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Oregon 'Pinot noir' grape anthocyanin enhancement by early leaf removal

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ABSTRACT

Complete cluster zone leaf removal of 'Pinot noir' was initiated at three separate pre-véraison growth stages (bloom, grain-pea size, and bunch closure) and maintained leaf free until harvest, for four growing seasons (2008–2011). Fruit anthocyanin composition was examined at harvest for the last two vintages (2010 and 2011) and compared to a control-no cluster zone leaf removal. Experiments were conducted at two commercially operating Oregon vineyards (site A = 420 rootstock/'Pinot noir' 115 scion and site B = 3309C rootstock/'Pinot noir' 777 scion). All clusters contained the five anthocyanins typically found in 'Pinot noir'. Leaf removal at bloom and maintained until harvest produced maximum anthocyanin accumulation in 'Pinot noir' grapes (site A = 85.24 mg/100 g and site B = 125.06 mg/100 g), compared to no leaf removal (control; site A = 57.91 mg/100 g and site B = 97.56 mg/100 g). Even leaf removal at bunch closure (last leaf removal initiation period) increased grape anthocyanin (site A = 73.22 mg/100 g and site B = 118.93 mg/100 g) compared to control, but total anthocyanins were lower than grapes from bloom leaf removal (first time period). Results differed slightly by vineyard site and rootstock/scion combination.

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1. Introduction

Leaf removal is a common canopy management method used to alter cluster microclimate, especially in a cool and wet climate where it can enhance air circulation, sunlight exposure, and berry temperature, while reducing *Botrytis* bunch rot infection (English, Thomas, Marois, & Gubler, 1989; Smart, Dick, Gravett, & Fisher, 1990). This practise is commonly used in western Oregon wine-grape growing regions, but to date there is no published research on how the timing of leaf removal influences Willamette Valley American Viticulture Area (AVA) berry quality. Though there are direct and indirect consequences to vine growth and berry quality due to this practise, growers continue to use leaf removal as a canopy management tool to improve cluster microclimate, reduce disease incidence/severity, increase fungicide spray penetration into the canopy, and decrease overall pesticide usage, etc. (English et al., 1989; Staff, Percival, Sullivan, & Fisher, 1997). Leaf removal can also alter yield, fruit composition, cluster morphology (open/loose cluster), whole vine photosynthesis capacity, and vine/root carbohydrate reserves amongst other physical and physiological effects (Bennett, Jarvis, Creasy, & Trought, 2005; Intrieri, Filippetti, Allegro, Centrinari, & Poni, 2008; Koblet, Candolfi-Vasconcelos, Zweifel, & Howell, 1994; Poni, Casalini, Bernizzoni, Civardi, & Intrieri, 2006; Staff et al., 1997). Undesired outcomes, such as reduced fruit set, fewer berries per cluster, decreased percentage of soluble solids, and delayed berry development have also been occasionally reported (Bennett et al., 2005; Chorti, Guidoni, Ferrandino, & Novello, 2010; Kliewer & Bledsoe, 1987; Lebon et al., 2008). Those studies, however, often involved varying levels of total canopy leaf removal; enough that changes in vine carbohydrate anabolism and catabolism were seen.

In Oregon, leaf removal is normally conducted between fruit set and véraison to increase sun exposure to the clusters (personal communication, P. Skinkis). Pre-véraison leaf removal is considered atypical in Oregon 'Pinot noir' production although there are reports regarding the benefits of leaf pulling at early growth stages from Italy (Intrieri et al., 2008; Poni, Bernizzoni, & Civardi, 2008; Poni, Bernizzoni, Civardi, & Libelle, 2009; Tardaguila, de Toda, Poni, & Diago, 2010), Slovenia (Lemut, Trost, Sivilotti, & Vrhovsek, 2011), and Greece (Kotseridis, Georgiadou, Tikos, Kallithraka, & Koundouras, 2012) on 'Barbera', 'Cabernet Sauvignon', 'Cariganan', 'Graciano', 'Lambrusco', 'Merlot', 'Pinot noir', and 'Sangiovese' cultivars. Since 'Pinot noir' is a cultivar known for its relatively low pigment (Liang, Owens, Zhong, & Cheng, 2011; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999; Nikfardjam, Mark, Avar, Figler, & Ohmach, 2006), vineyard management techniques with the potential to increase anthocyanin content in its berries would be valuable to Oregon grape growers. There has been no systematic evaluation to date on the impact of leaf removal at vine stages

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prior to véraison on Oregon winegrape quality, specifically anthocyanin composition.

