Research Article

Viticultural and Controlled Phenolic Release Treatments Affect Phenolic Concentration and Tannin Composition in Pinot noir Wine

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Abstract: Pinot noir fruit is often relatively low in the phenolics important for wine mouthfeel and color stabilization. Previous research has shown that leaf removal can influence the concentration and composition of Pinot noir fruit phenolics, but it is not clear to what extent these effects on grape composition are carried through the winemaking process. A novel thermal treatment, ‘Controlled Phenolic Release’ (CPR), has been demonstrated effective for increasing the phenolic concentration of Pinot noir wines, but there is currently limited information on the interaction between this process and viticultural practices. CPR is microwave heating of grape must to hasten phenolic extraction. This study

applied viticultural treatments to Pinot noir (no leaf removal; leaf removal at post-flowering and pre-veraison) in two commercial vineyards (A and B), and winemaking treatments in replicated microvinifications (control; CPR with skin contact; CPR with early pressing) were applied to fruit from vineyard A. Effects on grape and wine phenolics were examined using UV-visible spectrophotometry. Wine tannin composition and polymerization were assayed by methyl cellulose precipitation (MCPT) and high-performance liquid chromatography (HPLC). Viticultural treatments applied produced significant effects in vineyard A but not vineyard B. Winemaking treatments impacted the concentration of phenolics in wines, but there was no interaction between viticultural and winemaking treatments; effects were additive. Of scientific and practical significance was the finding that grape phenolic concentration determined from homogenate extractions of fruit did not reliably predict Pinot noir wine phenolics. Further, in wines, variation in tannin composition, the extent of tannin polymerization and tannin size was found between treatments. The novel findings here are that the viticultural treatment and the winemaking treatments were both significant and, being independent of each other, offered two separate ways to adjust phenolics in Pinot noir wine. Viticultural and winemaking treatments were also shown to affect tannin composition, with potential mouthfeel and color stability implications.

Key words: leaf removal, maceration extended, phenol, tannin, thermovinification

Introduction

The concentration of tannins and anthocyanins in red wine is associated with wine sensory effects, namely the perception of color and astringency (Smith et al. 2007, Damberg et al. 2012). In Pinot noir grapes, the concentration and distribution of phenolics offers a challenge for wine makers in that the total anthocyanin concentration is low compared with many other red grape varieties (Cliff et al. 2007). There are also a limited number of anthocyanin types in Pinot noir grapes (Cheynier et al. 2006), and all are non-acylated (Mazza et al. 1999). The acylation state influences anthocyanin color stability in food

and wine (Kirca et al. 2007, He et al. 2012), and non-acylated anthocyanins are vulnerable to discoloration under typical wine conditions such as: low pH, the presence of SO₂, and oxygen ingress (Cheynier et al. 2006). Red wine color stabilization occurs through formation of bisulfite-resistant pigments, of which a large component are pigmented polymers (McRae et al. 2012). Pigmented polymers result from complexing between anthocyanins, tannins and potentially other wine constituents (e.g. polysaccharides, proteins) (Peng et al. 2002, Hayasaka & Kennedy 2003, Bindon et al. 2016). Hence, anthocyanin and tannin concentration and composition are important for Pinot noir color stabilization, and for the evolution of wine mouthfeel. For example, changes in perceived astringency over time (Paissoni et al. 2018, Li & Duan 2019).

Pinot noir wine is typically low in tannin, compared with other well-studied red varieties. Analysis of 1325 commercial red wines encompassing five varieties concluded that Pinot noir wine had approximately half the tannin (as catechin equivalents) of Merlot and Cabernet Sauvignon (Harbertson et al. 2008). The physical distribution of tannin within Pinot noir grapes may contribute to this low wine tannin concentration since approximately 90% of total tannin is located in the seed and the remaining 10% in the grape skins (Kennedy 2008). Seed tannin tends to be slower to extract than skin tannin (Damberg et al. 2012), and has been associated with bitterness in red wine (McRae, Falconer, & Kennedy 2010, Damberg et al. 2012).

The challenge of managing winemaking for optimal phenolic composition in Pinot noir is exacerbated by the seasonal variability of cool climate growing conditions necessary for cultivation of ‘fine’ wine exemplars from this cultivar. Pinot noir originates from the Burgundy region in France, situated at latitude 47°N, with a mean growing season temperature (GST) of 15.5 °C (Jones et al. 2005). Under these climatic conditions, it can be difficult to reliably ripen fruit, as the combined effects of sugar accumulation, adequate anthocyanin and tannin concentration and the maintenance of sufficiently low pH. Furthermore, similarly cool regions like Tasmania, Australia (GST 15.4 °C), often experience late-season

rainfall and humidity which adds disease pressure to the challenge of Pinot noir production (e.g. powdery mildew, *Botrytis cinerea*).

