COOL CLIMATE WINEMAKING: EXOGENOUS TANNIN ADDITIONS IN RED HYBRID CULTIVARS

A Thesis

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Master of Science

by

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ABSTRACT

The addition of exogenous tannins has become a common practice in cool climate winemaking to increase tannin content in hybrid red wines. These additions, when made at recommended rates and times, often result in low tannin retention. Previous research has shown that various phenolic compounds have a mixed effect on lactic acid bacteria (LAB) growth and malolactic fermentation (MLF) success. This study examined the effect of exogenous tannin additions above recommended rates on two strains of LAB during MLF in hybrid sp. Corot noir, Noiret, and Marquette cultivars. A mixed effects model was used to determine the interactions of LAB and tannin additions on MLF, independently and together. In all cultivars there were interaction effects, varying among cultivars, indicating that LAB strain selection and tannin additions have an effect on MLF. Despite these effects, all lots finished MLF in two weeks or less, suggesting that the addition of the exogenous tannins studied may not impact completion of MLF when other conditions are optimal.

Further, little research has been performed on the timing of exogenous tannin additions in hybrid red wine. In 2013, wines were made from Maréchal Foch, Corot noir, and Cabernet Franc to compare the retention of exogenous tannins in interspecific hybrids and *Vitis vinifera*. In each cultivar a commercial exogenous tannin product containing ≈38% condensed tannin was added at a rate of 800 mg/L. Additions were made at each major processing step for a total of 3 additions treatments during the winemaking process. To determine the fate of tannins in each wine, a mass balance was performed tracking the loss and gain in tannin. With later additions, there was a progressive increase in retention for all cultivars, suggesting that adding tannin after alcoholic fermentation reduces the portion lost. Mass balance calculations also showed that 5-10 times more tannin was lost in the lees between post alcoholic fermentation (AF) and post

malolactic fermentation (MLF) samples than following MLF through eight months of aging. This suggests that in the hybrid red cultivars studied, later additions of exogenous tannin increase condensed tannin retention to levels comparable to that in *V. vinifera*.

The recommend dosage for exogenous tannins of 50-500 mg/L may not effectively increase condensed tannins. In 2013, wines were made from Maréchal Foch, Corot noir, and Cabernet Franc to compare the retention of exogenous tannins in interspecific hybrids and *Vitis vinifera*. After analyzing 12 commercial tannin products for condensed tannin concentration via HPLC, the highest, with a concentration 38%, was added at a rate of 400, 800, and 1200 mg/L after crush/before yeast inoculation. A separate portion of each cultivar was pressed off the skins immediately, fermented with 1600 mg/L of exogenous tannin, then back-blended post-fermentation with a control wine for a final theoretical concentration of 400 mg/L tannin addition. At bottling, tannin concentrations in all treatments were higher than the respective control, but none exceeded 50% retention. This suggests that high concentration additions of exogenous tannin increase the condensed tannins in hybrid red wines, but retention rates vary by cultivar.

BIOGRAPHICAL SKETCH

Alex Fredrickson is from Kennewick, Washington and graduated from the University of Idaho in 2012 with a B.S. degree in Food Science. He became interested in winemaking and enology while taking a wine microbiology and processing class from Washington State University, where he was able to make and study wine for the first time. Alex then worked as winemaking intern at E&J Gallo's Sonoma winery in Healdsburg, California where he developed a real passion for winemaking and the science behind the wine. He continued his winemaking experience with a cellar hand position at Church Road Winery in Napier, New Zealand where he learned in detail about real winemaking processes in the cellar as opposed to in the classroom or lab. To expand his knowledge, Alex decided to go back to school and get his M.S. from Cornell's Department of Food Science and Technology. Under the guidance of Anna Katharine Mansfield, he has helped the wine industry to better understand the addition of exogenous tannins to hybrid red wines. He has accepted a position in Eastern Washington with Duckhorn Wine Company's label Canvasback starting in August 2015, and has plans on working in Tasmania in 2016 with his wife.

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CHAPTER 1

EXOGENOUS TANNIN ADDITION AND LACTIC ACID BACTERIA STRAIN SELECTION: EFFECTS ON MALOLACTIC FERMENTATION

1.1 Abstract

The addition of exogenous tannins has become a common practice in cool climate winemaking to increase tannin content in hybrid red wines. Previous research has shown that various phenolic compounds have a mixed effect on lactic acid bacteria (LAB) growth and malolactic fermentation (MLF) success. This study examined the effect of exogenous tannin additions on two strains of LAB during MLF in hybrid cultivar sp. Corot noir, Noiret, and Marquette. A mixed effects model was used to determine the interactions of LAB and tannin additions on MLF, independently and together. In Corot noir there were interactions between LAB and tannin treatment for both malic acid degradation and lactic acid accumulation. Noiret and Marquette showed similar interaction for lactic acid accumulation, but not for malic acid degradation. Cell growth was affected by LAB treatment throughout MLF for all three cultivars, while an effect from tannin treatments was only seen in Noiret. LAB strain selection and exogenous tannin additions both had an independent effect, often throughout MLF. There were further interactions between LAB and tannin treatments, independent of time. Despite these effects, all lots finished MLF in two weeks or less, suggesting that the addition of the exogenous tannins studied may not impact completion of MLF when other conditions are optimal.

1.2 Introduction

In cool wet regions *Vitis vinifera*, and especially red cultivars, can be a challenge to grow due to large amounts of rainfall during the growing season and low temperatures in the winter (Newman 1986). These challenges have led to the use of interspecific hybrid cultivars more

suited for such conditions (Warmund et al. 2008). Because many hybrid cultivars have low tannin concentrations, exogenous tannin products are often used to increase total tannin content for quality and color stability (Manns et al. 2013). To increase tannin content in hybrid cultivars with low tannin, additions above the manufacture's recommended rates of 30-500 mg/l (Scott Laboratories 2013) are likely needed, as additions of 400 mg/l had little effect (Thomas 2013).

The conversion of malic acid to lactic acid, known as malolactic fermentation (MLF), can impart many sensory characteristics to wine. The organism responsible for MLF, *Oenococcus oeni*, is a Gram positive and microaerophilic lactic acid bacterium (LAB) (Bartowsky 2005). In cool climate regions where harvest often occurs before optimal ripening, MLF is may be used to reduce acidity and enhance biological stability (Lasik 2013).

Tannins are part of the large domain of phenolic compounds (Versari et al. 2013). The building blocks of tannins range from small, simple phenols including gallic and caffeic acids, to polyphenolic compounds that contain multiple phenolic rings, like catechin and epicatechin. Tannins in wine are described as high molecular weight phenolic mixtures (Waterhouse 2002). Condensed tannins are mixtures of polymers of flavonoids, and are most prevalent in wine, as they are derived from grape skin and seeds. Hydrolyzed tannins are gallic or ellagic acid based mixtures that do not occur naturally in grapes, but can be extracted from oak and exogenous tannin additions (Keulder 2006).

Exogenous tannins and LAB play important roles in hybrid red wine production, but their interactions in a wine matrix are poorly understood. *O. oeni* is an efficient bacteria that can thrive despite the challenges found in wine, such as high ethanol concentration, low pH, the presence of sulfur dioxide, and low nutrient availability (Wibowo et al. 1985). Even with the ability to grow in these conditions, other wine components can cause issues during MLF. Condensed tannins,

especially those with higher molecular weight, may negatively affect cell growth and viability, complicating MLF (Figueiredo et al. 2008). On the other hand, the monomeric flavanol catechin may have a stimulating affect on LAB (Alberto et al. 2001). Hydrolyzed tannins show a positive affect on *O. oeni* growth (Vivas et al. 2000), and gallic acid from hydrolyzed tannins has also been found to stimulate growth (Reguant et al. 2000).

This study was performed to determine whether the higher addition of exogenous tannins recommended for hybrid red cultivars and LAB strain selection have an effect on LAB growth, malic acid degradation, and lactic acid accumulation. This is important to understand, as conditions for MLF may be suboptimal in hybrid cultivars with higher titratable acidity and/or high alcohol concentrations. Little research has been performed to assess the interaction of exogenous tannins and the robust *O. oeni* stains typically used in cool climate winemaking.

1.3 Materials and Methods

Fruit. Three wine grape cultivars, Noiret (Branchport, NY), Marquette and Corot noir (Penn Yan, NY), were hand-harvested on 10/21/2014, 9/8/2014 and 10/6/2014, respectively. Approximately 450kg of each cultivar was crushed and divided into 18 fermentations with 20kg (Marquette) and 22kg (Corot noir and Noiret) of must per fermentation.

Winemaking. For all wine lots *S. cerevisiae* yeast strain GRE (*Lavin*) was rehydrated in 10ml water per 1g of dehydrated yeast with 0.3g/l of Go-Ferm Protect (*Lallemand*). After rehydration, all lots were inoculated with 264mg yeast/l. An addition of 144.1g of sucrose was added to each Corot noir lot to reach 20° Brix, and yeast assimilable nitrogen (YAN) additions were made as necessary. After additions were made, all lots were placed in a temperature-controlled cooler, held at 21°C. Caps were punched down two times per day over seven days of maceration, at which point all wine lots were at or below 0.6% residual sugar. Due to the small volume, the

wines were pressed by hand using a 7.6l large mesh container with press cloth and cheesecloth. Free run juice was collected for roughly ten minutes; then the must was pressed lightly until 11.35l of wine was collected, sampled, and transferred to carboys.

Experimental treatments. Tannin and LAB additions were made according to the experimental design shown in Table 1.1, resulting in a total of nine treatments performed in duplicate. Two exogenous tannin products were used: Tannin 1 (T1), containing hydrolyzed tannin, and Tannin 2 (T2), containing condensed tannin derived from white grape skins. Tannin products were added directly after pressing at a rate of 800 mg/l (9.08 g per 11.35l); each product was dissolved in ten times its weight of wine, then added back to the wine lot and thoroughly mixed. Post tannin addition samples were taken directly after mixing (Table 1.2).

Two LAB strains were used: LAB 1, a hardy strain often used in red hybrid winemaking, and LAB 2, a new, robust strain that can utilize malic acid in extreme wine conditions. LAB cultures were hydrated in 25°C DI water at a rate of 2.5g/100ml and lightly mixed with a stir bar for 15 minutes. Inoculations were added at a rate of 10mg/l. After MLF was complete, 60 ppm of SO_2 was added to each treatment wine. Wines without LAB additions received 60 ppm SO_2 to prevent MLF.

Table 1.1: Additions of exogenous tannin products and lactic acid bacteria (LAB) strains

Tannin treatment	**Control (no tannin)**	**Tannin 1 (T1)**	**Tannin 2 (T2)**
LAB treatment			
Control (no LAB)	No LAB x no tannin	No LAB x T1	No LAB x T2
LAB 1	LAB1 x no tannin	LAB1 x T1	LAB1 x T2
LAB 2	LAB2 x no tannin	LAB2 x T1	LAB2 x T2

Analyses. Analyses performed on treatments inoculated with LAB and uninoculated LAB controls varied slightly (Table 1.2).

Yeast Assimilable Nitrogen: A Chemwell 2910 multianalyzer with Software Version 6.3 (Awareness Technology, Palm city, FL) was used to measure yeast assimilable nitrogen (YAN) levels by enzymatic analyses (Unitech Scientific, Hawaiian Garden, CA). To amend low YAN levels, 5.0 g (0.25 g/kg) of Fermaid (Lallemand) and 8.4g (0.42 g/kg) of diammonium phosphate (DAP) was added to Marquette and 5.5 g (0.25 g/kg) of Fermaid (Lallemand) and 2.9 g (0.13 g/kg) of DAP to Corot noir 24hr after yeast inoculation, bringing YAN concentrations up to 273 and 200 mg/l, respectively.

***Exogenous tannins*:** Before additions, tannin products were analyzed for tannin concentration via HPLC solid phase extraction-phloroglucinolysis (Manns and Mansfield 2012). Samples were also analyzed via Adams-Harbertson Tannin Assay (Harbertson et al. 2002) to measure non-condensed tannins.

Microbial analysis: Separate 10ml wine samples were taken in sterile culture tubes for bacterial enumeration/organic acids, and 2.2ml centrifuge tubes for tannin analysis (Table 1.2). Microbiological dilutions were made with 9ml peptone (*Alpha Biosciences,* Baltimore, MD) water blanks until the estimated CFU/ml range was reached. Malolactic basal media (MLB media) was prepared according to Chiang (2008). Indigenous yeast populations of the must were determined using yeast extract peptone dextrose media (YPD) (*Alpha Biosciences*). Molten agar was poured over 1ml samples and mixed. Each dilution was performed in duplicate. Plates were inverted after solidifying and incubated aerobically at 30°C for three to five days.

Chemical analysis: Residual sugar (RS) levels were checked during fermentation using Clinitest tablets (Bayer, Etobicoke, ON, Canada). Organic acids were measured via HPLC analysis (*Agilent,* Palo Alto, CA). Tannin samples were frozen at -20°C until needed for analysis. Tannin concentration was determined via Adams-Harbertson Tannin Assay (Harbertson et al. 2002).

Table 1.2**:** Schedule of sampling and analyses in juice and wine

Sample Time	**Analyses in LAB[1]-inoculated samples**	**Analyses in uninoculated samples**
Must	YPD[2], MLB[3], organic acids, tannin[4]	YPD, MLB, organic acids, tannin
Post Alcoholic Fermentation	Tannin	Tannin
Post Tannin/LAB additions	MLB, organic acids, tannin	MLB, organic acids, tannin
2 days Post Inoculation	MLB and organic acids	None
4 Days Post Inoculation	MLB and organic acids	None
7 Days Post Inoculation	MLB and organic acids	None
MLF[5] completion	MLB, organic acids, tannin	MLB, organic acids, tannin

[1]Lactic acid bacteria
[2]Yeast growth on yeast extract peptone medium
[3]LAB growth on malolactic basal medium
[4]Tannin measured via Adams-Harbertson Tannin Assay (Harbertson et al. 2002)

***Statistical analysis*:** All statistical analysis was performed on JMP statistical software (SAS, Cary, NC). A mixed effects model was used with fixed effects of tannin treatment, LAB treatment, tannin treatment*LAB treatment, time point, tannin treatment*time point, LAB treatment*time point, and tannin treatment*LAB treatment*time point. A random sample-level effect was included.

1.4 Results

Wine analysis: At bottling, pH and TA were measured for all wines (Table 1.3.)

Exogenous tannin products. Analysis of T1 showed concentrations of 11 ppm (1.07% tannin) and 272 ppm (25.42% tannin) when measured with HPLC-Phloroglucinolysis and Adams-Harbertson, respectively. In contrast, T2 had concentrations of 179.9 ppm (17.81%) and 4.6 ppm (0.46%), respectively.

