Pruning effects on Pinot Noir vines in Tasmania (Australia)

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Summary

Effects of pruning on yield, three basic measures of fruit composition and cane carbohydrate concentration in *Vitis vinifera* cv. Pinot Noir vines were investigated in a cool climate wine area of Tasmania (Australia) from 2002 to 2004. Four pruning treatments comprising of 10, 20, 30 and 40 nodes per vine were imposed on 8-year-old vines. Bunch number, bunch weight, berry number and berry weight were measured. Cane samples were collected in the second year for tissue carbohydrate analysis. Fruit was analysed for pH, skin colour and sugar level. For each of the yield components measured there was a significant year effect but no interaction between year and pruning treatment. In each of the three years pruning to a higher bud number decreased the number of bunches per bud but no other yield component. Pruning treatment significantly affected starch content of the winter canes but not soluble carbohydrate levels. Pruning affected both pH and colour of fruit but not sugar concentration.

K e y   w o r d s : pruning, yield, carbohydrate.

Introduction

The yield of vines is dependent, in part, on the number of buds left on the vine at pruning. After bud burst, bunch number on the developing shoots determines potential crop, and leaf area determines how much crop can be ripened (Petrie *et al*. 2000). Lighter pruning, in general, increases yield but it may also be associated with negative effects on fruit quality such as lower total soluble solids and modified pH and titratable acidity (Jackson *et al.* 1984). Higher bud numbers left at pruning do not always give a linear yield response (Wolpert *et al*. 1983), and the vine tends to compensate by reducing the number of buds producing shoots, the number of bunches per shoot and average bunch weight. Not all reports indicate higher yields following lighter pruning (Wolpert *et al*. 1983), and similarly, some high-yielding cultivars have been reported to compensate for heavy pruning by increasing the numbers of fruitful basal buds that burst (Turkington *et al*. 1980). From the considerable literature on pruning levels and yield in other cultivars, it is clear that different cultivars respond differently to different levels of pruning, within the same vine management system.

Variable yield has been raised as a major management concern in some cool climate areas (Dunn *et al*. 2004), but there is little published information on yield behaviour in response to either environment or management. The present trials were intended to study the effect of different pruning severities on various components of yield. Reserve carbohydrates in overwintering vines and yield influences on three basic measures of fruit quality were also examined.

Material and Methods

Eight-year-old Pinot Noir (clone D5V12) vines, planted at 1.6 x 2.3 m spacing on a vertical trellis 1.8 m high with a base wire at 1 m in a commercial vineyard, were used. Prior to the experiment the vines had been pruned to a 4 cane, Scott Henry system, with two fruiting wires, at 1.0 and 1.15 m. Regular pruning had been to one (2 bud) basal spur, and 10 potentially fruitful buds on each of the 4 canes. Vineyard records indicated a relatively high, by local standards, average annual yield of 10 t·ha⁻¹, for 8 years.

In the winter of 2001 4 pruning treatments comprising of 10, 20, 30 and 40 buds per vine commenced, and in each treatment a single two-bud-spur was also retained. The bud numbers were obtained by reducing the number of 10 bud canes to 1, 2 or 3 with a 4-cane-control equivalent to the established pruning system. Five replicates were used in single vine plots. Pruning treatments continued through the following two seasons and measurements were taken in each of the 2002, 2003 and 2004 cropping seasons. At commercial harvest all bunches were collected from each cane and any fruit rising from the spur was collected and retained separately. Bunches were counted and harvested fruit placed in sealed plastic bags and stored at -18 °C before being thawed, and weighed to determine bunch weight. Berries were then removed, counted and mean berry weight per bunch calculated. Berries were sieved to determine the proportion of small seedless (≤5 mm diameter) and large berries (≥6 mm diameter). These sizes were chosen to separate sizes according to the berry size differential generally termed "hen and chickens" (May 2004).

