‘FRONTENAC’ RESPONSE TO LEAF REMOVAL AND TRAINING SYSTEMS & A MICROVINIFICATION AND DEACIDIFICATION BIOASSAY OF INTERSPECIFIC HYBRIDS (*VITIS* SPP.)

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Vineyard production and acid reduction microvinification experiments were conducted on interspecific hybrid grape cultivars in North Dakota. Training system and leaf removal effects on yield and quality for 'Frontenac' were assessed. Training system treatments included Geneva Double Curtain, High Cordon, Vertical Shoot Positioned, and 4-Arm Kniffin, and leaf removal treatments applied at bloom, post-bloom, veraison, and no removal. It was found that yield gains due to training system may be reached without negatively affecting fruit quality. The deacidification ability of biological and chemical treatments were assessed on the wines of 'Frontenac', 'La Crescent', and 'King of the North'. Biological treatments included *Saccharomycetes cerevisiae* (Maurivin B and 71B) and *Oenococcus oeni* (ER1A and EY2d), and the chemical deacification treatment cold stabilization. Greatest reduction of titratable acidity resulted from the combined biological and chemical treatments. This project and future research contributes to the optimization of grape growing and winemaking within our region.

ACKNOWLEDGEMENTS

I would like to thank all Northern Grape Gowers, Winemakers, and grape enthusiasts without you and your efforts I would not be here, working with a crop I love. You are the pioneering spirt that keeps our research alive.

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To my parents and family, you mean the world. Thank you for being my people. Thank you for your love. To my Goddaughter, Erin may you reach for your dreams and know that you are enough.

To Jake, the love of my life, my spit fire, my partner in crime, thank you for your support, your encouragement, your faith in me, and for never letting go of my clammy hand.

DEDICATION

To my Grandfather,

To my father,

To my mother,

To my husband,

To my self-doubts,

“For a seed to achieve its greatest expression, it must come completely undone.

The shell cracks, its insides come out and everything changes.

To someone who doesn’t understand growth, it would look like complete destruction.”

-Cynthia Occelli

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LIST OF ABBREVIATIONS

4AK.....Four Arm Kiffin

GDC .....Geneva Double Curtain

GDDs .....Growing degree days

HC.....High Cordon

KHT .....Potassium bitartrate

LAB.....Lactic acid bacteria

MLB.....Malolactic bacteria

MLF .....Malolactic fermentation

NC.....Non count

NCB .....Non-count basal

NCL.....Non-count latent

PEP.....Phosphoenolpyruvate

Pka.....Dissociation constant

PPFD .....Photosynthetic photon flux density

SS .....Soluble solids

VSP .....Vertical Shoot Positioned

CHAPTER 1. 'FRONTENAC' RESPONSE TO TRAINING SYSTEMS AND FRUIT ZONE LEAF REMOVAL

Abstract

Experiments conducted in 2013 and 2014 evaluated the effects of training system and leaf removal on yield and quality for 'Frontenac', an interspecific hybrid wine grape, at a research vineyard near Absaraka, North Dakota. The experiment was structured as a randomized complete block design with split-plot arrangement including four training system treatments (Geneva Double Curtain (GDC), High Cordon (HC), Vertical Shoot Positioned (VSP), and 4-Arm Kniffin (4AK)), four leaf removal treatments (bloom, post bloom, veraison, and no removal), and eight replicates. In 2013, 1428 growing degree days (GDDs) accumulated in the 155 days between frost events. In 2014, 1156 GDDs accumulated in the 121 days between frost events, 272 GDDs less than the year prior. Combined data analysis showed no significant differences in soluble solids (SS) and titratable acidity (TA) between trellis or leaf removal treatments. The pH in 2013 was significantly higher in VSP as compared to other treatments. In 2014, live nodes and total shoots were significantly greater in GDC and 4AK compared to HC and VSP. Additionally, GDC cluster number and yield were significantly greater in 2014 than 4AK and VSP. These findings suggest that 'Frontenac' SS accumulation and TA may not be affected by leaf removal or trellis system in North Dakota vineyards, yet yield gains due to training system may be reached without negatively affecting fruit quality.

Introduction

The recent and rapid expansion of the grape and wine industry in the Upper Midwest, with 13 licensed wineries and at least 40 vineyards in North Dakota alone since 2001 (2011

Strategic Vision and Direction Executive Summary-ND Grape and Wine Association), was made possible by the development and release of interspecific *Vitis* spp. hybrids during the 1990s. 'Frontenac', an interspecific hybrid with *V. riparia* parentage, was released from the University of Minnesota in 1996. 'Frontenac' is currently one of the most common wine grape cultivars in the Upper Midwest (Preston and Ganchiff, 2013) due to its cold hardiness, reliability and yield. Past studies involving interspecific hybrids with *V. riparia* parentage have demonstrated higher acidity and sugar contents, than traditional *V. vinifera* cultivars. 'Frontenac' follows this trend and averages 24.8 % soluble solids, a titratable acidity (TA) of 15.1 to 15.4 g/L, and an average pH of 2.9 at maturity (Mansfield, 2012). Additionally, 'Frontenac' poses a different acid profile at harvest with malic acid concentrations higher than old world norms. In a study of *V. riparia* cultivars, Kliewer et al. (1967) found that these grapes contained more malic acid (3.8 to 16.9 g/L) than tartaric acid (4.9 to 8.2 g/L), which is different than what has been noted in *V. vinifera*. Vos (2014) completed the first analysis of malic and tartaric acid concentrations in 'Frontenac' grapes. He found malic acid contents of 9.6 g/L, tartaric acid contents of 8.1 g/L, and a tartaric, malic acid ratio of 0.87. The recommended ranges of grape juice parameters for optimal red wine quality are a soluble solids between 20.5 and 23.5 % (Amerine et al., 1972), juice TA at harvest between 6.0 g/L and 8.0 g/L (Winkler et al., 1974), and pH between 3.4 and 3.5 (Amerine et al., 1972; Dami et al., 2005). 'Frontenac' soluble solids and TA are higher than recommended and the pH much lower. Further research is needed to learn viticulture practices that may better manage the acidity and sugar content of 'Frontenac', as improving grape quality is vital to the survival and sustainability of North Dakota vineyards and wineries. Several studies on *V. vinifera* have shown that improving irradiance at the fruiting zone can improved fruit quality (Archer and Strauss, 1989; Morrison and Noble, 1990; and Zoecklein et al., 1992).

However, interspecific hybrids with *V. riparia* lineage may react differently to standard practices used on *V. vinifera*. As such, this study examined the effects of training system and fruiting zone leaf removal on 'Frontenac' in North Dakota.

Grapevine training systems involve a manipulation of vine form and may lead to differences in total leaf area, the percentage of leaf area well-exposed to light, and the percentage of leaves located in the interior of the canopy (Katerji et al., 1994; Reynolds and Vanden Heuvel, 2009; Schultz, 1995; Smart et al., 1990). Consequently, the ability for a grapevine to photosynthesize efficiently depends upon its training system and the accompanying light microclimate of its leaves (Reynolds and Vanden Heuvel, 2009). Modifications in training may not only increase the amount of leaf area exposed to high-intensity direct radiation, (Smart, 1973; Smart et al., 1977) but may increase the interception of diffuse radiation (Smart, 1973) and improve the radiation microclimate of the remainder of the foliage (Smart et al., 1982). In addition, training may impact numerous other variables such as fruit bud differentiation, cluster exposure, vine water status, and leaf transpiration (Reynolds and Vanden Heuvel, 2009). Furthermore, training system structure that maximizes fruit sunlight exposure, especially in cool climates, can optimize berry growth and composition. Fruit in exposed portions of the canopy generally exhibit higher concentrations of sugars, anthocyanins, and total phenolics, as well as lower levels of malic acid, potassium, and juice pH compared with shaded fruits (Smart and Robinson, 1991). A number of studies have found fruit composition differences between different training systems and with the appropriate choice of training system, increases in yield and improvements in fruit composition and/or wine sensory have been reported (Bavougian et al., 2012; Cawthon and Morris, 1977; Couvillon and Nakayama, 1970; Howell et al., 1991; Huglin, 1977; Morris and Cawthon, 1980; Reynolds et al., 1995 and 1996; Shaulis et al., 1966).

Hence all aspects of vine growth, development, yield, and fruit composition may be affected by a modification in training (Reynolds and Vanden Heuvel, 2009).

However there are studies that have found regardless of training system there were no differences in fruit or wine composition (May et al., 1973; Peterlunger et al., 2002; Reynolds et al., 2004; Shaulis and May, 1971; van Zyl and van Huyssteen, 1980; Wolf et al., 2003). Martinson and Particka (n.d.) have stated that maintaining cluster exposure and avoiding shading may be more important than the training system as work at Coyote Moon Vineyards in 2013 in Clayton, NY in 'Frontenac', had a decrease of 2g/L TA in exposed clusters verses shaded clusters across all training systems. Macaulay and Morris (1993) reported lower pH and TA in sun-exposed fruit and in the wines made from them. Many other studies have found that shaded canopies produce fruit of lower sugar concentration and increased pH and TA content (Archer and Strauss, 1989; Coombe, 1959; Crippen and Morrison, 1986; Hunter et al., 1991; Morrison and Noble, 1990; Shaulis et al., 1966; and Zoecklein et al., 1992). The decreased sugar content in shaded fruit may result from a combination of a delay in maturation, or lower light intensity on source leaves (Smart et al., 1990), and lower berry temperature (Gaprindashvili, 1981; Percival et al., 1994). Increased pH levels in shaded berries have been associated with the accumulation of nitrates and potassium (Smart et al., 1990) as low light wavelengths 600nm/730nm in the canopy reduce the activity of the enzyme nitrate reductase which can lead to an accumulation of nitrate and potassium (Bledsoe et al., 1988). High TA levels in shaded fruit can be attributed to reduced malate degradation when berry temperatures are less than 30^0 C following veraison (Lakso and Kliewer, 1975 and 1978; Reynolds et al., 1986; Percival et al., 1994). Excessive shade also produces fruit with reduced aromatic, anthocyannin and monoterpene levels (Morrison and Noble, 1990). Thus, fruit zone leaf removal has been

researched to determine if it could be used to maintain cluster exposure and assist in berry ripening.

