

Spur pruning leads to distinctly different phenolic profiles of base sparkling wines than cane pruning

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Summary

Winter pruning is the principal method for regulating yield in viticulture. The aim of this work was to investigate the effectiveness of cane and spur pruning on yield, and on grape and wine composition. Cane and spur pruning were investigated in *Vitis vinifera* L. 'Pinot noir' and 'Chardonnay' vertically-shoot-positioned vines over three seasons. Effects on vine carbohydrates, yield components, leaf area, grape and base wine composition were determined. The canopies of spur pruned vines established more rapidly than cane pruned vines in the 2009/10 season, for both 'Pinot noir' and 'Chardonnay'. The canopies were denser under spur pruning than cane pruning. Pruning treatment had no effect on total yield for either cultivar in any of the three seasons. Total soluble solids (TSS) and titratable acidity were unaffected by pruning treatment, except in 2012 where TSS and pH were higher for spur pruned 'Chardonnay' vines. Apart from spur pruned 'Pinot noir' vine wood being higher in starch in the winter of 2011, overwintering starch and soluble sugar concentrations were not different between pruning treatments for 'Pinot noir' and 'Chardonnay'. Although not different in yield or basic fruit composition, fruit from spur pruned vines resulted in distinctly different phenolic profiles of base wines, with cane pruning appearing to negatively impact on the low molecular weight phenolics in the wine. The results presented here provide confidence that quality is not lessened, in fact could be improved, by shifting from the industry norm of cane to spur pruning for sparkling wine production in cool climates.

Key words: *Vitis vinifera*; pruning; sparkling wine quality; yield; carbohydrates.

Introduction

Pruning during winter when grapevines are dormant is an important cultural operation grapegrowers use to regulate yield. Pruning is a relatively simple and straightforward method that can be used to directly select the type of buds retained, as well as limit the number of buds per vine (MARTIN and DUNN 2000). Vines can be pruned leaving either a predominance of long canes (cane pruning) or short

spurs (spur pruning) on a perennial "cordon" structure. Despite some well documented advantages of spur pruning including more uniform shoot growth and higher capacity for the storage of reserves (BERNIZZONI *et al.* 2009), cane pruning continues to dominate in cool climate premium wine producing regions such as Tasmania. In such cool climates, where yields can be low, canes are preferred due to the most fruitful buds being retained, and less dense canopies being considered a lower disease risk, however such a preference is still based on anecdotal or observational evidence.

Stored carbohydrates are used by the vine in the spring to develop new shoots and the new canopy (VASCONCELOS *et al.* 2009), with a larger amount of old wood retained under the spur pruned system, it is expected that there is a higher capacity for storage of reserves as compared to a cane pruned system. There is little evidence of an effect on fruit or wine quality directly induced by pruning method, as opposed to final bud numbers and yield. JACKSON and LOMBARD (1993) reported that 'Pinot noir' aroma was reduced when vines were spur-pruned, despite yield and maturation being similar to cane-pruned vines.

Increased adoption of mechanized pruning is evident in sparkling wine vineyards and is driven by the need to reduce labor costs. To date mechanized spur pruning predominates in cool climates. Significant uncertainty exists regarding impacts on vine growth and the resulting fruit and wine chemistry. The aim of this study is to determine the influence of cane and spur pruning on vine carbohydrates, yield components, and leaf area as well as grape and base wine chemistry and spectral composition of *Vitis vinifera* L. cv. 'Pinot noir' and 'Chardonnay' for sparkling wine production. For the purpose of this study, the commonly used measures of pH, titratable acidity, total soluble solids (TSS) and UV spectra (JONES *et al.* 2014), will be the principal measures of fruit and wine composition. It was hypothesized that relatively greater canopy cover developed under spur pruning would lead to altered phenolic profiles in base wines.

Material and Methods

This study was conducted in 2010, 2011 and 2012 at a commercial vineyard planted with own-rooted 'Pinot noir' (clone D5V12) and 'Chardonnay' (clone I10V1) grape-

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vines in north east – south west oriented rows. The research site was located in the Coal River Valley, Tasmania (lat. 42° 45'S, long. 147° 23'E), Australia, and was planted in 1989. Vine spacing was 2.25 m x 1.5 m (inter- and intra-row respectively) and were trained to a vertical shoot positioned (VSP) trellis. Prior to the commencement of the study, vines were cane pruned to an average of 20 nodes per vine and with the exception of treatments, were managed commercially for the duration of the study.