The number of shoots that developed from the buds laid down was recorded at pruning. In each year, trunk circumference was measured 20 cm below the crown and trunk cross-sectional area was calculated.

At pruning in the second year (2002) cane samples were collected for tissue carbohydrate analysis. Two 1 cm seg-

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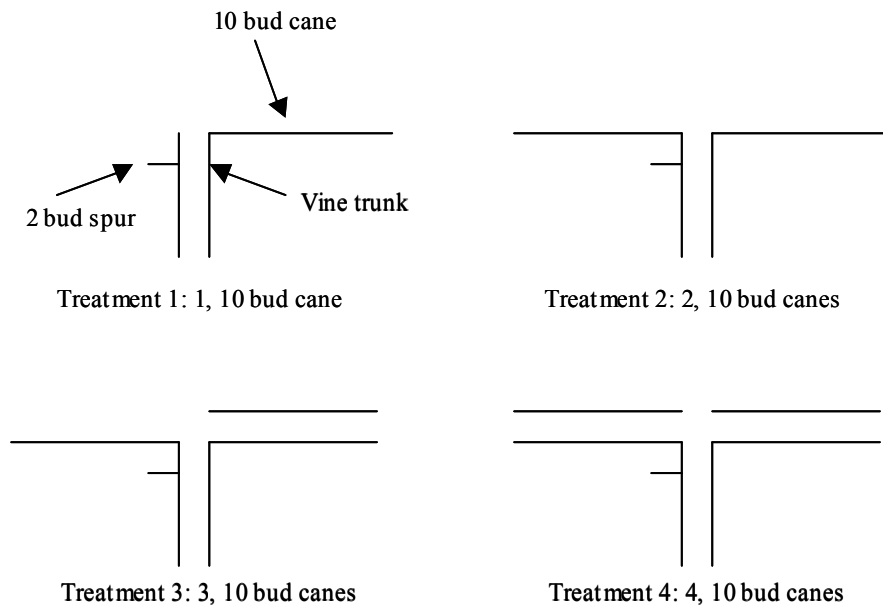
Diagram: Treatments imposed on vines, ranging from 10 to 40 bud canes.

ments were cut from the centre and apex of the right hand cane on each vine and the samples were stored at -18 ºC. Frozen samples were transferred to screw top vials and freeze-dried before being ground into a fine powder using a micro hammer mill fitted with a 1 mm sieve.

Carbohydrate analysis: Extraction and separation of carbohydrates from the freeze-dried cane tissue was based on the method of Lambrechts *et al.* (1994). Powdered freeze-dried samples (100 mg) were placed in centrifuge tubes and 5 ml of 80 % ethanol added, before incubation at 60 ºC for 1 h. The samples were then centrifuged for 10 min at 3000 rpm at 16 ºC (Beckman model J2-21 centrifuge). The supernatant was removed and stored and the pellet extracted twice more in the same way. The supernatants were then combined and stored at -18 ºC for soluble sugar assay and the pellets freeze-dried for later starch analysis.

The combined supernatant from the initial extraction was thawed, filtered and the ethanol removed using a rotary vacuum concentrator. The residue was made up to 2.5 ml with distilled water and washed twice with chloroform (5:8 v/v). The water phase was then used to determine soluble glucose, sucrose and fructose fractions using HPLC - MS. The column was a Waters High Performance Carbohydrate Cartridge, 4.6 mm x 250 mm, fitted with a guard cartridge of the same material. The mobile phase was 75 % methanol:25 % water, isocratic at 1.2 ml·min⁻¹. Sugars were detected by Atmospheric Pressure Chemical Ionisation mass spectrometry on a Finnigan LCQ ion trap MS. Retention times were determined from standard solutions, and calibration curves were generated over the expected concentration range.