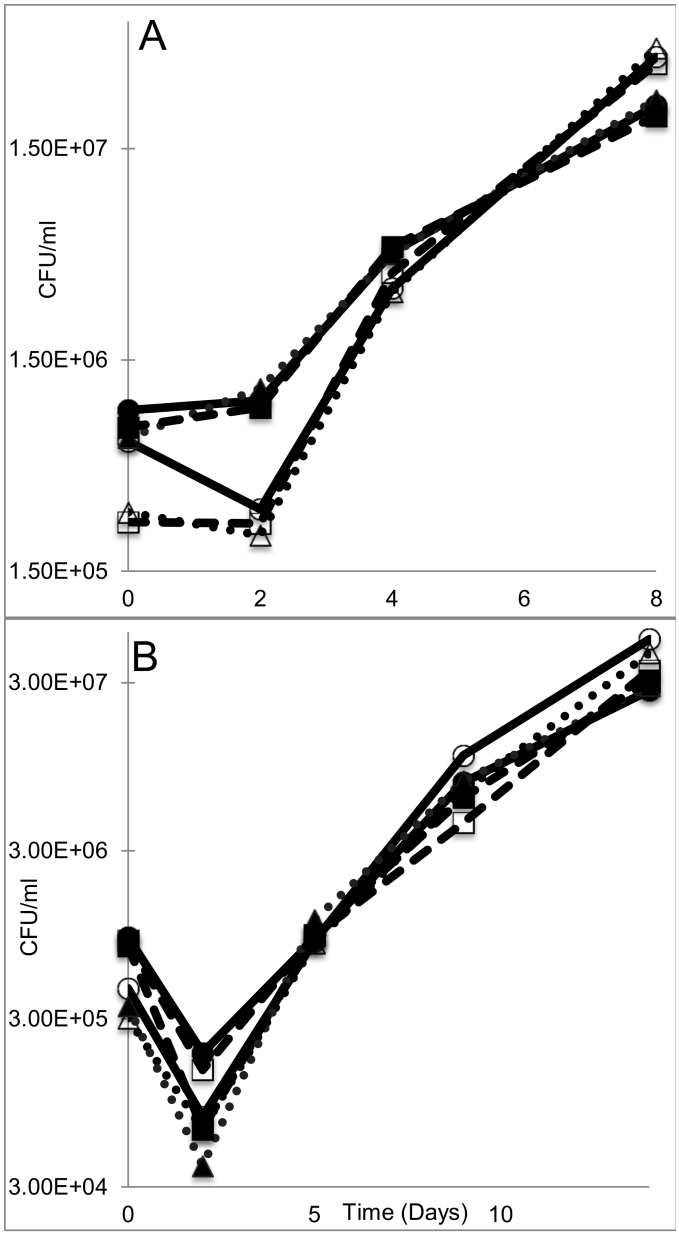

Figure 1.1: Time course of LAB growth for (A) Corot noir and (B) Noiret. LAB and Tannin treatments of (○) LAB 1 control, (●) LAB 2 control, (△) T1XLAB1, (▲) T1XLAB2, (□) T2XLAB1, (■) T2XLAB2.

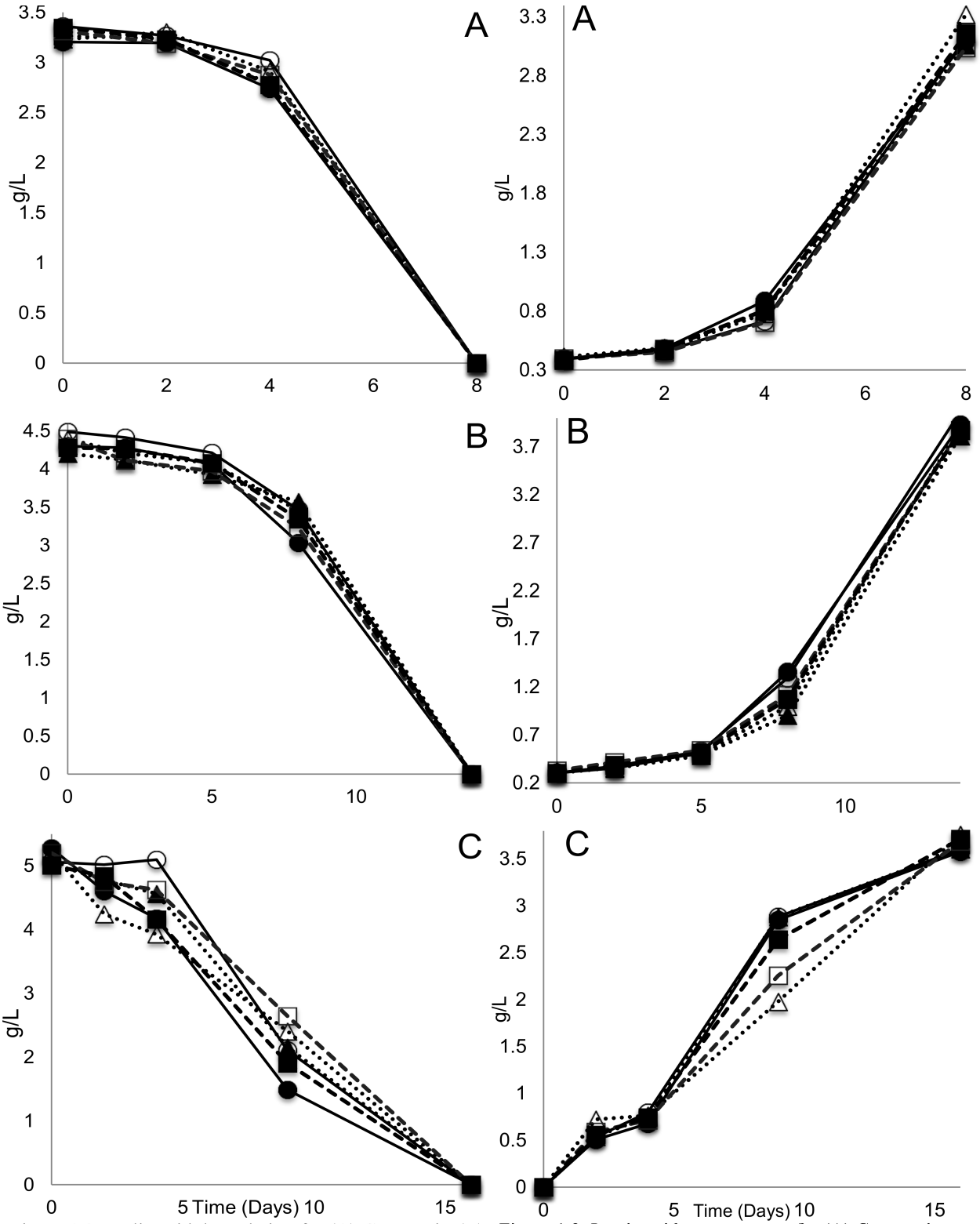

Figure 1.2: Malic acid degradation for (A) Corot noir, (B) Noiret, and (C) Marquette. LAB and Tannin treatments of (○) LAB 1 control, (●) LAB 2 control, (△) T1XLAB1, (▲) T1XLAB2, (□) T2XLAB1, (■) T2XLAB2. Each value is the mean of two replicates that are within a SD of ± 0.69 g/l.

Figure 1.3: Lactic acid accumulation for (A) Corot noir, (B) Noiret, and (C) Marquette. LAB and Tannin treatments of (○) LAB 1 control, (●) LAB 2 control, (△) T1XLAB1, (▲) T1XLAB2, (□) T2XLAB1, (■) T2XLAB2. Each value is the mean of two replicates that are within a SD of ± 0.23 g/l.

Table 1.3: Wine pH and titratable acidity (TA) [1] at bottling

	LAB[2] Inoculated wine		LAB Uninoculated Wine	
Cultivar	pH	TA (g/l)	pH	TA (g/l)
Marquette	3.65-3.76	6.4-6.8	3.53-3.68	7.0-8.3
Corot noir	3.67-3.76	5.8-6.3	3.69-3.73	5.9-6.1
Noiret	3.45-3.61	6.8-7.8	3.33-3.35	9.2-9.5

[1]Expressed as tartaric acid equivalents (TAE)
[2]Lactic acid bacteria

Table 1.4: Initial and final CFU/ml, malic acid, and lactic acid following malolactic fermentation (MLF) for all inoculated treatments.

Treatment	Initial CFU/ml	Final CFU/ml	Initial [MA[6]]	Final [MA]	Initial [LA[7]]	Final [LA]
Corot noir	Mean (SD[1])	Mean (SD)	Mean[8] (SD)	Mean (SD)	Mean[8] (SD)	Mean (SD)
LAB1[2] Control	6.18E5 (9.12E4)	4.08E7 (1.05E7)	3.4 (0.00)	0.0 (0.00)	0.4 (0.01)	3.1 (0.04)
LAB2[3] Control	8.68E5 (1.28E5)	2.40E7 (5.79E6)	3.2 (0.02)	0.0 (0.00)	0.4 (0.01)	3.1 (0.04)
T1[4] X LAB1	2.85E5 (1.48E5)	4.48E7 (8.35E6)	3.2 (0.01)	0.0 (0.00)	0.4 (0.01)	3.0 (0.00)
T1 X LAB2	2.56E5 (6.97E4)	3.78E7 (6.46E6)	3.3 (0.01)	0.0 (0.00)	0.4 (0.01)	3.1 (0.00)
T2[5] X LAB1	6.43E5 (5.07E5)	2.55E7 (2.60E6)	3.3 (0.06)	0.0 (0.00)	0.4 (0.01)	3.3 (0.01)
T2 X LAB2	7.20E5 (1.14E5)	2.14E7 (2.98E6)	3.4 (0.11)	0.0 (0.00)	0.4 (0.03)	3.2 (0.06)
Noiret						
LAB1 Control	4.55E5 (6.50E4)	5.45E7 (6.18E6)	4.5 (0.01)	0.0 (0.00)	0.3 (0.01)	4.1 (0.01)
LAB2 Control	9.18E5 (2.58E5)	2.70E7 (5.87E6)	4.3 (0.21)	0.0 (0.00)	0.3 (0.01)	4.0 (0.13)
T1 X LAB1	3.03E5 (1.48E4)	4.50E7 (7.91E6)	4.4 (0.13)	0.0 (0.00)	0.3 (0.01)	3.9 (0.05)
T1 X LAB2	3.58E5 (3.34E4)	2.88E7 (8.61E6)	4.2 (0.01)	0.0 (0.00)	0.3 (0.04)	3.8 (0.05)
T2 X LAB1	8.78E5 (7.79E4)	3.55E7 (1.06E7)	4.4 (0.08)	0.0 (0.00)	0.3 (0.02)	3.9 (0.00)
T2 X LAB2	8.15E5 (2.96E4)	3.10E7 (3.81E6)	4.3 (0.09)	0.0 (0.00)	0.3 (0.00)	3.9 (0.00)
Marquette						
LAB1 Control	3.85E6 (1.06E6)	7.93E6 (5.67E5)	5.1 (0.04)	0.0 (0.00)	0.0 (0.00)	3.6 (0.05)
LAB2 Control	5.03E6 (2.05E6)	3.88E6 (1.47E6)	5.3 (0.10)	0.0 (0.00)	0.0 (0.00)	3.6 (0.03)
T1 X LAB1	2.90E6 (9.67E5)	1.11E7 (9.12E5)	5.2 (0.22)	0.0 (0.00)	0.0 (0.00)	3.8 (0.33)
T1 X LAB2	1.15E6 (6.18E5	1.22E7 (2.79E6)	5.0 (0.00)	0.0 (0.00)	0.0 (0.00)	3.6 (0.08)
T2 X LAB1	3.35E6 (1.26E6)	5.23E6 (9.23E5)	5.2 (0.30)	0.0 (0.00)	0.0 (0.00)	3.7 (0.05)
T2 X LAB2	5.33E6 (2.27E6)	4.60E6 (1.14E6)	5.0 (0.08)	0.0 (0.00)	0.0 (0.00)	3.7 (0.28)

[1]Standard Deviation
[2]Lactic acid bacteria treatment 1
[3]Lactic acid bacteria treatment 2
[4]Exogenous tannin treatment 1
[5]Exogenous tannin treatment 2
[6]Malic acid
[7]Lactic acid
[8] expressed in mg/L

Malic acid. Initial malic acid concentrations were consistent among treatments within each cultivar, but among cultivars concentrations ranged from 3.3 to over 5 g/L (Figure 1.2). Malic acid in Corot noir was utilized quickly, and was completely depleted in less than 1 week.

In Marquette and Noiret, malic acid concentration began to decrease between 4 days and 1 week following inoculation, and was completely degraded by the end of week 2.

The three two-way interactions for Corot noir indicate that LAB growth (CFU/ml) was affected by exogenous tannin and LAB strains (Table 1.5). With Noiret and Marquette there was no effect from tannin or LAB treatments. In Marquette wines, however, LAB 2 seemed to reduce malic acid faster, as all LAB 2 treatments had lower malic acid concentrations after 1 week (Figure 1.1). No obvious trends were observed in Noiret.

Lactic acid. Early accumulation of lactic acid was evident in Corot noir and Noiret prior to LAB inoculation (Table 1.4). Final lactic acid concentrations ranged from 3 to 4 g/L, and from lowest to highest in T1 treatments < T2 treatments < control wines.

There was a three-way interaction between tannin treatment, LAB treatment, and time for Corot noir and Marquette (Table 1.5). In Noiret treatments, there was an interaction between tannin treatment and time. This trend was observed in both tannin treatments throughout MLF regardless of LAB strain (Figure 1.3).

Lactic acid bacterial growth. To better fit the mixed effects model, the LAB counts were transformed by taking the natural log. LAB growth data for Marquette was excluded, as initial sample plates lacked cycloheximide, and excessive yeast growth numbers resulted in overestimated plate counts. The negative controls and tannin controls had <100 CFU LAB in Marquette and Noiret after alcoholic fermentation, and LAB counts of 1E+03 and 7E+04 (figure 1.1) after MLF was complete in the inoculated lots. Organic acid profiles confirmed MLF did not occur in uninoculated lots.