Fruit zone leaf removal is one of the most frequently applied summer canopy management operations in winegrape growing (Bledsoe et al., 1988; Kliewer and Antcliff, 1970; Percival et al., 1994; Reynolds et al., 1996; Smart and Robinson, 1991; Zoeckleirn et al., 1992). Fruit zone leaf removal can be performed traditionally or early. Traditional leaf removal is conducted between fruit set and veraison and early leaf removal is conducted pre-bloom to fruit set (Smart and Robinson, 1991). Leaf removal pre-bloom is typically employed to improve canopy microclimate and to reduce yield, by reducing carbohydrate supply during flowering resulting in reduced fruit set and total sugar per berry (Caspari and Lang, 1996; Kliewer and Antcliff, 1970; Vasconcelos et al., 2009). Traditional leaf removal is commonly recommended to improve the canopy microclimate, and decrease disease incidence (Poni et al., 2006). Both methods are utilized to enhance berry quality, but depending on the cultivar, timing, and leaf removal severity, results vary (Poni et al., 2006).

Leaf removal on 'Sauvignon blanc' from fruit set to veraison with various defoliation rates was found to effectively reduce TA, malic acid, pH, and juice potassium in all leaf removal treatments with no effect on yield (Bledsoe et al., 1988). Similar results were found with basal leaf removal treatments on *V. vinifera* cultivars Bacchus, Pearl of Csaba, Schönburger, and Siegerrebe near veraison (Reynolds et al., 1995b). These cultivars had decreases in TA, pH, and potassium. Basal leaf removal in *V. vinifera* cultivars Graciano and Carignan at fruit set resulted in decreased malic acid concentration (Tardáguila et al., 2010). Interestingly, defoliation of 6 basal leaves per shoot pre-bloom in 'Sangioverse' caused a decrease in yield, increased soluble solids (SS) and total anthocyanins, and increased TA (Poni et al., 2006). Hence, not all attempts

to advance maturity or improve grape composition with leaf removal have been successful (Iland, 1988; Jackson and Lombard, 1993; Norton, 1987). Work done by Percival, Fisher and Sullivan (1994) in the Niagra region of Canada reported on leaf removal prior to veraison on *V. vinifera* and found no difference in SS, pH, and TA, and no reduction in yield. Therefore, leaf removal could be cultivar dependent. Three *V. vinifera* cultivars were compared by leaf removal treatments over 4 years. The cultivar Barbera had no significant differences in TA and pH, while cultivars Croatina and Malvasia di Candia aromatica had significant differences in TA (Bavaresco et al., 2008). A report by Portz et al. (2010) on 'Frontenac Gris' in Iowa found no significant differences in SS, pH, and TA with leaf removal conducted in early July. Similarly, leaf removal at veraison on 'Frontenac' by Wlordachak et al. (2009) in Illinois found no significant differences in SS, TA, and pH in leaf removal treatments.

Thus the intent of this experiment was to evaluate the effects of training system and leaf removal on 'Frontenac' grown in North Dakota. The effects of these practices are valuable and necessary for growers and winemakers in our young grape industry.

Materials and Methods

Experimental Site and Design

The University of Minnesota interspecific hybrid, 'Frontenac' was used to study the effects of training systems and leaf removal on vine performance and fruit composition over two years, 2013 and 2014. The research vineyard utilized was located at the North Dakota State University (NDSU) research station near Absaraka, ND (Lat: 46° 59' 22.0986" Long: -97° 21' 22.2222"). Soils at the site are Warsing sandy loam, fine-loamy over sandy and sandy-skeletal, mixed, superactive, frigid Oxyaquic Hapludolls with 0-2% slopes. One hundred twenty-eight

own-rooted ‘Frontenac’ vines were established in 2006, and spaced 2.6 m apart in rows 3.3 m apart. Rows were oriented north-south with 32 vines per row.

Vines were originally trained to the 4AK trellis system (Fig. 1). Then in 2010, three additional canopy-training systems were included GDC, HC, and VSP (Figs. 2-4). Training system treatments were arranged as a randomized complete block design, 8 replicates of the 4 training system treatments and 4 vines within each training system treatment, resulting in 16 vines per rep and 128 plants total. Fruit zone leaf removal treatments were arranged as a split-plot, with training system as the whole-plot, and leaf removal as the sub-plot, the four treatments included leaf removal at bloom, post bloom, veraison, and no removal as control. The treatments administered in the first year were re-administered to the same vines the second year.

Training Systems and Canopy Management

Vines in the HC system were trained to bilateral cordons 2 m aboveground (Fig. 3). Cordons extended in opposite directions (North-South) creating a slight overlap with adjacent vines. Shoots were combed downward three times during the growing season: three weeks post-bloom, four weeks post-bloom, and lastly at veraison. Vines in the 4AK system were trained to two bilateral cordons, one at 2 m aboveground, and the second at 1.5 m aboveground (Fig. 1). Shoots from both the upper and lower cordons were combed downward at the three times used for the HC. Vines in the VSP system were trained to bilateral cordons 1 m aboveground (Fig. 4). Shoots were tucked upward as needed between horizontally running catch wires throughout the summer. GDC vines (Shaulis et al., 1966) were trained to two bilateral cordons each 2 m aboveground with wires 0.6 m apart supported by post extensions (Fig. 2). Shoots were combed downward three times per season analogous with times for HC and 4AK.

