrane of the peach flesh cells. The stimulative tendency of sorbitol efflux across the tonoplast with the metabolic uncoupler, CCCP (Table 2), might be caused by its inhibitory effect on the uptake via a proton-sorbitol symport in the tonoplast. It has been suggested that activities [1] and the expression of genes and proteins [11, 17] of H$^+$-ATPase and H$^+$-PPase are regulated by ABA in some plant tissues. Recently, it was also indicated that a cDNA of hexose carrier protein expressed during ripening in grape berry contains potential ABA-responsive elements in the promoter sequence [8]. From these results, it is probable that ABA regulates sugar accumulation in peach flesh tissue by acting on these proteins which constitute the proton-sugar symport system.

In conclusion, it is indicated that sugar accumulation in peach fruit flesh is effectively stimulated by ABA at 10^{-5} M both *in vitro* and *in vivo*, and ABA stimulates uptake of sugars, especially sorbitol, into

the flesh by enhancing the carrier-mediated transport possibly across both the tonoplast and the plasma membrane. However, the mechanism of this regulation of the membrane transport by ABA is still obscure. Further studies are required to examine the effects of ABA on sugar accumulation in peach fruit at the molecular level.

Acknowledgement

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Soil moisture tension and nitrogen fertilization on cantaloupe melon: Part II

O. Perez-Zamora, M.R. Cigales-Rivero, M. Orozco-Santos and K.G. Perez-Castro

Campo Expt Tecoman, Programa Hortalizas, Apartado Postal Num 88, Tecoman 28100, Colima, Mexico
Campo Expt Tecoman, Programa Hortalizas, Tecoman 28100, Colima, Mexico
Univ Colima, Ctr Univ Invest & Desarrollo Agropecuario, Tecoman 28100, Colima, Mexico
Univ Guadalajara, Ctr Univ Sur, Ciudad Guzman, Jalisco, Mexico

Abstract

In 2002, a field experiment was carried out to increase yield and quality of export marketable Cantaloupe melon fruit (Cucumis melo L.) in a Typical Argisustoll soil of Colima, Mexico. Four N doses were applied to the soil (0, 80, 120 and 160 kg ha(-1)) and three target soil moisture tensions (10, 25, and 45 kPa) were used. During crop development, fertilization of 90 kg N ha(-1) was applied with irrigation to all the treatments from blossoming to first harvest. Application to the soil of 80 kg plus 90 kg N ha(-1) in the irrigation water had a positive effect on total fruit yield of categories 6 and 9.
Fruit yield response to soil moisture tension was linear; the highest yield was reached with 10 kPa of soil moisture tension. The soil water tension calculated for zero yield condition was 191 kPa. It was calculated that yield decreased at a rate of 0.406 Mg ha(-1) for each kPa of diminished soil moisture. The interaction N x soil moisture tension was significant (p less than or equal to 0.05) and affected fruit yield, as well as the number of aborted and matured fruits; however, when N was lacking, soil moisture did not affect yield. The application of 80 kg N ha(-1) (170 g total N) and 10 kPa moisture tension yielded 15 and 27% more fruit than the same N doses applied at 25 and 45 kPa of soil moisture tension. The application to the soil of 120 kg N ha(-1) (210 kg total N) at a tension of 10 kPa increased yield 13.5% and 24.6% more than at 25 and 45 kPa. Calculated yield with a multiple regression equation (R-2=0.96) was 81.1 Mg ha(-1) with N=142 kg ha(-1), and 10 kPa of soil moisture, which maximized (o)Brix content (10.0) and yield.

Influence of girdling and gibberellic acid on yield components, fruit composition, and vestigial seed formation of 'Sovereign Coronation' table grapes.

Reynolds, AG and de Savigny, C

Abstract

Vestigial seeds of 'Sovereign Coronation' table grapes frequently form partial seedcoats that are perceptible during consumption. This problem was addressed through cane/cordon girdling and gibberellic acid (GA(3)) sprays. 'Sovereign Coronation' vines were subjected to one of five treatments [untreated control; cane/cordon girdled; 15 ppm GA(3) at bloom (GA1); GA1 + 40 ppm GA(3) 14 days later (GA2); GA2 + 40 ppm GA(3) 14 days later]. GA(3) had no effect on yield or clusters per vine, but postbloom GA(3) treatments increased cluster and berry weights and reduced berries per cluster. Fruit maturity was not consistently affected by the treatments, although slight increases in Brix and pH and decreases in titratable acidity (TA) were associated with postbloom GA(3) treatments. Use of postbloom GA(3) applications reduced the number and weight of vestigial seeds with developed seedcoats, and reduced the number and weight of undeveloped seeds as well in 2 of 3 years. Girdling increased cluster and berry weights, decreased Brix and TA, and increased pH. Transpiration rate of leaves on girdled vines was also higher than control vines on one sampling date. Data suggest that use of bloom and postbloom GA(3) applications to 'Sovereign Coronation' may reduce the formation of perceptible vestigial seeds and thus improve the marketability of this cultivar.