Sugars were detected using negative ion adducts formed by post column infusion of 20 µl·min⁻¹ of 5 % formic acid in water. For the monosaccharides, tandem MS was used, with the M+ formate anion at 225.3 being isolated, activated at 25 % collision energy, and the subsequent

daughter at m/z 179 being further isolated and activated at 25 % collision energy with the final products at m/z 89, 119, 131 and 143 being used for quantitation. Sucrose was quantified by selected ion monitoring of the M+ formate anion at 387.3.

Starch levels were determined enzymatically using a total starch assay kit (Megazyme, Australia). The freeze-dried pellet retained from the soluble sugar assay was weighed into 20 ml glass test tubes with a 10.0 ml volumetric marking. Three ml of thermostable α-amylase in MOPS buffer (50 mM, pH 7.0) was added and mixed using a vortex mixer, prior to incubating at 100 ˚C for 6 min, vortexing vigorously again every 2 min. The tubes were then transferred into a 50 ˚C water bath and 4 ml of sodium acetate buffer (200 mM, pH 4.5) added followed by 0.1 ml of amyloglucosidase. The contents of the tube were vortexed and incubated at 50 ˚C for 30 min. After incubation the volume of the tube contents was adjusted to the 10.0 ml volumetric mark using distilled water. The tube was then vortexed and centrifuged at 3000 rpm for 10 min. Duplicate aliquots of the tube contents (0.1 ml) were transferred to the bottom of glass test tubes and 3.0 ml of GOPOD reagent (glucose oxidase, >12 000 units·l⁻¹; peroxidase, >650 units·l⁻¹; 4-aminoantipyrine, 0.4 mM) was added to each tube. The tubes were then incubated at 50 ˚C for 20 min and the absorbance read against a reagent blank at 510 nm.

Fruit quality: After weighing and size grading for yield component analysis, 20 randomly selected berries from each plot were stored at -18 °C for determination of pH, colour and total soluble solids.

A single 5 mm disc was excised from the side of each berry and the 20 discs for each plot combined and weighed. The skin discs were then freeze dried before being ground using a stainless steel mortar and pestle. The ground skins were extracted with 10 ml of cold 50 % methanol, adjusted to pH 1.0 and centrifuged at 9500 rpm for 20 min at 15 ºC. The supernatant was decanted and the precipitate extracted once

more in the same manner and the supernatants combined. The supernatants were then made up to a final volume of 50 ml and filtered through a membrane filter (0.45 µm). The total anthocyanins were assayed by measuring absorbance at 520 nm (MAZZA *et al.* 1999) and expressed as mg·g^{-1} of skins on a fresh weight.

To confirm that Pinot Noir only contained nonacylated anthocyanins (MAZZA 1999), analysis of anthocyanins in the skin extracts was performed using a Waters Alliance 2690 HPLC system equipped with a Waters 996 photodiode array detector. Separation was achieved on a Waters Xterra MS C18 column (5µm, 2.1 mm x 150 mm). For analysis, a flow rate of 0.3 ml.min^{-1} was used. Solvent A was methanol, and solvent B was 5 % (v/v) formic acid in water. A solvent gradient of 20:80 was used for 5 min followed by 40:60 at 23 min. A 20 µl sample was injected directly into the HPLC. Wavelengths from 200-600 nm were monitored, and chromatograms were generated at 520 nm.

The remainder of the berries from the bulk fruit sample for each plot was pressed and sieved to leave raw juice. The pH of the juice was then measured using a pH meter and total soluble solids measured as °Brix using a hand-held refractometer.

Experimental design and statistical analysis: All results were normally distributed (except for the proportion of viable buds) and untransformed data measured over the three years were analysed using a repeated measures ANOVA in the general linear models package of SPSS. Means were compared using least significant difference (LSD) calculated at P = 0.05 after the method of STEEL and TORRIE (1980).

For the cane carbohydrate data, there was no change in the 4 treatments by 5 replicates experimental design, but as samples were taken in one year only, repeated measures analysis was not required. These data were also normally distributed and were analysed using the univariate analysis of variance in the above package.

Linear regressions of treatment means for anthocyanins and pH were plotted against treatment means for the various yield components and vegetative growth also using SPSS.