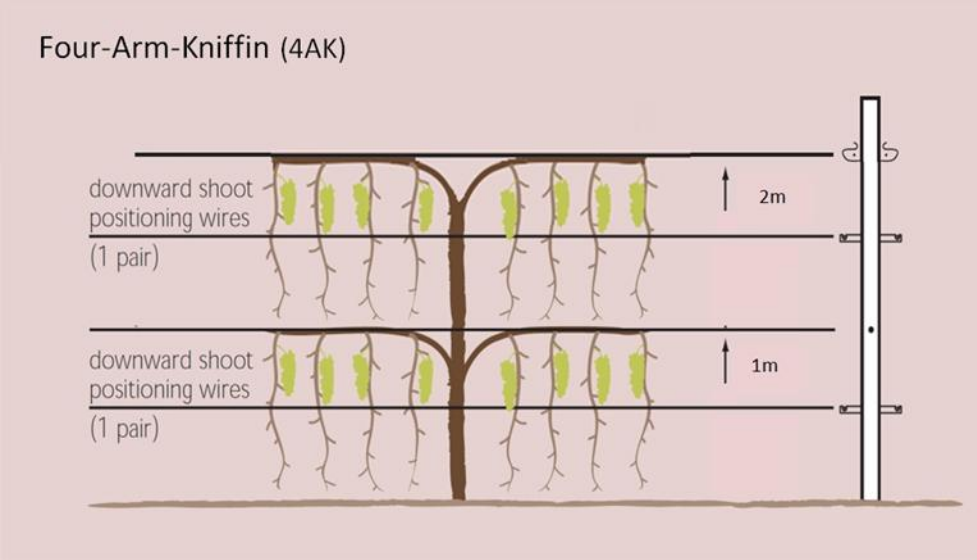

Figure 1. Drawing of the Four Arm Kniffin (4AK) training system

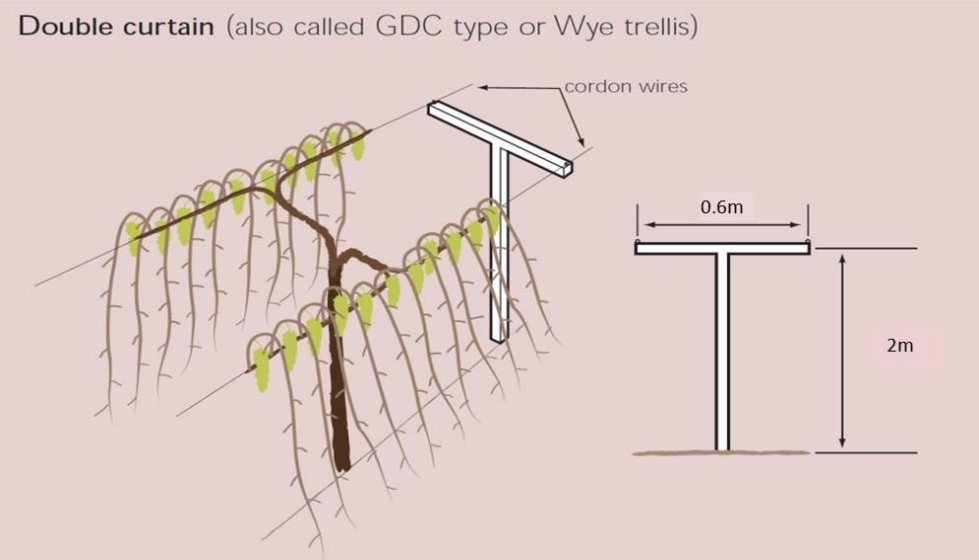

Figure 2. Drawing of the Geneva Double Curtain (GDC) training system

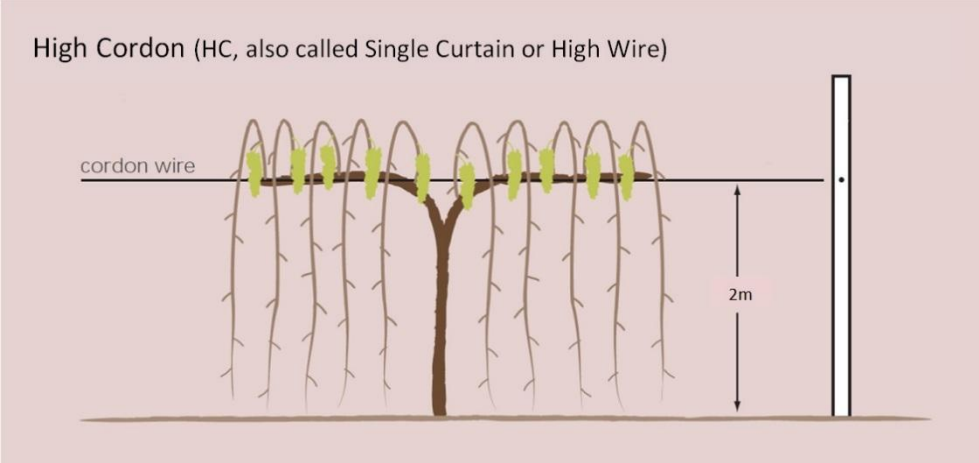

Figure 3. Drawing of the High Cordon (HC) training system adapted

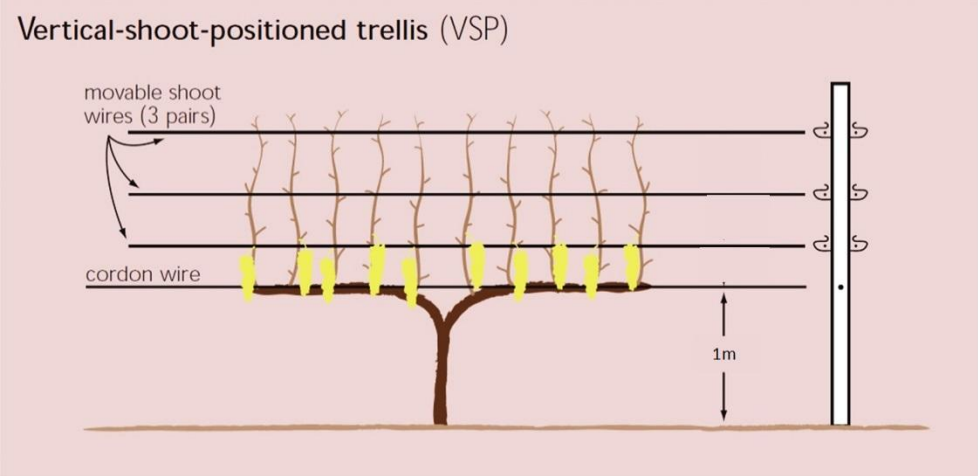

Figure 4. Drawing of the Vertical Shoot Positioned (VSP) training system

Vines were pruned in late spring to delay early bud break and decrease susceptibility to late spring frosts (Martin and Dunn, 2000; Ravaz, 1912; Shaulis,1971; Wolpert and Howell, 1984). Prunings of one-year-old canes were weighed to determine vine size. Balanced pruning was utilized to maintain balance between vegetative vigor and reproductive quality. The base node count was 30 and every additional 0.45kg of one-year-old pruning (1lb) added an additional 10 nodes with a maximum limit of 60 nodes/vine. Viable nodes were counted at bud burst, while

shoots per node and shoots per plant were counted close to bloom. Shoots were not thinned as to rejuvenate the cordon and potentially lessen gaps between spurs. Similarly clusters were not thinned. Annual petiole tests were used in the research vineyard to determine fertilizer applications. Weed, disease, and pest control were managed according to industry standards. Creeping red fescue grass was grown between rows as a ground cover. Shoot tips were only hedged if growth reached the soil surface.

Leaf Removal and Light Measurements

Canopy density of each training system was maintained during the growing season with shoot positioning appropriate for each training system. Leaf removal treatments were applied at bloom, three or four weeks post-bloom (once 289 GDDs accumulate post-bloom), and veraison. Leaves were removed from the basal three nodes on all shoots arising from the cordon and spurs. Photosynthetic photon flux density (PPFD), the photosynthetic active photons, wavelength range from 400 to 700 nanometers, emitted on a given target per second were measured in micromoles per square meter per second by a Line Quantum Sensor (Apogee Instruments, Logan, UT). The PPFD measurements of external solar radiation and internal cluster PPFD were taken prior to- and post-leaf removal of each vine. Percent transmittances were calculated by dividing the fruit-zone PPFD value by the ambient PPFD measured externally of the canopy. Measurements were taken from approximately one hour prior to solar noon and completed around one hour post solar noon at each leaf removal date: bloom, post bloom, and veraison.

Harvest Indices, Berry Composition and Vine Status

Fruit was sampled weekly from veraison to harvest. A 15-berry sample was randomly collected from each treatment replicate to monitor fruit composition by pH, soluble solids, and TA. At the final sampling date, fruit was harvested and weighed on a per plant basis for yield

results. Cluster weight was determined by weighing a random sample of three clusters per vine. Berry weight and diameter was determined by weighing and measuring a 100-berry sample from the three-cluster sample. Fruit characteristics were determined by a 15-berry sample per vine. Soluble solids were measured twice per sample by a portable pocket refractometer, (pal-1, ATAGO, Tokyo, Japan). Juice TA and pH were measured three times each per sample and were determined using standard methods with an Orion star A111 bench top pH meter (Thermo Scientific, Beverly, MA) (Iowa State University Extension and Outreach, 2013). The date of harvest each year was determined by inclement weather, availability of vineyard help, and fruit characteristics. Cane pruning weights, cordon lengths, and trunk diameter measurements taken each spring to determine vine size.

Statistical Analysis

Data was analyzed across years as split plot in time using Prox Mixed SAS statistical analysis software (SAS version 9.3, SAS Institute Inc., Cary NC). Differences were determined by pairwise t-tests, and significance of these differences were determined based on a 95% level of confidence for all comparisons.

Results

Data Interpretation

Of the variables measured only fruit characteristics, pruning weights, node viability, shoot number, cluster number, number of shoots per meter, and yield were significant for an interaction between training system and growing season. These variables (pH, soluble solids, TA, pruning weight, node viability, shoot number, cluster number, yield, codon length, shoots per meter) were separated by season for analysis and are explained below (Tables 2-8). All

other variables (retained nodes, cluster weight, berry count, berry weight, berry diameter) will be discussed in support of significant data results.

Variability between Seasons

In 2013, 1428 growing degree days (GDDs) base 10 C, accumulated in the 155 days between frost events, with the last spring frost date on the 12 May and the first fall frost date on the 13 Oct. Grapes were harvested on the 10 Oct. 152 days past the last spring frost event and with an accumulation of 1417 GDDs. In 2014, 1156 GDDs accumulated in the 121 days between frost events, with the last spring frost on the 16 May and the first fall frost on the 13 Sept. This was 34 days fewer between frost events and 272 GDDs less than the prior year. Since the September frost during 2014 did not cause complete leaf drop, clusters were left on the vine to ripen as they had not reached the desired harvest parameters. Clusters were left on the vine an additional 25 days and fruit were harvested on the 8 Oct. due to predicted freeze on the 9 Oct. which added 138 GDDs. This extension resulted in 146 days from the last spring frost until harvest and a cumulative 1294 GDDs. Due to the increase, the 2014 season was only 6 days and 123 GDDs less than 2013. Additionally, in 2013, 48 cm of rainfall was measured during the growing season. June had the most precipitation and August the least. July and August were the hottest months and September was warmer than the normal average. In 2014, 27.5 cm of rainfall was measured during the growing season. June had the most precipitation, while July, August, and October had the least. The warmest months were again July and August, but the summer of 2014 was cooler than average and cooler than the year prior. Furthermore, the 2013-2014 winter was ranked by the National Climatic Data Center as the 24th coldest winter of record for the state, with many locations in eastern North Dakota ranking much higher. The 2013-2014 winter also had below average snowfall, with many ground blizzards that consisted of no falling snow

but strong wind events. (NDAWN, 2014) (Figs. 5 and 6). Due to variability of years, post bloom leaf removal treatment application dates were based on GDDs. The amount of GDDs accumulated between 50% bloom and 50% veraison were similar between years. When half of the 30-year average GDDs were accumulated between bloom and veraison, which was approximately 289 GDDs, post-bloom leaf removal treatments were applied. This resulted in time differences for leaf removal treatments between years. Table 1. shows the differences in frost free days, days post spring frost until harvest, GDDs, and important industry and physiological dates for bud burst, bloom, veraison, and harvest.

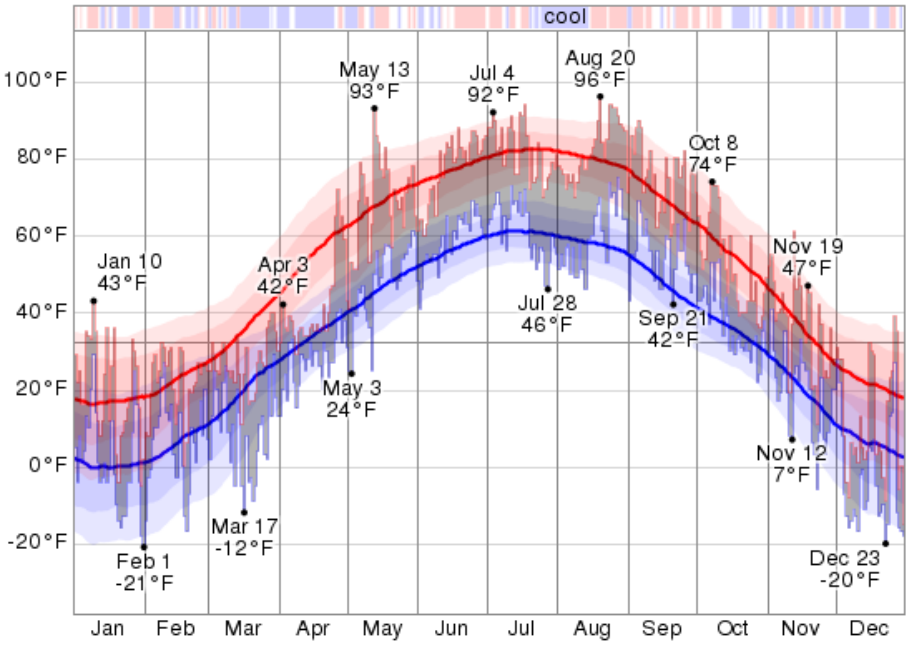

Figure 5. Historical weather record at Hector International Airport at Fargo, ND for 2013 Obtained from: (weatherspark.com/history/30234/2013/Fargo-North-Dakota-United-States).

