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IMPACT OF CROP LEVEL AND HARVEST DATE ON ANTHOCYANINS AND PHENOLICS OF RED WINES FROM ONTARIO

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ABSTRACT

Cabernet Sauvignon (CS) and Cabernet franc (CF) vines were subjected to two crop levels (full, half) and three harvest dates (earliest to latest; T0, T1, T2) over two vintages. Wines were analyzed for anthocyanins, phenolics, and proanthocyanidins. Crop level increased CS hue (2011, 2012), increased CS pH and reduced color intensity (2012), and reduced CF hue (2012). Harvest date had a greater effect than crop level, with many treatment interactions. Half crop (2011) increased three CS anthocyanins plus procyanidin B. Extended harvest increased eight compounds. Quercetin and (+)-catechin decreased in T1. Crop reduction (2012) increased malvidin-3-coumarylglucoside and (+)-catechin, but decreased petunidin and delphinidin-3-coumarylglucoside. Harvest date (2012) impacted all but two compounds, with highest anthocyanin concentrations in T1 wines. Gallic acid, (+)-catechin, and resveratrol increased with harvest date, while three phenols decreased. Half crop (2011) increased CF peonidin. Extended harvest increased four phenols while three others decreased. Crop reduction (2012) increased delphinidin-3-acetylglucoside, cyanidin-3-coumarylglucoside and caffeic acid; (-)-epicatechin and *p*-coumaric acid decreased. Several anthocyanins and phenols decreased between T0 and T2, nine of 13 anthocyanins decreased between T0 and T1, while others decreased from T1 to T2. Gallic acid and (+)-catechin increased with harvest date; (-)-epicatechin, *p*-coumaric acid, quercetin, and resveratrol decreased.

Key words: Cluster thinning, harvest date, proanthocyanidins, hydroxycinnamates, hydroxybenzoic acids, flavonoids

INTRODUCTION

Many vineyard factors impact development of polyphenolics (Downey et al. 2006). Temperature and light influence anthocyanin biosynthesis (Cortell et al. 2007; Holt et al. 2010; Di Profio et al. 2011a,b).

Cultural practices, e.g., crop level and harvest date, may also impact flavonoid analytes. Both influence flavonoids individually, particularly crop level (Guidoni et al. 2002; Keller et al. 2008; Di Profio et al. 2011a,b), and to a lesser degree harvest date (Pérez-Magariño and González-San José, 2004, 2006; Canals et al. 2005; Holt et al. 2010). Crop load (Ravaz Index), i.e. the ratio of crop size (yield) to vine size (weight of cane prunings) is among many factors that determine the ability of a vine to mature its fruit (Bravdo et al. 1985). Effects of crop level can differ depending if implemented through shoot thinning or dormant pruning (Reynolds et al. 1994, 2005; Holt et al. 2010) or by removing clusters (Mazza et al. 1999; Guidoni et al. 2002). Crop levels implemented by retaining additional shoots may result in shading, and shade effects are difficult to separate from those of crop size (Di Profio et al. 2011a,b). Fruit exposure-associated light and temperature effects influence anthocyanin and phenolics in red wine grape cultivars (Reynolds et al. 2005). Shade may be increased by early-season shoot thinning due to increased leaf size, whereas late season shoot thinning can lead to shortened internodes, reduced leaf size and lateral shoots, and better sunlight penetration into the canopy, ultimately increasing anthocyanins (Reynolds et al. 2005). This implies a positive relationship between soluble solids concentration (Brix) and anthocyanin synthesis (Di Profio et al. 2011a,b).

Influence of crop level adjustment depends upon vine phenological stage and viticultural treatment. Early cluster thinning is associated with greater cluster shading vs. veraison thinning, due to larger leaves, more lateral shoot growth, or higher overall vine vigor (Reynolds et al. 1994). Post-veraison cluster thinning eliminates effects associated with early shoot/cluster thinning because shoot growth has ceased, and is associated with increased Brix and anthocyanins (Di Profio et al. 2011a). In several red *Vitis vinifera* cultivars, crop reduction by cluster thinning resulted in increased Brix and anthocyanins, as well as total phenols and color intensity (Mazza et al. 1999; Downey et al. 2006; Guidoni et al. 2008). Impact of crop level adjustment is cultivar-dependent due to differences in anthocyanin biosynthesis capabilities (Guidoni et al. 2008). Cluster thinning can be more efficacious than basal leaf removal on anthocyanins and total phenols (Di Profio et al. 2011a), suggesting greater importance of source: sink ratios vs. cluster exposure in berry anthocyanin accumulation (Di Profio et al. 2011a,b).

Beneficial effects of crop reduction on anthocyanins and phenolics have been demonstrated extensively in Ontario (Reynolds et al. 2005; Di Profio et al. 2011a,b), although this may not be true elsewhere (Keller et al. 2008). Manipulations of sink to source ratios in grapevine by crop manipulation are known to affect total anthocyanins (Reynolds et al. 1994, 2005; Sun et al. 2010; Di Profio et al. 2011a), but their effects on individual anthocyanins have not been widely reported (Guidoni et al. 2002). Few studies have quantified individual phenolics impacted by crop manipulation (Guidoni et al. 2002; Pérez-Magariño and González-San José 2004; Di Profio et al. 2011b). Effects of how individual phenolics affect wine taste and mouthfeel and the role played by crop reduction are likewise noteworthy (Guidoni et al. 2002). Understanding viticultural impacts on accumulation of individual phenolics and anthocyanins could benefit wine quality (Pérez-Magariño and González-San José 2006).

Understanding which compounds are present at specific points of extended maturity and after crop manipulation could lead to quality enhancement. Harvest date and vineyard management may change berry size and composition, as well as subsequent wine composition. Phenolic development is influential in defining quality and maturity. Anthocyanin accumulation begins at veraison and continues through fruit maturation, although biosynthesis of individual anthocyanins occurs at different rates (Keller and Hrazdina 1998). Anthocyanin concentrations normally reach a plateau, but occasionally decline late in fruit maturation (Fournand et al. 2006). Anthocyanin accumulation initially begins with dihydroxylated anthocyanin glucosides (cyanidin, peonidin), followed by trihydroxylated anthocyanins (delphinidin, petunidin, malvidin) (Kennedy 2010). Unlike anthocyanins, there are two periods of flavonol biosynthesis in grape berries, the first around fruit set and the second 1-2 weeks after veraison until maturation (Downey et al. 2003b). Evolution of different phenolic families is strongly influenced by cultivar, as well as by cultural practices and terroir (Pérez-Magariño and González-San Jose 2006).

Few studies have investigated harvest date effects on wine color intensity. Wines made from more mature grapes generally had higher concentrations of free anthocyanins (Pérez-Magariño and González-San José 2004). During wine aging, free anthocyanins and flavanols decreased in conjunction with increased levels of anthocyanin derivatives—new pigments—responsible for maintaining color intensity and adding violet hues in aged wines. This suggested that composition of grapes harvested later than usual was crucial to quality aged wines in terms of color stability. Moreover, grapes that are allowed to desiccate prior to harvest may also exhibit increased concentrations of anthocyanins, other flavonoids, and proanthocyanidins (Rolle et al. 2009, 2013; Bindon et al. 2013; Figuerido-Gonzalez et al. 2013; Torchio et al. 2016). Delayed harvest is associated with higher Brix and ethanol; therefore, elevated ethanol facilitates anthocyanin and proanthocyanidin extraction, and decreases co-pigmentation, which consequently reduces color intensity (Canals et al. 2005). Increasing ethanol in the maceration media increases proanthocyanidin astringency. Work with Maréchal Foch wines (Sun et al. 2010) was consistent with Pérez-Magariño and González-San José (2004) in that higher intensities of blue or violet tones were detected in wines based upon later harvest dates, and those wines were also more “fruity”.

Another definitive reason for flavonoid research is to further understand the nature of berry maturation. There is widespread interest in late harvest and appassimento wine styles in Ontario. Fruit maturation determines grape quality, and time of harvest is an important factor in the making of quality wines (Perez-Magarino and González-San José 2006). Flavonoids contribute to likability and subsequent consumption of a particular wine style in terms of bitterness and astringency as well as hue, intensity and color (Hufnagel and Hofmann 2008). Co-pigmentation is important for understanding grape composition vs. wine color relationships, variation in color and pigment concentration between wines, and reactions involving anthocyanins during wine aging and stability (Boulton 2001). The purpose of this investigation was to

examine impacts of two crop levels, three harvest dates, and their interactions upon individual anthocyanins and phenolics in Cabernet franc and Cabernet Sauvignon wines in Ontario over two vintages.