There have been mixed results regarding leaf removal and grape anthocyanin quality, all confounded by varying experimental settings and other factors. For example, leaf removal at véraison inhibited anthocyanin accumulation in 'Kyoho' grapes (Kataoka, Sugiura, Utsunomiya, & Tomana, 1982), but leaves removed at fruit set increased anthocyanins in 'Pinot noir' grapes (Lemut et al., 2011) when compared to no leaf removal. In research conducted in Stellenbosch, South Africa (Hunter, De Villiers, & Watts, 1991a, 1991b), no differences were found in anthocyanin levels among the partially leaf removed (33% and 66%) 'Cabernet Sauvignon' grapes initiated at bud break, fruit set, pea size, or véraison. In Piacenza, Italy (Poni et al., 2008), 'Sangiovese' grapes grown in 120 L pots with leaves removed manually (first six basal leaves) at pre-bloom showed no difference in anthocyanin (expressed as per berry and 100 g of berries) compared to no leaf removed; however, they found that pre-bloom leaf removal increased anthocyanin levels in 'Barbera' and 'Lambrusco' over those of control grapes (Poni et al., 2009). Clearly, vineyard location, cultivar, rootstock/scion, timing of leaf removal, degree of leaf removal, frequency of leaf removal, growing season, training system, vine spacing, and root reserves are all factors influencing how leaf removal influences grape anthocyanin (Bennett et al., 2005; Chorti et al., 2010; Di Profio, Reynolds, & Kassimos, 2011a; Di Profio, Reynolds, & Kassimos, 2011b; Guidoni, Ferrandiono, & Novello, 2008; Hunter et al., 1991a, 1991b; Intrieri et al., 2008; Joscelyne, Downey, Mazza, & Bastian, 2007; Kemp, Harrison, & Creasy, 2011; Koblet et al., 1994; Kotseridis et al., 2012; Lemut et al., 2011; Lohitnavy, Bastian, & Collins, 2010; Mazza et al., 1999; Petrie, Trought, & Howell, 2000; Petrie, Trought, Howell, & Buchan, 2003; Poni et al., 2008, 2009; Tardaguila, Diago, de Toda, Poni, & Vilanova, 2008; Tardaguila et al., 2010).

Oregon is the major 'Pinot noir' grape-growing region in the USA, and its 2010 crop was valued at $63.2 million (Oregon Wine Board, OWB), making it a significant financial contributor to the state (>$2.7 billion in 2010; OWB). For such a high economic return crop, it is vital to know how implemented techniques might influence quality and if its benefits will offset increased production expenses. Most Oregon 'Pinot noir' vineyards require 20 h of labour and $270 per acre annually for manual leaf removal, but that significant cost may aid vineyard pest management by reducing the applications of fungicide that normally total $415 per acre annually (Julian, Seavert, Skinkis, VanBuskirk, & Castagnoli, 2008). The objective of this study was to determine if leaf removal around the cluster zone initiated at three different growth stages (bloom, grain-pea size, and bunch closure; all maintained leaf free until harvest), improved grape anthocyanin quality when compared to a control (no leaf removal) within the Willamette Valley AVA, USA.

2. Materials and methods

2.1. Cluster zone leaf removal treatments and fruit maturity indices

A leaf removal trial was conducted at two commercial vineyards located within the Willamette Valley AVA. At both locations, the trial was implemented in a randomised complete block design with each treatment replicated across six blocks, with plots consisting of eight vines (experimental unit). The two vineyard sites historically reached cluster ripeness about 5 weeks apart with site A ripening earlier (Table 1). This difference in ripening time is a result of climate and elevation differences between areas. Site A (Dayton, OR, USA) has an elevation of 120 m amsl (above mean sea level) while site B (Salem, OR, USA) is 37 km away and has an elevation of 245 m amsl. Site A vines were 'Pinot noir' 115 scion grapes grafted to 420A rootstock and planted in 1995. Site B vines were 'Pinot noir' 777 scion grapes grafted to 3309C rootstock, and planted in the late 1990s. Both vineyards were trained to Guyot training systems with north–south row orientation. These vineyards were managed under organic disease and nutrient practises, and both were non-irrigated sites. Three of the six blocks were examined for the fruit quality evaluation of this experiment.

The leaf removal treatments consisted of physically removing leaves around the clusters initiated at three vine growth stages (BBCH-EL, Biologische Bundesanstalt, Bundessortenamt and Chemical-Eichhorn and Lorenz, stages 65, 73, and 79; stages defined in Lorenz et al., 1994 and summarized in Table 1), and a no leaf removal was included as a control. Leaves were removed by starting at the base of the shoot up to the node just above the topmost cluster (total of 5–6 leaves per shoot removed). This resulted in a ~30 cm leaf free zone in the canopy, which was maintained throughout the entire growing season. There was no fruit thinning during the experiment so that leaf removal effects on fruit set and yield could be quantified. The experiment was conducted on the same vines for 4 years. All other vineyard management practises including disease management, nutrition, and hedging were conducted per standard commercial practises used at that vineyard operation.

To determine the influence of leaf removal on vine canopy size, yield, and fruit exposure, several vineyard measurements were taken during the growing season. The area of leaves remaining on the vines was quantified when each treatment was initiated (amount of leaves removed determined at this time point as well), and at véraison (once canopy vegetative growth cycle was completed), by a non-destructive method as described by Schreiner, Pinkerton, and Zasada (2012). A total of five shoots per plot were measured for whole shoot leaf area, and this was used with data on shoots per vine to estimate whole vine leaf area. The amount of sunlight received in the cluster zone was measured at solar noon on clear cloudless days at the onset of véraison using a ceptometer (LP-80, Decagon Devices, Pullman, WA, USA) positioned horizontally along the cluster zone of the vine, level, and parallel with the vine row. A total of three measures were taken per plot on the east side of the canopy within the north–south oriented vine rows. Whole vine yields were measured at harvest by removing all clusters per vine in each plot and recording cluster number and weight. Cluster weights, berries per cluster, and berry weight were determined from a seven-cluster composite, collected randomly from the harvest bins of each plot. The full viticulture aspect of this study will be available in a forthcoming publication.