Table 1.5: Mixed effects model (with F Ration and Prob > F statistics) of lactic acid bacteria (LAB) strains and tannin products throughout malolactic fermentation

	Malic acid		Lactic acid		CFU/ml**	
Interactions (effects)	F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F
Corot noir						
tannin treatment	0.0762	0.9275	8.6422	0.0171*	2.0911	0.2046
LAB treatment	12.8819	0.0115*	1.8626	0.2213	44.1329	0.0006*
tannin treatment*LAB treatment	5.2759	0.0476*	4.7728	0.0575	1.1372	0.3813
time point	771.5678	<.0001*	58627.16	<.0001*	762.0228	<.0001*
tannin treatment*time point	7.642	0.0027*	24.9776	<.0001*	2.0216	0.1155
LAB treatment*time point	23.3407	<.0001*	39.8141	<.0001*	31.2882	<.0001*
tannin treatment*LAB treatment*time point	2.8186	0.0734	5.9583	0.007*	0.8356	0.5583
Noiret						
tannin treatment	0.9077	0.4525	18.1681	0.0028*	2.9173	0.1303
LAB treatment	3.3727	0.1159	3.2389	0.122	5.1044	0.0646
tannin treatment *LAB treatment	3.6618	0.0913	0.1797	0.8399	0.1572	0.858
time point	112.8769	<.0001*	20890.7	<.0001*	1090.3620	<.0001*
tannin treatment *time point	2.0578	0.1101	19.458	<.0001*	3.9007	0.0045*
LAB treatment*time point	0.5286	0.6683	1.5664	0.2322	15.8994	<.0001*
tannin treatment*LAB treatment*time point	0.9838	0.4647	2.258	0.0844	1.1649	0.3594
Marquette						
tannin treatment	0.2031	0.8216	0.6049	0.5763	3.6228	0.0929
LAB treatment	2.1203	0.1956	1.5676	0.2571	3.7397	0.1013
tannin treatment*LAB treatment	2.1456	0.1982	1.2354	0.3554	2.4510	0.1667
time point	269.5141	<.0001*	1881.528	<.0001*	44.7148	<.0001*
tannin treatment *time point	2.2199	0.0887	5.804	0.0016*	2.1658	0.0685
LAB treatment*time point	2.5966	0.0842	12.0782	0.0001*	3.7908	0.0158*
tannin treatment*LAB treatment*time point	2.2451	0.0858	4.7675	0.0045*	2.3355	0.0516

*Significance at Prob > F less than 0.05

**The natural log was taken for a normalized distribution curve and better statistical analysis

Corot noir showed a more vigorous indigenous LAB population with CFUs around 2E+02 to 3E+02 after alcoholic fermentation (Figure 1.1). Efforts to stop LAB growth was not effective in this cultivar; uninoculated lots showed CFU counts from 3E+06 to 5E+06. Lactic acid concentrations were at 3.10, 3.20, and 3.32 g/l (for No LAB x no tannin, No LAB x Tannin 1, and No LAB x Tannin 2 respectively). Malic acid concentrations were all at 0 g/L. To determine if

the inoculated strain for each lot was the strain to complete the conversion, post-MLF wine samples were analyzed by a third party using implantation PCR. All samples were found to contain 100% of the inoculated strain except for a LAB1 control, which was estimated at just under 100%. Although the negative controls completed MLF with indigenous LAB, the inoculated LAB strains still dominated MLF in all treatments.

In Corot noir wines an interaction was found between LAB treatment and time (Table 1.5), which is evident in the close grouping of the two LAB strains throughout MLF (Figure 1.1). Similar interactions were found in Marquette (Table 1.5). Interactions of LAB treatments and time as well as tannin treatment and time in Noiret wines were also observed (Table 1.5).

Tannin concentration. Tannin concentrations did not differ within a cultivar until additions were made following alcoholic fermentation (Table 1.6). Tannin content in Marquette wines increased following exogenous additions, but dropped to a fraction of the initial concentration following MLF. Corot noir had a similar increase in tannin following AF and higher post-MLF retention. In Corot noir, T1 also resulted in higher final concentrations than T2. Noiret, typically seen as a having high tannin content for a hybrid cultivar (Springer and Sacks 2014), had concentrations more than double Marquette and Corot noir. Although indigenous tannin content was high, tannin additions resulted in increases similar to that in other cultivars. Overall, pronounced variability was observed in tannin concentration within treatment duplicates.

Table 1.6: Tannin concentration (mg/L) in Corot noir, Noiret, and Marquette measured via Adams-Harbertson (Harbertson et al. 2002)

	Sample time			
Treatment	Must	Post Alcoholic	Post tannin addition	Post Malolactic
Corot noir				
LAB1[1] Control	8.6	95.4 a	95.4a	45.4a
LAB2[2] Control	8.6	103.1a	103.1a	47.4a
Tannin1[3] X LAB1	8.6	115.1a	276.8b	195.9b
Tannin1 X LAB2	8.6	108.2a	305.8b	194.5b
Tannin2[4] X LAB1	8.6	117.8a	182.9c	96.1c
Tannin2 X LAB2	8.6	101.5a	146.8d	98.8c
Noiret				
LAB1 Control	23.9	270.1a	270.1ab	166.6a
LAB2 Control	23.9	224.7a	224.7a	114.0a
Tannin1 X LAB1	23.9	219.2a	381.5c	251.7c
Tannin1 X LAB2	23.9	227.5a	333.0bc	240.8bc
Tannin2 X LAB1	23.9	219.8a	276.2ab	171.9a
Tannin2 X LAB2	23.9	230.9a	278.1ab	175.6ab
Marquette				
LAB1 Control	65.5	92.0a	92.0a	5.1a
LAB2 Control	65.5	133.5a	133.5a	4.6a
Tannin1 X LAB1	65.5	125.5a	218.5a	21.4a
Tannin1 X LAB2	65.5	71.2a	120.5a	29.9a
Tannin2 X LAB1	65.5	131.4a	125.9a	5.9a
Tannin2 X LAB2	65.5	58.4a	159.3a	6.8a

[1]Lactic acid bacteria treatment 1
[2]Lactic acid bacteria treatment 2
[3]Exogenous tannin treatment 1
[4]Exogenous tannin treatment 2
Significant at P- < 0.05, within sampling time

1.5 Discussion

Exogenous tannin products. The two commercial tannins showed notable differences in tannin concentration depending on analysis. The discrepancies in T1 measurements may be because Adams-Harbertson measures total iron-reactive phenolics, which includes tannins and other phenolics not classified as tannins (Harbertson et al. 2002), while HPLC-phloroglucinolysis is more selective, measuring condensed tannins and excluding hydrolyzed tannins (Manns and Mansfield 2012). The very low Adams-Harbertson result for T2 is surprising for a product derived from grape skins, because condensed tannins are iron reactive phenolics which are measured in the assay. This could be due to the low concentration of condensed tannin, as the final absorbance of <0.1 AU is well below the lower limit of the Adams-Harbertson assay of 0.3 AU (Jensen et al. 2008).

Previous studies have varied greatly in the two measurements as well, with one study that looked at 38 different red cultivar grape skins reporting tannin levels measured by Adams-Harbertson ranging from 1.7-7.1 mg/g, and by HPLC-phloroglucinolysis ranging from 1.9-12.8 mg/g (Seddon and Downey 2008). A second study looking at 40 red wines measured tannin content by Adams-Harbertson in the range of 387-1655 mg/l, while HPLC-phloroglucinolysis had a range of 119-376 mg/l (Kennedy et al. 2006). With the large variation between the two analytical methods, it was important to select one analytical method for consistency, so Adams-Harbertson was used. Ultimately, 800mg/L additions of T1 and T2 were thought to have contributed 203.4 mg/L and 3.7 mg/L, respectively, of tannin to the treatment wines.

Malolactic fermentation. Selection of lactic acid bacteria strain can have a major impact on MLF, as alcohol tolerance, low pH viability, malic acid degradation rate, and SO_2

tolerance vary by strain (Solieri et al. 2010). As the two strains used in this study were chosen for their resilience, it is unsurprising that MLF was complete in all wines in two weeks or less (Figure 1.2). Further, both LAB strains MLF reached completion despite the presence of exogenous tannin (condensed or hydrolyzed) at relatively high addition levels. Also, the lactic acid accumulated prior to inoculation (Table 1.4) in Corot noir and Noiret suggests that MLF had started with indigenous bacteria. Despite this, it's likely that inoculated LAB strains dominated MLF, as implantation PCR results from Corot noir samples taking post MLF showed them to be the dominate strain.

The three-way interaction between tannin treatment, LAB treatment, and time for Corot noir and Marquette (Table 1.5) may indicate that the type of exogenous tannin and LAB strain can have an effect on MLF in certain hybrid cultivars. In Noiret, the interaction between tannin treatment and time (Table 1.5) demonstrates that the exogenous tannin product may have an effect on lactic acid accumulation throughout MLF.

The interactions between LAB treatment and time for Marquette and Corot noir wines (Table 1.5) indicate that LAB treatment may play an important role on cell growth. Also, interactions of LAB treatments and time as well as tannin treatment and time in Noiret wines (Table 1.5) suggest that LAB strain selection and tannin treatment both influence cell growth independently throughout MLF.

While malolactic conversion was completed in all wines, the interaction effects of tannin treatments on malic acid degradation and lactic acid accumulation indicate small differences in fermentations that may be magnified in less optimized fermentation conditions. In the mixed effects model, the three-way interaction (tannin, LAB, and time)

for Corot noir and Marquette was close to significance, suggesting that the tannin and LAB interaction occurs, but not throughout MLF.

The increased malic acid degradation with some T1 treatments (Figure 1.2) could be due to the stimulating effect of gallic acid, which for some LAB strains has been observed at concentrations of 50-200 mg/l (Alberto et al. 2001). Some interaction effects that led to inhibition could be due to condensed tannin (T2 treatments), especially if they were seed-derived, as these compounds have been shown to have an inhibitory effect on MLF at concentrations as low as 50-100 ppm (Vivas et al. 2000).

Bacterial growth. As with malic acid degradation and lactic acid accumulation, cell growth showed interaction effects, often with LAB treatments. An interaction with LAB strain and cell growth indicates different growth rates between LAB strains. These interactions can have a large impact on the speed of MLF, which may be either good or bad for winemakers, depending on stylistic goals. The fact that LAB1 strains grouped close together, but away from similarly grouped LAB2 strains, in Corot noir and (to some degree) in Noiret confirms that strain selection affects LAB growth. A faster growth rate for LAB can lead to increased ATP production, which can potentially lead to the production of more volatile acids and changes in wine aroma (Moreno-arribas and Mun 2011). Although MLF finished around the same time in all treatment wines, this interaction effect could play a role on MLF performance with less-hardy strains or in wines with low nutrients, pH, or temperature.

Tannin concentration. The increased concentration in wine tannin was lower than expected based on the tannin product analysis (Table 1.6). Though T1 was found to have a higher tannin content than T2 (as measured with Adams-Harbertson assay), wine

additions only resulted in a 100-200 mg/l increase immediately following addition. By the end of fermentation, tannin concentrations were 17-150 mg/l above the control. This is congruent with Harbertson et al. (2012), who suggested that the recommended exogenous tannin addition rates were too low, and caused little to no change in Merlot wines. In this study, large additions of exogenous tannin to hybrid cultivars with low tannin extractability (Springer and Sacks 2014), showed only small effects on the tannin concentrations within the wine.

Exogenous tannin additives play an important role in winemaking, especially in cool climate regions, but the right product and addition rate may vary by situation. Commercial products often give a recommended addition time and rate, and identify the species from which the tannin was extracted, but further parameters of use are often left for interpretation. These recommendations are typically for *Vitis vinifera* that contain higher concentrations of indigenous tannin and have higher tannin extractability (Springer and Sacks 2014). The recommended addition rates have had little effect on increasing final tannin concentrations in hybrid red wines (Thomas 2013). Without knowing how much condensed or hydrolyzed tannin is in an exogenous tannin product or the tannin extractability of a particular cultivar, it is hard to know how much the overall tannin concentration will increase in a particular hybrid wine.

1.6 Conclusion

Corot noir, Noiret, and Marquette cultivars show that higher addition rates of exogenous tannins and strain selection had various interaction effects on LAB growth, malic acid degradation, and lactic acid accumulation. Most of the interaction effects seen were cultivar specific. With the hybrid cultivars and LAB strains used in this study, MLF

is affected by higher exogenous tannin additions, but as all fermentations were complete in two weeks, practical implications are likely negligible.

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CHAPTER 2

TIMING EXOGENOUS TANNIN ADDITIONS FOR OPTIMAL RETENTION IN HYBRID RED WINE

2.1 Abstract

To effectively increase condensed tannin content in hybrid red wines, exogenous tannin additions made to the must may need to be added above the manufacturer-recommended dosage of 50-500 mg/L, and later additions have been found to increase tannin retention. In 2013, wines were made from Maréchal Foch, Corot noir, and Cabernet Franc to compare the retention of exogenous tannins in interspecific hybrids and *Vitis vinifera*. In each cultivar a commercial exogenous tannin product containing ≈38% condensed tannin was added at a rate of 800 mg/L. Additions were made at crush prior to yeast inoculation (M+), after the completion of alcoholic fermentation (AF+), or after the completion of malolactic fermentation (MLF+). To determine the fate of tannins in each wine, a mass balance was performed tracking the loss and gain in tannin (by weight) during the winemaking process. After eight months of aging, the retention of exogenous tannins was calculated using the theoretical condensed tannin addition (304 mg/L) and tannin measured in each wine. Maréchal Foch wines had tannin retention percentages of 12%, 26%, and 59% (M+, AF+, and MLF+, respectively). Corot noir evinced higher retention rates at 27%, 60%, and 73% for the same sample points, and Cabernet Franc higher still at 31%, 56%, and 80%. The progressive increase in retention suggests that adding tannin after alcoholic fermentation reduces the portion lost, likely as a result of decreased binding to cell wall material during wine processing. Mass balance calculations also showed that 5-10 times more tannin was lost in the lees from the period post alcoholic

fermentation (AF) to post malolactic fermentation (MLF) than from MLF to bottling, even when tannin additions were made after malolactic fermentation was complete. This suggests that in the hybrid red cultivars studied, later additions of exogenous tannin may increase condensed tannin retention to levels comparable to that in *V. vinifera*.

2.2 Introduction

V. vinifera grapevines perform best in warm and dry weather, with cool winters averaging at or above freezing temperatures (Cavalieri et al. 2003). In a cool, wet region like the New York Finger Lakes, where average winter temperatures drop below -4°C and average annual precipitation is 92 cm/year, it can be difficult for *V. vinifera* to produce high quality fruit (Mullins et al. 2011). *V. vinifera's* susceptibility to disease and lack of cold hardiness has led to the breeding of interspecific hybrid cultivars that can survive cold winters and ripen fruit in cool climate regions (Reisch et al. 2012).