Hence, winemakers need options to increase phenolic concentration in Pinot noir wine. Two options are: intervention in the vineyard to affect concentration of phenolics in fruit, and application of wine making interventions to adjust phenolic extraction and stabilization outcomes (Kennedy et al. 2006). Viticultural management techniques, such as leaf removal, are routinely applied to red grape varieties to influence berry color and phenolic concentration (Smart 1991, Diago et al. 2009). Trials investigating shading and exposing Pinot noir clusters have shown a reduction in flavonols in the fruit from shading, but little effect on anthocyanin concentration (Price et al. 1995, Cortell & Kennedy, 2006). A recent industry trend towards early season leaf removal, before flowering, has been examined in a range of varieties and was associated with an increase in anthocyanin concentration for Pinot noir (Lemut et al. 2011, Lee & Skinkis 2013, Sternad Lemut et al. 2015). However, Pinot noir leaf removal trials for sparkling wine production demonstrated that the anthocyanin response can be site-specific, with an anthocyanin increase at one site from early leaf removal and an anthocyanin decrease from early leaf removal at another site (Kerslake et al. 2013). Changes in Pinot noir fruit total tannin concentration (as epicatechin equivalents) in response to leaf removal has been reported, but was shown to vary according to the growing season (Kemp et al. 2011). In that study, there was no significant difference reported between two seasons of data for the mean degree of tannin polymerization, which is an indicator of color stabilization and may moderate wine astringency (McRae et al. 2010, McRae & Kennedy 2011).

Relatively few studies have examined whether Pinot noir grape composition responses to leaf removal translate through to differences in wine phenolics. A study undertaken in a relatively warm Pinot noir growing region (mean temperature in warmest month 23 °C) reported effects on wine flavonols, anthocyanins and hydroxycinnamic acid profiles, but the observed response varied substantially over two successive growing seasons (2009, 2010) (Lemut et al. 2011). In line with those findings, Cortell and

Kennedy (2006) reported reduced anthocyanin concentration from shaded cool-climate Pinot noir fruit that translated into effects in a model wine. Neither of those studies reported associated effects on tannin composition from leaf removal, but Kemp and others (2011) showed a seasonal effect of leaf removal on wine total tannin and monomer flavan-3-ols in wines made from leaf removal fruit. Taken together, these studies suggest that leaf removal can influence final Pinot noir wine phenolic concentration and composition, but the limited body of research to-date means we lack clarity on the magnitude of effects, whether effects are reliable between seasons, vineyards and regions, and also what the optimal timing is for leaf removal to have a desired impact on wine phenolics.

Recent reviews of phenolic extraction during maceration have highlighted the importance of skin contact time (e.g. extended maceration) as an effective mechanism available to winemakers to influence final phenolic concentration in red wine (Casassa & Harbertson 2014, Smith et al. 2015). Extended skin contact time, however, represents an impost on winery facilities during the high pressure logistical challenge of vintage. An earlier review concluded that six winemaking interventions were effective for increasing phenolics in red wine (Sacchi et al. 2005), and a more recent review which focussed on tannin extraction identified practices such as: addition of exogenous enzyme, submerged cap, intensive pumpover, extended maceration and thermovinification to consistently increase tannin concentration in red wines (Smith et al. 2015). Those authors, however, offered the caution that complex interactions between grape material, phenolic and non-phenolic wine matrix components such as tannins, mannoproteins, polysaccharides, and organic acids could result in tannin removal from wine in instances where the maceration process increased grape cell disruption (Smith et al. 2015). Two disruptive maceration processes commonly used by Pinot noir winemakers are extended maceration and peak fermentation temperature in excess of 30 °C (Haeger 2008). Thermal maceration was identified as effective for phenolic extraction in red winemaking (Sacchi et al. 2005) but is not commonly used in New World Pinot noir winemaking (Haeger 2008, Joscelyne 2009). A new method for Pinot noir maceration

called Controlled Phenolic Release (CPR) has proven effective for increasing the concentration of color and tannin compounds in finished wine (Carew et al. 2013, Carew et al. 2014). CPR involves heating grape must to 70°C by microwave and managed diffusion of phenolics by maintenance of peak temperature for a defined period. Few studies have examined grape to wine impacts on phenolics, particularly whether viticultural impacts are additive with phenolic impacts attributable to maceration treatments.

Because of its challenging phenolic profile, Pinot noir producers need a range of options to improve the phenolic profile of fruit in the vineyard, and to improve the efficiency and effectiveness of phenolic extraction in the winery. This study examined the impact on Pinot noir phenolic profile of viticultural intervention (leaf removal) in concert with winemaking by CPR.

Materials and Methods

Grapevines and viticulture. Two viticultural treatments were applied during the 2012/2013 season in two commercial vineyards in northern Tasmania, Australia (latitude 41°S). At both sites, own-rooted, *Vitis vinifera* L. cv. Pinot noir clone MV6 grapevines trained to a divided canopy on a Scott Henry trellis were cane pruned to 2 arms per vine, 10 nodes per arm (total of 20 nodes per vine).

At vineyard A, vines were 8 years old with vine spacing of 2.75 m x 1.50 m (inter- and intra-row, respectively) and received 1.5 ML/ha of drip irrigation. Soil at the vineyard was a well-drained brown clay loam over basalt. Vineyard B was 35 miles away from vineyard A. Vineyard B vines were 13 years old and spaced 2.25 m x 1.50 m (inter- and intra-row, respectively).

Table 1 shows weather data including daily minimum and maximum temperatures (°C), rainfall (mm) and total solar radiation (MJ/m²) for each site. Data was collected from ‘Patch Point’ daily meteorological data obtained from a proprietary ‘SILO’ database (White Hills) and from ‘SLIMS’ which is a proprietary database held by the Tasmanian Department of Primary Industries, Water and

Environment (Kayena). Growing season temperature (Hall and Jones, 2010), average daily radiation, and growing degree days with a base of 10°C (GDD10) (Amerine and Winkler 1944) were calculated.