Fruit for detailed composition analysis was collected at commercial ripeness, when a composite soluble solids reached ~22 °Brix for both years (2010 and 2011). Harvested clusters were stored at −80 °C until extraction and analyses. Ten clusters were collected per plot, and later a random selection of 50 berries were used for fractionation (into skin/pulp and seed fractions) and extraction (described in next section). Remaining frozen whole berries were powdered in liquid nitrogen using an IKA M20 Universal mill (IKA Works Inc., Wilmington, NC, USA). These whole berry powders were used for the fruit maturity measurements. One-hundred-berries weights (g), pH, TA (titratable acidity; expressed as g tartaric acid/100 g), and % soluble solids (°Brix-temperature compensated) were conducted as described in Lee and Finn (2007), with the exception of using a autotitrator T50 (Mettler Toledo Inc., Columbus, OH, USA) and InLab Expert Pro electrode (Mettler Toledo Inc.) for determining TA.

2.2. Reagents, chemicals, and standards

All chemicals, reagents, and standards used in this study were analytical or HPLC grade from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), unless indicated otherwise.

Table 1
Description of the leaf removal initiated at the three growth stages (all pre-véraison stages) and maintained throughout the entire growing season. A no leaf removal-control was included. Field experiments were conducted for four growing seasons (2008–2011) and detailed fruit component analyses were conducted at the final two growing seasons (2010 and 2011) at two commercially operating vineyards in Oregon, USA. Site A (Dayton, OR, USA) vines were 420A rootstock/'Pinot noir' 115 scion and site B (Salem, OR, USA) vines were 3309C rootstock/'Pinot noir' 777 scion. The two locations were approximately 37 km apart within the Willamette Valley AVA.

Timing of treatment initiated	BBCH-EL stage[a]	Description of the stages	2010		2011	
			Site A	Site B	Site A	Site B
Control (no leaf removal)	na	No leaves were removed during the entire season	na	na	na	na
Bloom	65	50% cap fall; 50% bloom	6/28/2010	7/13/2010	7/4/2011	7/18/2011
Grain-pea sized berries	73	3–5 mm diameter green berries	7/19/2010	8/4/2010	7/22/2011	8/8/2011
Bunch closure	79	Closed clusters (berries are touching)	8/4/2010	8/16/2010	8/8/2011	8/23/2011
Harvest dates			10/12/2010	10/26/2010	10/18/2011	11/1/2011

'na', not applicable.
[a] BBCH-EL (Biologische Bundesanstalt, Bundessortenamt and Chemical-Eichhorn and Lorenz) stage is defined in detail in Lorenz et al. (1994).

2.3. Fractionation and extraction for phenolic analyses

Berry fractions were liquid nitrogen (Norco Inc., Nampa, ID, USA) powdered, and extracted as previously described (Lee & Martin, 2009; Lee & Wrolstad, 2004) using an IKA M20 Universal Mill for skin/pulp fraction and a mortar and pestle for the seed fraction. Some minor modifications to the extraction method are described below. Briefly, liquid nitrogen berry powder (10 g used for skin/pulp and 3 g used for seed fractions) were extracted with 100% acetone, subsequently two additional extractions were done with 70% aqueous acetone (30:70 = water:acetone, v/v). Acetone was evaporated (using a RapidVap Vacuum Evaporation System set at 40 °C under vacuum; Labconco Corp., Kansas City, MO, USA) and extracts were re-dissolved in water (final volumes of 25 ml for skin/pulp fraction and 10 ml for seed fraction). These aqueous extracts were used for all phenolic spectrophotometric methods and anthocyanin HPLC analyses.

2.4. Spectrophotometric methods used for phenolic analyses

Total anthocyanins (TACY), total phenolics (TP), and total tannins (TT) were analysed for all extracts as described in Lee, Durst, and Wrolstad (2005), Waterhouse (2002), and Sarneckis et al. (2006). Absorbances were measured at 520 nm and 700 nm for TACY, 765 nm for TP, and 280 nm for TT. TACY was expressed as mg malvidin-3-glucoside/100 g (ε = 28,000 L/cm mol and molecular weight = 493.3 g/mol; detailed calculation described in Lee et al., 2005), TP was expressed as mg gallic acid/100 g, and TT was expressed as mg epicatechin/100 g. A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) was used for all three measurements. All samples were expressed in mg/100 g of whole berries (fresh weight, fw), but are referred to as mg/100 g for conciseness. Analyses were conducted in duplicate.

2.5. HPLC (High Performance Liquid Chromatography) conditions for individual anthocyanin separation

Analysis for anthocyanins using HPLC/DAD (diode array detector; for identification and quantification) and HPLC/DAD/MS (mass spectrometer; for identification) was used as per earlier published methods (Lee & Finn, 2007; Lee & Martin, 2009; Lee, Rennaker, & Wrolstad, 2008). Briefly, an Agilent HPLC 1100 (Agilent Technologies Inc., Palo Alto, CA, USA) was used for this investigation, and MS was used when needed. All quantification was done on a HPLC/DAD monitored at 520 nm. Anthocyanins were expressed as malvidin-3-glucoside (Polyphenols Laboratories AS, Sandnes, Norway). Anthocyanin peaks were identified based on retention time, UV–VIS spectra, external standards (when available), mother and daughter ions information, and prior published research (Lee & Martin, 2009).