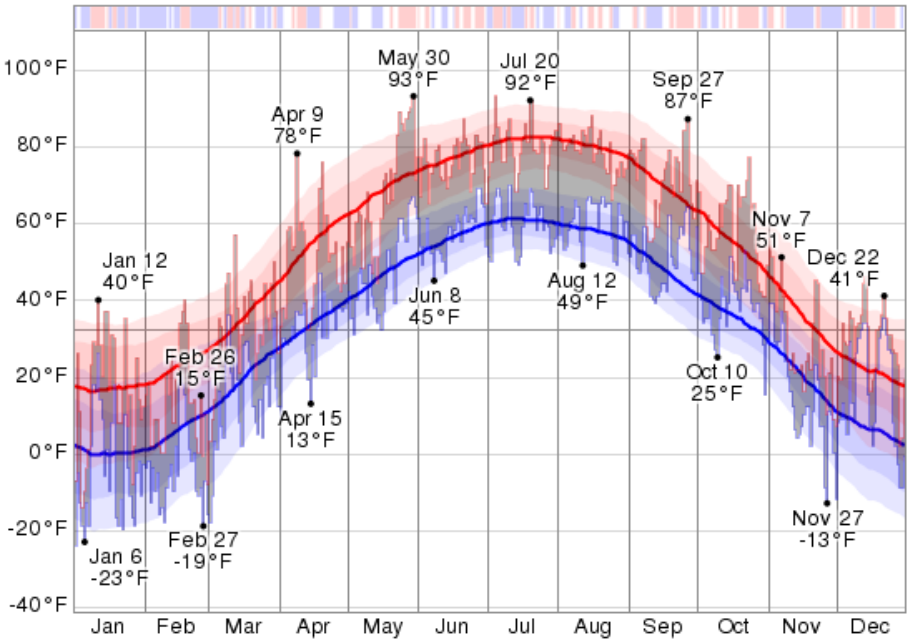

Figure 6. Historical weather record at Hector International Airport at Fargo, ND for 2014 Obtained from: (weatherspark.com/history/30234/2014/North-Dakota-United-States).

Table 1. Climatic and phenoligical data for 'Frontenac' grown near Absaraka, ND in 2013 and 2014.

Year	Frost Free Days[z]	GDDs[y]	Days till harvest[x]	Bud Burst[w]	Bloom[v]	Veraison[u]	Harvest[t]
2013	155	1428	152	May 29th	June 24th	Aug 19th	Oct 10th
2014	121	1156	146	May 27th	June 18th	Aug 14th	Oct 8th

[z]Days between last spring frost and first fall frost
[y] GDDs= Growing Degree Days (base 10C) accumulated in the frost free period
[x] number of days from last frost event till harvest
[w] when 50% of buds on a plant have burst, first leaf rolls back
[v] when vine is flowering and 50% of caps have fallen
[u] when 50% of the berries on a vine have softened and changed color
[t] date of harvest

Leaf Removal Treatments

No significant differences were found by leaf removal treatments on any variable tested on 'Frontenac' grown near Absaraka, ND in 2013 or 2014 (data not shown).

Training System Treatments

Fruit Characteristics

Fruit pH was significant in training systems within 2013. VSP had significantly greater pH than HC, GDC, or 4AK. Fruit pH was not affected by training system in 2014. Soluble solids and TA were not affected by training system in either year (Table 2).

Table 2. Effect of training system on pH, soluble solids and titratable acidity in 'Frontenac' in Absaraka, ND.

Treatment	pH [y]		Soluble solids [y]		Titratable acidity [y]	
	2013	2014	2013	2014	2013	2014
	--- -log[H+] ---		---- Brix ----		---- g/L ----	
HC [z]	3.20 b[x]	3.04 a	27.90 a	25.66 a	12.01 a	17.19 a
GDC	3.24 b	3.02 a	28.48 a	25.75 a	11.68 a	17.73 a
VSP	3.30 a	3.03 a	27.19 a	25.73 a	11.25 a	17.78 a
4AK	3.23 b	3.04 a	27.87 a	24.91 a	11.71 a	17.54 a

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin

[y] Fruit characteristics: pH, Soluble solids and titratable acidity were averages of a 15-berry sample per vine.

[x] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Pruning Weights and Node Viability

As mentioned earlier, plants were pruned using the balanced pruning method, a 30 node base count and an additional 10 nodes for every 0.45kg (1 lb) of one–year-old prunings. In 2013, pruning weights were under 0.45kg and all plants across treatments where pruned as close to 30 nodes as possible. The winter of 2014 was colder than average with wind storms and a lack of snow cover, bud death was documented in many vineyards across the upper Midwest. To account for possible bud death, additional nodes were kept to reduce plant stress and maintain similar live node counts. In 2014, balanced pruning had a base node count of 40, 10 nodes greater than 2013. The VSP plant growth from the 2013 season was great though not different,

but in 2014 pruning weights were significantly higher than all other training systems followed by 4 AK. As VSP pruning weights in 2014 were large enough to leave an additional 10 nodes per plant and attempts were made to keep the additional nodes. Unfortunately, due to winter winds and cold temperatures, much of the one-year-old wood was desiccated and dead, and node counts averaged 42.9 nodes per plant in the VSP treatments. Due to the lack of wood kept, no significant differences in nodes retained were found between training systems in either 2013 or 2014 (Table 3). Similarly, node viability was similar between years regardless of the increase in nodes left in 2014. Live node counts averaged 20 live nodes per plant in both 2013 and 2014. Of the nodes retained in 2013, approximately 70% were viable across all training system treatments, with no differences in viable nodes and node mortality for trellis treatments (Table 4). Greater node mortality occurred in 2014 than 2013. In 2014, GDC and 4AK had significantly higher node viability, averaging 56.9% viable, as compared to VSP and HC. Vines on VSP had significantly more node mortality compared to all other training systems, averaging 69% non-viable nodes.

Yield

In 2013, there were no differences in shoot numbers, subsequent clusters, cluster weights, and yield between training systems (Tables 5 and 6). In 2014, there were significantly more shoots with GDC and 4AK. In 2014, cluster numbers per treatment were significantly greater in GDC as compared to all other treatments. Vines in the GDC trellis had significantly higher yield compared to vines in 4AK and VSP trellises in 2014. However, cluster weights, average berry counts per cluster, berry weight and berry diameter were not significantly different between trellis treatments in 2014, or in 2013 (Tables 6 and 7).

Table 3. Effect of training system on average pruning weight and retained nodes per vine for 'Frontenac' in Absaraka, ND in 2013 and 2014.

Treatment	Pruning weight [z]		Retained nodes	
	2013	2014	2013	2014
	------- g/vine -------		------- number/vine -------	
HC [y]	62 a[x]	254 c	28.4 a	41.3 a
GDC	35 a	198 c	28.6 a	38.5 a
VSP	119 a	477 a	29.0 a	42.9 a
4AK	52 a	365 b	28.6 a	41.0 a

[z] weight of one year old prunings per vine
[y] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[x] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 4. Effect of training system on node viability post pruning per vine average in 'Frontenac' in Absaraka, ND in 2013 and 2014.

Treatment	Live nodes		Dead nodes	
	2013	2014	2013	2014
HC[z]	19.0 a[y]	17.8 b	9.4 a	22.6 b
GDC	20.0 a	23.9 a	8.5 a	16.3 c
VSP	20.6 a	15.0 b	8.7 a	28.4 a
4AK	21.1 a	22.6 a	7.4 a	19.4 bc

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 5. Effect of training system on shoot and cluster numbers per 'Frontenac' vine average in Absaraka, ND.

Treatment	Shoot number [y]		Cluster number [x]	
	2013	2014	2013	2014
HC [z]	20.9 a [w]	33.0 b	24.5 a	26.2 b
GDC	21.4 a	41.4 a	17.8 a	35.4 a
VSP	21.8 a	23.9 c	18.8 a	16.1 c
4AK	23.3 a	40.1 a	26.3 a	25.2 b

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[y] Average total shoot number per plant
[x] Average total cluster number per plant at harvest
[w] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 6. Effect of training system on cluster weights and yield per vine average in 'Frontenac' in Absaraka, ND.

Treatment	Cluster weight [y]		Yield [x]	
	2013	2014	2013	2014
	---------- g ----------		---------- Kg ----------	
HC [z]	52.76 a [w]	82.57 a	1.398 a	1.942 ab
GDC	40.21 a	79.32 a	0.832 a	2.627 a
VSP	51.34 a	73.76 a	1.160 a	1.166 c
4AK	47.52 a	82.94 a	1.373 a	1.915 b

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[y] Average cluster weight determined by averaging the weight of a random sample of three clusters per vine.
[x] Average weight of fruit harvested per plant.
[w] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 7. Effect of training system on average berry count per cluster, average berry weight, and average berry diameter in 'Frontenac' in Absaraka, ND.