Two leaf removal treatments were applied at both vineyards; no leaves were removed (noLR), or leaves were mechanically removed (LR) both post-flowering at E-L 26 on the Eichorn-Lorenz (E-L) Scale, and pre-veraison at E-L 34. For LR at vineyard A, leaves were removed on 21 December, 2012 and 21 January, 2013 using a Pellenc Leaf Remover (Pellenc, France). For LR at vineyard B, leaves were removed on 26 December, 2012 and 31 January, 2013 using an ERO double in-row leaf stripper (ERO, Germany).

The experiment was a randomised complete block design, with two treatments and four replicates. Each experimental block was a separate row in the vineyard and treatments were applied along the entire vineyard row with two vines per row used as measurement vines. For vineyard A, fruit was harvested on 3 April, 2013, 1 day ahead of commercial harvest. For vineyard B, fruit was harvested on 19 March, 2013, 1 day ahead of commercial harvest.

Grape analysis. One hundred berries per replicate from each vineyard were sub-sampled for fresh fruit compositional analysis, and another 100 berries per replicate were sub-sampled and frozen at -20°C for phenolic analysis.

Fresh berries were hand crushed and the juice was analysed for pH and titratable acidity (TA) (Crison, pH-Matic 23: Barcelona, Spain). Total soluble solids was measured using a digital hand-held pocket refractometer (Pocket PAL-87S: Atago, Japan). Grape total anthocyanins, total tannin and total phenolics were quantified by UV-visible spectrophotometry (Genesys 10S UV-Vis: Daly City, USA) (Iland 2000, Australian Wine Research Institute 2009), from frozen 100 berry samples prepared as previously described (Kerslake, Jones, Close, & Dambergs 2013) using homogenization of the thawed fruit at 8000 rpm for 20 seconds in a Retsch Grindomix GM200 homogenizer (Retsch GmbH and

Company, KG, Germany). Homogenized samples were then extracted in ethanol as per Iland (2000), and diluted 1 in 20 with 1M hydrochloric acid (HCl), prior to analysis via UV-visible spectrophotometry (Genesys 10S UV-Vis: Daly City, USA) (Iland 2000, Australian Wine Research Institute 2009).

Winemaking treatments. Due to processing and time constraints, only fruit from one of the vineyard trials was subjected to vinification. Vineyard A fruit was processed for small-scale experimental winemaking. Clusters from each viticultural treatment (LR, noLR) were randomly allocated to 1 kg lots for vinification by three winemaking treatments; control (ctl), Controlled Phenolic Release by microwave followed by fermentation on skins (CPR-skins) and Controlled Phenolic Release by microwave with pressing before initiation of alcoholic fermentation (CPR-press). There were four 1 kg replicates for each combination of treatments (n = 24). Control wines were made using the French Press method (Dambergs & Sparrow 2011, Carew et al. 2013), with submerged cap fermentation at 28°C for 7 days in Bodum™ coffee plungers, followed by press-off and cold settling at 4°C. Microvinification is widely used for wine research (Sparrow et al. 2015, Feng et al. 2019). A comparative trial of various scale microvinification methods by Sparrow and Smart (2015) concluded there were no significant differences in phenolic profile in Pinot noir wines made across a range of ferment sizes from 250 mL to 20 L, and that management of fermentation via controlled temperature conditions was key to authentic wine outcomes from small-scale, replicated fermentation for research. The French Press method is applied in controlled temperature conditions, and the volume to surface-area ratio of vessels is analogous to industry standard submerged cap fermenters.

CPR-skins wines were macerated following a previously published method (Carew et al. 2013), with grape must heated by microwave to 70°C and held at that temperature for 1 h, rather than the 10 min previously described. CPR-skins must was then cooled to 28°C, inoculated and fermented as described for the control treatment. CPR-press wines were macerated following a previously published method (Carew, Gill et al, 2014), with grape must heated to 70°C by microwave, held at that temperature for 1 hr,

juice pressed off from grape solids and then cooled to 28°C prior to inoculation and fermentation in 500 mL laboratory glass bottles (Schott Duran: Mainz, Germany) under airlock.

All wines were fermented using cultured yeast *Saccharomyces cerevisiae* EC1118 (Lalvin) that was rehydrated and applied according to the manufacturer's instructions. Wines were fermented to ≤ 2.5 g/L residual sugar, then cold settled at 4°C for 14 days and were not subjected to malo-lactic fermentation. Wines were stabilised at first racking with 80 mg/L total SO₂ and bottled under CO₂ cover to 50 mL amber glassware with wadded polypropylene capping. Wines were stored in the dark under ambient conditions at 22°C for six months prior to analysis. Ambient temperature storage was used to simulate the conditions wines would potentially be exposed to in a retail setting, as opposed to cellaring conditions.

Wine phenolics quantification. Wines were bottle aged for 6 months prior to analysis to allow for some in-bottle maturation, and thereby allow comment on medium-term stability of effects from the treatments applied. Analysis of wine phenolics at 6 months bottle age was by UV-visible spectrophotometry as previously described (Mercurio et al. 2007, Damberg et al. 2012, Carew et al. 2015). Nine phenolic parameters were examined in wines: total phenolics (colored and non-colored tannin and anthocyanin, and low molecular weight, non-pigmented phenolics); total pigment (total red color in the samples, including free anthocyanins and pigmented tannins); total anthocyanin (unbound and co-pigmented); color density (pigment saturation); hue (higher values more ruby/garnet, lower values more purple); non-bleachable pigment (stable color from pyranoanthocyanin formation, and anthocyanin and tannin complexing and has been correlated with pigmented tannin); SO₂-resistant hue (hue in the presence of high SO₂ concentrations, with low values indicating a greater concentration of purple-hued non-bleachable pigment); and tannin by the spectral method (pigmented and non-pigmented).