2.6. Statistical analysis

Statistical analysis was conducted using Statistica for Windows version 7.2 (StatSoft Inc., Tulsa, OK, USA). Difference between year (growing seasons) and among the treatment means were tested using analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) at $\alpha = 0.05$. Correlation was determined on the anthocyanin results obtained by the two methods.

3. Results and discussion

Our findings regarding all viticultural components (including disease incidents) of this study will be published in a forthcoming paper, but are briefly summarized below. The weather during the years of 2010 and 2011 was cooler than the 30-year average for the Willamette Valley. Both years had a wet spring and lower summer temperatures that delayed bud break, bloom, and harvest. The year of 2010 had ~500 mm of rainfall during the growing season, while 2011 had ~300 mm. This cool, wet weather created fruit set and disease concerns during both years. The 2010 season had an average of 52% fruit set across both sites, but in 2011 there was an 80% fruit set (considered a high-fruit set year). In 2011 only, bloom-time leaf removal decreased fruit set (22% reduction) compared to the control. The 10–14 day delayed ripening period led to harvests in mid to late October and early November for both years (Table 1). Temperatures for September and October throughout the Willamette Valley are typically cooler than other grape growing regions, and the following mean daily temperatures were recorded during fruit analyses years: 16.8 °C (2010) and 18.2 °C (2011) in September, and 11.5 °C in October (2010 and 2011). With the early leaf removal and the cool weather conditions for both seasons, no sun burnt fruit was observed during this study.

Site A had between 26% and 42% of leaves removed per shoot, while site B had 20% and 39%. Leaves taken off at bloom corresponded to a higher removal percentage since those leaves represented a larger percent of the total leaf area of the vine at the time in contrast to later leaf removal stages. When total vine leaf area was measured at véraison, there were no differences by treatment for the amount of vine leaf area; indicating adequate leaf area remained for proper vine development and fruit ripening. As a result, there were no differences observed for dormant pruning weight among leaf removal treatments.

There were only minor differences in the cluster weight and number of berries per cluster as a result of the leaf removal treatments. There were no differences in yield per vine at harvest for either site or year. Cluster weights were not different by treatment in 2011. In the low fruit set year of 2010 an influence from early season leaf removal on cluster size was seen. Cluster weight was lower at site A, with bloom leaf removal resulting in 55 fewer berries and a reduction in cluster weight of 43 g compared to other

treatments and control. While site B had fewer berries per cluster in the bloom treatment, there were no differences observed between cluster weights in 2010. During the better fruit set year of 2011, the only differences were found in site A, where the bloom time treatment had ~30 fewer berries per cluster, yet no reduction in final cluster weight or yield.

There was no difference from the two-way Tukey HSD of year × timing of treatment (site A, $p = 0.335$ and site B, $p = 0.636$), so results were summarized by each main effect with year and timing of treatment analysed separately (Tables 2 and 3).

3.1. Fruit maturity indices; TACY, TP, and TT results

Fruit maturity indices and simple spectrophotometric results are summarized in Table 2. In general, fruit maturity indices were more often different between the 2 years than among control and treatments applied. Berries from both sites in 2011 had higher berry weight, seed weight, seed count per berry, and TT. TACY was greater in 2010 for both vineyards. This increase in 100 berry weight, seed weight, and 100 berries seed count in 2011 are directly related to the higher relative fruit set observed in that year and are likely a result of the weather conditions prior to bloom for that season. Site B berries had significantly higher TP in 2011 compared to 2010, but site A berries exhibited no significant difference (Table 2).

Berries from site A did not differ by treatment or control in berry weight, seed count, pH, % soluble solids, or whole berry TT. TAs were the highest in control berries, and lowest in berries from leaf removed at bunch closure. Seed TTs were higher in berries when leaf removal was initiated at bloom (516 mg/100 g) compared to control (462 mg/100 g), although whole berry TT was not different. Skin/pulp fraction TT was not different among the treatments and control, ranging from 195 (control) to 234 mg/100 g (leaf removal initiated at bunch closure). Leaf removal at bunch closure had the lowest seed TT level (410 mg/100 g). Leaf removal initiated at bloom and when berries were grain-pea sized had the highest level of TACY and TP. Site A leaf removal initiated at bunch closure had the lowest seed weight (0.9 g lower than control) from 100 berries although the seed count was not found to be different.

In site B samples, only berries with leaf removal initiated at bloom and bunch closure had TACY at higher concentrations compared to the control. All other fruit maturity measurements (100 berries weight, seed weight from 100 berries, 100 berries seed count, pH, TA, and % soluble solids), TP, and TT did not differ compared to the control. Skin/pulp and seed fractions TT (only whole berries TT presented Table 2) did not differ with leaf removal treatments compared to the control.