Sugar accumulation **enhanced by osmoregulation in Satsuma mandarin fruit**
Yakushiji H, Nonami H, Fukuyama T, Ono S, Takagi N, Hashimoto Y
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Abstract:
The effect of water stress induced to enhance **sugar accumulation** in Satsuma mandarin (Citrus unshiu Marc,) fruit was investigated, Satsuma mandarin trees were subjected to water stress using mulch cultivation from late August to early December, In mulch treatment, soil was covered with double-layered plastic sheets that prevented rainfall from permeating the soil, but allowed water from soil to evaporate, The water status of soil, fine roots, pericarps, and juice vesicles was determined using the isopiestic psychrometer. As the severity of water stress increased, both water potential and osmotic potential of fine roots and pericarps significantly decreased in plants grown under mulch cultivation compared to well-watered trees, Although water potential and osmotic potential decreased, turgor of both roots and pericarps of the water stressed trees did not decrease under water stress conditions, Because turgor was maintained, osmoregulation occurred in Satsuma mandarin trees in response to water stress, The osmotic potential of juice vesicles in water-stressed fruit gradually decreased, and sugars accumulated in vesicle cells, Concentrations of sucrose, fructose, and glucose increased in fruit sap under water stress, and the acidity in the fruit juice increased, Furthermore, the total sugar content per fruit of water stressed trees was significantly higher than in fruit of well-watered trees, These results suggest that **sugar accumulation** in Satsuma mandarin fruit was not caused by dehydration under water stress but. rather that sugars were accumulated by active osmoregulation in response to water stress, When sugar components in osmoregulated fruit were analyzed, it was found that monosaccharides, i,e,, glucose and fructose, were largely responsible for active osmoregulation in fruit under mater stress conditions.

KeyWords   Plus:
LOW WATER POTENTIALS, OSMOTIC ADJUSTMENT, PRESSURE PROBE, GROWTH, PLANTS, TURGOR, STRESS, MAIZE, LEAF, STEM

Addresses:
EHIME UNIV,COLL AGR,MATSUYAMA,EHIME 790,JAPAN
FRUIT TREE RES STN,AKITSU BRANCH,AKITSU,HIROSHIMA 72924,JAPAN
EHIME PREFECTURE FRUIT TREE EXPT STN,MATSUYAMA,EHIME 79101,JAPAN

Abstract:

Mechanisms of **sugar accumulation** in response to drought stress in Satsuma mandarin (Citrus unshiu Marc.) fruit were investigated. Predawn leaf water potentials averaged -0.35 MPa for well-watered, -0.60 MPa for moderately drought-stressed, and -1.00 MPa for severely drought-stressed glasshouse-grown 3-year-old trees. Fruit peel turgor and fruit growth of the moderately drought-stressed trees recovered to a similar value to that of the well-watered trees. Photosynthetic rates and stomatal conductance of both moderately and severely drought-stressed trees were significantly lower than those of the well-watered plants. However, the total sugar content per fruit of moderately drought-stressed trees was the highest among the drought treatments. A C-13-labeling experiment showed that C-13 distribution in fruit grown under the moderately drought-stressed condition was the highest. These findings indicate that **sugar accumulation** in fruit was caused by an increase in translocation of photosynthates into fruit, especially into the juice sacs, under drought stress.

Author Keywords:

Citrus fruit, fruit growth, osmotic adjustment, low water potential, translocation, turgor, sink, photosynthates

KeyWords Plus:

LOW WATER POTENTIALS, OSMOTIC ADJUSTMENT, LEAVES, GROWTH, PHOTOSYNTHESIS, OSMOREGULATION, TRANSLOCATION, CITRUS, TURGOR, ROOTS

Addresses:

Yakushiji H, Natl Inst Fruit Tree Sci, Persimmon & Grape Res Ctr, Hiroshima 7292494, Japan

Natl Inst Fruit Tree Sci, Persimmon & Grape Res Ctr, Hiroshima 7292494, Japan

Ehime Univ, Coll Agr, Matsuyama, Ehime 7908566, Japan