Treatment	Berry Count [y]		Berry weight [x]		Berry diameter [w]	
	2013	2014	2013	2014	2013	2014
	--Berries/cluster--		-----g-----		----cm----	
HC [z]	65.9 a[v]	74.4 a	0.79 a	1.10 a	1.1 a[w]	1.1 a
GDC	52.7 a	75.0 a	0.74 a	1.05 a	1.0 a	1.1 a
VSP	63.1 a	67.9 a	0.80 a	1.08 a	1.0 a	1.1 a
4AK	59.9 a	74.2 a	0.77 a	1.11 a	1.0 a	1.1 a

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[y] Average berry count was determined from a random 3 cluster sample per vine
[x] Average berry weight from a 100 berry sample
[w] Average berry diameter from a 100 berry sample
[v] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Canopy Density

Canopy density was to be determined by use of a quantum line sensor measuring PPFD in the canopy before and after leaf removal treatments at three specific times throughout the summer. This was done to quantify radiation available due to intensity of leaf removal treatments and inherent differences of the training systems and shoot positioning. Similar work has been done by University of Nebraska in 'Frontenac' showing that higher transmittances occurred with vines trained as GDC and HC than vines trained as Smart-Dyson and VSP (Bavougian et al., 2012). In addition, vines on training systems with higher transmittances had fruit with lower TA concentrations, which agreed with findings by Smart and Robinson (1991), and Macaulay and Morris (1993) who observed higher TA concentrations in fruit from vines that were shaded. However, due to weather conditions on data collection days, data was unusable. Nonetheless, point quadrant data were collected in 2014 to supplement our understanding of the canopy density. Point quadrant is the use of a thin rod inserted into the fruit zone of the canopy

of a single vine 50 times, 25 from each side of the row with the rod parallel to the ground (Smart and Robinson, 1991). At each insertion, contacts with leaves and other vine parts are recorded. The data collected gives the ability to calculate percent gaps, leaf layer number, percent interior leaves, and percent interior clusters. These values were compared to optimum values to give an indication of canopy structure. These results are included in the discussion.

Additionally, shoots per meter of cordon data were calculated from total cordon length and total shoot number. Results suggest that vines trained to the 4AK trellis had a greater cordon length compared to vines on VSP or HC trellises, in both 2013 and 2014. Vines on the 4AK trellis also had the fewest shoots per meter compared to vines on GDC or HC trellises in both 2013 and 2014. Vines on the HC trellis had the most shoots per meter as compared to vines on the 4AK trellis in 2013, and HC had more shoots per meter than VSP or 4AK trellises in 2014 (Table 8).

Table 8. Effect of training system on average cordon length and average shoots per meter in 'Frontenac' in Absaraka, ND.

Treatment	Cordon length [y]		Shoots per meter [x]	
	2013	2014	2013	2014
	-------- m --------		---- Shoots/m ----	
HC [z]	1.834 c [w]	1.776 c	11.9 a	19.9 a
GDC	2.544 ab	2.418 ab	11.7 a	17.7 ab
VSP	2.301 bc	1.995 bc	9.4 ab	14.6 bc
4AK	2.973 a	2.840 a	8.1 b	13.1 c

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[y] Average cordon length measured in meters
[x] Average shoots per meter of cordon
[w] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Discussion

Leaf Removal

Leaf removal has been shown to affect yield, canopy microclimate, disease incidence, and impact fruit characteristics depending on timing, severity, location and cultivar (Arnold and Bledsoe, 1990; Crippen and Morrison, 1996; Dry, 2000; Hunter et al., 1991; Morrison and Noble, 1990; Percival et al., 1994; Tardaguila et al., 2008; Zoecklein et al., 1992). In the current study, many variables were analyzed for response to leaf removal treatments, yet significant differences were not found in response to leaf removal during either year.

Cultivars have been documented to respond differently to leaf removal treatments. Three *V. vinifera* cultivars were subjected to leaf removal treatments over 4 years, the cultivar Barbera had no significant differences in TA and pH, while cultivars Croatina and Malvasia di Candia aromatica had significant differences in TA (Bavaresco et al., 2008). Leaf removal at veraison on 'Frontenac' by Wlordachak et al. (2009) in Illinois found no significant differences in SS, TA, and pH with leaf removal treatments. A report by Portz et al. (2010) on 'Frontenac Gris' in Iowa found no significant differences in SS, pH, and TA with leaf removal conducted in early July. Additional research on shade leaf removal three weeks post bloom on 'Frontenac Gris' by Aipperspach (2013) at three vineyards across eastern North Dakota (near Buffalo, Clifford, and Wapheton) also found no influence on SS, pH and TA. According to the University of Minnesota, 'Frontenac Gris' was originally identified as a single bud sport cane found growing on a 'Frontenac' vine at the University of Minnesota Horticultural Research Center. 'Frontenac Gris vines' have shown the same good levels of disease resistance, vigor, productivity, high sugar levels and acidity as 'Frontenac' (Luby and Hemstad, 2006). Therefore, 'Frontenac' and 'Frontenac Gris' may act similarly to leaf removal treatments, with neither study having a

significant response. These prior studies on ‘Frontenac’ and ‘Frontenac Gris’ support our findings with lack of a significant response to leaf removal. As such, the cultivar ‘Frontenac’ may not respond to leaf removal treatments in these areas.

Leaf removal treatment response could be cultivar driven and/or location dependent. Work by Valenti, Ghiglieno, and Mattivi (2012) found that ‘Cabernet Sauvignon’ and ‘Sangiovese’ grown in different locations in Italy, Brisighella and Scansanco, subjected to the same leaf removal treatments had different results in all analytical parameters. Similarly, ‘Frontenac’ may respond to leaf removal in different areas, however as previously stated prior work in Illinois, Iowa, and North Dakota also found no response.

Interestingly, the early leaf removal treatment in our study did not affect yield components. This could be due to low severity of defoliation, the location, and or the cultivar’s possible inherent lack of responsivity to such treatment. Work done by Tardaguila et al. (2008), with early and late defoliation of 5 primary basal leaves per shoot had no effect on yield components. Interestingly, later work with early and late defoliation of 8 primary basal leaves per shoot had a 30 to 70% reduction in yield in early leaf removal (Tardaguila et al., 2010). Hence 3 basal nodes may not be severe enough to elicit a response. Additionally, both studies noted above had reductions in malic when leaves were pulled at fruit set. Individual acids were not collected in our study, and cannot be included.

As leaf removal treatments did not elicit a response in any parameter tested in our study all topics remaining in the discussion are based on training system treatment effects.

Training System

Fruit Characteristics

Training systems studies have found significant effects on fruit characteristics. This change may be due to increasing sunlight interception and temperatures to leaves and fruit (Smart and Robinson, 1991; Reynolds et al., 1995; Reynolds and Vanden Heuvel, 2009). The fruit characteristics pH, TA and soluble solids will be discussed below.

The pH and TA are determined by the concentration of acids within the grape. The major organic acids found are Acetic, Adipic, Ascorbic, Citric, Citramalic, Formic, Fumaric, Galacturonic, Glucuronic, Glutaric, Ketoglutaric, Lactic, Malic, Maleic, Malonic, Oxalic, Propionic, Pyruvic, Shykimic, Tannic, and Tartaric acids (Mato et al., 2005). The two most predominate acids in all stages of development and represent the most significant influences on the acidity and pH of juice are tartaric and malic acids and account for 69 to 92% of all acids within the grape berries and leaves (Lakso and Kliewer, 1975; Morris et al., 1983; Ruffner, 1982). Tartaric acid is a secondary product formed from the metabolism of glucose and ascorbate and its concentration remains relatively stable in the grape as it forms an insoluble salt that is not affected by catabolizable enzymes (Ruffner, 1982; Saito and Kasai, 1968). Malic acid is an active intermediate in grape metabolism, it is accumulated in the vacuole until berries undergo a metabolic shift at veraison and it is released from the vacuole. Malic acid is a potential source of carbon for respiration, gluconeogenesis, and other pathways (Ruffner, 1982). When the malic acid is metabolized, TA is reduced and influences the sugar-acid balance (Lakso and Kliwer, 1978; Ruffner, 1982). Though the exact biochemical and molecular mechanisms are yet to be understood for malic degredation, increased temperature post veraison results in increases malic degradation and temperature is considered the predominant factor mediating grape malate

content at maturity (Buttrose et al., 1971; Lakso and Kliewer, 1975 and 1978; Ruffner, 1982; Sadras et al., 2012; Sweetman et al., 2014). It is known that malate within the berry is synthesized from Phosphoenolpyruvate (PEP) carboxylase and degraded by the malic enzyme. The malic enzyme is much more heat stable than PEP carboxylase (Lakso and Kliewer, 1978). At high temperatures, 30^{o}C, after veraison, malic enzyme activity rises while PEP carboxylase activity declines (Ruffner et al., 1976). Work by Buttrose et al., (1971) with 'Cabernet Sauvignon' found lower concentrations of malic acid in berries developed at 30^{o}C post veraison as opposed to 20^{o}C. Another experiment with 'Shiraz' found a heated treatment of 30^{o}C, to have greater malic degradation as compared to the control treatment at 20^{o}C (Sweetman et al., 2014). Kliewer (1968) using temperature controlled growth rooms confirmed that cool regions typically produce grapes with higher concentrations of organic acids, the negative temperature correlation was demonstrated for malic acid, and its optimum temperature for malic accumulation was estimated to be 20^{o}C. If 30^{o}C is an optimal temperature for malic reduction post verasion, warm fall temperatures would be vital for optimal fruit ripeness and winemaking in North Dakota. However, 30^{o}C was only reached on 14 occasions in 2013 post veraison and only 4 times in 2014 post veraison (Figs. 7 and 8) (NDAWN 2014).

Perhaps this lack of heat caused a lack of response in pH and TA in 2014 and a mild reaction in 2013. It may be possible that a subtle amount of malic acid was degraded in 2013 resulting in a change in pH. The concentration term pH is a negative logarithmic concentration for free dissociated protons in solution, represents how much acid is in a solution (Boulton, 1980). Losses of small amounts of malic acid may have reflected in the pH. Titratable acidity however, is the concentration of free protons and undissociated acids in a solution that can react with a strong base and be neutralized (Boulton, 1980). The TA measurement represents acid

strength, and each acid component within the total titratable acidity has a different strength, its tendency to lose its proton. Tartaric acid is stronger than malic (Amerine et al. 1965). If tartaric acid content remains unchanged and the malic acid content slightly decreases the TA may have remained similar to a reading without malic acid degradation. This subtlety in grape response and the corresponding pH and TA readings may account for a nonsignificant difference in TA with a significantly higher pH with VSP in 2013.