Results

Although a seasonal yield effect was recorded (Fig. 1), with 2002 yields being significantly lower than in the two subsequent years, mean yield increased (P<0.05) with increased number of buds retained at winter pruning in each of the seasons. The yield increase was not proportional to the number of buds remaining, with a two-fold increase in total yield accompanying the four-fold increase in bud number from 10 to 40 buds in each of the three years. For each of the yield components measured, (bunch number, bunch weight, berry number and berry weight), there was a significant (P<0.001) year effect but there was no interaction (P>0.05) between year and treatments. Averaged over the three years there was a significant treatment effect (P<0.001) on bunch number per bud but there were no other treatment effects on the yield components (results not shown).
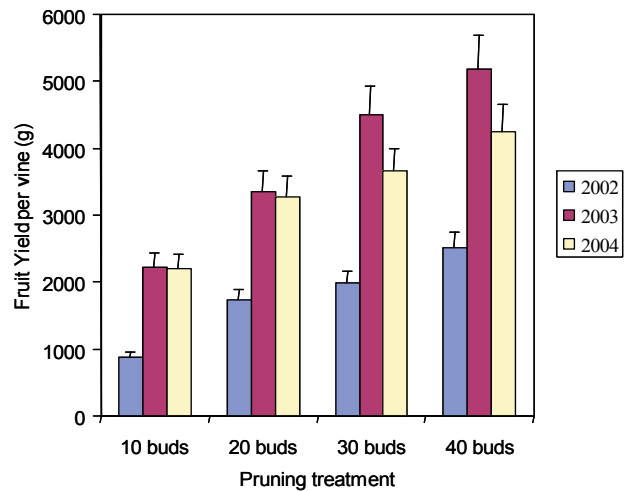

Fig. 1: Total fruit yield per vine at harvest for the 4 pruning treatments in the three years. Bars are standard errors of the means.
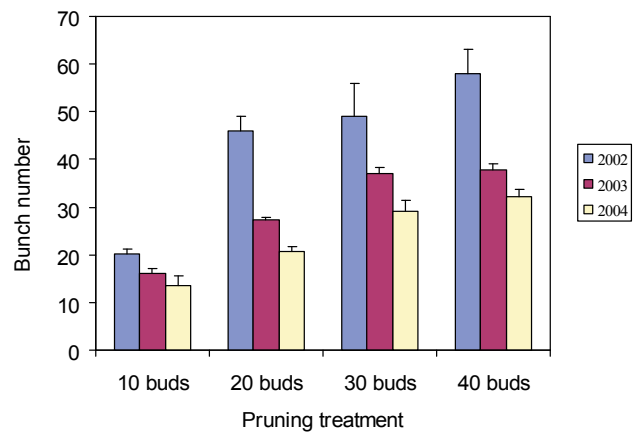

Fig. 2: Bunch number at harvest for the 4 pruning treatments. Vintages 2002-2004.

There was a significant increase in the number of bunches per vine with an increase in the number of buds remaining after pruning (Fig. 2), but the increase was not directly proportional to the number of buds retained. There was no significant difference (P>0.05) between the 10 bud (1.6 bunches per bud) and 20 bud (1.5 bunches per bud) treatments, but there was a significant reduction (P<0.05) to 1.2 bunches per bud for the 30 bud treatment and a further reduction to 1.0 bunch per bud for the 40 bud treatment. Overall, bunch number was markedly lower in 2003 and 2004 compared with the first year, 2002.

There was no significant (P>0.05) treatment effect on mean bunch weight, but there was a significant (P<0.05) year effect. Mean bunch weights were more than three times greater in 2003 and 2004 compared with 2002 for each of the 4 pruning treatments. Berry number and mean berry weight were not significantly affected by pruning (P>0.05) but showed a strong year effect (Tab. 1).

There were no significant treatment or year effects on pruning weights and although trunk cross-sectional area increased over the three years there was no treatment effect (results not shown).