MATERIALS AND METHODS

Site parameters

Experiments were conducted at Puglisi Vineyard, Niagara-on-the-Lake, Ontario (Latitude: 43.21°N; longitude: 79.10°W). Two separate 2-ha blocks of ≈10 year old Cabernet franc (CF; ENTAV clone 214) and Cabernet Sauvignon (CS; ENTAV clone 337) were chosen for study. Vines were grafted onto 3309 rootstock, trained to double Guyot with vertical shoot positioning, and spaced at 2.7 m x 1.2 m (row x vine), with permanent sod (every row) floor management. Soil series was imperfect to poorly drained Chinguacousy clay loam (Kingston and Presant 1989). Drainage tiles were installed prior to planting in every row at a ≈ 60 cm depth midpoint in each inter-row space. Nitrogen was not applied to the vines 2008-2011, but ≈ 600 kg/ha of muriate of potash (KCl; 0-0-60) was added in 2007. Pest control was consistent with local recommendations (OMAFRA 2010).

Experimental design

The treatments included two crop levels: full crop (FC) and half crop (HC) factorially combined with three harvest dates. Full crop treatments experienced no crop adjustment but half crop treatments were cluster-thinned at veraison to one basal cluster per shoot. In 2011 the date for the initial harvest treatment (T0) was 22 October 2011 for both cultivars, and subsequent harvest treatments occurred 3 weeks (07 November) and 6 weeks (06 December) thereafter (T1 and T2, respectively). In 2012, T0, T1, and T2 for CF were 2 October, 23 October, and 15 November, respectively, and for CS were 16 October, 8 November, and 27 November, respectively. Each block consisted of six adjacent rows of six-vine panels that comprised the crop level x harvest date treatment combinations. Treatment combinations were randomized within each block. Vines were cane pruned to three, eight-node canes plus two, two-node renewal spurs. Cane pruning weights were collected using a digital scale (Rapala, China) as an estimate of vine size. At harvest, clusters per vine were counted and yield per vine was recorded on a standard digital field scale (Mettler-Toledo 3200; Mettler, Zurich, Switzerland).

Vinification

Ten kg of fruit from each treatment replicate were combined from adjacent rows into three 20-kg fermentations for each treatment combination. Bins were inspected for damaged berries and clusters and sorted accordingly before being crushed and de-stemmed into 20-kg food-grade pails. Each treatment was inoculated with Lalvin EC-1118 (Lallemand, Montreal, QC) yeast at a rate of 0.4 g/L. Diammonium phosphate was added at 0.4 g/L prior to inoculation. All fermentations were conducted at 25°C in a temperature-controlled room. Punch down occurred twice daily over a 7-d period. After fermentation was

complete, wines were pressed in an Idropress 50 (Enoagricola Rossi, Calzaro, Italy) at 0.2 mPa. After pressing, wines were settled for 24 hr, racked, and inoculated at 0.01g/L with MBR Lalvin 31 *Oenococcus oeni* (Lallemant, Montreal, QC). Malo-lactic fermentation was carried out for ≈ 30 d at 23°C. Wines were thereafter cold stabilized for ≈ 30 d at -2°C, racked and stored at 12°C until pad filtration (0.45 μ) and bottling. Wines were sulfited at 30 mg/L following cold stabilization and at bottling.

Wine analysis

Titrateable acidity, pH, ethanol

Wine pH was measured with a Fisher Accumet Model 25 pH meter (Fisher Scientific, Mississauga, ON). Titrateable acidity (TA) was measured using a PC-Titrate automated titration system (Man-Tech Associates, Inc., Guelph, ON) by titrating a 5-mL wine sample to a pH 8.2 endpoint with 0.1 N NaOH. TA was reported in tartaric acid equivalents. Ethanol was measured by gas chromatography on an Agilent 6890 series GC (Nurgel et al. 2004).

Color

Wine samples (20 mL) were centrifuged at 10 000 g for 10 min in an IEC Centra CL2 centrifuge (International Equipment, Needham Heights, MA). Color analysis was based on a modified method provided by Mazza et al. (1999). Absorbance was measured on an Ultrospec 2100 pro UV/VIS spectrophotometer (Biochrom, Cambridge, UK). Samples were measured at 420 nm (maximum brown color) and 520 nm (maximum red/blue color) in 1-mm pathlength, 2-mL cuvettes. The reference blank for the wines contained 12% (v/v) ethanol and 10 g/L tartaric acid. Color analysis was calculated based on the following formulae: color intensity = $A_{520} + A_{420}$ (total absorbance); hue = A_{420}/A_{520} (ratio of the dominant wavelength).

Total anthocyanins

Total anthocyanins were measured using the pH shift method by measuring the differential absorbance at 520 nm between wines at pH 1.0 and 4.5 (Fuleki and Francis 1968). Buffer solutions of pH 1.0 and 4.5 were prepared as follows: pH 1.0 buffer consisted of 0.2M KCl/0.2N HCl (1/2.68; v/v); pH buffer 4.5 contained 1M sodium acetate/1M HCl/distilled water (1.67/1/1.5; v/v). Absorbance was measured with an Ultrospec 2100 pro UV/VIS spectrophotometer at 520 nm against respective pH buffer blanks. Total anthocyanin concentration was calculated based on a standard curve of malvidin chloride 3-glucoside using: Total anthocyanins (mg/L) = $(A_{pH1.0} - A_{pH4.5})/0.0042$.

Total phenols

Total phenols were analyzed using the Folin-Ciocalteu micro method (Waterhouse 2002). A standard curve was generated based on 0, 50, 100, 150, 250, and 500 mg/L gallic acid. Exactly 20 mL of each wine sample were centrifuged in a IEC Centra CL2 centrifuge for 10 min at 10 000 g. Wine samples were diluted 10-fold prior to their preparation in 10-mL test tubes. The addition of reactive solvents was done in 2-mL

plastic cuvettes consistent with [Waterhouse \(2002\)](#). They were subsequently left at 20°C for 2 hr in the dark. Absorbance of each solution was determined at 765 nm against a blank (i.e. 0 mL wine sample) using an Ultrospec 2100 pro UV/VIS spectrophotometer.

High-performance liquid chromatography

The Agilent 1100 Series HPLC system consisted of a micro vacuum degasser, micro autosampler, binary pump, thermostatted column oven, and a UV/VIS diode array detector (DAD). HP Chemstation software was used for the identification and quantification of individual analytes. Separation of the 2011 wine matrix analytes was accomplished with a reverse phase Supelcosil™ LC-18 HPLC column (5 µm particle size, L × I.D. 25 cm × 4.6 mm; Sigma-Aldrich, St. Gallen, Switzerland). Separation of 2012 wines was carried out using an Agilent Zorbax Stablebond SB-C₁₈ reverse-phase column (3.5 µm particle size; L × I.D. 50 mm × 4.6 mm; Agilent Technologies, Palo Alto, CA) with a Phenomenex SecurityGuard™ C-18 4 mm guard cartridge.

Maximum concentration for most anthocyanins and phenolics was estimated as 100 mg/L based upon previous experience, with quercetin, caffeic acid and *p*-coumaric acid maximum concentrations estimated as 20 mg/L ([Di Profio et al. 2011b](#)). Each standard was weighed on an M3 microanalytical balance (Mettler-Toledo, Zurich, Switzerland) and dissolved in methanol/acetic acid (9/1; v/v). Standards were passed through a 3-mL syringe (BD, Franklin Lakes, NJ) equipped with a 4-mm 0.45 µm syringe filter (Chromatographic Specialties, Brockville, ON) before analysis of each analyte. Analytes were made up in groups of cocktails of four to five standards based on compound type (anthocyanins, hydroxycinnamates, hydroxybenzoic acids, flavonoids, galloylated phenolics). Dilutions of 1:1, 1:2, 1:4 and 1:8 were run on the cocktails to create standard curves based on concentration (mg/L) and absorption (nm).