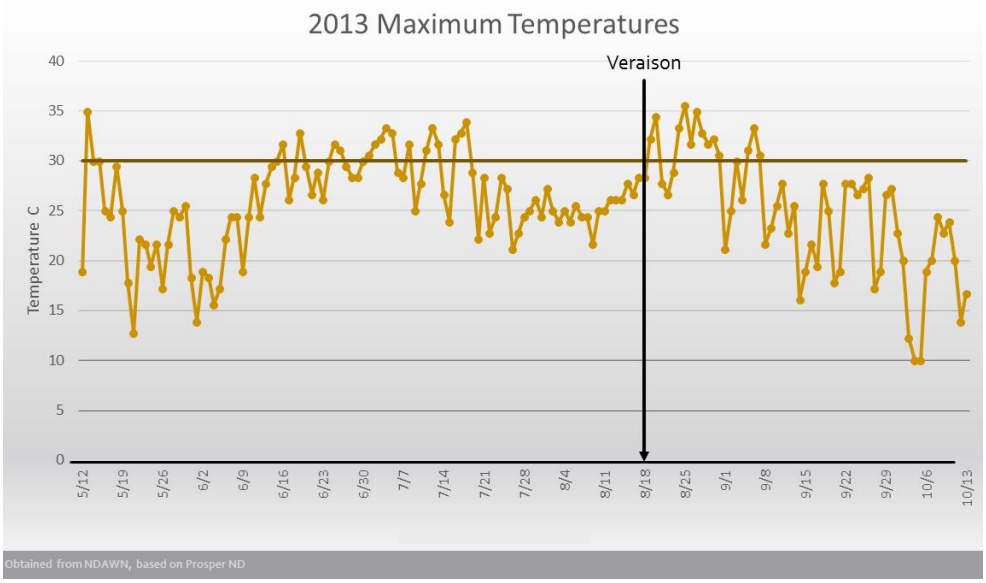

Figure 7. Maximum temperatures in 2013 growing season post frost obtained from NDAWN at the Prosper, ND weather station.

On the contrary, a differing theory would be that increases in pH are due to shading. Smart (1987) stated that shading and low 600nm/730nm wavelength ratios in the canopy reduce the activity of the enzyme nitrate reductase which can lead to an accumulation of nitrate and potassium in the shoots and fruit (Bledsoe et al., 1988; Percival et al., 1994). Potassium acts as a buffer as it affects the solution by binding to acids and decreasing the acid strength in solution (Maculay and Morris, 1993). The pH of grape juice results from the balance between anionic forms of organic acids and the major cations. Therefore alteration of the concentration of any of

these factors affects the final pH of the juice. High concentrations of potassium in juice decrease the concentration of free acids in juice resulting in an overall increase in the pH (Kodur, 2011). Hence, potassium does not lessen the amount of acids in the solution, just the availability of those protons. However, the protons can still be dissociated by a strong base, so the TA remains unchanged. The increased pH and lack of response in TA could be due to increased shade and potassium in VSP. Interestingly a high concentration of potassium in the berry may decrease the rate of malic acid degradation, by impeding transfer of malic acid from the vacuole storage pools to the cytoplasm, the site of malic acid respiration (Hale, 1977).

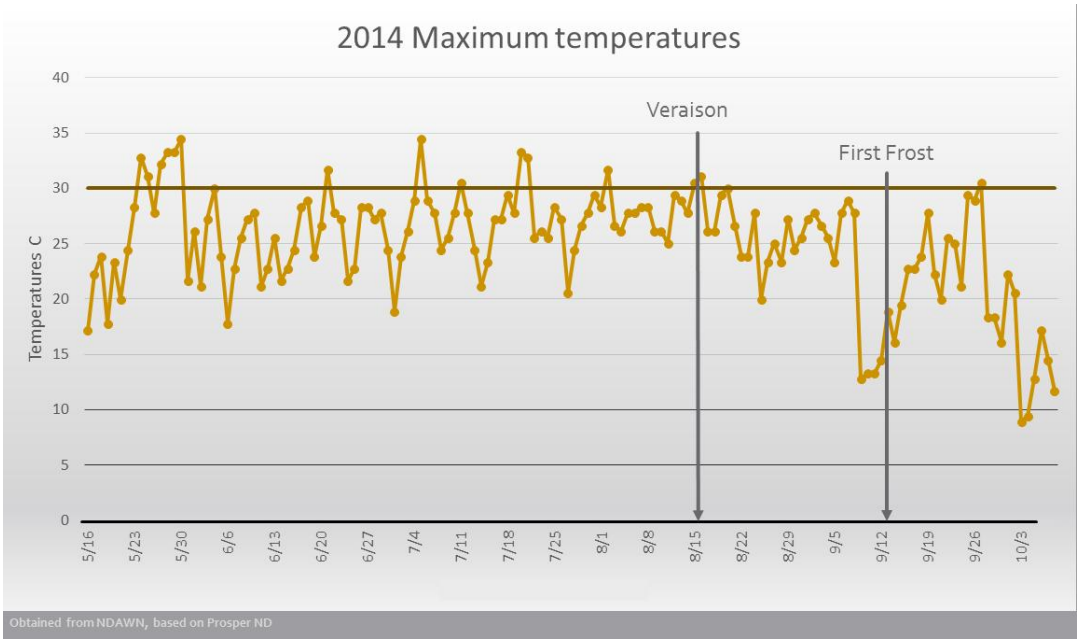

Figure 8. Maximum temperatures in 2014 growing season post frost obtained from NDAWN at the Prosper, ND weather station.

Another theory for lack of response in fruit characteristics to treatments could be no difference in light infiltration between treatments, and/or that the canopy is so open it is already supersaturated that treatments cannot illicit a response due to a predisposed high light infiltration

condition. As increases in light infiltration in plants with low canopy density may not significantly affect grape sugar, acidity and color (Bavaresco et al., 2008).

To validate treatment effects PPFD by Line Quantum Sensor was collected, but the data was unusable due to clouds and irregular light measurements. Therefore, point quadrant data was collected once in 2014 to give some insight in canopy structure. Point quadrant data allows one to look at percent gaps in the canopy, leaf layer numbers, percent interior leaves and percent interior clusters (Smart and Robinson, 1991). Percent gaps are optimum between 20-40%, leaf layer number optimally between 1.0-1.5 or less, interior leave percentage optimally less than 10%, and percent interior cluster less than 40% for an optimal canopy (Smart and Robinson, 1991). It was found that no significant differences were found between trellis systems across all measurements however, some treatments were found to be outside of optimal ranges (Table 9). The HC and 4AK systems had less than optimal percentage gaps within the canopy, potentially resulting in increased shading compared to GDC and VSP. Only VSP had the correct amount of interior leaves, as all other trellis systems were greater than the optimal value, potentially resulting in a dense canopy. Interior clusters and leaf layer numbers were inside the optimal ranges, with VSP having the most optimum value. According to optimal values, VSP was in the correct canopy values for all parameters.

Hence an increase in pH in 2013 in VSP due to potassium and increased shading may be unlikely however neither specific acids nor potassium content were measured in this study. However, point quadrant data suggests that training systems implemented were close to optimal values, with some room for improvement.

Work by Bavougian et al., (2012) near Crete, NE in ‘Frontenac’ found increases in yield and brix and decreases in TA when trained to GDC as compared to HC, SD, and VSP in 2008.

But in 2009 only increases in yield as compared to VSP and HC were found, with no significant differences in Brix, TA, or pH. The results of our study are consistent with those of Martinson and Particka (n.d.) with the Northern Grapes Project near Clayton NY at Coyote Moon Vineyards. They found increased 'Frontenac' yields Top Wire Cordon (TWC) compared to VSP, but no significant differences were found in fruit chemistry between their training systems in either 2012 or 2013.

Table 9. Point quadrant data collected on 'Frontenac' in Absaraka, ND in 2014.

Treatment	Gaps [z]	Leaf layer [y]	Interior leaves [x]	Interior clusters [w]
	---%---	Leaf number	----%----	-----%-----
HC [v]	18.6 a[u]	1.3 a	19.5 a	24.4 a
GDC	22.2 a	1.2 a	18.5 a	25.2 a
VSP	28.6 a	0.9 a	5.8 a	6.6 a
4AK	18.2 a	1.3 a	14.1 a	30.9 a

[z] Average percentage of the canopy that is open and free of plant material per plant (optimum value between 20-40%)
[y] Average leaf layer number per plant is the number of leaves intercepted by a potential beam of sunlight (optimum value 1.0-1.5 or less)
[x] Average percentage of leaves interior to the canopy per plant (optimum value < 10%)
[w] Average percentage of clusters interior to the canopy of the plant (optimum value < 40%)
[v] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[u] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Due to the mixed response of 'Frontenac' with training systems treatments in past studies, I would assume that our plants were not light saturated, that increased light infiltration is possible, and that malic acid degradation may be the most likely cause for the drop in pH. Trellis system and leaf removal treatments are largely impacted by severity of application, timing, weather and location. Our work supports that at Clayton NY and differs from the findings in Nebraska. 'Frontenac' may be better suited to southern growing regions if a change in acidity is desired.

Pruning Weights and Node Viability

Training system treatments and canopy management were more intensely regulated during the years of the study, than years prior. The less intense management in the years prior to the experiment may account for lack of difference between training system pruning weights in 2013. Pruning weights in 2014 were significantly different between training systems and were greatest in VSP. The increased growth in VSP could be due to its vertical nature as downward positioning of a grape vine reduces vigor. Downward shoot positioning reduces vine growth, cane diameter and lowers pruning weights due to a narrowing of the xylem vessels reducing sap flow and lower hydraulic conductivity associated with a reduction in stomatal conductance of leaves (Schubert et al., 1996; Smart et al., 1982; Lovisolo and Schubert, 2000). Since the VSP vines were the only vines to be positioned vertically this could account for a larger pruning weight in 2013 and a significantly greater pruning weight in 2014 compared to the other training treatments. Additionally, large diameter canes are less winter hardy and more vigorous, vigorous canopies will often grow late into the fall and shoots/buds will not harden off well nor be fruitful (Wilwerth et al., 2014). This could account for greater node mortality in VSP in 2014 compared to all other cultivars.