Tannin compositional analysis. For each wine replicate at 6 months bottle age, a 4 mL sample was loaded onto a solid phase extraction (SPE) cartridge and isolated following the method of Kassara and Kennedy (2011). Isolated tannin was subjected to acid-catalysed depolymerization in the presence of phloroglucinol. Released tannin subunits as flavan-3-ol components and phloroglucinol adducts were analysed by a published HPLC protocol (Kennedy and Jones 2003), and quantified as epicatechin equivalents using (-)-epicatechin as the quantitative standard (Sigma Aldrich, St Louis, USA). The HPLC protocol involved two 300 x 7.5 mm, 5 μ m PLgel columns in series: 500 Å and 104Å (Varian Inc., Mulgrave, Victoria, Australia) sheathed by a PLgel guard column. Samples in methanol were diluted with 4 volumes of mobile phase (N,N-dimethylformamide with 1% v/v glacial acetic acid, 5% v/v water, 0.15 M lithium chloride) and a 20 μ L injection volume was applied. Columns were maintained at 60°C with a sample flow of 1 mL/min and elution monitored from 280 – 520 nm. Tannin isolate analysis by gel permeation chromatography followed a published protocol (Kennedy and Jones, 2003) with the modifications reported in Bindon and Kennedy (2011). The percentage of tannin subunit yield from phloroglucinolysis as a proportion of total tannin concentration provided the measure “percent conversion yield,” which represents the proportion of total tannin that was depolymerizable.

In total, four tannin compositional measures were applied to tannin isolated from wine samples: % tri-hydroxylated (epigallocatechin, prodelphinidin) subunits (%triOH); % galloylated subunits (%gall); mean degree of polymerization (mDp); and molecular size at 50% elution by gel permeation chromatography (50%GPC). GPC was used as an indicator of the median size of tannin polymers in each wine sample, mDp provided a measure of the mean number of polyphenol subunits in wine tannin polymers, % trihydroxylation has been proposed as an indicator of tannin of skin origin, and % galloylation has been proposed as an indicator of seed-origin of tannin (Kassara & Kennedy 2011).

Statistical analysis. Juice and wine chemistry, and phenolics and tannin composition data were analysed in the statistics package R (version number 3.1.0) using one-way and two-way ANOVA with post-hoc analysis as required by Tukey's honestly significant difference (HSD) test with significance at $p \leq 0.05$.

Results and Discussion

Fruit composition and phenolics. Weather conditions during the growing season were comparable between the two vineyard sites, however total heat accumulation (cumulative GDD₁₀) was lower than the long-term regional average as was rainfall (Table 1). Table 2a shows that basic fruit phenolics and maturity at vineyard A were not significantly impacted by leaf removal (LR), with the exception of total phenolics concentration, which was 12 % higher in fruit from the LR treatment. Table 2b shows no statistically significant impact of vineyard treatments at vineyard B on any of the fruit parameters reported. A seasonal- and site-specific response in total phenolics concentration was reported by Lee and Skinkis (2013), with an increased concentration associated with early- and mid-season leaf removal at one site, and no response to the same treatments at another site. However, in that study an increase in total anthocyanin concentration in fruit was observed at both sites associated with leaf removal, which was not observed in our study. However, our findings did concur with other studies in Shiraz and Pinot Noir which demonstrated that defoliation has little effect on grape total anthocyanins (Cortell & Kennedy 2006, Price et al. 1995, Ristic et al. 2007).

Given cluster exposure did not impact upon fruit phenolics, tannin and anthocyanins, a comparison was made between the two vineyards for these three parameters. Total phenolics and

anthocyanins were significantly higher at vineyard B with no difference in total tannin. When comparing seasonal weather conditions as in Table 1, there was negligible difference between GST, cumulative GDD₁₀ or average daily radiation, therefore it could be expected that there would be a minimal response if these weather parameters alone were the primary drivers. This indicates there are other site-specific factors which drive these phenolic measures, potentially local climatic, geology, pedology and phenology characteristics, as identified in the study in the Côte de Nuits appellation (Roullier-Gall 2014).

The measure of total phenolics in fruit may not offer useful insight into phenolic effects because this is a ‘catch all’ measure which does not distinguish between, for example, anthocyanin, tannin and low molecular weight non-pigmented phenolic compounds (Dambergs et al. 2012). Hence, it is difficult to infer whether the significant increase in total phenolics from LR treatment that was observed in vineyard A fruit (Table 2a) was associated with a possible trend toward higher values for anthocyanin and tannin with LR in that fruit (Table 2a), or if the significantly higher total phenolics result for vineyard A fruit infers the LR treatment yielded fruit with a greater concentration of low molecular weight non-pigmented phenolics. By way of example, hydroxycinnamic acids were reported by Sternad Lemut and others (2015) to increase after early leaf removal.

Tables 2a and 2b show that LR was not associated with a significant difference in total tannin concentration in fruit from both vineyard sites, compared with noLR treatment. This lack of a treatment effect contrasts with previous research reporting increased total tannin as a result of cluster exposure in Pinot noir (Price et al. 1995, Cortell & Kennedy 2006). In a climate similar to that of our study, Kemp and others (2011) also reported no difference in total Pinot noir fruit tannin concentration when leaves were removed as early as 7 days after flowering, and Lee and Skinkis (2013) also reported no significant response in terms of tannin to leaf removal treatments applied to Pinot noir vines from flowering onwards. Both studies, however, reported variation in mean total fruit tannin between vintages.