Thirty vines of each cultivar were randomly selected from two separate vineyard blocks (one for 'Pinot noir', one for 'Chardonnay'). Fifteen vines of each cultivar were converted to spur pruned in June of 2009, and the remaining fifteen maintained as cane pruned. All vines were pruned to 20 buds each. Spur pruned vines had ten, two bud spurs and cane pruned vines had two, ten bud canes. Weather data was obtained from the closest Bureau of Meteorology site (Hobart Airport, Tasmania).

Canopy assessment occurred three times during the first season (November, December and January) then once in subsequent seasons (January). The technique used was the modified point quadrat method for estimating canopy structure of grapevines as described by PONI *et al.* (1996). Five vines per treatment, for each cultivar, were randomly selected to perform the assessment.

A sample of 30 leaves (sampling across all leaf ages) per cultivar were picked from approximately fifteen different vines during each canopy assessment, scanned on a high resolution desktop scanner and analyzed using the computer image analysis program WinFOLIA Basic (Regent Instruments Inc., Canada) to determine total leaf area (cm²) from which the average leaf area (m²) was calculated.

Yield and cluster number were recorded at harvest and average cluster weights calculated. Prior to crushing, a 200 berry sample was taken and was weighed fresh for mean berry weight data. A further 200 berry sample was frozen for later phenolic analysis. Berries were sampled from the top, middle and bottom of the clusters. Fruit was pressed in a flatbed whole cluster water bladder press and a juice sample taken for total soluble solids (TSS) analysis using a handheld refractometer (Vintessential manufactured product FG103/113, China) to measure °Brix, which was expressed as °Baume (°Be; °Brix ÷ 1.8) (HAMILTON and COOMBE 2004). Titratable acidity (TA) and pH were measured on fresh juice using a 785 DMP Titrimo autotitrator (Metrohm, Switzerland).

For each treatment, standard protocol winemaking was used for each batch of fruit, according to the method of KERSLAKE *et al.* (2013). Wine samples were clarified by centrifugation, diluted 1/10 in 1M HCl, then scanned from 200-800 nm with a Genesys 10S (Thermo, USA) spectrophotometer to collect phenolic spectral fingerprints (Kerlake *et al.*, 2013). Total phenolics were calculated using absorbance at 280 nm (ILAND 2000). Caffeic acid (Sigma Aldrich) was prepared as a 100 µg·L⁻¹ solution in 50 % ethanol, then diluted 1/10 in 1M HCl for scanning.

Before pruning in winter 2010 and 2011, cane wood samples were obtained. For the cane pruning treatment, the second shoot from the arm to the right hand side of

the trunk was removed, and for the spur pruned vines, the shoot arising from basal bud of the second spur on the cord on the right hand side of the trunk was selected. Wood samples were frozen at -20 °C, freeze-dried and then stored at -20 °C for later extraction and analysis of carbohydrates.

After drying, cane samples were cut into 1 cm segments. Samples were ground to be able to pass through a 1 mm sieve using an IKA Cutting Mill (A11 basic Analytical mill) followed by a Retsch MM200 Ball Mill. Extraction and separation of carbohydrates from the freeze-dried cane tissue was based on the method described by JONES *et al.* (2013), where starch levels were determined enzymatically using a total starch assay kit (model K-TSTA; Megazyme Pty Ltd, Sydney, Australia (AOAC Method 996.11, AACC Method 76.13).

For soluble sugar extraction, using a 5 mL Hamilton syringe with a removable needle, the 9 mL of combined supernatant liquid was filtered through a 0.45 µm filter into a glass test tube. The filter was rinsed with approx. 0.5 mL of 80 % ethanol. 50 µM of trehalose standard (250.3 mg·25 mL⁻¹) was added to give a final concentration of 200 ppm. The test tubes were placed in a boiling water bath and the liquid evaporated to less than 1 mL. This was made up to 2.5 mL with distilled water and filtered through a SepPak Accell plus CM Cartridge into a 1.5 mL Eppendorf centrifuge tube. The tubes were centrifuged at 10,000 rpm for 10 min, using an Eppendorf bench top centrifuge. The supernatant liquid was filtered through a 0.45 µm and a 0.2 µm filter in tandem, into an HPLC vial. All samples were filtered and frozen and analysed for soluble fructose, glucose and sucrose, using HPLC-MS according to the method reported in HEAZLEWOOD *et al.* (2006).