The increase of node mortality in VSP trained vines may be due to increased vegetative growth in 2014 and potential lack of hardening prior to first frost, or due to injury later in the winter when rapid and large winter temperature fluctuations occurred. Cordons within the VSP system are the closest to the ground and this proximity could result in increased soil radiation in warm days in the winter. Without snow cover, VSP could potentially be subjected to more frequent micro warming and cooling compared to the other training systems, resulting in decreased bud acclimation. In late winter, warm temperatures can promote bud deacclimation,

and buds are injured when temperatures return rapidly to normal subzero conditions (Fennell, 2004). As vines deacclimate, some of the changes inside the cells that allowed them to survive very cold temperatures are reversed. The vascular plugs are digested by enzymes, allowing water to move into proximity of the buds. Hormone levels that kept the cells dormant decline and some of the cryoprotectants that helped dehydrate the cells are metabolized. This allows the cells to rehydrate and freeze at higher temperatures (Ker and Brewster, 2011). Water starts to move into the roots and trunk as storage starches are metabolized into sugars in the xylem. (Wolpert and Howell, 1984). Visual notes from both years seemed to note shoot collapse and cordon collapse was much more frequent in 2014, especially in VSP, which potentially signifies injury to the conductive tissues of the vine. When numerous cells are damaged, the structure and function of the vine can be impaired, injury to phloem and xylem of the cane can restrict movement of water and nutrients, and this can lead to shoot collapse (Wilwerth et al., 2014). The combination of all factors may help explain vine response to the VSP training system.

After the winter of 2014, vines on the GDC and 4AK systems had significantly higher node viability as compared to vines on VSP and HC. This may be due to training system as GDC and 4AK are classified as divided canopy systems, but 4AK is vertically divided and GDC horizontally divided. Divided canopy systems were designed to reduce vigor and improve sunlight exposure (Smart and Robinson, 1991). Increases in light penetration into the canopy can increase periderm formation and also increase carbohydrate storage promoting a greater freezing tolerance (Reynolds and Heuvel, 2009; Wolpert and Howell, 1986). These factors increase cold hardiness and may explain the increased viability of nodes in GDC and 4AK.

Yield

There were no differences in yield in 2013 but there were statistical differences in yield between training systems in 2014. Typically, differences in yield between training systems are due to use of divided canopies, as increases in yield are due to increased amount of nodes and shoot numbers (Reynolds and Heuvel, 2009; Shaulis and May, 1971). However in our instance, there were no differences in nodes retained statistically across training systems in 2014 (Table 4). Vines on GDC and 4AK systems did have the highest amount of live nodes and the greatest amounts of shoots statistically (Table 4 and 5). This could be due to increased cordon length and possible latent node viability, as both GDC and 4AK have the longest cordons (Table 8). However when looking into plant yield, vines on GDC had significantly greater yield and total cluster numbers compared to vines on 4AK even though there were no statistical differences in shoot number between the two treatments (Tables 5 and 6). Furthermore, vines on GDC had a statistically greater yield than vines on the VSP training system, but this was not statistically greater than HC, and HC had the shortest cordon length in both 2013 and 2014. To understand differences, expansive data on bud and subsequent shoot types was taken in 2014.

Shoots were assessed individually to determine if they were derived from count buds, a bud 'counted' during pruning that in optimal conditions would be fruitful, or non-count, a shoot arising from the basal node or a latent dormant bud, historically less fruitful and not included in counts during spring pruning (Sanchez and Dokoozlian, 2005). Additionally, we noted if the shoot was primary, secondary or tertiary. Grapes have compound buds with the primary bud as the most fruitful and the tertiary bud as the least fruitful or no fruit at all, as shown in figures 9 and 10 (Sanchez and Dokoozlian, 2005). It was noted that in non-count basal buds, vines on 4AK and GDC systems did not differ in shoot number, and both were greater than vines on the

VSP system. The number of primary basal shoots counts did not differ in vines in GDC and 4AK systems, but GDC had more primary shoots than 4AK. Secondary basal buds were not statistically different in any of the training systems. Tertiary basal buds did not differ between vines on GDC and 4AK systems, while slightly more tertiary buds occurred with the 4AK system. This indicates that for non-count basal buds, the same number of shoots arose in 4AK and GDC. However, observationally it was found that vines on GDC had more primary shoots while vines on 4AK had more tertiary shoots (Table 11). In non-count latent shoots, again vines on 4AK and GDC had the most shoots, but they were not statistically different in number. Both primary and secondary latent shoot numbers did not statistically differ between training systems. However, vines on 4AK and GDC systems had the highest amount of tertiary, resulting in more shoots for these training systems. So, in both latent and basal non-count nodes, vines on 4AK and GDC systems had a higher amount of shoots (Tables 10, 11, 12, and 13). This trend also occurred for count shoots (Table 14). In count shoots vines on GDC and 4AK had the highest amount and were not statistically different from each other. Though not significant, vines on GDC systems had slightly more primary and secondary shoots than vines on the 4AK system. However, vines on the 4AK system had statistically greater amounts of tertiary count shoots than GDC. Though vines on GDC and 4AK systems did not statistically differ in number of count shoots, vines on GDC tended to have more fruitful count shoots and vines on 4AK had statistically more tertiary shoots, and less fruitfulness. This supplemental data collected in 2014 seems to show that divided canopies can increase shoot number, but that not all divided canopies are equal as 4AK had decreased fruitfulness due to type of shoot.

These differences in shoot type and fruitfulness may be due to temperature differences and exposure to sunlight (Baldwin, 1964; Buttrose, 1969; Sanchez and Dokoozlian, 2005).

Visual differences in vitality of the upper and lower cordons were observed throughout the 2014 summer and during spring pruning, with the lower cordon having less growth and fruit. Shoot data was not separated on upper and lower cordons, but the lack of snow cover and possible increases in radiation from the uncovered soil in the winter may have resulted in more varied temperature and increased bud deacclimation and injury in the lower cordon. Increased shading of the lower cordon due to shoot positioning of the 4AK also may have limited the lower cordon's fruitfulness and impacted the training system's yield. Work by May et al. (1976), Sanchez and Dokoozlian (2005), and Corzo (1978) has shown increased shading decreases bud fruitfulness during the following season.

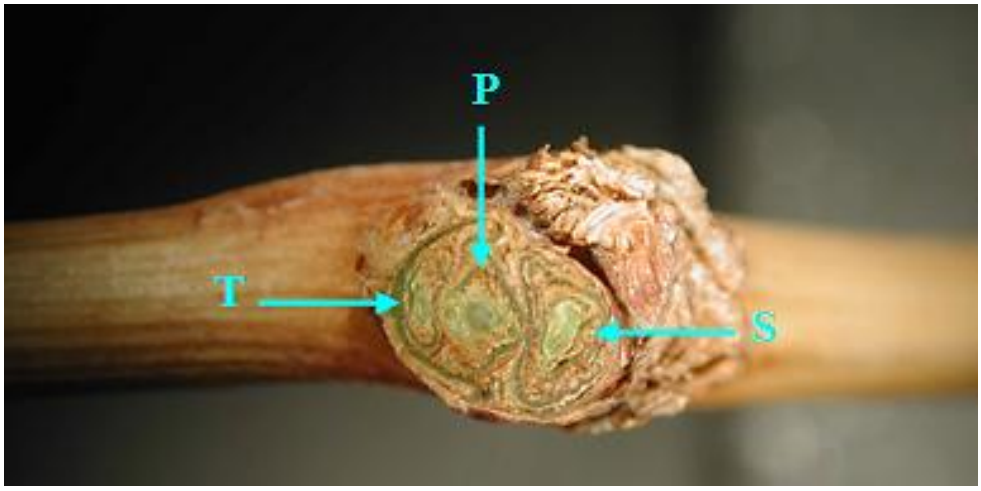

Figure 9. Compound *Vitis* bud, composed of the primary bud (P), secondary bud (S), and tertiary bud (T). Photo credit to Drs. Harold Larsen and Horst Caspari, Colorado State University.

Figure 10. Shoots arising from Primary, Secondary and Tertiary buds in 'Frontenac' and the respective fruitfulness, from left to right (Primary, Secondary, Tertiary) at research vineyard in Absaraka, ND 16 June 2014.

Table 10. Effect of training system in 2014 on bud data; average node viability and average shoot number in Absaraka, ND on 'Frontenac'.

Treatment	Nodes retained [y]	Live nodes [x]	Dead nodes [w]	Total shoots [v]
HC [z]	41.2 a[u]	18.5 bc	15.9 c	33.0 b
GDC	40.3 a	24.2 a	19.3 bc	41.4 a
VSP	42.9 a	14.6 c	22.5 b	23.9 c
4AK	41.0 a	21.6 ab	28.3 a	40.1 a

[z] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[y] Average of nodes retained post pruning per plant
[x] Average of nodes with live growth post pruning per plant
[w] Average of nodes without live active growth post pruning per plant
[v] Average total number of shoots per plant
[u] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 11. Effect of training system in 2014 on non-count basal bud data, primary, secondary and tertiary shoots in Absaraka, ND on 'Frontenac'.

Treatment	NCB total shoot[z]	NCB primary[y]	NCB secondary[x]	NCB tertiary[w]
HC[v]	13.9 a[u]	2.6 a	5.6 a	5.5 a
GDC	13.3 a	2.5 a	5.2 a	5.4 ab
VSP	7.3 b	0.9 b	3.2 a	3.2 b
4AK	15.6 a	1.6 ab	6.5 a	7.4 a

[z] Average Non-count basal=NCB shoots arising from the basal bud per plant, historically these buds are less fruitful and not included in node count in spring pruning
[y] Average of Non-count basal=NCB primary shoots per plant, most fruitful shoot
[x] Average of Non-count basal=NCB secondary shoots per plant
[w] Average of Non-count basal=NCB tertiary shoots per plant, least fruitful shoot
[v] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[u] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 12. Effect of training system in 2014 on non-count latent bud data, primary, secondary and tertiary derived shoots in 'Frontenac' in Absaraka, ND.