Kemp et al. (2011) reported no difference in 'Pinot noir' berry TT, using the same methylcellulose precipitation method, in two mechanical leaf removal treatments initiated at 7 and 30 days post-flowering compared to no leaf removal. They also reported that differences were likely due to different growing seasons and not the leaf removal treatments. Our 'Pinot noir' whole berry TT values (603.0–734.3 mg/100 g) were probably higher than Kemp et al. (2011; 334–403 mg/100 g), due to the differences in extraction solvent and methods employed (liquid nitrogen powdering and aqueous acetone extraction *versus* their aqueous ethanol homogenisation with homogenizer) and means of treatment application (manual *versus* mechanical leaf removal).

TACY values from this study were similar to previous Oregon 'Pinot noir' berry TACY reports utilising the same pH differential method (Lee & Martin, 2009; Lee & Rennaker, 2011). TP and TT values in this study were higher than amounts reported earlier (Lee & Martin, 2009; Lee & Rennaker, 2011); these differences likely result from differences in extraction solvent and method, in addition to the dissimilar environmental and viticultural factors the berries were grown under.

3.2. Individual anthocyanins

All 'Pinot noir' grape anthocyanin profiles (Table 3) were within the established profile previously reported (Lee & Martin, 2009; Lee & Rennaker, 2011; Lemut et al., 2011; Mazza et al., 1999); glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (in the order of elution; five individual anthocyanins), with malvidin-3-glucoside as the main 'Pinot noir' anthocyanin (>54.8%) in both rootstock/scion combinations.

TACY values (Table 2) were positively correlated ($r = 0.993$, $n = 48$; $p \leqslant 0.05$), and the trend was in agreement with total anthocyanins obtained by HPLC (Table 3), as previously noted (Lee & Finn, 2007; Lee & Rennaker, 2011; Lee et al., 2008). Anthocyanin values obtained by HPLC were 1.1–1.5 times greater than TACY values due to dissimilarity between the analysis methods (Lee & Finn, 2007; Lee & Rennaker, 2011; Lee et al., 2008). Besides experimental settings in the field contributing to a range of reported values, differing extraction procedures and analytical methods can further differentiate measurements (Lee & Rennaker, 2011; Lee et al., 2008). All these experimental factors should be taken into consideration when comparing primary and secondary metabolites assessments from one report to another.

Site A samples from 2010 were higher in all five individual and total anthocyanins compared to 2011, although in samples from site B the trend was mixed (delphinidin-3-glucoside and petunidin-3-glucoside were higher in 2011 compared to 2012; peonidin-3-glucoside and malvidin-3-glucoside were higher in 2010 compared to 2011) and total anthocyanins were not significantly different between years.

In samples from site A, besides peonidin-3-glucoside (no significant difference), the four other individual anthocyanins and total anthocyanins were higher (1.3–1.5 times) in berries from leaf removal treatments compared to control. Leaf removal initiated at bloom at site B resulted in higher levels of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, and total anthocyanins (1.3 times higher) compared to control. These differences in anthocyanins were not due to berry weight (not significantly different; Table 2).

Berries individual anthocyanin proportions (Table 3) depended on vineyard (each site had different rootstock/scion combination). It is interesting to note that leaf removal can alter the individual anthocyanin proportion; berries from site A leaf removal treatments had a higher proportion of delphinidin-3-glucoside compared to control berries, although this difference was not observed in berries from site B. In general, both sites' berries from leaf removal treatments had lower proportions of peonidin-3-glucoside compared to the control. These observed differences in anthocyanins were not due to berry weight/size (Table 2).

Comparing our findings to that of other leaf removal trials is not straightforward due to differences in initial timing of leaf removal, level of cluster zone exposure, cultivar, yield, vineyard location, climate and weather during the growing season, but general comparison follows. Tardaguila et al. (2010), Poni et al. (2009), and Lemut et al. (2011) found increased anthocyanin levels in 'Barbera', 'Carignan', 'Graciano', 'Lambrusco', and 'Pinot noir' by removing leaves at stages pre-bloom and during fruit set. Mazza et al. (1999) reported that basal leaf removal at bloom in their 'Pinot noir' grown in Okanagan Valley (Osoyoos, British Columbia, Canada) were higher in anthocyanins than control during one growing season, but not different the following year of the study; although, it is unclear if the cluster zones were maintained free of leaves up until harvest as in our study. In Niagra-on-the-Lake, Ontario, Canada, Di Profio et al. (2011a) reported no difference in anthocyanins

Table 2
Results of the fruit maturity indices (TA expressed as g of tartaric acid/100 g) and spectrophotometric measurements (TACY expressed as mg of malvidin-3-glucoside/100 g; TP expressed as mg of gallic acid/100 g; TT expressed as mg epicatechin/100 g). Values following the mean in parenthesis are standard errors. Different lower case letters within the column under year (n = 12 per year) and timing of leaf removal treatment (n = 6 per treatment) initiated were significantly different ($p \leqslant 0.05$).