Before analysis by HPLC, all wine samples were thawed at room temperature for 24 hr. The 100-mL wine samples were shaken vigorously by hand, and 20 mL of each sample were centrifuged in a IEC Centra CL2 centrifuge for 10 min at 10 000 g. Samples were subsequently syringe filtered through Millex-GV Filter (0.22 µm, 33 mm; EMD Millipore, Billerica, MA) into vials for HPLC analysis.

All 2011 samples were analyzed according to the following direct injection HPLC method. Mobile phase solvents (all HPLC grade) were: (A) aqueous 0.2% trifluoroacetic acid and (B) methanol/ acetonitrile/ trifluoroacetic acid (60/39.8/0.2; v/v/v). The gradient was (%B): 0 to 20% (0-70 min), 20 to 40% (70-110 min), 40 to 100% (110-150 min), and 100% (150-165 min), at a constant flow rate of 1.0 mL/min. Post run time was for 15 min, for a 180-min total run time. Mobile phases for 2012 samples was aqueous 0.2% trifluoroacetic acid (TFA) (solvent A), and HPLC-grade acetonitrile and 0.2% TFA (solvent B), as per [Ibern-Gómez et al. \(2002\)](#) and [Di Profio et al. \(2011b\)](#). Flow rate was 1.0 mL/min. The gradient was (%B): 5% (0-15 min), 35% (15 min), 100% (16-25 min), and 5% (26 min). Post-run time was 10 min for a 36-min total run time. Injection volume was 10 µL and column temperature was 30°C. Best selectivity by DAD was

achieved at the following extinction wavelengths: 525 nm (anthocyanins), 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids), 280 nm (flavonoids and their respective galloylated esters), and 365 nm (quercetin).

Analyses of 2011 wines focused upon galloylated derivatives (Supplementary Table S1). T2 wines were not analyzed in 2011 due to their high level of oxidation. Standards used for HPLC were as follows: (–)-catechin gallate, (–)-epicatechin gallate, (–)-gallocatechin, *p*-coumaric acid, caffeic acid, gallic acid, protocatechuic acid ethyl ester (Sigma-Aldrich, St. Gallen, Switzerland); quercetin, (+)-catechin, (–)-epicatechin, protocatechuic acid, procyanidin B1, malvidin-3-O-glucoside, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside chloride (Extrasynthèse, Genay Cedex, France); petunidin 3-O-glucoside, (Fluka, St. Gallen, Switzerland). Only five galloylated compounds were detected in 2011 wines [(–)-catechin gallate, (–)-gallocatechin, protocatechuic acid, protocatechuic acid ethyl ester, procyanidin B1] consequently they were not quantified in 2012. Analyses of 2012 wines focused upon acetylated and coumarylated anthocyanins (Supplementary Table S2). These anthocyanins were identified in 2012 wines by comparison of peak retention times with those in the literature (Ibern-Gómez et al. 2002; Di Profio et al. 2011b), and were quantified using concentrations of equivalent non-acylated anthocyanins. All solvents used for HPLC analysis (methanol, acetonitrile and trifluoroacetic acid) were HPLC grade (≥99.9%; Sigma-Aldrich, Chromasolv®). Deionized water was obtained from a Milli-Q water purification system (EMD Millipore).

Statistical analysis

SAS statistical software package (SAS 9.3; SAS Institute, Cary, NC) conducted with GLM procedure was employed for statistical analysis. Data were analyzed by analysis of variance (ANOVA). Multiple comparisons of individual means were done using Duncan’s multiple range tests at $p \leq 0.05$.

RESULTS

Basic wine composition, Cabernet Sauvignon

Crop level in 2011 did not affect any wine composition variables except hue, which was greater in HC wines (Table 1). In 2012, HC were higher than FC wines in pH and hue and slightly lower in color intensity (Table 1). In harvest date treatments in 2011, TA, intensity, anthocyanins, and phenols decreased relative to harvest date, whereas pH, ethanol, and hue increased. Anthocyanins increased in T1, but decreased in T2 (Table 1). In 2012, TA, pH, and hue were highest in T2 wines, and intensity, anthocyanins and phenols were lowest. However, unlike 2011, anthocyanins and color increased in T1 wines relative to T0 (Table 1). Crop level x harvest time interactions with standard deviations are shown in Figure 1. Harvest date x crop level interactions occurred in 2011 for intensity and phenols whereby HC reduced intensity and phenols for T1 (Figure 1 A,B). However, significance levels were relatively low, and they did not show trends substantially

different from the main effects. Interactions were found in 2012 for pH, intensity, hue, anthocyanins, and phenols (Figure 1 E-I). In FC wines pH and hue varied little between T0 and T1, followed by a substantial increase in T2, while color intensity, anthocyanins, and phenols increased between T0 and T1 and then declined in T2. In HC, increases in pH and hue in T2 wines were not observed, and declines in color intensity, anthocyanins, and phenols were not as pronounced as in FC wines.

Basic wine composition, Cabernet franc

Reducing crop level in 2011 decreased ethanol and increased color intensity (Table 2). In 2012, only hue was impacted in terms of a slight decrease in HC wines (Table 2). Extended harvest date in 2011 led to reductions in TA, ethanol, intensity, anthocyanins, and phenols, although differences were observed only in T2 for all but ethanol. Extended harvest time led to increases in pH and hue (T2 only for hue) (Table 2). Extended harvest time in 2012 increased TA (T2 only), pH, ethanol, intensity (T1 only), and hue (T2 only), decreased TA in T1, and lowered anthocyanins in T2 (Table 2). Crop level x harvest time interactions (2011; Figure 2) occurred for TA and ethanol; however, significance levels were quite low, and there was little difference from main effects other than an elevation in ethanol in HC/T2 wines (Figure 2 A,B). An interaction was found in 2012 for hue, suggesting that HC wines experienced a greater increase in hue in T2 than their FC counterparts (Figure 2C).

HPLC analysis of individual phenolic analytes

Cabernet Sauvignon

HC in 2011 increased petunidin, peonidin, malvidin, and procyanidin B (Table 3). Delayed harvest increased peonidin, malvidin, gallic acid, caffeic acid, catechin gallate, procyanidin B1, protocatechuic acid, and protocatechuic acid ethyl ester (Table 3). Quercetin and (+)-catechin both decreased in T1. In 2012, crop reduction increased malvidin-3-coumarylglucoside and (+)-catechin, but decreased petunidin and delphinidin-3-coumarylglucoside (Table 4). Harvest date impacted all but two compounds (Table 4). In most cases responses of anthocyanidins was parabolic, with highest concentrations in T1 wines; exceptions were peonidin-3-acetylglucoside and petunidin-3-coumarylglucoside. Among phenols, gallic acid and (+)-catechin increased with delayed harvest, caffeic acid, (-)-epicatechin, and quercetin decreased, and resveratrol displayed a parabolic trend. Interactions in 2011 (Figure 1) were apparent for gallic acid and quercetin whereby gallic acid increased in FC wines in T1 to a greater degree than in HC wines, while quercetin increased substantially in HC/T0 wines but not in FC wines (Figure 1 C,D). In 2012 there were 12 crop level x harvest date interactions including six anthocyanins and (-)-epicatechin (Figure 1 J to P). In all cases, anthocyanins showed parabolic trends relative to harvest date, with highest concentrations in T1 wines. However, values in the HC/T2 wines were considerably lower than in the FC/T2 wines (Figure 1 J to O). This trend was also apparent for (-)-epicatechin but not to the same degree (Figure 1P).