Although interspecific hybrids have allowed more regions to produce red wines, many hybrid reds have low tannin concentrations (Sun et al. 2011a). The use of standard winemaking practices to improve tannin extraction and content in a wine has been largely unsuccessful (Manns et al. 2013), suggesting that some compound unique to hybrid cultivars prevents tannin extraction, retention, or both. The fact that hybrid products often have a fraction of the tannin found in *V. vinifera* wines may indicate increased binding to cell wall material or higher crude protein concentrations in hybrid cultivars (Springer and Sacks 2014b, Springer et al. 2015). Grape cell walls are mainly composed of polysaccharides (90% by weight), but also contain phenolic compounds and proteins that are stabilized by ionic and covalent linkages (Bautista-Ortín et al. 2015, Nunan et al. 1997). During alcoholic fermentation, these materials can bind to tannins and limit

extractability (Bindon et al. 2010). Since concentrations of compounds with tannin-binding capability vary by cultivar, it seems likely that tannin concentration would vary, too.

Commercial exogenous tannins have become an increasingly popular method of improving tannin concentration, but there is little reported work on the extensive product range available, leaving winemakers with questions about what products to use and when to use them (Hill and Kaine 2007). Exogenous tannins are derived from plant material, and can be organized into two groups: condensed tannins (from grape skins and seeds) and hydrolyzable tannins (from oak or other wood), with condensed playing a larger role in winemaking (Canuti et al. 2012). Condensed tannins originate with monomeric flavan-3-ols of catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin-gallate that form oligomers and polymers. These tannins vary in size, which is expressed as mean degree of polymerization (mDP). In grapes and wine, condensed tannin size ranges from 2-40 mDP (Gerós et al. 2012).

The recommended dosage of exogenous tannin products vary, ranging from 30-500 mg/l. To date, most of these recommendations have been devised for *V. vinifera,* rather than hybrid, cultivars (Scott Laboratories 2013). Although the addition of exogenous tannin products has been shown to increase tannin concentration, a recent study suggested that the maximum addition rate (400 mg/l) of a grape-derived tannin product with 23.4% condensed tannin to three hybrid cultivars had little effect on the final tannin concentration of the wine (Thomas 2013). Addition timing is another factor that has been understudied, especially in hybrid cultivars. One study did examine additions post alcoholic fermentation (PA) and reported increased tannin retention

compared to additions made to the must (Parker et al. 2007). The concern for later additions is the possibility of detrimental sensory characteristics arising from plant extraction artifacts remaining in exogenous tannin products (Harbertson et al. 2012).

The purpose of this study was to further investigate the addition of exogenous tannin in hybrid red cultivars. In particular, we examined the impact of additions made at various times during the winemaking process to determine whether there is an optimal addition time to maximize tannin concentration in the finished wine. Further, this study was performed to follow the loss and gain of tannin throughout the winemaking process and help identify the processing points at which tannin is lost from the wine solution.

2.3 Materials and Methods

Cultivar selection and harvest. The fruit from hybrid cultivars Maréchal Foch and Corot noir, along with *V. vinifera* Cabernet Franc, were obtained from the American Viticulture Area-designated Finger Lakes of New York in 2013. Maréchal Foch was mechanically harvested in Penn Yan, NY, USA on September 20, 2013; Corot noir hand-harvested in Romulus, NY, USA on October 8, 2014, and Cabernet Franc hand harvested in Lansing, NY, USA on October 16, 2014. Approximately 20 kg of each cultivar was crushed and destemmed mechanically (Rossi e Cama, Prospero, Pleasantville, NY) to yield 25 kg of must. Using a 2L graduated cylinder, 1.6 L of must was measured and added to eight 4L polycarbonate containers.

Winemaking. All juice lots received 50 ppm SO_2 (from a 103 g/L solution made by dissolving potassium metabisulfite in water). For all wine lots, *S. cerevisiae* yeast strain GRE (Lalvin, Petaluma, CA, USA) was rehydrated in 10ml water per 1 gram of dehydrated yeast with 0.3g/l of Go-Ferm Protect (Lallemand, Petaluma, CA, USA). After

rehydration, all lots were inoculated at a concentration of 264mg/l. Yeast assimilable nitrogen (YAN) additions were made as necessary using diammonium phosphate (DAP) and Fermaid K (Lallemand) to bring total YAN up to 200 mg/l. Two hundred and fifty grams of sucrose was added to each Corot noir lot to reach 20°Brix. Fermentation took place in an air-conditioned room held at 21°C. After seven days of maceration with twice-daily cap mixing, the wines were pressed in a fine mesh strainer to yield just over 1L of wine, which was transferred into 1L Pyrex media bottles. The pomace was frozen for later analysis. Lactic bacteria strain Alpha (Enoferm, Petaluma, CA, USA) was hydrated in 25°C DI water at a rate of 2.5g/100ml and lightly mixed for 15 minutes. Using that solution, wines were inoculated at a rate of 10 mg/l, resulting in a 400µL addition to each media bottle. All samples were placed in a 16°C cooler for malolactic fermentation (MLF). Organic acids were measured to determine initial malic acid concentration. After two weeks, organic acids were measured to determine if MLF was complete. Maréchal Foch and Corot noir lots finished in two weeks, while all the Cabernet Franc lots took three to four weeks to finish MLF. After MLF was complete, 50 ppm SO_2 was added to each lot. Each wine was then filtered under vacuum with Whatman no. 1 filter paper (GE Healthcare, Little Chalfont, United Kingdom) and a Büchner funnel. After filtering, the wines were placed in a 2°C cooler for cold stabilization. After eight months of aging, the wines were racked, and the lees were centrifuged to remove as much liquid as possible and frozen for later analysis.

Mass balance. To track the progression of tannins during the winemaking process, a mass balance was performed by taking the weight of each lot after every winemaking step, addition made, or sample taken. The mass at each sampling point was multiplied by

the concentration of tannin (mg/L) in the juice/wine to get the total tannin weight. Lees and pomace were also weighed throughout. The amount of tannin lost during specific winemaking stages was subsequently calculated using the total mass of tannin in wine and byproducts (lees and pomace).

Exogenous tannin treatments. From previous studies, an exogenous tannin product was chosen that had 38% condensed tannin, the highest concentration in the products tested, when measured via HPLC. Tannin additions of 800 mg/L were made and tannin analysis samples were taken during the winemaking process (Table 2.1). Exogenous tannin additions were made by thoroughly mixing the tannin product in ten times its weight of wine, then adding the solution to the wine lot. Samples for tannin analysis were taken in 2ml aliquots and frozen in 2.2ml centrifuge tubes for later analysis.

Table 2.1: Exogenous tannin additions during major winemaking processes and sampling schedule for tannin analysis

Tannin addition time	Tannin sample points
Control (no tannin addition) (C)	Must (M)
Must (M+)	Post tannin addition (PT)
Post alcoholic fermentation (AF+)	Post alcoholic fermentation (AF)
Post malolactic fermentation (MLF+)	Post malolactic fermentation (MLF)
	Post cold stabilization (CS)
	eight months of aging (8MA)

*All treatments performed on duplicate fermentations

Percent retention was calculated by subtracting the control (C) tannin from measured tannin at bottling to yield the amount of condensed tannin derived from exogenous tannin additions (T_e). The theoretical concentration of added condensed tannin concentration was 38% of the exogenous tannin addition of 800 mg/l, or 304 mg/l. The

formula ($T_e/304_{mg/l}$)*100 gave the percentage of exogenous tannin remaining in the wine after winemaking was complete.

Analysis. ***Yeast Assimilable Nitrogen.*** A Chemwell 2910 multianalyzer with Software Version 6.3 (Awareness Technology, Palm city, FL, USA) was used to measure YAN levels by enzymatic analyses (Unitech Scientific, Hawaiian Garden, CA, USA). Cabernet Franc YAN levels were below 200mg/l, so 0.25g/kg of Fermaid K was added to each ferment 24hr after yeast inoculation.

Exogenous tannins. Prior to additions, the exogenous tannin product was analyzed for condensed tannin concentration via a modified HPLC solid phase extraction-phloroglucinolysis method (Manns and Mansfield 2012). Samples were also analyzed via Adams-Harbertson Tannin Assay (Harbertson et al. 2002) and methylcellulose precipitable tannin assay (Sarneckis et al. 2006).

Chemical analysis. Residual sugar (RS) levels were checked during fermentation using Clinitest tablets (Bayer, Etobicoke, ON, Canada). Organic acids were measured via HPLC analysis (*Agilent,* Palo Alto, CA), and tannin concentration using modified HPLC analysis method (Manns and Mansfield 2012).

Pomace and lees analysis. A portion (10% by mass) of pomace and all the lees from each lot were freeze-dried and retained for future analysis. One quarter of the dried pomace was ground into a powder using a Geno Grinder (SPEX sample prep, Metuchen, NY, USA) and all the lees was ground into a powder by hand. One half gram of each sample was extracted with 20ml of 70% acetone (Sigma, St. Louis, MO, USA) overnight. The solution was filtered with Whatman no. 1 filter paper (GE Healthcare, Little Chalfont, United Kingdom) and the acetone solvent was evaporated with nitrogen. Water

was added to the sample to reach a final dilution of 20ml, and 1ml of the dilution was measured for condensed tannin via modified HPLC solid phase extraction-phloroglucinolysis (Manns and Mansfield 2012). To determine the mass of tannin in each sample, the tannin concentration was multiplied by the 20ml water solution (.02L) to get the weight of tannin analyzed (mg). Using weight ratios, the total mass of tannin in the lees and pomace was determined.

Statistical analysis: All statistical analysis was performed on JMP statistical software (SAS, Cary, NC, USA), and mean comparisons were completed using Tukey-Kramer HSD.

2.4 Results

Exogenous tannin product. The exogenous tannin product was found to have 387 mg/l (37.99%) condensed tannin when measured via HPLC, 509 mg/l (49.88%) via Adams-Harbertson, and 916 mg/l (88.82%) via the methylcellulose precipitable tannin assay.

Tannin concentration over time. As expected, the tannin content increased immediately after tannin addition in all cultivars, and additions made following MLF resulted in the highest final tannin concentrations among treatments (Figure 2.1). Tannin additions to must had by far the least impact on tannin concentration, but later additions showed increasing concentrations in the final wine.

This was especially evident in the low tannin cultivar Maréchal Foch, where tannin concentration in M+ was 143% higher than C after eight months of aging; at the same sample point, AF+ increased tannin concentration by 245% and MLF+ showed an even larger increase (567%) (Figure 2.1). Corot noir had higher overall tannin concentrations than the Maréchal Foch but retained less tannin with each addition.

Tannin concentrations increased 109% in M+, 239% in AF+, and 291% in MLF+ when sampled after eight months of aging (Figure 2.1). In Cabernet Franc, a lab error resulted in the loss of the in the MLF+ treatment, so the wine tannin concentration measurement of the control at MLF was used as a reasonable estimate. Cabernet Franc M+ increased 149% over C at 8MA, AF+ had an increase of 274%, while the MLF+ resulted a steady increase of 374% (Figure 2.1).

Percent tannin retention. Percentage of tannin retained from exogenous tannin additions, like tannin concentration, increased with later addition time (Figure 2.2). Maréchal Foch tannin retention was below 26% for M+ and AF+, while the latest addition (MLF+) retained over twice as much (Figure 2.2). Similarly, Corot noir had 27% tannin retention for M+, while AF+ and MLF+ retention was above 60% (Figure 2.2). Tannin retention in Cabernet Franc continually increased with later tannin additions, with MLF addition retaining 80% of the tannin (Figure 2.2).

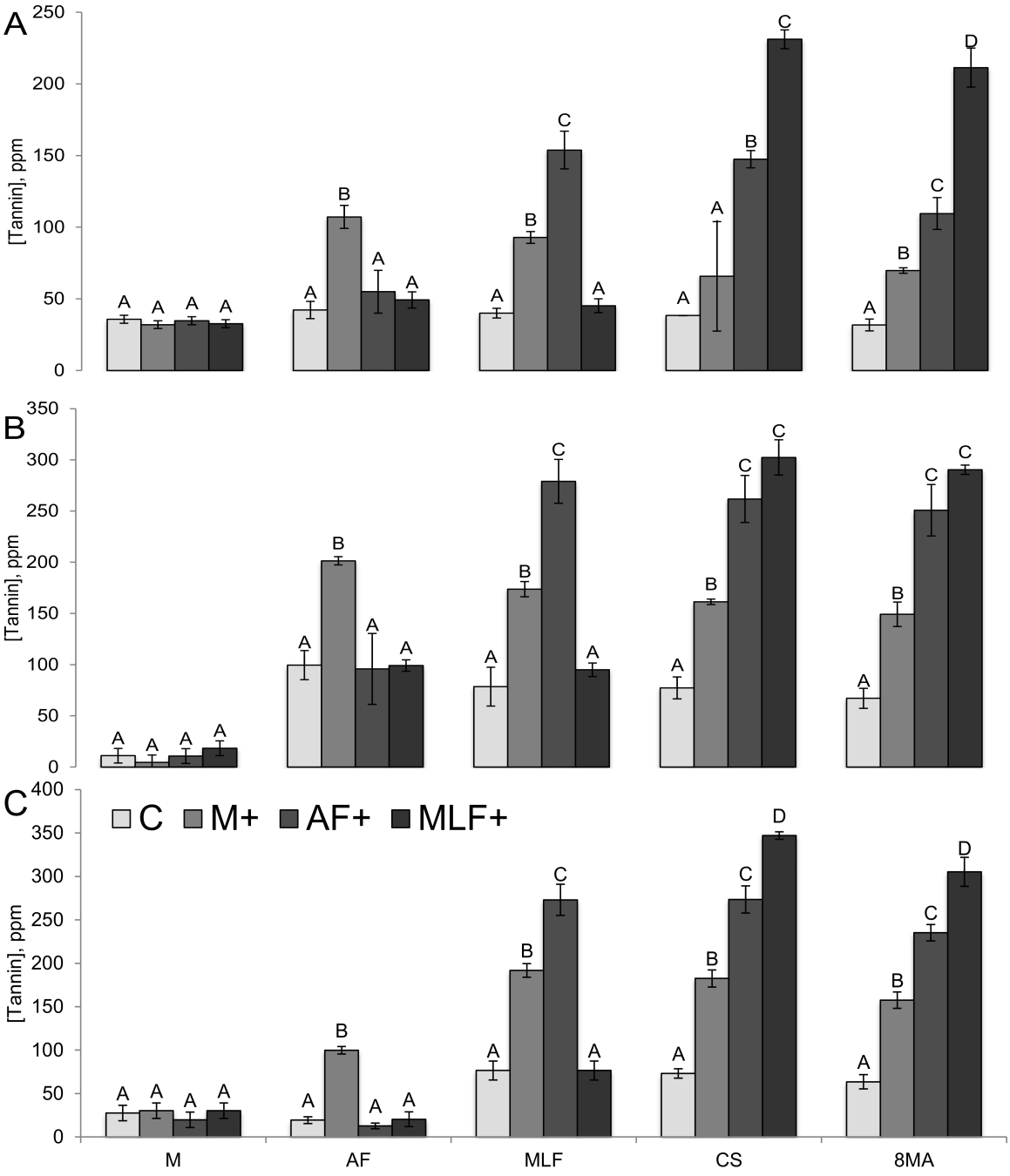

Figure 2.1: Tannin concentration during major winemaking stages in (A) Maréchal Foch, (B) Corot noir, and (C) Cabernet Franc. Tannin additions of 800 mg/l made at must (M+), post alcoholic fermentation (AF+), or post malolactic fermentation (MLF+). A control (C) of no tannin addition was done as well. Tannin analysis sampling points performed via: HPLC solid phase extraction-phloroglucinolysis (Manns and Mansfield 2012) were: must (M), Post alcoholic fermentation (AF), post malolactic fermentation (MLF), post cold stabilization (CS), and eight months of aging (8MA). Significant at $p < 0.01$, within each processing stage.