Several of these previous studies utilised different tannin analysis methods to the current study which could account for the discrepancies between studies treatment effects on total tannin. Based on these results from our study (Tables 2a and 2b), and previous studies, LR treatment could be concluded to be an unreliable method for increasing the concentration of color and tannin compounds in Pinot noir fruit.

Of both scientific and practical significance was the finding that fruit phenolics analysis by the homogenate method (Iland 2000; Australian Wine Research Institute, 2009) did not reliably predict Pinot noir wine phenolic concentration. This discrepancy has also been reported for Shiraz and Cabernet Sauvignon (Bindon et al 2014). Further, the methods used to quantify total tannin did not provide insight into variation between treatments in the apparent source of tannins (composition), and the impact that variation in tannin composition may have had on the extent of tannin polymerization and size of tannin polymers in wines. These findings on tannin composition and polymerization may demonstrate that viticultural and winemaking interventions could affect wine mouthfeel and color stability, in different ways to simply impacting phenolic concentration. An important conclusion from this research is the limitation of a currently accepted method for quantifying grape phenolics by homogenization (Tables 2a and 2b), and the limited relevance of those results for describing wine phenolic concentration. This highlights the need for more effective, realistic measures with predictive power for wine outcomes. The Australian Wine Research Institute's wine-like extraction protocol, which aims to measure extractable (under winemaking-like conditions) rather than total tannin and color (Bindon et al. 2014), may address the grape analysis shortcoming highlighted in our research findings.

A lack of Pinot noir grape composition response to defoliation treatments does not always lead to a lack of response in Pinot noir wines. Cortell and Kennedy (2006) found that although there was little effect of shading on the anthocyanin content of Pinot noir grapes, wines made from shaded fruit were significantly lower in anthocyanins than wines made from exposed fruit. This was similar to findings by

Price et al. (1995) who found no difference in berry anthocyanins, however they found that Pinot noir wines from exposed fruit had more anthocyanins than shaded fruit. Price and others (1995) related this to berry size variation and the resulting difference in juice to skin ratios, however Cortell and Kennedy (2006) suggest that anthocyanins from exposed fruit were more extractable than anthocyanins from shaded fruit. Leaf removal, or defoliation, of grapevines has been demonstrated to relatively severe to elucidate a response in anthocyanins 65% reduction in leaf area = not significant reduction in anthocyanins (Pallioti 2013), likely due to photosynthetic compensation in retained leaves (Poni 2000).

In the following section, we describe the impacts of LR on the concentration and composition of wine phenolics, and discuss the potential importance of impacting phenolics for wine characteristics.

Wine phenolics and tannin composition. Analysis by two-way ANOVA showed no interaction between the viticultural treatments applied at vineyard A and winemaking treatments applied to fruit from vineyard A for total phenolics (Table 3) or for tannin composition (Table 4) in wines at 6 months bottle age. The lack of interaction between these viticultural and winemaking treatments suggests that the significant effects observed were additive, indicating potential intervention points for phenolic and tannin concentration adjustment in both the vineyard and winery.

Leaf removal influence on wine phenolics and tannin. Leaf removal treatment was associated with significant differences in total pigment, color density and non-bleachable pigment concentration in wines. Total pigment and non-bleachable pigment were 17% higher in wines made from LR fruit, and this higher stable color result was reflected in the 15% higher color density observed in LR wines at 6 months bottle age (Table 3).

The lack of a significant difference for total phenolic concentration in LR wines (Table 3) contrasted with the observations from fruit analysis (Tables 2a and 2b) and supports research that has led to questioning of the predictive value of commonly used methods for quantifying phenolics in red grapes (Bindon et al. 2014). The work of Bindon and others (2014) emphasised the distinction between total

available phenolics in grape material, and total phenolics extracted under winemaking conditions. Application of a wine-like extractive analysis (Bindon et al. 2014) to the leaf removal fruit, instead of the homogenate method used in our study, might have delivered a different result in terms of the impact of viticultural treatment on grape total phenolics concentration; further research specific to Pinot noir would be required to verify this assertion.

Phenolic compounds are generated in red wine grapes as a response to light exposure (Price et al. 1995) and the leaf removal regime applied in this study was implemented by the Vineyard Manager with a target to increase fruit exposure by approximately 75% at each vineyard. Cluster exposure at key phenological stages is important (e.g. timing of leaf removal, as opposed to cumulative light exposure) as tannin precursors are synthesised during the first phase of berry growth, and anthocyanins accumulate during the ripening phase (Boss et al. 1996). Based on the data in Table 3, leaf removal post-flowering and pre-veraison could be concluded as effective for increasing stable color in Pinot noir wine at 6 months bottle age, however our results were not able to elucidate the mechanism which underpins these observations at the grape level. An alternate hypothesis would be that leaf removal had no impact on the synthesis and accumulation of phenolics, but rather changed the relative extractability of phenolics. This hypothesis would suggest physical cellular and sub-cellular effects with relevance to the retention or release of phenolics during fermentation.