Cabernet franc

Reducing crop level in 2011 increased peonidin but no other compounds were impacted (Table 5). Extended harvest time increased gallic acid, procyanidin B1, (-)-epicatechin, and procatechuic acid ethyl ester, while peonidin, (+)-catechin, and *p*-coumaric acid decreased (Table 5). In 2012, two anthocyanins (delphinidin-3-acetylglucoside, cyanidin-3-coumarylglucoside) and caffeic acid increased with crop reduction, while (-)-epicatechin and *p*-coumaric acid decreased (Table 6). As with 2012 CS, nearly all anthocyanins and phenols were impacted by harvest date. In nearly all cases there were decreases between T0 and T2; of 13 anthocyanins quantified, nine showed decreases between T0 and T1 as well as from T1 to T2, while others displayed only a decrease from T1 to T2 (Table 6). As in CS, gallic acid and (+)-catechin increased with delayed harvest date, with decreases in (-)-epicatechin, *p*-coumaric acid, quercetin, and resveratrol. There were no interactions in 2011 for individual anthocyanins and phenols. However, there were eight crop level x harvest date interactions in 2012 (Figure 2), of which five involved anthocyanins and three were associated with phenols (Figure 2 D to K). Malvidin and delphinidin-3-coumarylglucosides in HC/T2 wines were considerably lower than in FC/T2 wines (Figure 2 E,H). Both petunidin-3- and delphinidin-3-acetylglucosides were much higher in HC/T1 wines than in FC/T1 (Figure 2 F,G). Responses of (+)-catechin were not substantially different than the main effects (Figure 2I). Caffeic acid was much higher in FC/T2 than in HC/T2 wines (Figure 2J). (-)-Epicatechin was much higher in FC/T0 wines than in all other treatment combinations (Figure 2K).

DISCUSSION

Crop level

Common harvest indices, e.g. Brix, TA, pH may be insufficient in some circumstances for accurate determination of fruit maturity if their evolution does not coincide with the development of secondary metabolites such as anthocyanins and phenolics (Di Profio et al. 2011a,b). Consequently, quantification of anthocyanins and phenols are worthy of consideration. Their contribution to wine color and to other organoleptic characteristics of wines such as bitterness and astringency make these analytes of utmost importance (Pérez-Magariño and González-San Jose 2004 2006).

There were no crop level effects on ethanol or TA in either cultivar in both vintages (Tables 1,2). Crop level can be important in pH management, and overcropping may delay fruit maturity, which reduces pH. Considering that the vines were likely in balance (all were < 10 for both cultivars in both seasons) with respect to crop load (Moreno Luna 2014; Lefebvre et al. 2015), the lack of pH differences between treatments (except for one instance) was not surprising. Normally, TA decreases and pH increases with veraison cluster thinning (Di Profio et al. 2011a). Reduced crop level increased color intensity for 2011 CF and inexplicably reduced intensity in 2012 CS. Intensity can be influenced primarily by anthocyanin content, but also by temperature, ethanol, pH, SO₂, percentage of polymers vs. monomers, co-pigmentation, and other factors (Somers and Evans 1979). Hue was increased by reducing crop in CS in both seasons, but was

reduced in 2012 CF. Lack of change in total anthocyanins and phenols in the crop level treatments is consistent with others (Kennedy et al. 2000; Downey et al. 2006).

Crop reduction is often effective for increasing phenols and general fruit quality (Reynolds et al. 1994; Mazza et al. 1999; Guidoni et al. 2002). The exact relationship between cluster thinning and increased phenolic development is not fully understood. A few assumptions persist, relating source: sink ratios as the determining factor leading to increased levels of anthocyanins, color intensity, and phenolics (Guidoni et al. 2002). Yield reductions resulted from shoot thinning in Reynolds et al. (2005), and due to cluster thinning at veraison in Di Profio et al. (2011a,b) occurred. Both studies demonstrated positive relationships between Brix and anthocyanins. This is noteworthy, given the possible reduction in photosynthetic capabilities of shoot-thinned vines vs. cluster thinning at veraison with no canopy adjustment. Overall, secondary metabolites, such as phenolics, may be synthesized in the grape berry, yet are apparently influenced by carbohydrate partitioning.

Cluster thinning of CS in Ontario increased six phenolics (gallic acid, epicatechin, catechin, caffeic acid, *p*-coumaric acid, quercetin) in one growing season, and all analytes increased except catechin and quercetin the following season (Di Profio et al. 2011b). Catechin likewise increased with reduced crop level for 2012 CS (Table 4). Reduced crop level had no impact on most phenolics in one season in CF, but gallic acid and epicatechin increased the following year (Di Profio et al. 2011b). No differences were found for 2011 CF for these analytes (Table 5), but caffeic acid increased, and both catechin and epicatechin decreased with reduced crop level (Table 6).

This demonstrates the significant impact of season and site on development of the same cultivars from the same region. In this case, 2011 mean temperatures were consistently between 15 and 25°C throughout September until 15 October, when they decreased to < 15°C, with periods < 5°C. Substantial rainfall events between 10 and 20 mm occurred late September to early October and sporadic events < 15 mm occurred throughout October. In 2012, a hot and dry growing season ended with mean temperatures > 15°C throughout most of September and October, with decreases in mean temperatures to < 10°C in late October. Rainfall was sporadic throughout September and October with two noteworthy events > 15 mm on 24 September and 24 October plus a period of precipitation (15-25 mm daily) between 27 October and 3 November (Vine and Tree Fruit Innovations Network 2013). Di Profio et al. (2011b) observed in one vintage that all individual anthocyanins increased, but the following year, malvidin and petunidin did not increase for CS with crop reduction despite consistent field and winemaking practices. In CF, cluster thinning increased all anthocyanins except for cyanidin in one vintage, and the following year, cyanidin and malvidin did not increase. Petunidin, peonidin, and malvidin increased with crop reduction for 2011 CS (Table 5), but petunidin- and delphinidin-3-coumarylglucoside decreased, and malvidin-3-coumarylglucoside increased in

335 2012 (Table 4). Only peonidin increased for 2011 CF (Table 5) and two acylated anthocyanidins increased in
336 2012 (Table 6).

337 Several authors have concluded that anthocyanin composition is primarily determined by cultivar
338 (Brossaud et al. 1999), environmental conditions (Spayd et al. 2002) and rate/stage of maturity (Ryan and
339 Revilla 2003). The variability in anthocyanin accumulation is consistent with results observed in previous
340 studies in relation to environmental conditions from season to season (Guidoni et al. 2002; Cortell et al.
341 2007; Di Profio et al. 2011b). Temperatures > 35°C typically decrease anthocyanin accumulation, while
342 sunlight exposure has a positive linear effect on anthocyanin biosynthesis (Spayd et al. 2002). Few
343 individual anthocyanins decreased in any of the crop level treatments for both cultivars except for petunidin
344 in 2012 CS. However, cluster thinning can sometimes lead to increased anthocyanins (Cortell et al. 2007;
345 Guidoni et al. 2008; Di Profio et al. 2011b). Three anthocyanins increased in 2011 HC CS (Table 3), but only
346 peonidin increased in CF (Table 5). Few crop level effects were apparent in the warm 2012 season. This
347 suggests that cluster thinning can override temperature effects in cool seasons to increase anthocyanins.

348 There were also few crop level effects on individual phenolics (Tables 3-6). Procyanidin B1 increased in
349 2011 CS and catechin increased in 2012 CS with reduced crop, while caffeic acid increased in 2012 CF
350 along with decreases in *p*-coumaric acid and epicatechin. These findings are inconsistent with Kennedy et al.
351 (2000), who indicated that extractable flavan-3-ol monomers decreased most rapidly with increasing fruit
352 maturity, followed by procyanidin extension units; however, only a few flavan-3-ol and procyanidins were
353 analyzed in this study, whereas many more were quantified by Kennedy et al. (2000). Flavan-3-ols and
354 procyanidins could have a greater sensory impact than galloylated tannins, considering that astringency and
355 bitterness decrease with increasing fruit maturity (Downey et al. 2006). This is noteworthy, because
356 winemakers have interest in developing a means to predict wine tannin amount and composition from fruit
357 analysis. Initial fruit proanthocyanidins determine the eventual skin and seed proanthocyanidins extracted
358 into wine (Cortell et al. 2005). Differences in anthocyanins and phenols can occur between cultivars in terms
359 of harvest date and crop level, and there are also intrinsic differences in composition within cultivars to
360 complicate matters further (Pérez-Magariño and González-San José 2004).

361 The lack of response to crop level reduction in this trial may be explained by considering vine balance
362 (Keller et al. 2008). The vines could have developed sufficient canopy size to support their crop before it was
363 reduced at veraison. A commonly-accepted definition of vine balance is the Ravaz Index, which is the ratio
364 of yield vs. previous year's pruning weights. Indices between 4 and 10 are indicative of vines that are in
365 balance (Bravdo et al. 1985). In CS the Ravaz Indices were 5.0 vs. 4.0 in 2011 and 9.3 vs. 5.9 in 2012 for FC
366 and HC, respectively; in CF they were 4.4 vs. 3.1 in 2011 and 7.8 vs. 5.5 in 2012 for FC and HC,
367 respectively (Moreno Luna 2014; data not shown). Because these indices are within or below the desired
368 range for all cropping treatments, it is unlikely that reducing crop would have impacted berry, must or wine
369 composition.