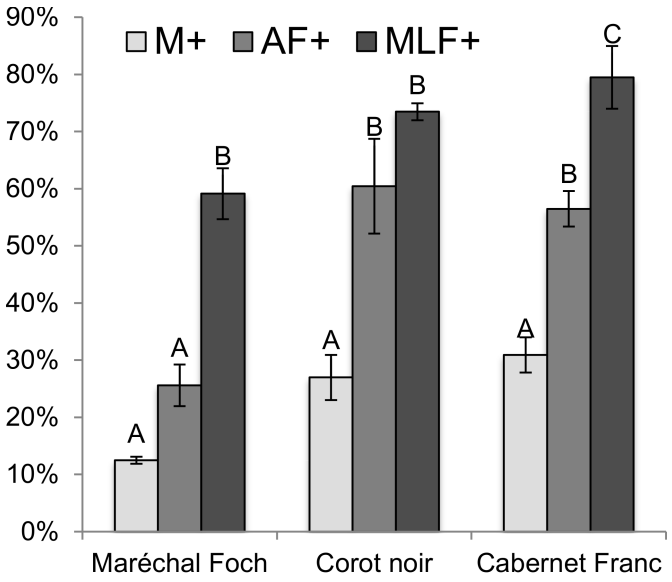

Figure 2.2: Percentage of exogenous tannin retention at bottling. Tannin additions of 800 mg/l made at must (M+), post alcoholic fermentation (AF+), or post malolactic fermentation (MLF+). Significant at $p < 0.01$, within each cultivar

Mass balance. A large portion of the exogenous tannin additions were lost either early in the winemaking process or quickly after the tannin addition was made, and decreased only slightly after that (Table 2.2-4). Since mass balance was monitored by weight, the M and PT at must values may be overestimated (9-64 mg/l for the must and 474-843 mg/l for PT at must), as the skin and seeds add to the mass, resulting in a higher calculated tannin mass. The pomace tannin concentration was excluded from this dataset because calculated tannin concentrations were inconsistent, likely due to the small sample size. Due to these discrepancies, the mass balance is used to compare among specific processing steps, namely AF and MLF+lees (MLF+L), MLF/CS and 8MA+lees (8MA+L).

Table 2.2: Tannin mass (mg) during Maréchal Foch winemaking.

Treatment	Sample	Wine	Lees	PT[1]	Total
Control	Must	64.8	--	--	64.8±7.9
	AF[2]	47.3	--	--	47.3±6.9
	MLF[3] + lees	44.8	27.0	--	71.8±17.1
	CS[4]	41.3	--	--	41.3±0.1
	8MA[5] + lees	34.1	7.2	--	41.3±9.3
M+[6]	Must + PT	57.0	--	637.3	694.3±21.6
	PA	120.0	--	--	120.0±9.1
	MLF + lees	103.6	67.5	--	171.2±15.4
	CS	69.8	--	--	69.8±40.1
	8MA + lees	74.2	5.6	--	79.7±4.4
AF+[7]	Must	60.1	--	--	60.1±2.1
	PA + PT	61.5	--	159.5	221.0±23.5
	MLF + lees	170.6	98.0	--	268.5±13.6
	CS	159.2	--	--	159.2±0.2
	8MA + lees	118.5	9.6	--	128.1±16.8
MLF+[8]	Must	57.4	--	--	57.4±3.9
	PA	55.3	--	--	55.3±6.1
	MLF + lees	50.5	35.7	--	86.2±5.2
	MLF + PT	50.5	--	217.9	268.4±6.1
	CS	246.0	--	--	246.0±4.5
	8MA + lees	224.9	33.3	--	258.2±15.9

[1]Post tannin addition
[2]Post alcoholic fermentation
[3]Post malolactic fermentation
[4]Post cold stabilization
[5]Eight months aging
[6]Must addition
[7]Post alcoholic fermentation addition
[8]Post malolactic fermentation addition

Table 2.3: Tannin mass (mg) during Corot noir winemaking.

Treatment	Sample	Wine	Lees	PT[1]	Total
Control	Must	19.5	--	--	19.5±17.4
	AF[2]	112.8	--	--	112.8±16.0
	MLF[3] + lees	88.8	36.4	--	125.2±21.7
	CS[4]	82.7	--	--	82.7±9.5
	8MA[5] + lees	71.9	2.1	--	74.0±8.7
M+[6]	Must + PT	7.9	--	843.3	851.3±32.3
	PA	227.5	--	--	227.5±6.1
	MLF + lees	196.1	55.5	--	251.6±13.0
	CS	175.0	--	--	175.0±2.2
	8MA + lees	161.6	4.4	--	166.1±13.6
AF+[7]	Must	18.6	--	--	18.6±14.3
	PA + PT	108.6	--	246.1	354.7±39.4
	MLF + lees	314.0	61.3	--	375.3±24.9
	CS	283.7	--	--	283.7±25.7
	8MA + lees	271.8	13.5	--	285.3±28.1
MLF+[8]	Must	32.2	--	--	32.2±0.1
	PA	111.8	--	--	111.8±6.3
	MLF + lees	107.0	40.4	--	147.4±7.5
	MLF + PT	107.0	--	269.8	376.9±26.0
	CS	327.7	--	--	327.7±17.7
	8MA + lees	314.7	21.5	--	336.2±5.2

[1]Post tannin addition
[2]Post alcoholic fermentation
[3]Post malolactic fermentation
[4]Post cold stabilization
[5]Eight months aging
[6]Must addition
[7]Post alcoholic fermentation addition
[8]Post malolactic fermentation addition

Table 2.4: Tannin mass (mg) during Cabernet Franc winemaking.

Treatment	Sample	Wine	Lees	PT[1]	Total
Control	Must	48.8	--	--	48.8±2.6
	AF[2]	21.8	--	--	21.8±4.6
	MLF[3] + lees	85.8	64.7	--	150.5±12.8
	CS[4]	78.9	--	--	78.9±6.4
	8MA[5] + lees	68.5	0.9	--	69.4±9.5
M+[6]	Must + PT	53.1	--	474.1	527.2±59.7
	PA	112.6	--	--	112.6±5.2
	MLF + lees	215.5	97.6	--	313.1±12.9
	CS	196.9	--	--	196.9±10.1
	8MA + lees	169.8	2.5	--	172.3±9.7
AF+[7]	Must	34.9	--	--	34.9±35.2
	PA + PT	14.6	--	303.6	318.3±6.1
	MLF + lees	306.2	88.4	--	394.6±24.0
	CS	295.2	--	--	295.2±17.8
	8MA + lees	253.9	7.2	--	261.1±11.2
MLF+[8]	Must	53.7	--	--	53.7±0.1
	PA	23.1	--	--	23.1±9.5
	MLF + lees	--*	52.7	--	138.5±13.2
	MLF + PT	--*	--	284.1	370.0±16.6
	CS	376.7	--	--	376.7±2.8
	8MA + lees	331.3	10.5	--	341.8±

[1]Post tannin addition
[2]Post alcoholic fermentation
[3]Post malolactic fermentation
[4]Post cold stabilization
[5]Eight months aging
[6]Must addition
[7]Post alcoholic fermentation addition
[8]Post malolactic fermentation addition
* This sample was lost due to lab error, so tannin concentrations from C were used as an estimate for this time point.

2.5 Discussion

Exogenous tannin product. Exogenous tannin additions play an important role in red hybrid winemaking, but it is important to understand how much tannin is in the commercial product being used and what rate should be used for each specific cultivar. Previous research showed that exogenous tannin products contained 12-48% tannin when measured via the Adams-Harbertson tannin assay, and that manufacturer-recommended addition rates had little impact on wine tannin (Harbertson et al. 2012). In contrast, this study used a product with 38% tannin concentration at a higher addition rate, and tannin concentrations increased in every treatment.

Tannin concentration over time. Low extractability makes it difficult to achieve desired tannin content in Maréchal Foch (Springer and Sacks 2014b, Sun et al. 2011b), so improving exogenous tannin effectiveness is important for improving wine quality. The relatively high proportion of *V. riparia* and *V. rupestris* in Maréchal Foch parentage (Jackson 2014) may be responsible for the cultivar's low extractability, as *V. riparia* wines, in particular, have been found to have extremely high protein content (Springer et al. 2015), and likely higher tannin binding capability. Maréchal Foch is also known as an early ripening grape that can be overcropped (Sun et al. 2011b), both conditions which could result in lower indigenous tannin. Tannin concentrations in Maréchal Foch were lower than other cultivars (Figure 2.1), due both to low indigenous wine tannin (Manns et al. 2013, Sun et al. 2011b) and potentially to the tannin-binding capacity that effects low extractability.

Corot noir, which typically has low indigenous tannin (Sun et al. 2011a), was found to have more than twice as much indigenous tannin as Maréchal Foch and

Cabernet Franc at 8MA (Figure 2.1). The higher tannin content is hard to understand given Corot noir's extremely diverse ancestry, but it is less *V. riparia* based than Maréchal Foch (Reisch et al. 2006). Tannin concentrations increased from M+ to AF+ and only slightly from AF+ to MLF+ when sampled after eight months of aging (Figure 2.1). This indicates that for this cultivar, later additions beyond AF may not enhance tannin retention as much as earlier additions. The higher indigenous tannin content resulted in Corot noir having tannin concentrations similar to Cabernet Franc rather than Maréchal Foch, the other hybrid cultivar.

Cabernet Franc typically contains high tannin concentrations (Brossaud et al. 1999) in the berry, and the difference between Cabernet Franc and hybrid cultivars can be even higher in finished wine (Springer and Sacks 2014b). Given previous research, the tannin concentration in Cabernet Franc was expected to be the highest of the test cultivars, and the majority of exogenous tannin added was expected to stay in the wine, especially at or after AF. The C wines were actually very similar to the Corot noir samples, which may have been due to fruit quality, as the Cabernet Franc was picked early due to extensive botrytis infection, which can result in lower tannin extractability (Bindon et al. 2014). The low tannin concentration at PA, and subsequent increase at MLF, was likely due to oxidized samples, as all the PA samples yellowed visibly in storage. The later additions increased final tannin content, following a similar trend as the other cultivars.

Despite some variation among cultivars, the common trend suggested that the later the tannin addition, the higher the tannin concentration at bottling (Figure 2.1). Even with these additions and increases, tannin content in all the finished wines was below the

V. vinifera average tannin content of 544 mg/l observed by Harbertson et al. (2008) in a survey of major world growing regions. Although the tannin content in this study is lower than in warmer growing regions, when compared to tannin content in cool climate regions (Manns et al. 2013, Sun et al. 2011a, b) the exogenous tannin additions had a large impact on final tannin concentration.

When comparing *V. vinifera* Cabernet Franc to hybrid cultivars, early additions resulted in the highest increase in tannin (Figure 2.1). On a whole, the later the addition resulted in less of a difference among the different cultivars. Cabernet Franc and Corot noir had similar tannin concentrations at 8MA for all addition times, while Maréchal Foch was lower (Figure 2.1). Previous research has shown that actual wine tannin measured among the three cultivars showed Corot noir falling between Maréchal Foch and Cabernet Franc (Springer and Sacks 2014b). With normal tannin concentrations for the hybrid cultivars and a lower than previously measured tannin content for Cabernet Franc, similar tannin concentrations in Corot noir and Cabernet Franc is reasonable. Also, when purified grape seed tannin was added to interspecific hybrid wines including Maréchal Foch, 56% (on average) of the tannin was lost, while only 34% of tannin was lost from a collection of *V. vinifera* wines that included Cabernet Franc (Springer and Sacks 2014a). The difference between Cabernet Franc and Maréchal Foch was similar to that observed in this study.

Tannin retention. Because Maréchal Foch has the lowest indigenous tannin concentration, any increase from exogenous tannin additions is important to wine quality. Additions made early in the winemaking process yielded a low tannin retention percentage (Figure 2.2). The finished wine from the MLF+ treatment had over 4-fold

more tannin than the control, and tannin retention was comparable to the other cultivars. This suggests that exogenous tannin additions made later in the winemaking process in Maréchal Foch can overcome binding material enough to increase tannin concentration and retention. One study showed that the size of cell wall material has a significant effect on tannin adsorption, with smaller particle size resulting in more tannin falling out of solution (Nelson 2011). If cell wall material is breaking down more in hybrid than *V. vinifera* cultivars during AF, there could be more and smaller cell wall material present after AF. While skin breakage was not monitored in this study, visual observation suggested that Maréchal Foch skins were often broken down into smaller fragments at AF, while Corot noir and Cabernet Franc typically had whole intact skins. This could explain the low tannin retention in AF+ treatments in Maréchal Foch as more skin breakdown resulted in smaller particles and more exposed cell wall material.

Tannin retention was more than twice as high in Corot noir's AF+ treatment than in Maréchal Foch. Even though these cultivars have similar tannin extractability (5.7% for Maréchal Foch and 6.6% for Corot noir) (Springer and Sacks 2014b), there is likely less cell wall material and protein in Corot noir wines following alcoholic fermentation (with the skins staying intact), leading to less tannin bound and higher total tannin concentration at bottling. The MLF+ resulted in only a small increase over the AF+ (13%) suggesting that waiting to make a tannin addition later than AF may not result in a higher final tannin concentration in Corot noir wines.

Cabernet Franc tannin retention increased with later additions (AF+ was 25% higher than M+, and MLF+ was 24% than AF+), suggesting that even a *V. vinifera*

cultivar like Cabernet Franc can benefit from later exogenous tannin additions to help overcome binding issues that may occur early in the winemaking process.

Muscat Bailey, a hybrid cultivar with *V. labrusca* ancestry, was found have tannin concentrations significantly lower than in *V. vinifera* cultivars Cabernet Sauvignon, Merlot, and Zweigeltrebe (Ichikawa et al. 2011). This mirrors results found in the current study, where the French American hybrid Maréchal Foch had the lowest tannin retention, and the Neo-American hybrid Corot noir had much higher tannin retention, with concentrations more like the *V. vinifera* Cabernet Franc.