The pattern of difference associated with leaf removal that we observed in these young trial wines may be promising for longer term color stability as an analysis of a 30 year and a 50 year series of Shiraz wines showed that wine color density was more closely correlated with concentration of pigmented polymers (non-bleachable pigment) than anthocyanin concentration, after the first two years of bottle aging, and that non-polymerised anthocyanins did not contribute significantly to visible color in wines by four years bottle age (McRae et al. 2012). Given the tendency of some Pinot noir wines to lose color with bottle aging, the early stable color results reported here at 6 months bottle age may justify the cost of leaf

removal during the growing season as a means to build color and color stability, particularly in vineyard blocks with a history of producing wine with poor color or poor longer term color stability.

The observed lack of significant difference in fruit tannin concentration between leaf removal treatments (Tables 2a and 2b) was also apparent in wines, with no significant difference observed in wines between the two leaf removal treatments for the tannin measure applied in this study (total tannin by spectroscopy).

While differences in total wine tannin concentration between leaf removal treatments were non-significant (Table 3), Table 4 shows several differences in the tannin composition of wines measured at 6 months bottle age. From the phloroglucinolysis results, four tannin compositional measures are presented for tannin isolated from wine samples, namely the percentage of tri-hydroxylated (epigallocatechin, prodelphinidin) subunits (%triOH) and galloylated subunits (%gall), the mean degree of polymerization (mDp); and molecular size at 50% elution by gel permeation chromatography (50%GPC).

Leaf removal treatment was a main effect for four out of the five tannin composition measures presented (Table 4). The mean mass conversion ratio for LR wines was 4% lower than for noLR wines, possibly associated with greater incorporation of pigment within the tannin structure, as discussed previously, which may have indicated more of the tannin in LR wines was more derived (i.e. less able to be depolymerized). A more derived tannin structure might potentially impact wine sensory quality, given the established association between red wine aging and perceived astringency attributed to changes in tannin structure (McRae & Kennedy, 2011, McRae et al. 2012).

The difference in the size of wine tannin polymers as measured by gel permeation chromatography (50% GPC) was not significantly different between treatments, however tannin mDp was slightly lower in noLR wines compared with LR wines. This was possibly associated with an increased contribution of larger skin tannin in LR wines, reflected in higher tannin %TriOH with a corresponding drop in in %gall. This may have been due to a higher grape skin tannin concentration associated with

increased light exposure after leaf removal, but may alternately reflect differences in skin tannin extractability resulting from the LR treatment. Retention of tannin in wine is a function of both its relative extractability, and net loss through selective re-adsorption to grape solids and/or macromolecules during alcoholic fermentation. Recent research showed wine tannin composition in Pinot noir may have been impacted by preferential re-adsorption of seed tannins to grape pulp, compared with tannin of skin origin (Sparrow et al 2015). LR treatment may have altered the physiochemical qualities of grape solids in a way that altered relative tannin extractability, or (re)adsorption properties of the grape for tannin, however this was not explored in our study, but is an area identified for future investigation.

The mDp values for wines in our study sit within the range of mDp reported in the literature for young Pinot noir wines which is 5.0 – 8.6 subunits (Aron & Kennedy 2007; Kemp et al 2011). Wine tannin polymers in aged wine are generally larger and less astringent than those of young red wine (McRae et al 2012) however, polymers with a higher mDp have been associated with more astringent mouthfeel qualities in model wines (Vidal et al 2003). Further investigation of the hydrophobicity of subunits from LR and noLR wines might have offered insight into the mechanism of mouthfeel effects associated with these apparently differing polymer constituents; McRae and others have established that a greater number of hydrophobic subunits may confer less mouthfeel astringency (McRae et al 2013) but it is not clear if the changes in tannin composition observed here would be substantive enough to significantly impact sensory properties.

Given the important role of tannin in Pinot noir mouthfeel and color stabilization, these viticultural effects on tannin composition may influence wine sensory quality and aging potential. The volumes of wine made in this trial precluded formal sensory analysis so future research should include sensory assessment of wines, given the tannin composition effects observed here. Further investigation may also be warranted to better understand if observed tannin composition effects were a contributing factor for the greater concentration of non-bleachable pigment observed in LR wines at 6 months bottle

age. If so, this might suggest the viticultural treatment could be applied to generate a tannin composition associated with more effective color stabilization.

Winemaking treatments influence on wine phenolics and tannin. The two controlled phenolic release (CPR) winemaking treatments applied in this study were under investigation as alternate maceration approaches to manage Pinot noir wine phenolics concentration and tannin composition, compared with standard vinification on skins (control). There were no interactions between viticultural and winemaking treatments so therefore winemaking treatment results have been discussed separately from viticultural treatment results. Winemaking treatment was a main effect for all nine of the phenolic parameters examined in wines at 6 months bottle age (Table 3), and for all tannin composition measures, apart from percent conversion ratio which was not significantly different amongst winemaking treatments (Table 4). The three winemaking treatments were associated with significant differences in some phenolics concentration and tannin composition measures, and those results are shown in Tables 3 and 4. In the following section, we consider each of the two CPR treatments applied separately as possible alternatives to ‘standard’ winemaking as represented by the control winemaking treatment.

The CPR-skins treatment involved must being subjected to CPR and then fermented on skins for seven days. Table 3 shows that, compared with control wines, the CPR-skins wines had significantly higher total phenolics, non-bleachable pigment, color density, and total tannin. The difference between control and CPR-skins wines for these parameters was substantial; CPR-skins wines had almost two-fold the non-bleachable pigment and total tannin of control wines. It is notable that the concentration of anthocyanin and total pigment in control and CPR-skins wines was not significantly different. Taken together with the observed effect of CPR on non-bleachable pigments this may indicate that the composition of compounds in the ‘total pigment’ fraction of wines differed between treatments, and this may have resulted in precocious development of stable pigmented products.