	Levels	100 Berries weight (g)	Seed weight (g) from 100 berries	100 Berries seed count	pH	TA (g/100 g)	% Soluble solids (°Brix)	TACY (mg/ 100 g)	TP (mg/ 100 g)	TT (mg/ 100 g)
Site A (Dayton, OR, USA) vines were 420A rootstock/'Pinot noir' 115 scion										
Year	2010	112.0 (2.8) a	5.3 (0.2) a	121 (2) a	3.55 (0.02) b	0.82 (0.02) a	22.9 (0.1) b	42.3 (1.7) b	552.8 (12.4) a	648.6 (18.4) a
	2011	125.1 (2.0) b	6.9 (0.2) b	171 (3) b	3.47 (0.02) a	0.89 (0.01) b	21.2 (0.2) a	27.7 (1.3) a	555.2 (7.7) a	708.8 (17.8) b
Timing of treatment	Control (no leaf removal)	126.4 (3.2) a	6.4 (0.2) b	150 (12) a	3.49 (0.03) a	0.94 (0) c	21.6 (0.4) a	28.1 (2.9) a	521.2 (10.4) a	657.0 (20.6) a
	Bloom	114.5 (4.7) a	6.5 (0.2) b	148 (12) a	3.53(0.03) a	0.85 (0.02) b	22.2 (0.4) a	39.5 (4.2) c	591.8 (14.6) b	734.3 (32.7) a
	Grain-pea sized berries	118.8 (4.4) a	6.0 (0.4) ab	144 (13) a	3.47 (0.02) a	0.85 (0.02) b	22.3 (0.3) a	37.7 (3.6) bc	560.1 (7.3) ab	679.3 (26.9) a
	Bunch closure	114.5 (4.1) a	5.5 (0.4) a	141 (9) a	3.55 (0.04) a	0.79 (0.03) a	22.2 (0.6) a	34.7 (3.0) b	542.9 (5.7) a	644.1 (20.9) a
Site B (Salem, OR, USA) vines were 3309C rootstock/'Pinot noir' 777 scion										
Year	2010	101.9 (3.1) a	4.5 (0.2) a	120 (4) a	3.47 (0.02) b	0.84 (0.02) a	22.7 (0.2) b	52.4 (2.1) a	554.7 (8.3) a	603.0 (19.3) a
	2011	116.3 (0.5) b	5.8 (0.2) b	140 (5) b	3.23 (0.03) a	1.10 (0.04) b	21.4 (0.1) a	50.6 (1.3) a	617.2 (10.2) b	705.0 (15.2) b
Timing of treatment	Control (no leaf removal)	108.0 (6.6) a	5.0 (0.3) a	128 (6) a	3.38 (0.06) a	0.97 (0.07) a	21.8 (0.3) a	45.4 (2.1) a	565.0 (9.8) a	642.6 (30.7) a
	Bloom	112.0 (4.8) a	5.7 (0.5) a	139 (8) a	3.35 (0.07) a	0.98 (0.08) a	22.5 (0.4) a	54.7 (2.1) b	607.0 (15.4) a	678.4 (29.6) a
	Grain-pea sized berries	110.3 (3.4) a	5.1 (0.3) a	129 (6) a	3.34 (0.06) a	0.96 (0.07) a	21.8 (0.3) a	52.4 (1.8) ab	591.6 (19.4) a	623.9 (45.0) a
	Bunch closure	106.2 (3.6) a	4.8 (0.4) a	122 (9) a	3.33 (0.06) a	0.97 (0.07) a	22.1 (0.4) a	53.4 (2.3) b	580.2 (25.4) a	671.1 (22.5) a

Table 3
Results of individual anthocyanin obtained by HPLC (concentrations expressed as mg of malvidin-3-glucoside/100 g; the proportions, in italic type, reported in %). All concentrations are in 100 g of fresh weight. Different lower case letters within the column for year ($n = 12$ per year) and timing of leaf removal treatment ($n = 6$ per treatment) initiated were significantly different ($p \leqslant 0.05$). Values following the mean in parenthesis are standard errors. Di-OH (hydroxylated) anthocyanins can be calculated by summing cyanidin-3-glucoside and peonidin-3-glucoside. Tri-OH can be calculated by summing delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside.