Harvest date

Delayed harvest led to substantial reductions in cluster and berry weights in both cultivars due to desiccation (Moreno 2014). Cluster weight (g) ranges were: Cabernet franc: 117.4 to 83.9 (T0 to T2; 2011) and 89.8 to 39.9 (2012); Cabernet Sauvignon: 91.9 to 71.4 (2011) and 79.4 to 83.6 (2012). Brix levels in both cultivars concomitantly increased substantially with delayed harvest; ranges were: Cabernet franc: 23.6 to 27.4 (2011) and 23.4 to 27.4 (2012); Cabernet Sauvignon: 22.6 to 26.5 (2011) and 24.4 to 28.2 (2012) (Moreno 2014). Extended harvest date therefore followed the expected trend in wines of both cultivars (Tables 1,2; Figures 1,2), where TA decreased while pH and ethanol increased (Pérez-Magariño and González-San José 2004, 2006). TA decreases and pH increases with later harvest dates in 2011 could have been influenced by climatic factors for both cultivars. The 2011 summer was very warm but there was substantial autumn rainfall (Vine and Tree Fruit Innovations 2012; <http://www.weatherinnovations.com>), and therefore dilution might explain the TA reductions. Furthermore, malate decreases with maturity, and may have continued to decrease after normal commercial harvest. In the hot and dry 2012 season, the slight increase in TA in T2 may have been due to desiccation and concentration. A decrease in total anthocyanins was apparent in T2 for both CS (2011, 2012; Table 1) and CF (2011 only; Table 2), while there was also an increase in T1 in 2011 CS. Increases in anthocyanins for CS in T1 could be attributed to berry desiccation, whereby changes in skin weight: total berry weight ratio might have incurred (Kennedy et al. 2002). Decreases in both cultivars for these analytes could be attributable to dilution effects caused by harvest rainfall, but also because grapes become physiologically compromised with extended maturity (Kennedy 2010). Additionally, wine total phenols and anthocyanins can be very different from the grapes due to structural features within each family of compounds (anthocyanins and proanthocyanidins) influencing extractability; e.g., lower extraction rates for coumarylated anthocyanins and tannins with a high degree of polymerization (Fournand et al. 2006). Amounts of wine flavanols may differ among treatments by about twice as much as in the fruit, as flavanols are poorly soluble in a wine solvent system (Kennedy et al. 2002).

Several individual anthocyanins increased between T0 and T1 in CS in both vintages, but many decreased with extended harvest date in CF (Tables 3-6). During berry maturation, malvidin- and peonidin-3-glucosides usually increase, while other anthocyanidin monoglucosides tend to decrease at the end of maturation. This is likely because malvidin- and peonidin-3-glucosides are the final products of anthocyanin pathway biosynthesis (Canals et al. 2005). This could explain why malvidin and peonidin increased in 2011 CS. However, effects of extended fruit maturity on individual anthocyanins are not well understood nor extensively researched. Reduced peonidin in 2011 CF and decreased quercetin for 2011 CS may be explained by late season fungal infections (which, despite sorting, were still present to a limited degree on the fruit), which can decrease concentrations of individual anthocyanins (Piermattei et al. 1999).

In CS, astringent compounds, e.g. protocatechuic acid, caffeic acid, gallic acid increased with extended harvest, yet only caffeic acid exceeded sensory threshold (Tables 3,4). Individual compounds produce unique

405 astringent and bitter characteristics, but they interact in a wine matrix (Hufnagel and Hofmann, 2008). It is
406 unknown whether some of these analytes exceed threshold concentrations in berries and subsequent wine. In
407 CF, astringent compounds, e.g. gallic acid increased with later harvests, while *p*-coumaric acid decreased
408 (Tables 5,6). Both compounds were below sensory thresholds (Hufnagel and Hofmann 2008). Among
409 galloylated derivatives, procyanidin B1 was affected in CS by an extended harvest date, but was below
410 threshold concentrations (Table 3). Other bitter and astringent compounds, (+)-catechin and procatechuic
411 acid ethyl ester, responded to later harvests. Catechin decreased relative to harvest date in 2011 CS and CF
412 but increased in both cultivars in 2012. CS did not show appreciable decreases in epicatechin, whereas in
413 2011 CF it increased with later harvest, consistent with Pérez-Magariño and González-San José (2004). They
414 determined that wines associated with initial harvest dates were generally higher in catechin than those from
415 final harvests. Results are also consistent with Jordão et al. (2001), who suggested that flavan-3-ol monomers
416 decreased in the latter stages of grape maturation. Bitter and astringent compounds, e.g. (+)-catechin, (-)-
417 epicatechin, procyanidin B1, were below threshold except procatechuic acid ethyl ester, which increased
418 with later harvests.

419 Through different harvest dates, the intent was to gain a better understanding of which phenolics changed
420 over the course of fruit development. In CF, no galloylated tannins decreased; however, ethyl esters and
421 oligomers increased. This information would lead to the assumption that seed tannins, which contain
422 galloylated derivatives, were unchanged by later harvest date or lower crop level (Jackson 2009). The
423 opposite may occur in cultivars such as CS, considering (-)-catechin gallate increased with extended harvest
424 date. This is consistent with Pérez-Magariño and González-San José (2004), who found an increase in
425 galloylation in wines associated with late harvests. A greater than three-fold molar increase in galloylated
426 derivatives was measured between wines from a low vigor vs. a high vigor plot (Cortell et al. 2005).
427 Assuming that lower vigor hastened fruit maturity (Cortell et al. 2005, 2007), and a later harvest date
428 likewise resulted in more mature berries, both situations had similar consequences. Although there were no
429 changes in most flavan-3-ols with crop reduction in either cultivar in this trial, wine flavan-3-ol monomers
430 were observed to increase in an Oregon Pinot noir vineyard with increases in grapevine vigor (Cortell et al.
431 2005). Procyanidin B1 increased in both cultivars with delayed harvest date, and also with reduced crop level
432 for CS. This is consistent with Pérez-Magariño and González-San José (2004), who reported higher flavan-3-
433 ol dimers in wines associated with later harvest dates.

434 CONCLUSIONS

435 Cluster thinning and harvest date influenced evolution of individual phenolics of young red wines.
436 Harvest date had a greater impact than cluster thinning on both CS and CF. There was no increase in total
437 phenols and anthocyanins in either cultivar due to cluster thinning, possibly because vines were initially
438 balanced with respect to vine size and yield. There were numerous changes in the composition of individual

anthocyanins and proanthocyanidins as a result of cluster thinning and harvest date. CS demonstrated a greater response to cluster thinning than CF for individual phenolics. Responses to crop level and harvest date differed between phenolic families as well as between cultivar and vintage. Most anthocyanins increased relative to harvest date in CS but many decreased in CF. However, most phenolics increased in both cultivars with delayed harvest. Highest concentrations of many phenolics did not coincide with the maximum Brix accumulation. Further research is needed to determine if there is consistency across vintages as to which individual analytes are impacted the greatest from extended harvest date. Consequently, this relationship could draw insight into which compounds improve astringency and bitterness with increasing fruit maturity. It seems clear that extended harvest date produced grapes with better anthocyanin and phenolic composition. Furthermore, these results show that the length of time that clusters are left on the vines must be carefully monitored, because some beneficial effects of extended harvest date can be lost.