Bunch weight, berry number, and berry weight of Pinot Noir across pruning treatments as influenced by year. There was no significant interaction between treatment and years for any of these yield components

	Year			
	2002	2003	2004	LSD
Bunch weight, g	41.2	129.9	144.7	23.2
Berry number	85.3	128.6	142.1	28.3
Mean berry weight, g	0.50	1.0	1.0	0.23

The number of viable buds (as a percentage of total in each treatment) was heavily skewed and analysis of variance was not carried out, but there was no apparent pattern between years or treatments. Treatment means across years were similar for all treatments with around 10 to 20 percent of buds failing to develop as shown in Tab. 2. There was a significant interaction ($P<0.05$) between years and treatments for the mean number of bunches per viable bud with greater bud numbers generally resulting in fewer bunches per viable bud. In the 10 bud treatment there was no significant difference in bunch number per bud across the three years, with the 20 and 40 bud treatments showing a marked decline from 2002 to 2003 (Tab. 2).

There was a significant ($P<0.05$) pruning treatment effect on the starch content of winter canes as shown in Tab. 3. Canopies with 10 and 20 buds per vine had a starch content significantly higher than the 30 and 40 bud canopy treatments, but there was no difference in starch content between the 30 and 40 bud treatments. Starch as a percentage of the total extracted carbohydrates was also significantly higher ($P<0.05$) in the 10 than the 20 bud treatments.

Fructose, glucose and sucrose showed no significant differences ($P>0.05$) between pruning treatments (results not shown).

Nonacylated anthocyanins, delphinidin 3-monoglucoside-*p*-coumarate, cyanidin 3- monoglucoside-*p*-coumarate, petunidin 3-monoglucoside-*p*-coumarate, peonidin 3-monoglucoside-*p*-coumarate, malvidin 3-monoglucoside-*p*-coumarate were present in the skin extracts, but no acylated anthocyanins were detected. There was a significant ($P<0.05$) treatment effect on both, pH and total anthocyanins, but not on sugar content (Tab. 3).

Across all treatments there was a significant ($P<0.001$) negative regression ($R^2 = 0.64$) between berry number per bunch and total anthocyanins (Fig. 3).

There were no other significant regressions between quality and yield or growth parameters, but there was a significant ($P<0.05$) positive regression between pH and total soluble solids (Fig. 4).

T a b l e 2

Percent budburst and bunches per viable bud for the 4 pruning treatments over three experimental years. Percent budburst was not statistically analysed (see text). There was a significant interaction between years and treatment for bunch data, and the LSD = 0.33 ($P = 0.05$) for comparisons within the table

Treatment no. of buds	2002		2003		2004	
	Bud burst %	Bunches per viable bud	Bud burst %	Bunches per viable bud	Bud burst %	Bunches per viable bud
1	100	2.01	92	1.80	78	2.06
2	88	2.38	85	1.63	83	1.65
3	85	1.26	86	1.01	80	1.61
4	87	1.83	86	1.09	87	1.09

T a b l e 3

Level of starch (mg·g⁻¹) on a dry weight basis in the cane during dormancy (from 2002) and anthocyanins (mg·g⁻¹ of berry skins FW), pH and total soluble solids (TSS; °Brix) of mature fruit (mean from 2002, 2003 and 2004) as affected by pruning treatments. ns = not significant

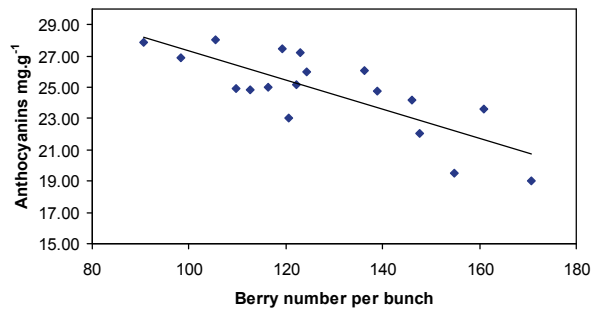|	Number of buds retained				
	10	20	30	40	LSD (P = 0.05)
Starch (mg·g⁻¹)	40.3	52.7	8.1	8.2	11.5
Anthocyanins (mg·g⁻¹)	4.11	5.24	6.09	5.41	0.79
pH	3.46	3.49	3.33	3.33	0.10
TSS (°Brix)	20.68	20.53	19.74	20.28	ns