	Levels	Delphinidin-3-glucoside	Cyanidin-3-glucoside	Petunidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3-glucoside	Total anthocyanin	*% Delphinidin-3-glucoside*	*% Cyanidin-3-glucoside*	*% Petunidin-3-glucoside*	*% Peonidin-3-glucoside*	*% Malvidin-3-glucoside*
Site A (Dayton, OR, USA) vines were 420A rootstock/'Pinot noir' 115 scion												
Year	2010	6.65 (0.71) b	1.54 (0.14) b	6.78 (0.63) b	19.31 (0.45) b	56.71 (2.45) b	90.98 (4.25) b	*7.1 (0.5) b*	*1.7 (0.1) a*	*7.3 (0.4) b*	*21.5 (0.7) a*	*62.5 (0.6) a*
	2011	2.93 (0.36) a	0.83 (0.11) a	3.22 (0.35) a	15.22 (0.93) a	36.11 (2.08) a	58.31 (3.21) a	*4.8 (0.4) a*	*1.4 (0.1) a*	*5.3 (0.3) a*	*26.5 (1.6) b*	*61.9 (1.2) a*
Timing of treatment	Control (no leaf removal)	2.42 (0.50) a	0.73 (0.11) a	2.82 (0.54) a	16.14 (0.84) a	35.81 (4.65) a	57.91 (6.35) a	*4.0 (0.4) a*	*1.3 (0.1) a*	*4.6 (0.4) a*	*28.9 (2.2) b*	*61.2 (1.6) a*
	Bloom	6.65 (1.28) c	1.45 (0.30) b	6.64 (1.16) c	17.73 (1.79) a	52.77 (5.64) c	85.24 (9.65) c	*7.4 (0.7) c*	*1.6 (0.2) a*	*7.5 (0.6) c*	*21.2 (1.9) a*	*62.2 (1.5) a*
	Grain-pea sized berries	5.78 (1.12) c	1.37 (0.20) b	5.95 (1.04) c	17.99 (1.15) a	51.11 (5.15) bc	82.21 (8.51) bc	*6.7 (0.7) bc*	*1.6 (0.1) a*	*7.0 (0.6) bc*	*22.4 (1.1) a*	*62.3 (0.6) a*
	Bunch closure	4.31 (0.63) b	1.18 (0.20) ab	4.59 (0.61) b	17.19 (1.55) a	46.00 (3.91) b	73.22 (6.49) b	*5.7 (0.4) b*	*1.5 (0.2) a*	*6.1 (0.3) b*	*23.6 (1.3) a*	*63.0 (1.4) a*
Site B (Salem, OR, USA) vines were 3309C rootstock/'Pinot noir' 777 scion												
Year	2010	10.79 (1.15) a	2.42 (0.29) a	10.07 (0.90) a	21.26 (1.01) b	72.35 (3.19) b	116.89 (5.85) a	*9.0 (0.6) a*	*2.0 (0.1) a*	*8.5 (0.4) a*	*18.4 (0.7) b*	*62.1 (1.0) b*
	2011	18.30 (1.14) b	2.80 (0.18) a	14.51 (0.72) b	15.51 (0.66) a	61.71 (2.07) a	112.84 (3.63) a	*16.2 (0.8) b*	*2.5 (0.1) b*	*12.8 (0.4) b*	*13.8 (0.4) a*	*54.8 (0.9) a*
Timing of treatment	Control (no leaf removal)	10.25 (1.77) a	1.88 (0.13) a	9.16 (1.28) a	17.23 (1.14) a	59.04 (3.65) a	97.56 (5.00) a	*10.4 (1.6) a*	*1.9 (0.1) a*	*9.3 (1.1) a*	*17.9 (1.7) b*	*60.4 (1.5) a*
	Bloom	16.71 (1.69) b	3.20 (0.36) b	13.91 (1.00) b	20.21 (2.24) a	71.03 (4.99) a	125.06 (6.82) b	*13.6 (1.7) a*	*2.5 (0.2) a*	*11.3 (1.0) a*	*15.9 (1.2) ab*	*56.6 (1.7) a*
	Grain-pea sized berries	15.51 (2.60) b	2.46 (0.34) ab	13.03 (1.62) b	17.09 (0.94) a	69.82 (3.45) a	117.91 (4.73) ab	*13.0 (2.0) a*	*2.1 (0.2) a*	*11.0 (0.2) a*	*14.5 (0.7) a*	*59.4 (2.7) a*
	Bunch closure	15.71 (2.24) b	2.90 (0.28) ab	13.05 (1.37) b	19.07 (2.12) a	68.23 (4.32) a	118.93 (5.72) b	*13.3 (1.9) a*	*2.4 (0.2) a*	*11.0 (1.1) a*	*15.9 (1.3) ab*	*57.3 (2.1) a*

(spectrophotometric method) between control and basal leaves removed at ~1 cm berry size stage in 'Merlot' and 'Cabernet franc' grapes. They observed an increase in anthocyanins in basal leaf removed for 'Cabernet Sauvignon' compared to control in two of three growing seasons examined.

Anthocyanins can be summarized into di-hydroxylated (di-OH; sum of cyanidin-3-glucoside and peonidin-3-glucoside) and tri-hydroxylated (tri-OH; sum of delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucsoide) based on the anthocyanin $C_6C_3C_6$ backbone B-ring substitution via the biosynthetic pathway (Tarara, Lee, Spayd, & Scagel, 2008). Berries from site A had no difference in di-OH anthocyanins. Its tri-OH anthocyanins were significantly higher in berries from leaf removal treatments compared to control (control = 41.05 mg/100 g < leaf removal initiated at bunch closure = 54.85 mg/100 g < leaf removal initiated at grain-pea sized berries = 62.85 mg/100 g < leaf removal initiated bloom = 66.06 mg/100 g). The proportion of tri-OH anthocyanins in site A samples were higher in treatments where leaf removal was initiated at bloom (77%) and grain-pea sized berries (76%) compared to the control (70%). In berries from site B, di-OH anthocyanins were the highest in leaf removal at bloom compared to the control (control = 19.10 mg/100 g < leaf removal initiated at grain-pea sized berries = 19.56 mg/100 g < leaf removal initiated at bunch closure = 21.93 mg/100 g < leaf removal initiated bloom = 23.41 mg/100 g). Site A tri-OH anthocyanins were higher in all berries from leaf removal treatments compared to the control. Site B's proportion of tri-OH anthocyanins were higher when leaves were removed at grain-pea sized berries (83%) compared to the control (80%). Increases in concentration and the proportion of tri-OH anthocyanins in the leaf-removed berries resulted in a more intensely purple (darker hue; He & Giusti, 2010; Mizuno, Hirano, & Okamoto, 2006) coloured berry, in contrast to the higher di-OH levels (di-OH anthocyanins are redder) of control berries. These berry factors influence their resulting wines by the colour they ultimately impart (Di Profio et al., 2011b).