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LITERATURE CITED

- Bindon, K., Varela, C., Kennedy, J., Holt H., and Herderich, M., 2013. Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon. 1. Grape and wine chemistry, Food Chem. **138**:1696-1705.
- Boulton, R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. Am. J. Enol. Vitic. **52**:67-87.
- Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. 1985. Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon. Am. J. Enol. Vitic. **36**:132-139.
- Brossaud, F., Cheynier, V., Asselin, C., and Moutounet, M. 1999. Flavonoid compositional differences of grapes among site test plantings of Cabernet franc. Am. J. Enol. Vitic. **50**:277-284.
- Canals, R., Llaudy, M.C., Valls, J., Canals, J.M., and Zamora, F. 2005. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. J. Agric. Food Chem. **53**:4019-4025.
- Cortell, J.M., Halbleib, M., Gallagher, A.V., Righetti, T.L., and Kennedy, J.A. 2005. Influence of vine vigor on grape (*Vitis vinifera* L. Cv. Pinot noir) and wine proanthocyanidins. J. Agric. Food Chem. **53**:5798-5808.
- Cortell, J.M., Halbleib, M., Gallagher, A.V., Righetti, T.L., and Kennedy, J.A. 2007. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. J. Agric. Food Chem. **55**:6575-6584.
- Di Profio, F., Reynolds, A.G., and Kasimos, A. 2011a. Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet Sauvignon. I. Yield and berry composition. Am. J. Enol. Vitic. **62**:139-151.

- 475 Di Profio, F., Reynolds, A.G., and Kasimos, A. 2011b. Canopy management and enzyme impacts on Merlot,
476 Cabernet franc, and Cabernet Sauvignon. II. Wine composition and quality. *Am. J. Enol. Vitic.* **62**:152-
477 168.
- 478 Downey, M.O., Harvey, J.S., and Robinson, S.P. 2003a. Analysis of tannins in seeds and skins of Shiraz
479 grapes throughout berry development. *Austral. J. Grape and Wine Res.* **9**:15-27.
- 480 Downey, M.O., Harvey, J.S., and Robinson, S.P. 2003b. Synthesis of flavonols and expression of flavonol
481 synthase genes in the developing grape berries of Shiraz and Chardonnay (*Vitis vinifera* L.). *Austral. J.*
482 *Grape and Wine Res.* **9**:110-121.
- 483 Downey, M.O., Dokoozlian, N.K., and Krstic, M.P. 2006. Cultural practice and environmental impacts on
484 the flavonoid composition of grapes and wine: A review of recent research. *Am. J. Enol. Vitic.* **57**:257-
485 268.
- 486 Figueiredo-Gonzalez, M., Cancho-Grande, B., and Simal-Gandara, J. 2013. Effects on colour and phenolic
487 composition of sugar concentration processes in dried-on- or dried-off-vine grapes and their aged or not
488 natural sweet wines. *Trends in Food Science & Technology* **31**:36-54.
- 489 Fournand, D., Vicens, A., Sidhoum, L., Souquet, J., Moutounet, M., and Cheynier, V. 2006. Accumulation
490 and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *J.*
491 *Agric. Food Chem.* **54**:7331-7338.
- 492 Fuleki, T., and Francis, F.J. 1968. Quantitative methods for anthocyanins. *J. Food Sci.* **33**:266-274.
- 493 Guidoni, S., Allara, P., and Schubert, A. 2002. Effect of cluster thinning on berry skin anthocyanin
494 composition of *Vitis vinifera* cv. Nebbiolo. *Am. J. Enol. Vitic.* **53**:224-226.
- 495 Guidoni, S., Ferrandino, A., and Novello, V. 2008. Effects of seasonal and agronomical practices on skin
496 anthocyanin profile of Nebbiolo grapes. *Am. J. Enol. Vitic.* **59**:22-29.
- 497 Hufnagel, J.S., and Hofmann, T. 2008. Orosensory-directed identification of astringent mouthfeel and bitter-
498 tasting compounds in red wine. *J. Agric. Food Chem.* **56**:1376-1386.
- 499 Holt, H.E., Birchmore, W., Herderich, M.J., and Iland, P.G. 2010. Berry phenolics in Cabernet Sauvignon
500 (*Vitis vinifera* L.) during late-stage ripening. *Am. J. Enol. Vitic.* **61**:285-299.
- 501 Ibern-Gómez, M., Andrés-Lacueva, C., Lamuela-Raventós, R.M., and Waterhouse, A.L. 2002. Rapid
502 HPLC analysis of phenolic compounds in red wines. *Am. J. Enol. Vitic.* **53**:218-221.
- 503 Jordão, A.M., Ricardo-da-Silva, J.M., and Laureano, O. 2001. Evolution of catechins and oligomeric
504 procyanidins during grape maturation of Castelhão Francês and Touriga Francesa. *Am. J. Enol. Vitic.*
505 **52**:230-234.
- 506 Keller, M., and Hrazdina, G. 1998. Interaction of nitrogen availability during bloom and light intensity
507 during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol.*
508 *Vitic.* **49**:341-349.
- 509 Keller, M., Smithyman, R.P., and Mills, L.J. 2008. Interactive effects of deficit irrigation and crop load on
510 Cabernet Sauvignon in an arid climate. *Am. J. Enol. Vitic.* **59**:221-234.
- 511 Kennedy, J.A., Matthews, M.A., and Waterhouse, A.L. 2000. Changes in grape seed polyphenols during fruit
512 ripening. *Phytochemistry* **55**:77-85.
- 513 Kennedy, J.A., Matthews, M.A., and Waterhouse, A.L. 2002. Effect of maturity and vine water status on
514 grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **53**:268-274.

- 515 Kennedy, J.A. 2010. Wine colour. Pp. 73-104. In: A.G. Reynolds (ed.). Managing Wine Quality. Vol. 1:
516 Viticulture and Wine Quality. Oxford: Woodhead Publications.
- 517 Kingston, M.S., and Presant, E.W. 1989. The Soils of the Regional Municipality of Niagara. Ontario
518 Institute of Pedology, Rep. 60 Vol. 1 and 2.
- 519 Lefebvre, R., Reynolds, A.G., and Di Profio, F. 2015. Crop load and harvest date have minimal impact on
520 bud cold hardiness and cane carbohydrate levels of four grapevine cultivars. *Vitis* **54**:189-93.
- 521 Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. 1999. Anthocyanins, phenolics, and color
522 of Cabernet Franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem.* **47**:4009-
523 4017.
- 524 Moreno Luna, L.H. 2014. Impact of crop level and hang time on the composition of four wine grape
525 cultivars from the Niagara Region. MSc Thesis, Centre for Biotechnology, Brock University, St.
526 Catharines, ON.
- 527 Nurgel, C., Pickering, G.J., and Inglis, D.L. 2004. Sensory and chemical characteristics of Canadian ice
528 wines. *J. Sci. Food Agric.* **84**:1675-1684.
- 529 Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA). 2010. Guide to Fruit Production
530 2010-2011. Publication 360. Ontario Ministry of Agriculture, Food and Rural Affairs. Toronto, Ontario.
- 531 Pérez-Magariño, S., and González-San José, M.L. 2004. Evolution of flavanols, anthocyanins, and their
532 derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening.
533 *J Agric Food Chem* **52**:1181-1189.
- 534 Pérez-Magariño, S., and González-San José, M.L. 2006. Polyphenols and colour variability of red wines
535 made from grapes harvested at different ripeness grade. *Food Chem.* **96**:197-208.
- 536 Piermattei, B., Piva, A., Castellari, M., Arfelli, G., and Amati, A. 1999. The phenolic composition of red
537 grapes and wines as influenced by *Oidium tuckeri* development. *Vitis* **38**:85-86.
- 538 Reynolds, A.G., Price, S.F., Wardle, D.A., and Watson, B.T. 1994. Fruit environment and crop level effects
539 on Pinot noir. I. Vine performance and fruit composition in British Columbia. *Am. J. Enol. Vitic.* **45**:452-
540 459.
- 541 Reynolds, A.G., Molek, A., and de Savigny, C. 2005. Timing of shoot thinning in *Vitis vinifera*: impacts on
542 yield and fruit composition variables. *Am. J. Enol. Vitic.* **56**:343-356.
- 543 Rolle, L., Giacosa, S., Segade, S.R., Ferrarini, R., Torchio, F., and Gerbi, V. 2013. Influence of different
544 thermohygrometric conditions on changes in instrumental texture properties and phenolic composition
545 during postharvest withering of 'Corvina' winegrapes (*Vitis vinifera* L.). *Drying Technology* **31**:549-564.
- 546 Rolle, L., Torchio, F., Giacosa, S., and Gerbi, V. 2009. Modifications of mechanical characteristics and
547 phenolic composition in berry skins and seeds of Mondeuse winegrapes throughout the on-vine drying
548 process. *J. Sci. Food Agric.* **89**:1973-1980.
- 549 Ryan, J., and Revilla, E. 2003. Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at
550 different stages of ripening. *J. Agric. Food Chem.* **51**:3372-3378.
- 551 Somers, T.C., and Evans, M.E. 1979. Grape pigment phenomena: Interpretation of major colour losses
552 during vinification. *J. Sci. Food Agric.* **30**:623-633.
- 553 Sun, Q., Sacks, G., Lerch, S., and Vanden Heuvel, J.E. 2010. Impact of shoot thinning and harvest date on
554 yield components, fruit composition, and wine quality of Marechal Foch. *Am. J. Enol. Vitic.* **62**:32-41.