Analytical canopy data was analyzed non-parametrically using Mann-Whitney U test. Yield, fruit and wine data was analyzed by analysis of variance (ANOVA) in SPSS. Spectral data was analyzed by principal component analysis (PCA) using The Unscrambler, version 10.1 (Camo, Norway). Principal component analysis was performed on raw spectra using a wavelength range of 230-430 nm at one nm intervals. Linear discriminant analysis of pruning treatments was performed using the PCA scores.

Results

The three seasons differed climatically; the 2011 season was cooler than the 2010 and 2012 seasons, and the 2012 season was drier than the other two seasons (Tab. 1).

Table 1

Hobart Airport weather station data for the three experimental seasons

	Vintage		
	2010	2011	2012
Mean January temp °C	23.8	22.7	23.7
Growing degree days (Oct-Apr)	1291.1	1110	1247.8
Growing season rain (mm) (Oct-Apr)	331.6	345.4	296.6

The canopies of spur pruned vines established more rapidly than cane pruned vines in the 2009/2010 season, for both 'Pinot noir' and 'Chardonnay', as indicated by the number of effective insertions on the three measuring dates (Tabs 2 and 3). For both cultivars there were no gaps recorded on any of the measurement dates under the spur pruning system, whereas under cane pruning there were a considerable number of gaps recorded for the November and December dates. The canopies were denser under spur pruning as indicated by the higher number of leaf contacts for 'Pinot noir', however for 'Chardonnay' the number of leaf contacts were not different for the latter two dates. The final canopy appeared to be similar in all three seasons for both cultivars (Tabs 2, 3 and 4).

Pruning treatment had no effect on total yield for either cultivar in any of the three seasons ('Pinot noir' mean total yields were 2.6 (cane pruned 2.8 and spur pruned 2.5) in 2010, 3.3 (cane pruned 3.4 and spur pruned 3.2) in 2011 and 1.8 kg·vine⁻¹ (cane and spur pruned both 1.8) in 2012;

and 'Chardonnay' total yields were 1.45 (cane pruned 1.4 and spur pruned 1.5) in 2010, 2.3 (cane pruned 2.2 and spur pruned 2.4) in 2011 and 0.85 kg/vine (cane pruned 0.8 and spur pruned 0.9) in 2012). For 'Pinot noir' there was a greater number of clusters under spur pruning in 2011 and 2012, but there was no difference between cane and spur pruning in 2010 (Tab. 5). In all three seasons, 'Pinot noir' cluster weights were lower under spur pruning. For 'Chardonnay' a similar trend was observed, with more clusters in all seasons, and in 2010 cluster weights were lower under spur pruning (Tab. 5).

Basic fruit quality was unaffected by pruning treatment, except in 2012, where TSS were lower and pH was higher for spur pruned 'Chardonnay' vines ('Pinot noir' mean TSS was 18.9 (cane pruned 18.9, spur pruned 19.0) for 2010, 17.1 (cane pruned 17.4, spur pruned 16.8) for 2011, and 19.9 (cane pruned 20.0, spur pruned 19.8) for 2012; and 'Chardonnay' mean TSS was 18.1 (cane pruned 18.2, spur pruned 18.1) for 2010, 17.4 (cane pruned 17.5,

Table 2

Canopy parameters calculated for 'Pinot noir' grapevines in 2009/2010 season. Data represent the means of five vine-replicates for the fruiting zone. (¹Calculated as percent of total insertions per vine (40) resulting in any contact)

Date	Pruning	Effective insertions (%)	Leaf contacts	Cluster contacts	Gap %	Leaf number	Average leaf area (cm ²)	Total leaf area (m ²)
25 Nov	Spur	100	103	6	0	98	141.3	13.85
	Cane	77	65	7	23	71	151.6	10.76
	Sig.	0.008	0.008	ns	0.008	0.008	0.008	ns
22 Dec	Spur	100	120	6	0	264	121.8	32.15
	Cane	80	78	8	20	204	144.2	29.41
	Sig.	0.008	0.008	ns	0.008	0.008	0.008	ns
28 Jan	Spur	100	117	8	0	180	131.9	23.74
	Cane	97	96	11	3	156	160.8	25.09
	Sig.	0.032	0.008	ns	0.008	0.008	0.008	ns