Mass balance. Understanding when and where tannin is lost during the winemaking process can be important for understanding how to optimize retention from exogenous tannin additions. The mass balance helps show the amount of tannin that was gained and lost in the different cultivars (Table 2.2-4). In MLF lees, Maréchal Foch lost more tannin from AF+, even though the PT increase at PA was similar for all cultivars. When broken up into processing steps, the mass balance gives a better picture of where tannin is lost. Within each cultivar there is more tannin in the lees at the MLF sample point then after eight months aging, even in treatments where exogenous tannin was added after MLF. It may be possible that the tannin went from large quantities of phenolic compounds towards homogeneous polymerization until precipitation occurred and tannin fell out of solution (Gómez-Plaza et al. 2004). The tannin content could have also changed as polymeric tannins underwent spontaneous cleavage of interflavanic bonds (Vidal et al. 2002), resulting in less measurable tannin in the lees after aging. This decrease in tannin concentration indicates that tannin additions made later in the winemaking process can

result in higher retention percentages, as there is less time for homogenous polymerization to occur before the wines are bottled.

Percent recovery of total tannin content averaged 75%, compared to 93.5%-100.8% in earlier mass balance studies (Nelson 2011, Schultz 2009). Tannin recovery in this work was potentially hampered by wine matrix changes caused by adding exogenous tannins at different winemaking stages. Further, a full understanding of skin-to-seed ratio is required to accurately measure pomace tannin. Because hybrid cultivar grape skins break down more rapidly than *V. vinifera* during fermentation, measuring pomace tannin can be difficult, requiring steps beyond the scope of this work. Further, tannin fractions bound to cell wall material, which may be a source of unrecovered tannin, were not measured in this analysis as in previous studies (Nelson 2011, Schultz 2009).

2.6 Conclusion

Inadequate tannin concentration is a concern for producers of red hybrid wines, but exogenous tannin products provide the potential to increase final tannin content. Tannin concentration does increase from exogenous tannin additions made both above the manufacturer's recommendation and later in the winemaking process, especially if additions are made after alcoholic fermentation. Tracking changes in tannin concentration in three cool climate wine grape cultivars suggested that more tannin is lost in lees removed following malolactic fermentation than in lees recovered after eight months of aging. This gives an indication that all wines contained less binding material later in the winemaking process, yielding a higher retention of later tannin additions.

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CHAPTER 3

ADDITION RATE OF EXOGENOUS TANNIN FOR OPTIMAL RETENTION IN HYBRID RED WINES

3.1 Abstract

Winemakers often add exogenous products to increase tannin content in red hybrid wines, but the recommend dosage of 50-400 mg/L may not effectively increase condensed tannins. In 2013, wines were made from Maréchal Foch, Corot noir, and Cabernet Franc to compare the retention of exogenous tannins in interspecific hybrids and *Vitis vinifera*. After analyzing 12 commercial tannin products for condensed tannin concentration via HPLC, the highest, with a concentration 38%, was added at a rate of 400, 800, and 1200 mg/L after crush/before yeast inoculation. A separate lot of each cultivar was also pressed off the skins immediately, fermented with 1600 mg/L of exogenous tannin, then back-blended post-fermentation with a control wine for a final theoretical concentration of 400 mg/L exogenous tannin product. At bottling, tannin concentrations in all treatments were higher than their respective controls. Tannin retention rates varied by cultivar, ranging from 19-24% in Maréchal Foch wines, 25-43% in Corot noir, and 34-48% in Cabernet Franc. The back-blended wines had similar tannin retention rates to those with 400 mg/L additions in Corot noir and Cabernet Franc, but higher tannin retention in Maréchal Foch (75 mg/L with a 400 mg/L addition and 93 mg/L in the back-blended wines). Though concentrations of condensed tannins were higher in all treatment wines, none exceeded 50% retention. This suggests that high concentration additions of exogenous tannin increase the condensed tannins in hybrid red wines, but retention rates vary by cultivar.

3.2 Introduction

Cool climate winemaking has become more popular in the US within the last decade. In 2007, cool climate states accounted for 7.51% of the USA's bottled wine production; in 2014 that number jumped to 13% (TTB, 2010, 2015). This increase belies regional challenges, however, as cool climates show lower fruit yields per acre and higher costs of production (Doloreux et al. 2015). The Finger Lakes AVA has a cool, humid continental climate with precipitation averaging 92 cm/yr (Mullins et al. 2011), about 60% of which falls between April and October. Along with humidity and precipitation, the AVA averages only 2,200-2,300 degree days (Newman 1986). This increases the difficulty of growing *V. vinifera* grapevines that originate from a Mediterranean climate (Cavalieri et al. 2003) and perform best in warm and dry weather. Due to the challenging growing conditions, breeding programs have been producing disease-resistant and cold tolerant hybrid cultivars that can perform well in cool and wet regions (Reisch et al. 2012).

In red winemaking, a common concern is low polyphenol content, and especially tannin concentration, with one cool climate study reporting tannin content (Tarko et al. 2010) $1/5^{th}$ of that measured in a warmer climate (Harbertson et al. 2008). Red hybrid cultivars also have inherently lower tannin concentrations, ranging from 25 to 125 mg/l, with the exception of Noiret, which averages 354 mg/l (Springer and Sacks 2014). In addition to low tannin concentrations, hybrid cultivars also have tannins with lower mean degree of polymerization (mDP). Mean degree of polymerization is the tannin polymer subunit average, which is generally around 32 mDP in skin tannins and 10 mDP in seed tannins in *V. vinifera* cultivars (Harbertson et al. 2008, Thomas 2013). For hybrid

cultivars the mDP can much lower, with a 2011 study reporting mDP range of 2.26-3.21 for three hybrid cultivars wines studied (Manns et al. 2013).

Processing methods traditionally used to improve tannin concentration, like cold soaking and hot pressing, have shown little to no effect on tannin concentration in red hybrid wines (Manns et al. 2013). The addition of commercial enological tannins has been found to improve tannin concentration in finished wines (Bautista-Ortín et al. 2007), but these additions made little sensory impact on wines unless large additions were made late in the winemaking process (Harbertson et al. 2012). Commercial tannin products are marketed for all facets of the winemaking process, but typically those intended to increase retention are sold as fermentation tannins, and are recommended for additions made at crush or during alcoholic fermentation (Scott Laboratories 2013).

Tannin binding is an important consideration in hybrid red cultivars, as it can remove both indigenous and added tannin from solution. Tannin binding can relate to a number of measurements, including crude protein, and protein both within the flesh as well as the skin. One means of calculating tannin binding within a specific cultivar is to assess the distribution coefficient of free vs. bound tannin (**K**[free tannin]/[bound tannin]). This coefficient can be determined by adding an exogenous tannin with a known tannin concentration and measuring how much of the exogenous tannin binds to the cell wall material of grape skins or flesh. The lower the coefficient is, the more tannin is bound to the skin or flesh. In a recent study, French-American hybrid cultivars were found to have an average binding coefficient of 8.1, the neo-American hybrid cultivars had an average of 13.2, and the *V. vinifera* cultivars had an average of 22.1 (Springer and Sacks 2014).

Tannin binding can also relate to grape cell wall material. Cell wall material contains mostly polysaccharides (90% by weight), of which cellulose and pectin account for 30-40% (Bautista-Ortín et al. 2015). Cell walls also contain phenolic compounds and proteins that play important roles in winemaking (Nunan et al. 1997). Tannin has the ability to bind to this cell wall material, limiting the extractability of tannin into the wine during maceration (Bindon et al. 2010).

Manufacturers' recommended dosage of exogenous tannin is typically between 5-400 mg/l for *V. vinifera* cultivars, with some manufacturers including a recommendation for red non-*V. vinifera* of up to 600 ppm (Scott Laboratories 2013). A recent study examining cool-climate hybrids and tannin additions made at the top of the manufacturer's recommended dosage of 400 mg/l (specified for the product) showed only slight increases in final wine tannin concentrations (Thomas 2013). Parker (2007) found that adding grape seed-derived exogenous tannins at a rate of 200 mg/l (measured at 40% tannin) prior to fermentation in Shiraz had no impact on the finished wine. Keulder (2006) found that the addition to must of exogenous tannins much higher than the manufacturer's recommendation (1000 mg/l) resulted in higher tannin concentration than the control, but made no difference in other, lower additions. In both studies, ineffective tannin additions were at or below 500 mg/l. The composition of the tannin product used was not reported in these studies.

Commercial exogenous tannins are natural products extracted from various botanical sources (Versari et al. 2013) and are separated into two groups: hydrolyzable tannins, which are absent in grapes but which arise in traditional wine processing from oak aging, and condensed tannins, which are abundant in grape seeds and skins (Moreno-

Arribas and Polo 2009). Since grape tannins are the most common and prevalent in wine, this research focused on commercial condensed tannin products. Content of exogenous tannin products is not regulated, but manufacturers typically provide botanical source and recommended addition time, though not tannin percentage. One study of various exogenous tannin products on the market found the tannin content to range from 12-48% (Harbertson et al. 2012).

In an effort to optimize tannin retention in hybrid red wines, this study examined the impact grape-derived condensed tannin additions at levels well above those recommended in manufacturer literature. To that end, various tannin additions were made to two hybrid red wine cultivars, Maréchal Foch and Corot noir, and one comparison *V. vinifera,* Cabernet Franc. In addition, to test the hypothesis that higher tannin retention could be achieved if grape solids are not present, tannin additions were also made to a juice fraction of each cultivar and back blended with a traditionally-produced red wine.

3.3 Materials and Methods

Exogenous tannin. Eleven different exogenous tannin products, derived from either grape skins or seeds, were obtained commercially. Each product was added to water to make a 1000 mg/l solution and analyzed for tannin concentration via HPLC solid phase extraction-phloroglucinolysis (Manns and Mansfield 2012), Adams-Harbertson tannin assay (Harbertson et al. 2002), and the methylcellulose precipitable tannin assay (Sarneckis et al. 2006). Solubility was tested by adding 800 mg/l of the powdered product to a solution of water, 200 g/l sugar (50:50 glucose and fructose), and 10% ethanol which was mixed for ten minutes and analyzed for tannin via HPLC (Manns and Mansfield

2012). Product stability was measured by adding 800 mg/l of exogenous tannin to model wine (3 g/l of tartaric acid, 1 g/l of malic acid, 12% ethanol, and adjusted to pH 3.5 with sodium bicarbonate) and model juice (3 g/l of tartaric acid, 1 g/l of malic acid, 100 g/l glucose, 100 g/l fructose, and pH 3.5 with sodium bicarbonate), then mixing for ten minutes. The solutions were then held at room temperature in darkness without further mixing, and samples were taken at addition, after one day, and 13 days, and were analyzed for tannin concentration via HPLC (Manns and Mansfield 2012).

Cultivar selection and harvest: Fruit from Maréchal Foch, Corot noir, and Cabernet Franc was obtained from various vineyards within the Finger Lakes AVA. Maréchal Foch was mechanically harvested in Penn Yan, NY, USA on September 20, 2013; Corot noir hand-harvested from Romulus, NY, USA on October 8, 2014, and Cabernet Franc hand harvested from Lansing, NY, USA on October 16, 2014.

Winemaking. The fruit was crushed, divided equally into three 300kg lots, and placed into 114 L containers. The containers were mixed, and 42 kg of must was taken and pressed using a hydraulic press and press cloth, yielding 24 kg of pressed juice to be used for fermentations without skin contact. The juice was evenly distributed into two five-18.9l carboys and 25 ppm SO_2 was added.

With the remaining fruit, 7 kg of must was taken from each container and added to 10, 28 L stainless steel pots, totaling to 21 kg of much for each lot. 50 ppm SO_2 was added, and all fermentations were performed in duplicate.

For all wine lots, *S. cerevisiae* yeast strain GRE (Lavin) was rehydrated in 10ml water per 1g dehydrated yeast with 0.3g/l of Go-Ferm Protect (Lallemand, Petaluma, CA, USA). After rehydration, all lots were inoculated at a concentration of 264mg/l. Cabernet

Franc YAN levels were below 200mg/l, so 0.25g/kg of Fermaid K (Lallemand) was added to each ferment 24hr after yeast inoculation. In Corot noir, 250g of sucrose was added to reach 20°Brix. Maréchal Foch and Cabernet Franc had soluble solids of 23.9 and 21.2°Brix, respectively.

Fermentation took place in a temperature-controlled cooler held at 21°C. Caps were punched down twice daily over 7 days of maceration, at which point all wine lots were at or below 0.6% residual sugar. Due to the small volume, wines were pressed by hand using a 7.6l mesh container with a press cloth and cheesecloth. Free run juice was collected for roughly ten minutes; then the must was pressed until 11.4l of wine was recovered and transferred to carboys. Lactic acid bacteria strain Alpha (Enoferm, Petaluma, CA, USA) was hydrated in 25°C DI water at a rate of 2.5g/100ml and lightly mixed for 15 minutes, then added to all wines at a rate of 10mg/l. All wines were placed in a 16°C cooler for malolactic fermentation (MLF), which was completed in two weeks in Maréchal Foch and Corot noir and three to four weeks in Cabernet Franc. After MLF was complete (as determined by organic acid concentration), 50 ppm SO_2 was added, and all lots were placed in a 2°C cooler for cold stabilization and storage. At bottling, SO_2 concentration was adjusted to 30ppm as necessary and racked into standard 750mL glass bottles (Waterloo Container, Waterloo, NY, USA) with screw-top closures (Scott Labs, Petaluma, CA, USA). Wines were stored at 21°C until needed for further analysis.

Treatments. The exogenous tannin product that had the highest measured tannin was used for a series of additions. Rates for additions made to the must were: Control (C) with no tannin added, 400 mg/l (400), 800 mg/l (800), and 1200 mg/l (1200). Tannin additions of 1600 mg/l were made to pressed juice, which was ultimately combined in a

blend of 75% control wine and 25% juice fermentations to produce a final theoretical addition of 400 mg/l of the tannin product (Blend). This trial was designed to determine whether tannin additions made in a wine matrix without skin contact would impact tannin retention. Additions were made by thoroughly mixing the tannin product in ten times its weight of juice, then adding the solution to the whole lot.

Sampling protocol. All wines were sampled at must (M), post tannin addition (T), post alcoholic fermentation (AF), post malolactic fermentation (MLF), post cold stabilization (CS), and at bottling (BT). Samples for tannin analysis were taken in 2 ml aliquots and frozen in 2.2ml centrifuge tubes until needed.

Analysis. ***Yeast Assimilable Nitrogen.*** A Chemwell 2910 multianalyzer with Software Version 6.3 (Awareness Technology, Palm city, FL, USA) was used to measure YAN levels by enzymatic analyses (Unitech Scientific, Hawaiian Garden, CA, USA).