Tannin compositional analysis indicated that the higher tannin outcome in CPR-skins wines may, in part, have been attributable to greater proportional extraction of seed tannin; as demonstrated by the approximately 25% higher %gall in tannins isolated from the CPR-skins wines compared with control wines from that fruit (Table 4). Control winemaking was associated with significantly higher mDp, compared with CPR-skins wines. As previously noted, galloylated tannins have been associated with astringency in model red wines (Vidal et al. 2003) so this may have implications for the palatability of CPR-skins wines.

Similar to the results observed for noLR versus LR wines (Table 4), the molecular size of tannin in CPR-skins wines by 50%GPC was not significantly different to that of control wines, despite the lower mDp. Should the observed differences in tannin composition have been associated with increased seed extraction, it would have been expected that tannin size measures might have been more clearly differentiated due to established differences in hydrodynamic volume associated with tannin origin in the grape (Kennedy & Taylor 2003). In other words, a greater extraction of seed tannin than skin tannin associated with the treatments applied, would have been predicted to result in smaller molecular weight tannin polymers (ie. lower 50%GPC values).

CPR-press treatment wines were made by subjecting grape must to CPR and then pressing juice off skins prior to fermentation. In the current study, Control and CPR-press wines did not differ significantly in concentration of total phenolics, tannin, non-bleachable pigment, or color density (Table 3), suggesting the CPR-press treatment was somewhat analogous to submerged cap alcoholic fermentation on skins for 7 days in terms of color development. This was also demonstrated in that CPR-press wines were significantly lower in total pigment (17%) and anthocyanin (63%) than control wines but had attained an equivalent level of non-bleachable pigment (Table 3). This suggests that, as for CPR-skins wines, there may have been precocious formation of stable color in CPR-press wines. The hue value for CPR-press wines was significantly lower than the control indicating more purple-hued wines. The low

SO₂-resistant hue values suggested that the purple hue of the CPR-press treatment coincided with a significantly higher concentration of purple pigmented polymers. Analysis of these wines over a longer bottle age period would establish whether CPR-press treatment wines, having attained this comparatively high early concentration of stable purple color, might maintain this apparent ‘young’ purple coloration over time. The stable color may be related to ethyl bridged compounds (e.g. malvidin-3-glucoside dimer) (Pissarra et al. 2003, Sanchez-Ilarduya et al. 2012) and pigments formed through direct condensation (e.g. flavanyl-vinyl pyranoanthocyanins) (Mateus et al. 2003, Cheynier et al. 2006). Future analysis of CPR-press wines by liquid chromatography mass spectrometry or electrospray mass spectrometry is recommended to examine for differences in concentration of these compounds (Hayasaka et al 2007; Pissarra et al 2003). Such future research might establish which compounds and formation mechanisms are important in contributing to the purpleness of CPR-press wines. Tannin composition varied between control and CPR-press wines (Table 4). CPR-press wines had a higher relative proportion of trihydroxylated tannins than control wines which, as discussed previously, may point to a greater contribution of skin-derived material. CPR-press wines had limited contact time with seeds, and seed tannins tend to extract during the latter part of on-skins fermentation. There were also notable differences in the size of tannin polymers in CPR-press wines, compared with control wines, suggesting tannin polymers were smaller. This was associated with a lower mDp in CPR-press wines from LR treatment, than control wines, and significantly lower 50%GPC in CPR-press wines than control for the noLR wines. This again highlights that tannin origin within the grape (skin versus seed) has implications for tannin hydrodynamic volume (molecular size) which is correlated with, but not necessarily dependent upon, the absolute molecular mass. Longer term bottle aging and sensory assessment of wines would provide insight into the impact of these polymer differences on wine mouthfeel, tannin condensation and color stability.

The data presented herein shows that CPR may offer two potential winemaking options: 1. wine that is phenolically similar to control wine, but fermented without pomace (hence approximately 40% saving in tank space, no cap management required), and 2. wines with relatively high total tannin and non-bleachable pigment concentration after fermentation for a relatively short period of 7 days (hence achieving highly extracted, phenolic-rich wine from a short occupation of tank space).

CPR-skins winemaking for Pinot noir is unlikely to be widely adopted as it results in highly extracted wines, that are potentially too phenolically intense and concentrated to represent this variety. The more likely application of CPR-skins winemaking for Pinot noir would be in generating blending components, particularly in vineyards or seasons where fruit color or tannin accumulation is poor.

The industry potential of the CPR-press results reported here, also need to be interpreted conservatively as a previously published study in which we compared control and CPR-press wines from two parcels of fruit vinified in the same way as this study demonstrated that the relative impact of control and CPR-press on wine phenolics could vary by parcel of fruit (Carew et al. 2014). CPR-press treatment may therefore offer production efficiencies by allowing wines to be pressed off early and liberating winery tank space but further development of this approach is required. For example, further investigation of the hold time parameters (duration of time must is held at high temperature prior to pressing off) for CPR-press and the relative impact of various viticultural practices on phenolics extractability. In addition to those research questions, wider uptake of CPR-press as a winemaking technique would depend on additional research on the sensory quality and long-term development of wine character for wines made in this way.

Conclusion

Managing the extraction of phenolic compounds from grape solids into juice during alcoholic fermentation is key to producing the best possible quality Pinot noir wine under the tight time constraints of vintage. The vagaries of cool climate growing conditions for Pinot noir, and capital cost of winery equipment and capacity mean that vineyard managers and winemakers need options to more accurately and efficiently achieve the desired phenolic character in wines. We reported herein on two intervention options for adjusting phenolic concentration and composition in Pinot noir wines, and demonstrated that those two interventions might be applied to achieve *additive* effects on wine phenolic concentration.