Table 3

Canopy parameters calculated for 'Chardonnay' grapevines in 2009/2010 season. Data represent the means of five vine-replicates for the fruiting zone. (¹Calculated as percent of total insertions per vine (40) resulting in any contact)

Date	Pruning	Effective insertions (%)	Leaf contacts	Cluster contacts	Gap %	Leaf number	Average leaf area (cm ²)	Total leaf area (m ²)
25 Nov	Spur	100	92	2	0	55	145.75	8.02
	Cane	65	56	3	35	51	149.63	7.63
	Sig.	0.008	0.008	ns	0.008	ns	ns	ns
22 Dec	Spur	100	116	8	0	264	123.08	32.49
	Cane	80	92	7	20	201	142.28	28.60
	Sig.	0.008	ns	0.008	0.008	0.008	ns	0.008
28 Jan	Spur	100	147	10	0	276	117.54	32.44
	Cane	100	103	8	0	216	134.58	29.07
	Sig.	ns	ns	0.008	ns	0.008	ns	0.008

Table 4

Canopy parameters calculated for 'Pinot noir' and 'Chardonnay' grapevines in the 2011 and 2012 seasons. Measurements were taken on the 18th January 2011 and 21st January 2012. Data represent the means of five vine-replicates for the fruiting zone. (¹Calculated as percent of total insertions per vine (40) resulting in any contact)

		Year	Effective insertions (%)	Leaf contacts	Cluster contacts	Gap %	Leaf number	Average leaf area (cm ²)	Total leaf area (m ²)
Pinot noir	Spur	2011	100	117	9	0	176	131.9	23.74
	Cane	2011	98	96	10	2	162	160.8	21.82
		Sig.	<0.01	<0.01	ns	<0.05	<0.01	<0.01	ns
	Spur	2012	100	123	8	0	171	127.4	22.63
	Cane	2012	99	91	8	1	159	163.4	20.09
		Sig.	<0.05	<0.01	ns	<0.01	<0.01	<0.01	ns
Chardonnay	Spur	2011	100	147	10	0	262	117.54	32.44
	Cane	2011	100	142	8	0	253	120.3	31.45
		Sig.	<0.05	<0.01	ns	<0.01	<0.01	<0.01	ns
	Spur	2012	100	142	8	0	253	120.3	31.45
	Cane	2012	100	107	9	0	225	136.4	28.51
		Sig.	ns	<0.01	ns	ns	<0.01	<0.01	ns

Table 5

Influence of pruning treatment on yield and yield components of 'Pinot noir' and 'Chardonnay' in each of the three experimental seasons

		Cluster number			Cluster weight (g)		
		2010	2011	2012	2010	2011	2012
Pinot noir	Cane pruned	23	26	17	123	131	106
	Spur pruned	25	32	21	101	102	85
	Sig.	ns	<0.005	<0.05	<0.001	<0.05	<0.05
Chardonnay	Cane pruned	13	21	13	105	105	57
	Spur pruned	19	26	20	79	90	48
	Sig.	<0.001	<0.01	<0.001	<0.01	ns	ns

spur pruned 17.4) for 2011 and 20.2 (cane pruned 20.4, spur pruned 20.0) for 2012) ('Pinot noir' mean pH was 3.0 (cane pruned 2.9, spur pruned 3.1) for 2010, 2.9 (cane and spur pruned 2.9) for 2011, and 3.0 (cane pruned 3.1, spur pruned 2.9) for 2012; and 'Chardonnay' mean pH was 2.9 (cane pruned 3.0, spur pruned 2.9) for 2010, 3.0 (cane and spur pruned 3.0) for 2011 and 3.0 (cane pruned 3.0, spur pruned 3.1) for 2012). TA was unaffected by pruning treatment in both cultivars ('Pinot noir' mean TA was 12.4 (cane pruned 13.2, spur pruned 11.6) for 2010, 14.5 (cane pruned 14.7, spur pruned 14.4) for 2011, and 11.5 (cane pruned 11.2, spur pruned 11.9) for 2012; and 'Chardonnay' mean TA was 12.3 (cane pruned 12.4, spur pruned 12.2) for 2010, 14.6 (cane and spur pruned both 14.6) for 2011 and 13.8 (cane pruned 14.1, spur pruned 13.5) for 2012). Base wine total phenolics was also unaffected by pruning treatment, except for 'Pinot noir' the first year, where base wines made from spur pruned vines were higher in total phenolics ('Pinot noir' mean total phenolics was 2.97 (cane pruned 2.81, spur pruned 3.13) for 2010, 6.1 (cane pruned 5.85,