Fig. 3: Relationship between berry number per bunch and antho-cyanins (mg·g^{-1} of berry skins FW), averaged over 2002, 2003 and 2004. y = -0.0931x + 36.649. R^2 = 0.62.
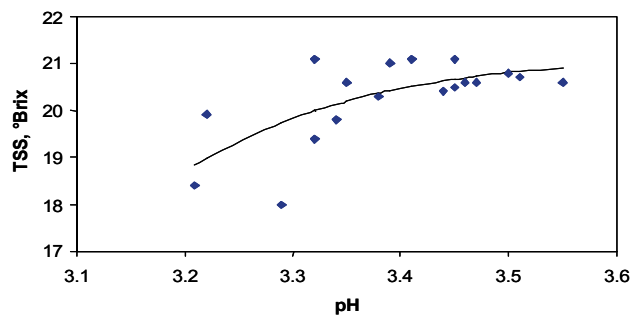

Fig. 4: Relationship between total soluble solids (TSS) and pH in berries, averaged over 2002, 2003 and 2004. R^2 = 0.47. y = 21.21(1-e$^{-6.09(x-2.85)}$).

Discussion

Industry generally accepts that Pinot Noir produces two bunches per bud and bunch number per bud would be expected to remain relatively constant as number of buds decreased rather than the significant increase observed here. The budburst and mean bunches per viable bud data indicate that the effect on bunch number was almost certainly due to variation in the number of bunches developing from viable buds, rather than any increase in "blind" buds at higher bud numbers.

These results concur with the findings of CLINGELEFFER and SOMMER (1994), who found a similar trend in a pruning experiment on Cabernet Sauvignon vines, ranging from 2 buds to 14 buds per vine. Bunches per bud decreased from 1.74 at the 2 bud treatment to 0.83 at the 14 bud treatment. Their results also demonstrated within-vine control of budburst and showed the strong influence of number of buds on percentage budburst. The authors did not comment on numbers of bunches per viable bud.

Trials conducted in New Zealand with 4 different cultivars by JACKSON and STEANS (1983-1984) suggested that yield increased with bud number up to a specific level, likely to be dependant on the cultivar and district. It was suggested that above this level, a further increase in bud number would be expected to have a limited effect on yield since the photosynthetic ability of the canopy would be reaching capacity, or the potential of the soil and roots to provide further water and nutrients would be nearing exhaustion.

Chardonnay and Gewürztraminer vines used in their study showed no peak in production, with yield paralleling bud numbers up to the treatment maximum of 84 buds per metre of row. Sauvignon Blanc yields increased until bud numbers reached 48 per metre, after which only marginal increases occurred. Müller Thurgau yields levelled off at about 40 buds per metre and Cabernet Sauvignon showed no response to increased buds per metre. In this study, it appears the Pinot Noir vines (clone D5V12) may be approaching the threshold yield level, with a pronounced decrease in the proportional increase in fruit yield per vine and the number of bunches per vine from 30 to 40 buds.

The carbohydrate analyses confirmed that bud number at pruning had an effect on stored starch in the following winter with starch levels being reduced by heavier crop load, but there was no effect on soluble carbohydrates. Starch levels were also comparable with the SOMMER and CLINGELEFFER (1995) study and there was no significant difference in vine size as trunk cross-sectional area or pruning weight in response to treatment. Consequently, carbohydrate concentration differences reflect real differences in total reserves in response to treatment, as it appears the vines compensated for the differences between pruning treatments. Values for total soluble carbohydrates were consistent with results of SOMMER and CLINGELEFFER (1995), and these figures were also within the range reported by RÜHL and ALLEWELDT (1990) and RÜHL and CLINGELEFFER (1993).