Priority future research to build from the findings reported here would include winemaking on a larger scale (beyond microvinification) and further analysis of the long-term development of wine color (stability) from the viticultural and winemaking treatments we applied. Larger scale winemaking would allow for consideration of the relative mouthfeel effects of the range of tannin compositions observed in wines in this study. For example, further investigation of the properties of wine tannins associated with the maceration treatments could establish their potential impact on wine mouthfeel, the extent of folding in these polymers, impact on availability of hydrophobic sites for binding with salivary proteins, and resulting potential to mediate astringency perception (McRae & Kennedy 2011).

Recent research also highlights differences in macromolecules other than tannin (polysaccharide, protein) in wines from alternate winemaking (Bindon et al. 2016). Extending this research would provide insight into the relative impact of viticultural treatments (eg. leaf removal) in combination with novel winemaking approaches (eg. CPR) to further elucidate the practical potential of novel viticulture and winemaking for managing wine macromolecular composition and more accurately controlling the sensory outcomes of Pinot noir winemaking.

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Table 1 Weather conditions during the grape growing season (October 2012 – April 2013) and the long-term regional average for Northern Tasmania, Australia (LTA; 1995-2019). GST = growing season temperature, GDD₁₀ = growing degree day base 10.

Vintage	Kayena 2012/13	White Hills 2012/13	Regional LTA
GST (°C)	15.6	15.5	15.3
Average daily radiation (MJ/m ²)	20.0	20.0	17.0
Total cumulative GDD ₁₀	1186	1187	1134
Total rainfall (mm)	262	283	347

Table 2a Phenolics concentration and composition measures for fruit with two viticultural treatments at vineyard A.

	Total soluble solids (°Be)	Titrateable acidity (g/L)	pH	Total phenolics (AU/g)	Total anthocyanins (mg/g)	Total tannin (mg/g)
No leaves removed	13.0	8.89	3.30	1.15 a ^a	0.60	6.89
Leaves removed	13.4	8.39	3.29	1.31 b	0.70	7.93

^aDifferent lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($p < 0.05$).

Table 2b Phenolics concentration and composition measures for fruit with two viticultural treatments at vineyard B.

	Total soluble solids (°Be)	Titrateable acidity (g/L)	pH	Total phenolics (AU/g)	Total anthocyanins (mg/g)	Total tannin (mg/g)
No leaves removed	12.6	8.30	3.31	1.33	0.84	7.73
Leaves removed	12.4	8.39	3.25	1.36	0.88	7.90

^aDifferent lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($p < 0.05$).

Table 3 Main effects on phenolics concentration in 6 month old Pinot noir wine from two viticultural treatments at vineyard A, and three winemaking treatments by modified Somers measure and total tannin by spectral method.

	Total phenolics (AU)	Total pigment (AU)	Total anthocyanins (mg/L)	Color density (AU)	Hue	SO ₂ – resistant hue (AU)	Non-bleachable pigment (AU)	Total tannin (g/L)
Viticultural treatment								
No leaves removed	24.3	4.25 a ^a	27.3	6.21 a	0.94	1.17	1.73 a	0.71
Leaves removed	25.2	5.16 b	33.9	7.28 b	0.92	1.15	2.08 b	0.76
Winemaking treatment								
Control	23.2 a	4.67 a	43.4 a	5.90 a	0.96 a	1.24 a	1.50 a	0.60 a
CPR-skins	33.8 b	5.57 a	32.4 ab	8.22 b	0.96 a	1.17 a	2.37 b	1.33 b
CPR-early press	17.2 a	3.87 b	16.1 b	6.10 a	0.86 b	1.06 b	1.84 a	0.29 a
Two-way ANOVA (P values)^b								
Leaf removal	0.67	0.04	0.41	0.003	0.33	0.33	0.008	0.67
Winemaking	>0.001	0.01	0.03	>0.001	0.003	>0.001	>0.001	>0.001
LR x Winemaking	0.73	0.60	0.79	0.35	0.41	0.29	0.65	0.77

^aDifferent lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($p < 0.05$).^bTwo-way ANOVA results for main effects and interactions.

Table 4 Main effects on tannin composition in 6 month old Pinot noir wine from two viticultural treatments at vineyard A, and three winemaking treatments.

	Mass Conversion Ratio (%)	Trihydroxylated tannin (%)	Galloylated tannin (%)	Mean degree of polymerization (mDp)	Molecular mass at 50% GPC (g/mol)
Viticultural treatment					
No leaves removed	62 a ^a	22 a	3.7 a	6.2 a	1822
Leaves removed	58 b	25 b	3.2 b	7.5 b	1940
Winemaking treatment					
Control	58	22 a	3.4 a	7.8 a	1971 a
CPR-skins	52	23 a	4.4 b	6.7 b	2012 a
CPR-early press	59	25 b	2.7 c	6.1 b	1660 b
Two-way ANOVA (P values)^b					
Leaf removal	0.02	>0.001	>0.001	>0.001	0.20
Winemaking	0.28	>0.001	>0.001	>0.001	>0.001
LR x Winemaking	0.99	0.10	0.07	0.94	0.90

^aDifferent lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($p < 0.05$);^bTwo-way ANOVA results for main effects and interactions.