spur pruned 6.35) for 2011, and 3.0 (cane pruned 3.39, spur pruned 2.58) for 2012; and 'Chardonnay' mean total phenolics was 2.83 (cane pruned 2.69, spur pruned 2.96) for 2010, 5.8 (cane pruned 6.07, spur pruned 5.6) for 2011 and 4.89 (cane pruned 4.91, spur pruned 4.87) for 2012) as seen in Tab. 6. Freezing of the homogenate was thought not to

Table 6

Influence of pruning treatment on total phenolics (AU/g) of 'Pinot noir' and 'Chardonnay' base wines in each of the three experimental seasons

		2010	2011	2012
Pinot noir	Cane pruned	2.81	5.85	3.39
	Spur pruned	3.13	6.35	2.58
	Sig.	<0.05	ns	ns
Chardonnay	Cane pruned	2.69	6.07	4.91
	Spur pruned	2.96	5.60	4.87
	Sig.	ns	ns	ns

have impacted on the total phenolics results, as comparable studies have shown that freezing for up to 3 months has no significant effect on total phenolics (CYNKAR *et al.* 2004). Using phenolic results obtained from frozen berries was considered valid (OLLARTE MANTILLA *et al.* 2013).

Principal component analysis of UV-visible spectra was used to determine the major drivers for wine phenolic composition related to treatment effects. In all cases, treatment clusters were evident for both 'Pinot noir' and 'Chardonnay' base wines. As shown in the example given in figure 1a; PC2 separates the treatments for 2010 'Chardonnay' base wines. Principal component 2 had positive loadings for 260 nm, strong negative loadings at 300 and 330 nm (note: < 230 nm is a region that tends to be off-scale at the dilutions used). Treatment clustering with PCA implies that low 260 nm absorbance and high 300 and 330 nm absorbance is associated with the spur pruning treatment. Caffeic acid is a freely soluble hydroxycinnamate phenolic compound in grapes (VERETTE *et al.* 1988). The caffeic acid UV spectrum has a trough at 260 nm and overlapping peaks at 295 and 325 nm (Fig. 2). Some wavelength shift and changes in relative absorbance at these wavelengths can occur, related to pH, concentration and the presence of other compounds (BELAY 2012), but these wavelengths agree well with the PCA loadings in Fig. 1, implying that

the spur-pruned treatment enhances hydroxycinnamate concentrations. Note that neither the wine nor caffeic acid showed peaks at 280 nm, a wavelength commonly used to estimate total phenolics (Fig. 2). The examples of wine spectra in Fig. 2 show clear absorbance differences at 330 nm related to treatment, but when all replicates are examined together this can be over-ridden by individual sample differences in juice extraction rates, requiring PCA to tease out the treatment related effect. If discriminant analysis is used in combination with PCA scores to identify samples by treatment, in most cases 8/8 samples were correctly identified, but in the case of 2010 'Pinot noir' and 2012 'Chardonnay' 7/8 samples were correctly identified by treatment type.

The starch concentration was higher for spur pruned 'Pinot noir' vines when measured in the winter of 2011. All other starch and soluble sugar concentrations were not different between pruning treatments for 'Pinot noir' and 'Chardonnay', however varied between seasons ('Pinot noir' mean starch was 74.7 mg·g⁻¹, fructose was 24.6 mL·g⁻¹, glucose was 26.8 mL·g⁻¹, and sucrose was 23.3 mL·g⁻¹ in 2010. 'Chardonnay' mean starch was 79.4 mg·g⁻¹, fructose was 24.6 mL·g⁻¹, glucose was 26.8 mL·g⁻¹, and sucrose was 23.5 mL·g⁻¹ in 2010. In 2011 'Pinot noir' mean starch was 58.4 mg·g⁻¹ (cane pruned 53.2, spur pruned 64.1), fructose