Chemical analysis. Residual sugar (RS) levels were checked during fermentation using Clinitest tablets (Bayer, Etobicoke, ON, Canada). Organic acids were measured using the chromatographic conditions in AOAC method 986.13 via HPLC analysis (*Agilent,* Palo Alto, CA) (AOAC 2000). Tannin concentration was determined via HPLC (Manns and Mansfield 2012).

Sensory analysis. Sensory analysis was performed in individual sensory booths with white light. Panelists were given 30ml wine samples in ISO tasting glasses (covered with plastic lids) which were labeled with 3-digit codes and presented in an order randomized using a Latin Square.

To evaluate tannin concentration, 33 panelists (16 female & 17 male, with an average age of 40yrs and an age range of 21-71yrs) used free choice sorting with multi-

dimensional scale analysis (MDS) (Tang et al. 2002). Each panelist came to three sessions, one per cultivar. The four treatments (control, 400, 800, and 1200 mg/l) with fermentation duplicates were provided in a flight of eight wines. Panelists used their own criteria to group similar wines together in one to four groups. Wines grouped together were assigned a 1, and wines in different groups received a 0.

To determine whether the blended wine differed from the 400mg/L must addition, a triangle test was performed with a group of 30 panelists (14 female, 16 male, with an average age of 39yrs and an age range of 26-65yrs).

Statistical analysis. Mean comparison of tannin concentrations was performed using Tukey-Kramer HSD. The triangle test analysis and mean comparison was performed on JMP statistical software (SAS, Cary, NC, USA). The multidimensional scale analysis was performed using the PROC MDS in SAS (SAS, Cary, NC, USA). An R^2 between 0.90 and 1 and badness-of-fit (stress value) between 0 and 0.15 indicate a good fit of the model to the data and significance of the plot (Preszler et al. 2013).

Table 3.1: Tannin content from grape derived enological tannin products measured in duplicate via SPE-P[1], A-H[2], and MCP[3].

Tannin Product	SPE-P [Tannin]	SPE-P Tannin %	A-H [Tannin]	A-H Tannin %	MCP [Tannin]	MCP Tannin %
1	387.5	38.0%	508.8	49.9%	916.2	89.8%
2	358.0	33.5%	483.9	45.2%	812.8	76.0%
3	298.3	29.2%	321.1	31.5%	516.2	50.6%
4	291.4	28.6%	375.3	36.8%	605.9	59.4%
5	239.0	23.4%	277.1	27.2%	347.2	34.0%
6	230.0	21.3%	195.3	18.1%	364.5	33.7%
7	218.1	20.6%	270.9	25.6%	367.9	34.7%
8	197.4	18.8%	408.9	38.9%	654.1	62.3%
9	198.9	18.4%	440.5	40.8%	712.8	66.0%
10	191.9	17.8%	217.4	20.1%	292.1	27.0%
11	179.0	16.9%	128.8	12.1%	195.5	18.4%
12	86.9	8.0%	164.6	15.2%	323.1	29.9%

[1]HPLC solid phase extraction-phloroglucinolysis (SPE-P) (Manns and Mansfield 2012)
[2]Adams-Harbertson Tannin Assay (A-H) (Harbertson et al. 2002)
[3]Methylcellulose precipitable tannin assay (MCP) (Sarneckis et al. 2006)

3.4 Results

Exogenous tannin products: The analysis of commercial enological tannins showed a wide range of tannin concentrations (Table 3.1). Tannin product 1, which is derived from 100% grape seed extract (*V. vinifera*), had the highest tannin concentration in all three analyses and was subsequently used for all tannin additions. The mDP for tannin product 1 was found to be 4.63.

Table 3.2: Indigenous tannin concentration (mg/l)[1] for major processing steps

	Processing Step			
	AF	MLF	CS	BT
Cultivars				
Maréchal Foch	43.8	47.5	54.4	46.0
Corot noir	110.3	101.8	98.9	79.3
Cabernet Franc	45.8	176.3	177.8	142.0

[1]Average from duplicate fermentations

Tannin concentration. With few exceptions, exogenous tannin additions increased condensed tannin concentration across cultivars at all sampling points (Figure 3.1).

Maréchal Foch had the lowest tannin overall, with indigenous concentrations ranging from 43.8-54.4 mg/l (Table 3.2). Additions increased tannin concentrations in all treatments, and tannin content decreased in all lots as the wines aged (Figure 3.1). Tannin concentrations doubled in treatment 400 post AF, tripled in 800, and increased by almost 300% with the 1200 mg/l addition. By bottling, tannin concentrations had decreased by 36-52% in all wines. The mDP of the tannin treatments BT was slightly above the control (Table 3.3).

Indigenous tannin content was higher in Corot noir (Table 3.2). Corot noir also showed a greater increase in tannin concentrations, with the difference among treatments 400 and 800 almost 3X higher at bottling (Figure 3.1). Further, the decrease in tannin concentration was much smaller for 800 and 1200 treatments throughout processing, with reductions of only 14-20% by BT. The 400 treatment had a higher reduction of 48%, which mostly occurred from AF to MLF. The mDP BT was slightly lower in the 400 addition when compared to C (Table 3.3).

Cabernet Franc had the highest tannin concentration throughout, including indigenous tannin (Table 3.2). Control tannins at bottling were almost double that of Corot noir, and 100 mg/l more than Maréchal Foch (Table 3.2). Despite these differences in indigenous concentration, the increase from tannin addition was similar to Corot noir BT for 800 and 1200, while 400 increased tannin concentration more than in either of the hybrid cultivars. The mDP at bottling was the highest as well (Table 3.3).

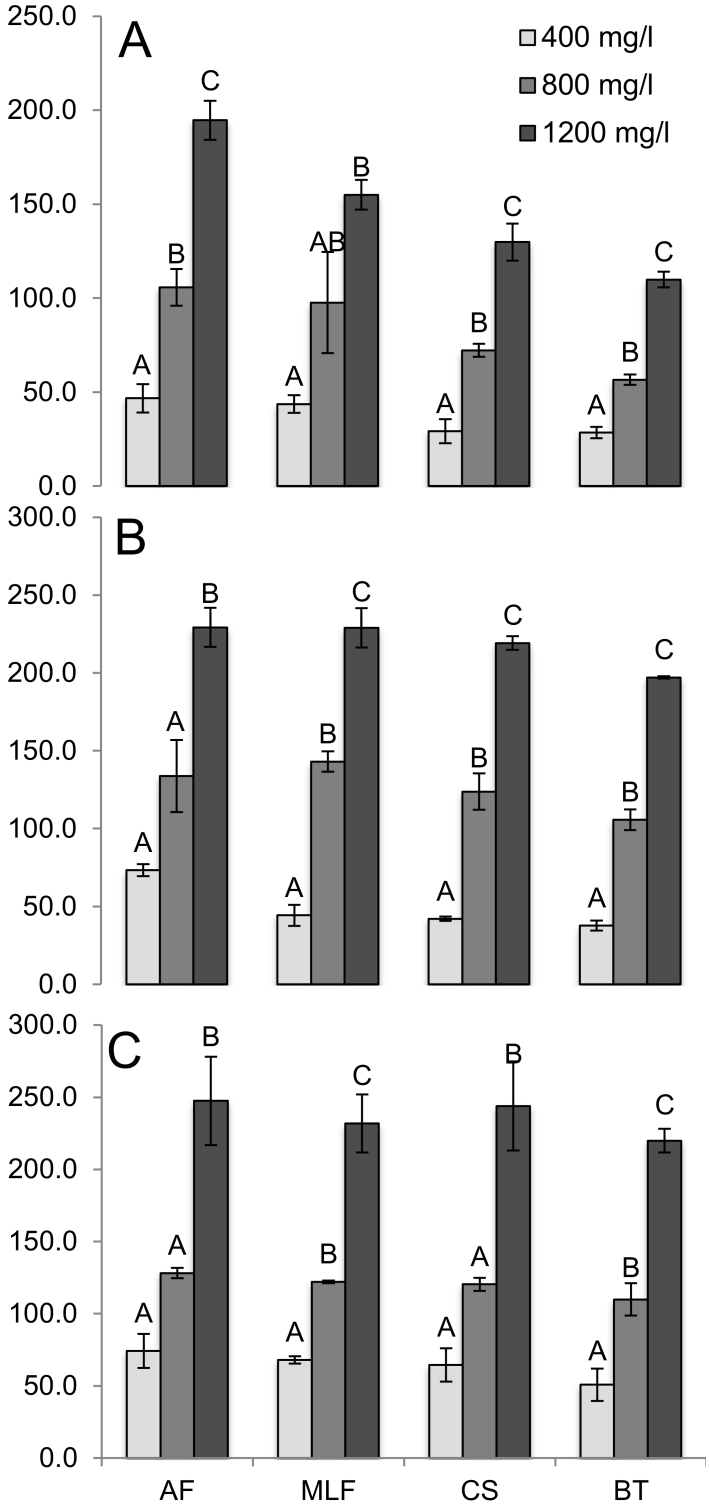

Figure 3.1: Tannin concentration (mg/l) from exogenous tannin (final - control) during major winemaking stages in (A) Maréchal Foch, (B) Corot noir, and (C) Cabernet Franc cultivars. Significant at $p < 0.01$, within each processing stage

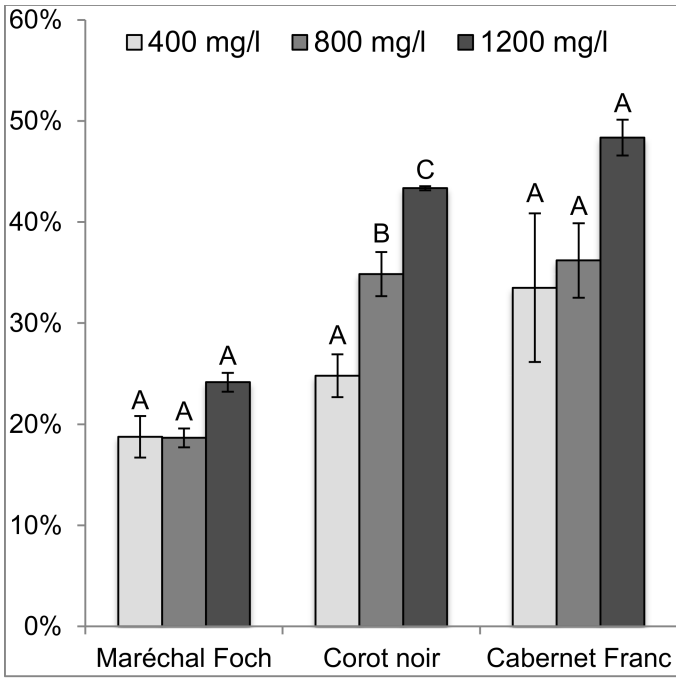

Figure 3.2: Percentage of exogenous tannin remaining in the wine (BT). Significant at p <0.01, within each processing stage

Tannin retention was calculated by subtracting the tannin concentration of the control from that of each tannin treatment at bottling, giving the amount of condensed tannin in the wine that came from the exogenous tannin addition (T_e). The theoretical concentration added was 38% of the weight of the exogenous tannin addition (T_i). Thus, the calculation $(T_e/(T_i*0.38))*100$ gave the percentage of exogenous tannin remaining in the wine. Maréchal Foch, having a low indigenous tannin concentration, showed insignificant retention, as tannin concentration did not increase (Figure 3.2). Additions to Corot noir resulted in a progressive increase in tannin retention, while Cabernet Franc retention was high for all three additions (Figure 3.2).

Table 3.3: Average mDP of cultivars and tannin treatments BT.

	Treatment			
	C	400	800	1200
Cultivars				
Maréchal Foch	2.77a[1]	2.98a	3.23a	3.33a
Corot noir	3.97a	3.75a	4.21a	4.59a
Cabernet Franc	4.23a	4.35a	4.57ab	4.97b

[1]Different letters within a column show difference at $p<0.01$

Sensory evaluation. Multi-dimensional scale analysis charts helped to visualize sensory differences in wines. The closer two treatments are, the more sensorially similar panelists found them to be. Panelists considered all treatments for Maréchal Foch and Cabernet Franc different (Figure 3.3). For Corot noir, treatments 400 and 800 were similar to each other, but different from C and 1200.

Exogenous Tannin: Blended vs. Must Addition. Initially, the Blend treatment resulted in higher tannin concentrations than the 400 treatment, but as the wines aged, these differences decreased (Table 3.4). Maréchal Foch was the only cultivar to show any difference at bottling, although it was relatively small. Cabernet Franc and Corot noir wines showed no difference at bottling, though tannin concentration among Corot noir treatments varied considerably with a standard deviation of 9.2.

Sensory analysis showed that panelists could distinguish between Corot noir treatments (P-value <0.05), but did not indicate a difference in Maréchal Foch and Cabernet Franc treatments (data not shown.) The difference seen in Corot noir may have been due to the large variability among the samples. If the Corot noir replicate with higher tannin concentration was chosen, it may have been easier to notice the difference between that and the 400 treatment.