555 Spayd, S.E., Tarara, J.M., Mee, D.L., and Ferguson, J.C. 2002. Separation of sunlight and temperature
556 effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. **53**:171-182.

557 Torchio, F., Urcan, D.E., Lin, L., Gerbi, V., Giacosa, S., Segade, S.R., Pop N., Lambri M., and Rolle L.
558 2016. Influence of different withering conditions on phenolic composition of Avana, Chatus and
559 Nebbiolo grapes for the production of 'Reinforced' wines. Food Chem. **194**:247-256.

560 Vine and Tree Fruit Innovations. 2013. Weather Innovations Incorporated. www.vineinnovations.com.
561 Accessed 22 August 2013.

562 Waterhouse, A.L. 2002. Determination of total phenolics. Current Protocols in Food Analytical Chemistry,
563 11.1.1-11.1.8. Hoboken, New Jersey: John Wiley and Sons, Inc.

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For Review Only

Table 1: Impacts of viticultural treatments on Cabernet Sauvignon wine composition, Niagara-on-the-Lake, ON 2011-2012.

Factor	Titrateable acidity (g/L)	pH	Ethanol (% v/v)	Intensity ^a	Hue ^a	Total anthocyanins (mg/L)	Total phenols (mg/L)
2011							
Crop level							
Full	5.99	3.68	12.4	0.747	0.825	287.5	2202.4
Half	5.99	3.68	12.3	0.723	0.894	275.9	2102.8
Significance ^b	NS	NS	NS	NS	**	NS	NS
Harvest date^c							
T0	6.44 a	3.45 c	11.5 c	0.898 a	0.649 b	336.1 b	3106.1 a
T1	6.22 b	3.59 b	12.4 b	0.793 b	0.687 b	392.8 a	2095.6 b
T2	5.32 c	4.00 a	13.2 a	0.515 c	1.242 a	116.1 c	1256.1 c
Significance ^b	***	***	****	***	***	***	***
Interaction ^b	NS	NS	NS	**	NS	NS	*
2012							
Crop level							
Full	6.51	3.36	14.8	0.647	0.606	427.3	2156.9
Half	6.61	3.47	14.7	0.600	0.656	403.6	2018.8
Significance ^b	NS	**	NS	*	***	NS	NS
Harvest date^c							
T0	6.30 b	3.42 ab	14.2	0.637 b	0.583 b	443.1 b	2150.0 a
T1	6.49 b	3.36 b	15.4	0.727 a	0.573 b	553.2 a	2333.3 a
T2	7.04 a	3.48 a	14.6	0.448 c	0.791 a	167.4 c	1626.3 b
Significance ^b	*	NS	NS	***	****	****	**
Interaction ^b	NS	**	NS	***	***	*	**

^a Hue: A420/A520. Intensity: A520 ± A420.

^b *, **, ***, ****, NS: Significant at $p \leq 0.05$, 0.01, 0.001, 0.0001, or not significant, respectively. Means in columns within years followed by different letters are significantly different, $p \leq 0.05$, Duncan's multiple range test.

^c Treatments: Normal harvest (T0), harvest three weeks after T0 (T1), harvest three weeks after T1 (T2).

Table 2: Impacts of viticultural treatments on Cabernet franc wine composition, Niagara-on-the-Lake, ON 2011-2012.

Factor	Titrateable acidity (g/L)	pH	Ethanol (% v/v)	Intensity ^a	Hue ^a	Total anthocyanins (mg/L)	Total phenols (mg/L)
2011							
Crop level							
Full	5.64	3.80	13.6	0.449	1.00	175.1	1862.7
Half	5.75	3.81	14.0	0.568	1.01	168.8	1898.4
Significance ^b	NS	NS	NS	*	NS	NS	NS
Harvest date ^c							
T0	6.16 a	3.51 c	12.7 b	0.594 a	0.76 b	244.2 a	2287.0 a
T1	6.04 a	3.73 b	14.3 a	0.534 a	0.76 b	235.0 a	2388.8 a
T2	4.89 b	4.18 a	14.3 a	0.397 b	1.51 a	36.71 b	1053.5 b
Significance ^b	***	***	***	**	***	****	***
Interaction ^b	*	NS	**	NS	NS	NS	NS
2012							
Crop level							
Full	5.75	3.51	14.7	0.340	0.817	186.2	1378.8
Half	6.01	3.50	15.1	0.349	0.758	213.8	1511.9
Significance ^b	NS	NS	NS	NS	*	NS	NS
Harvest date ^c							
T0	5.92 b	3.41 b	13.9 c	0.322 b	0.629 b	254.1 a	1341.7
T1	5.48 c	3.59 a	15.0 b	0.397 a	0.672 b	220.4 a	1541.7
T2	6.44 a	3.52 a	16.3 a	0.300 b	1.196 a	88.15 b	1456.3
Significance ^b	**	***	**	***	****	****	NS
Interaction ^b	NS	NS	NS	NS	*	NS	NS

^a Hue: A420/A520. Intensity: A520 ± A420.

^b *, **, ***, ****, NS: Significant at $p \leq 0.05$, 0.01, 0.001, 0.0001, or not significant, respectively. Means in columns within years followed by different letters are significantly different, $p \leq 0.05$, Duncan's multiple range test.

^c Treatments: Normal harvest (T0), harvest three weeks after T0 (T1), harvest three weeks after T1 (T2).

Table 3: Impacts of viticultural treatments on individual anthocyanins and phenol compounds (mg/L) in Cabernet Sauvignon wines, Niagara-on-the-Lake, ON 2011.

Compound	Full crop	Half crop	Significance ^a	Harvest T0 ^b	Harvest T1	Significance ^a	Interaction ^a
Delphinidin	16.50	18.78	NS	16.50	18.77	NS	NS
Petunidin	43.15	48.81	*	43.83	48.13	NS	NS
Peonidin	3.21	3.66	**	3.27	3.60	*	NS
Malvidin	92.20	101.49	**	91.92	101.77	**	NS
Gallic acid	34.87	31.53	NS	26.88	39.52	***	*
(+)-Catechin	64.24	59.72	NS	78.79	45.17	***	NS
Caffeic acid	3.45	3.29	NS	7.47	15.93	**	NS
(-)-Epicatechin	0.00	12.21	NS	12.11	0.00	NS	NS
<i>p</i> -Coumaric acid	0.88	0.21	NS	4.46	2.28	NS	NS
Quercetin	1.55	2.99	NS	3.52	1.02	*	*
Gallocatechin	20.54	0.00	NS	0.00	20.54	NS	NS
Catechin gallate	11.39	12.02	NS	7.48	15.93	*	NS
Procyanidin B1	37.27	56.00	*	31.19	62.08	***	NS
Protocatechuic acid	4.11	4.27	NS	2.58	5.80	**	NS
Protocatechuic acid ethyl ester	5.64	4.43	NS	2.47	7.61	***	NS

^a *, **, ***, NS: Significant at p 0.05, 0.01, 0.001, and not significant, respectively.

^b Treatments: Normal harvest/control (T0), harvest three weeks after T0 (T1).

Table 4. Effects of crop level and harvest date on anthocyanins and phenols in Cabernet Sauvignon wines, Niagara-on-the-Lake, ON, 2012.