The differences in anthocyanin content between clusters of control and leaf removal treatments observed in this study are in the concentration range of mg/100 g (site A = 15.31–27.33 mg/100 g and site B = 20.35–27.50 mg/100 g in differences; Table 3). To visualise the extent of this colour enhancement by leaf removal: the cranberry juice cocktail familiar to most consumers/researchers contains only 1.31–1.36 mg/100 ml of anthocyanins by the pH differential method (expressed as cyanidin-3-glucoside) or 0.54–1.08 mg/100 ml by HPLC (Lee, 2013; Lee et al., 2005, 2008). Since the human eye can detect such minute amounts of anthocyanin, and the threshold for some (cyanidin-based) anthocyanins are as low as 0.09–0.36 mg/100 ml (Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002), it's simple to envision how an increase of >15 mg/100 g concentration could intensify the colour of 'Pinot noir' wine.

Studies conducted in other regions, with leaf removal at later stages of berry development, found differing results. In Kyoto, Japan, leaf removal at véraison had negative results, where 'Kyoho' grapes' (*Vitis vinifera* × *Vitis labruscana* Bailey) anthocyanin accumulation (units were absorbance; spectrophotometric method) was lower compared to no leaf removal (Kataoka et al., 1982). Guidoni et al. (2008) removed 50% of the leaves around 'Nebbiolo' clusters at 5 weeks after bloom and found no difference in anthocyanins (determined by HPLC) compared to the control. Joscelyne et al. (2007) reported no significant difference in the anthocyanin content of wine made from leaf pulled (at 4 weeks post fruit set around fruit zone and canopy lifted by 60 cm) *versus* the control (commercial practises; did not report if any or some leaf pulling occurred) 'Cabernet Sauvignon' and 'Shiraz' grapes grown in Iraak, Victoria, Australia.

In this study, there was a 1.7 to 2.4-fold increase in the sunlight reaching exposed clusters compared to the control at sites B and A, respectively, during both years (measured once at véraison during solar noon using a ceptometer). The percent ambient sunlight reaching the cluster zones was similar among the leaf-removed treatments, which was expected since the same amount of leaves were removed from all leaf removal treatments and then maintained leaf free. Berries from this study (% tri-OH anthocyanin in leaf-removed treatments > % tri-OH anthocyanin in control) displayed the opposite trend to what was reported in Tarara et al. (2008; % tri-OH anthocyanin in *Sun* 'Merlot' berries < % tri-OH anthocyanin in *Shade+1*; these two treatments had similar berry temperature and were only different in solar exposure), although, based on concentration, the trends of di-OH and tri-OH anthocyanins were similar. This discrepancy might be due to the differences in experimental vineyard location, cultivar, vine size/vigour, microclimate, berry temperature, solar radiation, and vineyard management practises between the two studies (current work *versus* Tarara et al., 2008). Furthermore, the Willamette Valley has cooler daytime temperatures in comparison to eastern Washington where the work of Tarara et al. (2008) was conducted. The temperature differences between exposed clusters and the control might be a factor in their anthocyanin accumulation since it is well known that sun-exposed berries have different day (and sometimes night) berry temperatures compared to shaded, and this could influence metabolite accumulation (Bergqvist, Dokoozlian, & Ebisuda, 2001; Chorti et al., 2010; Tarara et al., 2008). Unfortunately, monitoring berry solar exposure and temperatures during the entire experiment period were beyond the scope of this study.

Although, anthocyanin biosynthesis does not occur until véraison, 'Pinot noir' berries can benefit from early leaf removal, possibly altering the primary metabolite accumulation, which affect the later season metabolite biosynthesis (Bennett et al., 2005; Candolfi-Vasconcelos, Candolfi, & Kobelt, 1994; Lebon et al., 2008). The possible photosynthesis compensation by the remaining leaves following leaf removal (Candolfi-Vasconcelos et al., 1994; Hunter & Visser, 1988; Petrie et al., 2003; Poni et al., 2008, 2009) needs to be further investigated. Composition analyses were conducted on the berries from the last two growing seasons, from vines receiving the same leaf-pulling treatments for four consecutive years, and the accumulative effect of the leaf removal on the vine wood reserve should be taken into consideration when interpreting these results for vineyard practise implementation. Though the similarity in dormant pruning weights, after 4 years, between all vines, demonstrated sufficient leaf area remained to support vine growth, development, and nutrient reserves.

4. Conclusion

Based on our findings from 'Pinot noir' grapes obtained from two Willamette Valley AVA vineyards (different rootstock/scion combination and vine age) for two growing seasons, leaf removal initiated at bloom and maintained free of leaves until harvest is a recommended canopy management practise if Oregon winegrape growers desire the highest achievable levels of anthocyanins in their 'Pinot noir' grapes. Leaf removal did not alter most fruit maturity indices (berry weight, pH, and % soluble solids) compared to no leaf removal-control. If growers cannot obtain a crew early in the season to conduct leaf removal around the cluster zone at bloom, they can still achieve similar results with leaf removal between bloom and bunch closure to increase 'Pinot noir' anthocyanin accumulation.

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