The higher reserve carbohydrate levels in the 10 and 20 bud canes compared with the 30 and 40 bud canes corresponded with higher bunches per bud overall and per viable bud in these treatments the following year.

PETRIE et al. (2000) showed that despite uncropped vines having a larger leaf area compared with cropped vines, uncropped vines did not produce a higher total dry weight than the cropped vines and therefore concluded that in uncropped vines carbon assimilation was sink-limited. It was suggested by FLORE and LAKSO (1989) that sink limitation might have been caused by end-product inhibition of photosynthesis. In cropped vines, PETRIE et al. (2000 a) measured similar photosynthetic rates in all cropping level treatments after veraison, suggesting that carbohydrate supply was source-limited. In the present study starch reserves showed a marked increase when fruit load was decreased with bud number reduction from 30 to 20, but there was no further increase with a further decrease in bud number.

EDSON et al. (1993) found that while management practices can influence partitioning of carbohydrate to fruit or vegetative growth, it is difficult to increase the absolute amount of dry matter produced through management practices. However, BENNETT et al. (2000) reported that reduced over-wintering carbohydrates due to defoliation of Chardonnay vines caused a reduction in both, inflorescence number per bud (shoot) and the flower number per inflorescence. The authors suggested that vines should be managed both for the current crop and for subsequent crops in order to maintain sufficient carbohydrate reserves for balanced growth and cropping from year to year. This result concurs with the results from the present trial, where fewer bunches per bud were apparent in treatments with lower overwintering starch concentrations.

In addition to the yield and yield/bud effects, pruning treatments also had a significant impact on two of the three measured aspects of basic fruit quality. Anthocyanin levels were lower in berry skins from vines pruned to low bud numbers, and there was a small but significant decrease in fruit pH at high bud numbers. Importantly however, there was no significant effect on total soluble solids, suggesting that the treatment effects impacted directly on the fruit quality parameters measured rather than indirect effects related to delayed fruit maturity at higher crop loads. These results, suggesting that some components of wine quality were enhanced in vines pruned to give higher yields, are contrary to a popular view that quality and yield are negatively correlated as reported in previous studies for the same production region (Farquhar 2002).

The decrease in anthocyanin content with increasing berry number suggests that (in this experiment) colour development may be related more to internal shading within the bunch than to shading within the canopy. In the present trial, there was no treatment effect on pruning weight suggesting that canopy shading was not influenced by treatment. This result concurs with Rojas-Lara and Morrison (1989) who reported that anthocyanin accumulation in the fruit was affected more by shading within the cluster than by canopy shading.

The significant decrease in pH between the 10/20 bud and 30/40 bud treatments is not consistent with the literature. In most studies such as that conducted by Jeong (2004), larger more shaded canopies resulted in a higher pH. Smart *et al.* (1988) and Archer and Strauss (1989) also reported an increased pH due to within-canopy shading of Cabernet Sauvignon vines. The change in pH with no apparent change in canopy again suggests that canopy architecture is not limiting basic fruit quality attributes in the vines of this study.

In summary, no relationship between fruit yield and quality (measured as anthocyanin content and pH) was recorded, contrary to some publications (Farquhar 2002, Kliewer 1970, 1977) and in agreement with others (Hunter and de la Harpe 1987). Hunter and de la Harpe (1987), using a similar range of bud numbers to the present study, showed a significant effect on colour in one year but not in the subsequent year. Although further investigation is needed, these results indicate that management of quality within this range of bud numbers does not need to be based on target yields. However, the cumulative effect of pruning to high bud numbers may result in depletion of starch reserves and have long-term effects on both, yield and quality.

Acknowledgements

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