Compound	Full crop	Half crop	Significance ^a	Harvest T0 ^b	Harvest T1	Harvest T2	Significance ^a	Interaction ^a
Delphinidin	48.39	50.12	NS	25.37 b ^c	93.91 a	18.08 b	****	NS
Cyanidin	39.60	38.53	NS	20.55 b	70.75 a	19.32 b	****	*
Petunidin	1.41	0.78	**	0.91	1.26	1.11	NS	NS
Peonidin	11.40	10.25	NS	5.15 b	20.47 a	4.86 b	****	*
Malvidin	330.59	285.55	NS	147.35 b	533.98 a	210.30 b	****	*
Delphinidin-3-acetylglucoside	8.56	9.99	NS	4.94 b	18.80 a	1.51 b	****	NS
Cyanidin-3-acetylglucoside	2.34	1.81	NS	1.46 b	3.15 a	1.38 b	**	NS
Petunidin-3-acetylglucoside	14.46	13.96	NS	6.27 b	25.05 a	9.88 b	****	*
Peonidin-3-acetylglucoside	2.64	2.10	NS	4.27 a	0.82 b	1.83 b	****	NS
Malvidin-3-acetylglucoside	115.85	98.14	NS	49.16 b	187.14 a	73.56 b	****	*
Delphinidin-3-coumarylglucoside	4.05	3.15	**	1.73 b	6.34 a	2.29 b	****	NS
Cyanidin-3-coumarylglucoside	1.53	1.39	NS	0.59 b	2.31 a	1.50 ab	**	NS
Petunidin-3-coumarylglucoside	1.63	2.15	NS	0.71 b	2.32 a	3.02 a	**	NS
Peonidin-3-coumarylglucoside	----	----	--	----	----	----	----	----
Malvidin-3-coumarylglucoside	22.06	32.38	*	9.40 b	40.02 a	34.75 a	***	*
Gallic acid	25.19	23.72	NS	21.21 b	22.43 b	32.38 a	***	NS
(+)-Catechin	19.67	28.75	**	15.90 b	29.66 a	28.50 a	**	NS
Caffeic acid	18.46	13.70	NS	22.99 a	13.33 b	9.85 b	*	NS
(-)-Epicatechin	56.50	54.68	NS	60.99 a	63.39 a	35.81 b	***	**
<i>p</i> -Coumaric acid	0.75	0.65	NS	0.61	0.78	0.72	NS	NS
Quercetin	6.22	7.31	NS	10.20 a	7.57 a	0.43 b	*	NS
Resveratrol	0.41	0.29	NS	0.24 b	0.53 a	0.25 b	**	NS

^a *, **, ***, ****, NS: Significant at $p \leq 0.05, 0.01, 0.001, 0.0001$, or not significant, respectively.

^b Treatments: Normal harvest/control (T0), harvest three weeks after T0 (T1), harvest six weeks after T0 (T2).

^c Means in rows followed by different letters are significantly different, $p \leq 0.05$, Duncan's multiple range test.

Table 5: Impacts of viticultural treatments on individual anthocyanins and phenol compounds (mg/L) in Cabernet franc wines, Niagara-on-the-Lake, ON, 2011.

Compound	Full crop	Half crop	Significance ^a	Harvest T0 ^b	Harvest T1	Significance ^a	Interaction ^a
Delphinidin	8.30	9.73	NS	9.54	8.59	NS	NS
Petunidin	25.49	31.78	NS	31.60	26.19	NS	NS
Peonidin	2.40	2.99	**	2.96	2.47	*	NS
Malvidin	75.57	81.45	NS	84.60	73.17	NS	NS
Gallic acid	36.17	37.62	NS	29.15	44.05	**	NS
(+)-Catechin	46.80	57.48	NS	64.68	41.09	***	NS
Caffeic acid	2.68	2.07	NS	2.74	2.01	NS	NS
(-)-Epicatechin	12.05	14.24	NS	0.00	25.28	***	NS
<i>p</i> -Coumaric acid	0.43	0.49	NS	0.96	0.00	***	NS
Quercetin	9.18	2.47	NS	4.21	7.02	NS	NS
Gallocatechin	0.00	30.20	NS	4.56	26.02	NS	NS
Catechin gallate	0.99	3.94	NS	2.97	2.13	NS	NS
Procyanidin B	23.72	18.28	NS	12.23	28.81	*	NS
Protocatechuic acid	7.80	4.65	NS	3.16	8.90	NS	NS
Protocatechuic acid ethyl ester	7.46	6.66	NS	2.73	10.99	***	NS

^a *, **, ***, NS: Significant at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively.

^b Treatments: Normal harvest/control (T0), harvest three weeks after T0 (T1).

Table 6. Effects of crop level and harvest date on anthocyanins and phenols in Cabernet franc wines, Niagara-on-the-Lake, ON, 2012.

Compound	Full crop	Half crop	Significance ^a	Harvest T0 ^b	Harvest T1	Harvest T2	Significance ^a	Interaction ^a
Delphinidin	2.73	3.80	NS	4.33 a ^c	3.97 a	0.60 b	***	NS
Cyanidin	3.76	4.93	NS	6.56 a	4.57 b	0.70 c	***	NS
Petunidin	0.55	0.57	NS	0.77 a	0.61 a	0.16 b	***	*
Peonidin	1.50	1.83	NS	2.32 a	1.74 b	0.56 c	***	NS
Malvidin	52.62	60.25	NS	80.29 a	55.78 b	21.63 c	****	*
Delphinidin-3-acetylglucoside	1.44	1.65	*	1.78 a	2.00 a	0.50 b	****	**
Cyanidin-3-acetylglucoside	0.40	0.31	NS	0.41	0.34	0.29	NS	NS
Petunidin-3-acetylglucoside	1.25	1.53	NS	2.01 a	1.32 b	0.57 c	***	*
Peonidin-3-acetylglucoside	3.21	3.83	NS	4.99 a	3.59 b	1.19 c	***	NS
Malvidin-3-acetylglucoside	17.18	19.26	NS	27.37 a	17.81 b	5.10 c	****	NS
Delphinidin-3-coumarylglucoside	0.78	0.91	NS	1.54 a	0.61 b	0.17 c	****	***
Cyanidin-3-coumarylglucoside	0.21	0.29	*	0.33 a	0.34 a	0.00 b	***	NS
Petunidin-3-coumarylglucoside	0.39	0.37	NS	0.58 a	0.39 b	0.08 c	***	NS
Peonidin-3-coumarylglucoside	----	----	----	----	----	----	----	----
Malvidin-3-coumarylglucoside	4.31	5.03	NS	6.44 a	5.09 b	1.39 c	****	NS
Gallic acid	28.98	29.85	NS	23.19 c	29.63 b	38.43 a	***	NS
Catechin	14.55	12.53	NS	8.57 b	15.80 a	17.61 a	****	*
Caffeic acid	14.24	17.45	**	11.11 b	26.17 a	7.46 c	****	****
Epicatechin	73.75	39.27	****	93.70 a	41.42 b	23.35 c	****	****
Coumaric acid	2.27	1.91	*	2.10 b	3.05 a	0.62 c	****	NS
Quercetin	3.55	5.30	NS	6.04 a	5.42 a	0.53 b	**	NS
Resveratrol	0.27	0.26	NS	0.43 a	0.28 b	0.00 c	****	NS

^a *, **, ***, ****, NS: Significant at p 0.05, 0.01, 0.001, 0.0001, or not significant, respectively.

^b Treatments: Normal harvest/control (T0), harvest three weeks after T0 (T1), harvest six weeks after T0 (T2).

^c Means in rows followed by different letters are significantly different, $p \leq 0.05$, Duncan's multiple range test.

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Figure 1. Significant interactive effects between crop level and harvest date for Cabernet Sauvignon wines, Puglisi Vineyard, Niagara-on-the-Lake, ON, 2011-2012. 2011: A: Color intensity; B: Total phenols; C: Gallic acid; D: Quercetin; 2012: E: pH; F: Color intensity; G: Hue; H: Total anthocyanins; I: Total phenols; J: Cyanidin; K: Peonidin; L: Malvidin; M: Petunidin-3-acetylglucoside; N: Malvidin-3-acetylglucoside; O: Malvidin-3-coumarylglucoside; P: Epicatechin.

Figure 2: Significant interactive effects between crop level and harvest date for Cabernet franc wines, Puglisi Vineyard, Niagara-on-the-Lake, ON, 2011-2012. 2011: A: Titratable acidity; B: Ethanol; 2012: C: Hue; D: Petunidin; E: Malvidin; F: Delphinidin-3-acetylglucoside; G: Petunidin-3-acetylglucoside; H: Delphinidin-3-coumarylglucoside; I: Catechin; J: Caffeic acid; K: Epicatechin.

For Review Only