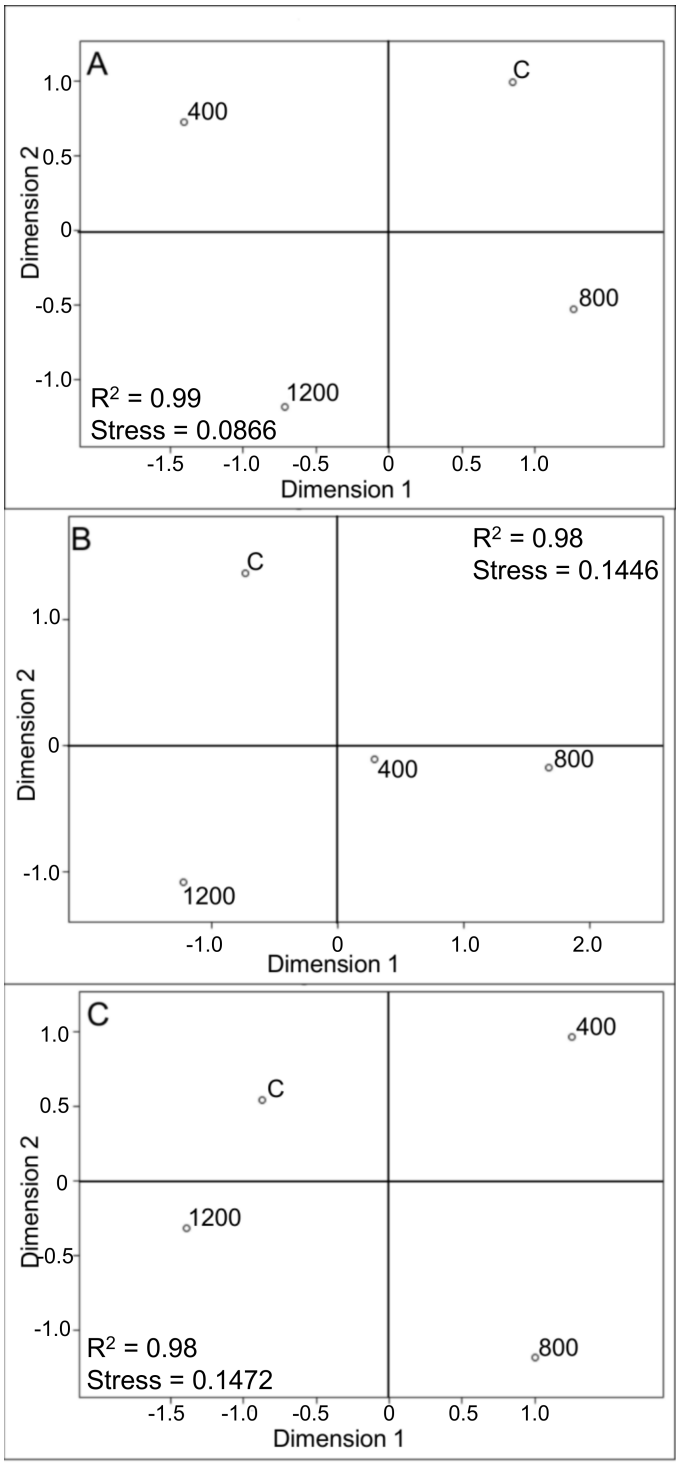

Figure 3.3: Multi-dimensional scale analysis chart for (A) Marèchal Foch, (B) Corot noir, and (C) Cabernet Franc

Table 3.4: Tannin concentration (mg/l) at major winemaking stages for 400 mg/l exogenous tannin added at must and 75% control/25% 1600 mg/l exogenous tannin addition to juice (400 mg/l addition) blend after alcoholic fermentation

Cultivar	Treatment	PA/Post Blend	MLF	CS	BT
Maréchal Foch	400	90.4 a[1]	91.1 a	83.6 a	74.5 a
	Blend	113.7 b	105.7 a	116.6 b	93.4 b
Corot noir	400	183.6 a	146.0 a	140.9 a	116.9 a
	Blend	181.1 b	180.9 b	168.0 b	144.4 a
Cabernet Franc	400	120.1 a	244.2 a	242.3 a	192.8 a
	Blend	140.9 b	234.4 b	234.4 a	193.1 a

[1]Different letters within a column show difference at $p<0.01$

3.5 Discussion

Exogenous tannin products. For winemakers to effectively use exogenous tannins in cool climate winemaking they must know the composition or amount of tannin in the product. The knowledge of tannin percentage helps winemakers make effective decisions on additions. An earlier review of eight different tannin products, both condensed and hydrolyzed (Harbertson et al. 2012) found a concentration range of 193-425 mg/l CE (in a 1000 mg/l solution), or 12-48% tannin (measured via Adams-Harbertson Tannin Assay). This agrees with the range of 129-509 mg/l found in this study, confirming that a large portion of exogenous tannin products is not tannin. The low percentage of tannin could be due to certain thermal processing steps during the extraction and production of exogenous tannins which may cause changes in the tannin concentration (Versari et al. 2013).

A preliminary trial of tannin solubility in different solutions raised the concern that additions in must (with high sugar content) may result in less tannin dissolved (See supplemental data). Harbertson (2013), however, showed that increasing sugar content actually improved solubility of tannin-protein complex, and that in normal wine conditions, sugar concentration would have negligible effects on protein-precipitable tannins. Hernandez-Jimenez (2011) showed

that increased ethanol concentrations improved proanthocyanidin extraction. There are likely small solubility effects when adding exogenous tannins at different winemaking stages, but their impact seems negligible.

Indigenous tannin concentration. The indigenous tannin concentration for Maréchal Foch BT of 46 mg/l (Table 3.2) was in range with previous results of 29-59 mg/l (Sun et al. 2011a). In contrast, Corot noir's indigenous tannin concentration at BT (79.3 mg/l) was slightly higher than previous results of 42-64 mg/l (Sun et al. 2011b), and the Cabernet Franc indigenous tannin at BT (142.0 mg/l) was only slightly lower than the 150-200 mg/l reported in recent work (Springer and Sacks 2014). Because tannin concentrations in grapes vary by year, site, and climate (Preszler et al. 2013, Scheiner et al. 2011), these differences are within an expected range for the region.

Impact of tannin addition on concentration and retention. Exogenous tannins can be lost during a wine's life through precipitation, adsorption to cell wall material, and incomplete solubility (Versari et al. 2013). Such loss is evident in the tannin retention percentages shown at bottling (Figure 3.2). Tannin loss varies by cultivar, and the relative retention rates observed in Maréchal Foch, Corot noir and Cabernet Franc mirror the tannin extractability of these cultivars as defined by Springer and Sacks (2014).

In Maréchal Foch, all additions resulted in increased tannin concentration at AF, but a steady decline occurred throughout the winemaking process (Figure 3.1). This could be an indication that tannin is less stable in Maréchal Foch wines, meaning tannin could still be bound for a longer period after post tannin addition. Maréchal Foch, which has a tannin extractability of (6%), had both lower tannin concentrations and retention than Corot noir or Cabernet Franc (Figures 3.1, 3.2). The flesh of this cultivar is reported to have one of the higher concentrations

of crude protein (119 mg/g), and when exogenous tannin is added to Maréchal Foch cell wall material, a large portion is bound (Springer and Sacks 2014). In a wine matrix, this binding results in precipitation of the tannin-protein agglomerate, reducing tannin concentrations in the wine; any cell wall material remaining in the wines after pressing may contribute to the continued decrease in tannin content. This was evident as the Maréchal Foch skin pomace was observed to be in pieces, as opposed to a whole, intact grape skins. As addition rates increased, the retention percentage stayed the same (Figure 3.2), likely because tannin concentrations had not yet reached a level to quench binding activity. The progressive increase in tannin concentration, however, may indicate that binding mechanisms can ultimately be overcome with increasingly larger additions.

In contrast to Maréchal Foch, tannin concentrations in Corot noir treatments were similar throughout the winemaking process, with only a slight loss from AF through BT (Figure 3.1.) Further, Corot noir was unique in retaining larger portions of exogenous tannin as addition concentration increased (Figure 3.2). This difference suggests that increasing additions were reaching a saturation point, where binding or precipitation decreased. A distribution coefficient of free vs. bound tannin found Corot noir to be between that of Maréchal Foch and Cabernet Franc (Springer and Sacks 2014). Differences in tannin retention could be that there is less cell wall material or protein in the must and/or after AF, resulting less tannin lost to binding. In this study after AF, Maréchal Foch skins had broken down and were in pieces, while the Corot noir and Cabernet Franc skins were mostly intact. This could be an indication of tannin binding as well.

Like Corot noir, Cabernet Franc tannin concentrations stayed similar from AF to CS, and showed a slight loss from CS to BT (Figure 3.1). Cabernet Franc had the highest tannin

concentration (both with indigenous and added tannins) (Table 3.2) as well the highest mDP (Table 3.3) in this study. Cabernet Franc was similar to Maréchal Foch in that the percentage of exogenous tannin in the wine at BT was similar among addition rates, but unlike Maréchal Foch, the retention was high throughout (Figure 3.2). Since the percentage of exogenous tannin that remained in the wine didn't change, it's possible that the wine wasn't affected as much by cell wall binding or precipitation. Crude protein within the grape flesh (98.3 mg/g) was much lower than Maréchal Foch, while the distribution coefficient of free vs. bound tannin is almost twice as high when compared to Corot noir (Springer and Sacks 2014). The higher tannin content in Corot noir compared to Maréchal Foch could be due to less cell wall material and protein content in the wine.

Mean degree of polymerization. The addition of exogenous tannins to hybrid cultivars did not have an effect on mDP, even though there was hope that with the use of *V. vinifera* tannin the mDP would increase. There was likely no increase because the mDP of T1 was 4.63, only slightly above that of the control wines. The low mDP of the product may have resulted from the source (grape seeds) and tannin extraction method. Particularly in Maréchal Foch, the lower tannin retention of exogenous tannins may have caused a smaller increase in mDP as well. Cabernet Franc showed an increase in mDP with higher additions, likely due to the higher retention rates.

Sensory evaluation Results for this study did show that tannin additions between 400-1200 mg/l all had an impact on the sensory properties (Figure 3.3). In a previous study with 200 mg/l additions, both pre- and post-fermentation additions showed minor differences in astringency (Parker et al. 2007). As panelists in this study reported differences in all but one treatment (Figure 3.3), additions above 200 mg/l may be needed to affect tannin related sensory

characteristics. On the other hand, large condensed tannin additions (800 mg/l) after alcoholic fermentation have been reported to result in an increase in earthy flavors and bitterness while lower additions (60-300 mg/l) also resulted in higher ratings of color intensity, red color, and sourness over the control wines (Harbertson et al. 2012). This indicates that even in wines where tannin additions increase tannin concentration slightly, sensory differences observed could have been from characteristics not influencing mouth feel.

Exogenous Tannin: Blended vs. Must Addition. The similarity in tannin concentrations in blended and direct-addition wines is similar to that reported in a previous study comparing a blended wine with 400 mg/l additions made before, after, and during fermentation in hybrid cultivars (Thomas 2013). The difference observed in Maréchal Foch treatments (Table 3.3) may not be relevant if sensory differences are small; though panelists did not report a difference in this trial, the sample size was insufficient to claim that no sensory difference exists. It is notable that the Maréchal Foch treatments retained different tannin concentrations throughout the winemaking process, and that Corot noir was similar to Maréchal Foch, except that variation among treatments in Corot noir resulted in no significant difference at bottling between the blended treatment and the 400 mg/l addition at must treatment. Both Maréchal Foch and Corot noir treatments had large differences which equilibrated over time. This may be because cell wall material or protein was still present in the wine after blending, so tannin was still being actively bound. Cabernet Franc treatments were similar after MLF, indicating that these samples are affected less by cell wall material or protein. The sensory difference panelists reported in the Corot noir may have been due to the variability in tannin concentration among the duplicate fermentations.

3.6 Conclusions

The addition of exogenous tannins at fermentation is a potential method to increase tannin content in hybrid red wines, but cell wall absorption and precipitation may result in the loss of up to 81% of additions made. Winemakers working with red hybrid wines will likely need to make exogenous tannin additions much higher than those recommended by product manufacturers to aid retention. Additions made to juice and blended with traditionally produced red wines show similar tannin retention rates to those made directly to must; as this method is more time consuming, it is unlikely to be widely adopted. As tannin extractability and binding capacity varies by cultivar, and the content of exogenous tannin additions varies by product and even production lot, it is currently difficult for wine producers to accurately predict the impact of tannin additions on hybrid red wine quality.

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Supplemental Data

Tannin product solubility and stability. The solubility of exogenous tannin in water was 290.7 mg/l, so with 304 mg/l of tannin added, 95.6% went into solution. In 200 g/l sugar solution, 272.6 mg/l (89.7%) of tannin went into solution. In a 10% ethanol solution, 302.6 mg/l (99.5%) of tannin went into solution. Not unexpectedly, tannins had lower solubility in model juice. Once in solution, the tannin product was stable over the 13-day test period (Table 3.5).

Table 3.5: Stability of 800 mg/l exogenous tannin added to model wine and juice for 13 days. Different letters within a column show significant at $p < 0.01$ between each sample day.

	[Tannin]
Model Juice 0 day	242.5 A
Model Wine 0 day	299.5 B
Model Juice 1 day	259.1 A
Model Wine 1 day	297.2 B
Model Juice 13 days	237.3 A
Model Wine 13 days	299.9 B

CHAPTER 4
FUTURE WORK

Exogenous tannins have been a useful tool in the production of red wine for winemakers in cool climate regions, and to some degree, in all grape-growing regions in the world. Despite their wide acceptance, there are still many concerns that need to be addressed. First are the reported discrepancies among tannin labeling and actual tannin content in commercial products (Versari et al. 2013). Since tannin is usually extracted with heat or solvents, more than just tannin is obtained from the source, resulting in a product with only 12-48% tannin (Harbertson et al. 2012). Most commercial tannin products do not list the amount of tannin contained, but instead, add descriptions about increasing mouth feel or complexity to wines. This gives the impression that the product is composed mostly of tannin, which can be misleading to winemakers. A key to future work in exogenous tannin additions is educating winemakers about what these products actually contain. This could be as simple as winemakers measuring their products for tannin content. The A-H assay is a common tool in many wineries, but winemakers choose not to take advantage of these procedures as they are only using the lab for wine analysis, and not analysis of wine products. Measuring the tannin content of these products will empower the winemaker to make better decisions on how much tannin to add, maximizing mouth feel and quality. If an increasing number of wineries measured their products and determined tannin concentrations, this could potentially put pressure on manufactures to either label tannin percentages on exogenous tannin products or give detailed descriptions of the content of these products.

Chapter 1 investigated the interactions between LAB and tannin, and whether this relationship plays an important role in winemaking. However, more needs to be done to better understand the severity of these interactions in a hybrid wine matrix. Rather than just making a

prophylactic tannin addition of 800 mg/l, additions need to be made based on the percentage of tannin within the products. So, if the product being used contains 30% tannin and a winemaker wants to make an addition of 750 mg/l of tannin, they need to add 2,500 mg/l of the tannin product. Although this seems excessive, this would give a great indication of the extreme side of how tannin interacts with LAB and if this is a significant issue for MLF. Also, a diverse selection of LAB strains needs to be used for this study. Most winemakers know that not all LAB strains are created equal, each having its own strengths and weaknesses, so studying a wide range will help us better understand these interactions.

Chapter 2 and 3 help give a good understanding that hybrid cultivars with low indigenous tannin can increase tannin concentrations by either additions 2-3 times the manufacture's recommendation rate or additions made late in the winemaking process. Once again, knowing the percentage of tannin within a product can allow winemakers to make more specific additions. With each of these larger additions, however, there is potential for changing the sensory quality of a wine. Performing a descriptive analysis for these studies is imperative for knowing how these wines have been influenced, either positively or negatively, which in turn will allow winemakers to make an informed decision on whether or not to add large amounts of tannin to their wines. If making large tannin additions to wines results in sensory properties that are not desirable, looking for a way to reduce the binding capacity may be the next step. While techniques would have to be developed, it would be interesting to see if removing red skins and seeds from the juice, bentonite fining or pasteurizing the juice to remove/precipitate proteins, and then adding the juice back to the skins/seeds for fermentation would increase the tannin content without having to make exogenous tannin additions. It is important to keep investigating ways to improve tannin content in hybrid red cultivars while maximizing quality.

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