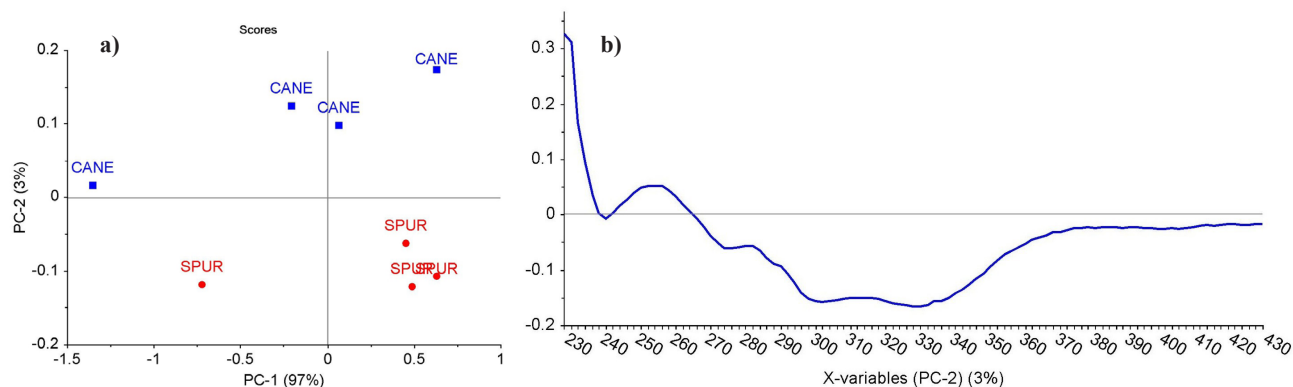


Fig. 1: Spectral analysis of 'Chardonnay' wine from the 2010 season. **a)** Scores plot for Principal Component Analysis (PCA), 230-430 nm. **b)** Loadings Plot for Principal Component 2 from PCA, 230-430 nm.

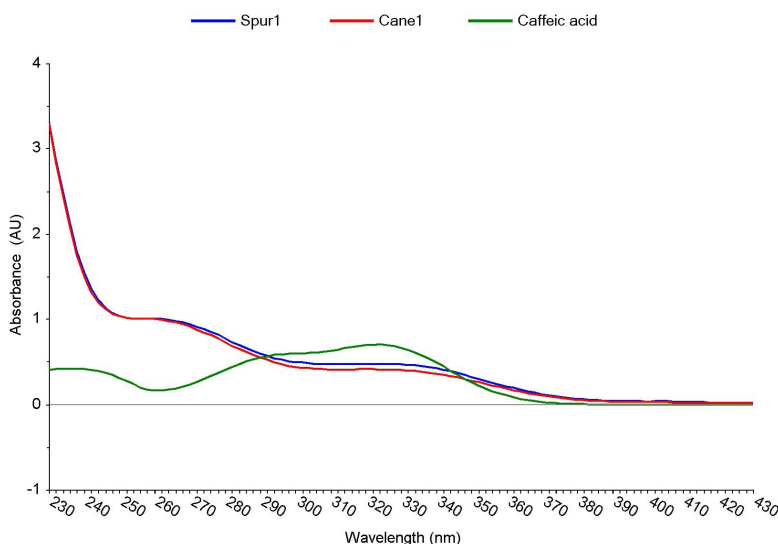


Fig. 2: Spectral analysis of cane and spur pruned 2010 'Chardonnay' base wines; 10 µg·L⁻¹ caffeic acid.

(2005) showed that inadequate reserves resulted in slower shoot development, fewer inflorescences per shoot, fewer flowers per inflorescence, and reduced vine yield. SANCHEZ and DOKOOZLIAN (2005) reported that fruitfulness could be more easily optimized in canopy systems that encourage uniform shoot development and light exposure, which would likely result in higher net carbon assimilation and available photosynthates at the time of fruit bud differentiation.

Conclusion

The current study indicates that spur pruning results in higher quantities of hydroxycinnamates developed in base wines, rather than the more bitter flavonoids, as indicated by spectral phenolic fingerprints, when compared to cane pruning. Depending on the wine style required by sparkling producers, the texture and mouthfeel imparted by hydroxycinnamates (KEMP *et al.* 2015) may or may not be desirable and pruning method could be used to regulate their concentrations. The next step is to impose the common practice of leaf removal in the fruiting zone to test if the canopy can be sufficiently manipulated to achieve a further improved wine quality, and to conduct formal sensory analysis of wines. If leaf removal of spur pruned vines results in superior quality wines, the economic benefit of being able to mechanize pruning and leaf removal, without compromising wine quality, may be attractive to cool climate growers.

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