Treatment	NCL total shoot[z]	NCL primary[y]	NCL secondary[x]	NCL tertiary[w]
HC[v]	1.8 bc[u]	0.0 a	0.4 a	1.4 bc
GDC	3.0 ab	0.1 a	0.3 a	2.5 ab
VSP	1.3 c	0.0 a	0.1 a	1.1 c
4AK	3.3 a	0.0 a	0.3 a	2.9 a

[z] Average Non-count latent=NCL shoots arising from latent dormant bud per plant, historically these nodes are less fruitful and not included in node count in spring pruning
[y] Average of Non-count latent=NCL primary shoots per plant, most fruitful shoot
[x] Average of Non-count latent=NCL secondary shoots per plant
[w] Average of Non-count latent=NCL tertiary shoots per plant, least fruitful shoot
[v] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin
[u] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 13. Effect of training system in 2014 on non-count shoots derived from basal bud or latent node in 'Frontenac' in Absaraka, ND.

Treatment	Non count total shoot count[z]	NC basal total count	NC latent total count
HC [y]	16.0 a[x]	13.9 a	1.8 bc
GDC	16.6 a	13.3 a	3.0 ab
VSP	8.8 b	7.3 b	1.3 c
4AK	19.0 a	15.6 a	3.3 a

[z] Average Non count total shoot counts per plant, Non count=NC includes basal bud shoots and latent node shoots

[y] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin

[x] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 14. Effect of training system in 2014 on count shoots; primary, secondary, and tertiary derived shoots in 'Frontenac' in Absaraka, ND.

Treatment	Count total shoots[z]	Count primary[y]	Count secondary[x]	Count tertiary[w]
HC [v]	19.4 ab[u]	3.9 bc	6.6 bc	8.9 ab
GDC	24.3 a	6.6 a	9.4 a	7.8 b
VSP	15.1 b	2.3 c	5.1 c	7.5 b
4AK	23.0 a	4.6 ab	7.9 ab	10.5 a

[z] Average count total shoots per plant, historically these shoots arise from nodes that are more fruitful and are included in node count in spring pruning

[y] Average of count primary shoots per plant, most fruitful shoot

[x] Average of count secondary shoots per plant

[w] Average of count tertiary shoots per plant, least fruitful shoot

[v] Abbreviations HC=High Cordon, GDC=Geneva Double Curtain, VSP=Vertical Shoot Positioned, 4AK=four-Armed-Kniffin

[u] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Canopy Density

As stated above, point quadrant data was collected in 2014 to help offer some insight into the canopy structure. Point quadrant data allows us to look at percent gaps in the canopy, leaf layer numbers, percent interior leaves and percent interior clusters (Smart and Robinson, 1991). Percent gaps are optimum between 20-40%, leaf layer number optimum at 1.0-1.5 or less, interior leave percentage less than 10%, and percent interior cluster less than 40% for an optimal canopy (Smart and Robinson, 1991). It was found that no significant differences were found between trellis systems across all measurements however some treatments were found to be outside of optimal ranges (Table 9). Vines on HC and 4AK systems had less than optimal percentage gaps within the canopy, potentially resulting in increased shading compared to vines on GDC and VSP. Only VSP vines had the correct amount of interior leaves, while all other trellis systems resulted in greater than the optimal value, and potentially a dense canopy. Interior clusters and leaf layer numbers were inside the optimal ranges with vines on VSP having the most optimum. According to optimal values, VSP resulted in the correct canopy values for all parameters.

Interestingly, though VSP had the most optimal canopy structure according to these parameters, yield was not increased in this treatment, as vines on GDC and HC had the greatest yield. Canopy structure and light infiltration data would be useful in tandem to find the best fit for a desired outcome. For example, vines on VSP showed better canopy structure in 2014 and had an increase in pH in 2013, however these vines also had vigorous growth in 2013 and severe dieback in 2014. Vines on GDC and HC had increased yield potentially due to increases in sunlight without any negative fruit characteristic effects. Lastly, vines on 4AK had a higher amount of shoots after the hard winter of 2013-2014, similar to vines on GDC, but it had lesser

yields and lacked an effect on fruit quality. As such, the effects of canopy structure and training system effects are notable and with proper use and continued research may assist grape growers throughout North Dakota and the upper Midwest.

Conclusion

Canopy management practices gave increased yield in 'Frontenac' without negatively affecting quality, in the years of our study at the Absaraka research vineyard. Further studies on the effects of canopy management practices to improve fruit quality need to be completed to develop a standard set of recommended viticultural practices for this cultivar and others to optimize fruit quality for winemaking. Increasing the geographical range of this study and additional seasons will aid in reducing the influence of uncontrollable outside variables. Additionally, measurements of potassium concentration, individual acid profile analysis, and proper canopy light infiltration data would give an improved picture of treatment effects and physiological response.

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CHAPTER 2. ACID PROFILE ANALYSIS OF MICROVINIFIED DEACIDIFIED COLD CLIMATE HYBRID GRAPE WINES BY HPLC

Abstract

High acidity is a general characteristic of wine grapes grown in northern regions. Too much acid is problematic, as it can result in unbalanced and unpleasant wines. Present research investigated the deacidification ability of biological and chemical treatments on cold climate hybrid grape wines. The 2013 and 2014 vintages of 'Frontenac', 'La Crescent', and 'King of the North' hybrids, grown near Absaraka and Linton, North Dakota were microvinified and deacidified. Biological treatments included the selected wine yeast species *Saccharomycetes cerevisiae* (Maurivin B and 71B) and bacteria starter culture of *Oenococcus oeni* (ER1A and EY2d) and their capacity to reduce the concentration of malic acid. The ability of the chemical deacidification treatment (cold stabilization) to reduce the concentration of potassium bitartrate, the naturally occurring salt of the grape's tartaric acid, was also determined. Wines were analyzed by HPLC. As expected titratable acidity (TA) of all treatments were significantly lower than that of the control, with greatest reduction resulting from the combined biological and chemical treatments. Wine TA was at most reduced by 59% with Maurivin B, malolactic fermentation (MLF), and cold stabilization, followed by 55% reduction with 71B, MLF and cold stabilization. Yeasts were not significantly different in malic concentrations post MLF, but prior to MLF, Maurivin B resulted in significantly less malic acid compared to 71B. Better peak separation was achieved through sulfonic acid buffered with sodium sulfate and a silica column. Future studies should involve modifying the current conditions to achieve better resolution. This

project and future research will contribute to the optimization of winemaking within our region, and to the production of sustainable high-quality wines.

Introduction

Wine is composed of more than one thousand compounds including, alcohols, carbohydrates, polyphenols, aldehydes, ketones, enzymes, pigments, vitamins, minerals, organic acids, and other not yet identified compounds (Conde et al., 2007). As such, wines are an immensely complex chemical matrix and the factors that distinguish a great wine from the ordinary are still not well understood. However, it is known that all good wines are properly balanced in alcohol, sweetness, tannin, and acidity. Alcohol for viscosity, heat and body, sweetness to balance, soften and highlight fruit flavors, tannin giving structure, astringency, and longevity, and acidity giving tartness, color, clarity, stability, increased aromatics, oxidation rate and biological stability (Koone et al., 2014; Gawel et al., 2007). It has been stated that no component in wine has such extensive and important functions as acidity (Milisavljevic, 1971). Too much acid results in struggling or stuck yeast and malolactic bacteria fermentations, and a sour, sharp, green, and acidulous taste (Alexandre and Charpentier, 1998). Too little acidity results in dull colored wine, a reduction in aromatics, unstable microbe conditions, and bland, flat and flabby wines (Hudelson, 2010). Hence, the amount of acid is very important to the quality of wine, too little or too much across the spectrum is undesirable. Therefore, acidity is carefully managed.

Wine acidity is derived from both the grape and the fermentation process, with the grape being the main contributor. Differences in environmental factors, vineyard management, climate, the cultivar grown, fermentation techniques, stability choices, and winemaker preferences affect the amount of certain acids present and the overall organoleptic experience

(Attia et al., 2004; Bagajewicz et al., 2007; Becker, 1977; Buttrose et al., 1971; Conde et al., 2007; Davis et al., 1985; Ewart, 1987; Fleet, 1993; Hale and Buttrose, 1974; Hunter and Visser, 1990; Jackson, 1991; Kliewer and Gates, 1987; Koblet, 1985; McCarthy and Cirami, 1990; Munyon and Nagel, 1977; Rankie et al., 1971; Saayman and Viljoen-Bloom, 2006; Sepulveda and Kliewer, 1986; Sequin, 1986; Shaulis and May, 1971; Smart, 1982; Volschenk et al., 2006; Winkler, 1954). In regions such as ours with shorter growing seasons and fewer growing degree days (GDDs), high acidity is common, similar to the high acidity found in the famous regions of Burgundy, Champange, Alsace Districts of France, the Piedmont region of northern Italy, the Rhine, and Mosel Valleys in Germany (Becker, 1985, Winkler et al., 1974). However, the grapes grown in our regions are inherently different due to their parentage and typically express acidity levels higher than old world norms.

Cultivars grown in our regions derive their cold-hardiness genes from *Vitis riparia*, a wild grape species native to North America, which have been introgressed into a *Vitis. vinifera* genetic background. These cultivars have expanded the wine grape growing region and although this new, cold climate wine industry is poised for growth, *V. riparia* based cultivars differ from other wine grape cultivars in viticultural and enological ways (Mansfield et al., 2014; Rolfes et al., 2012). These differences require modification of viticultural cultural practices and enological winemaking techniques. Acid reduction is one such enological technique, employed on local *V. riparia* based cultivars to make balanced high quality wine, as it is imperative to the growing wine industry in North Dakota, the surrounding area, and other nontraditional grape growing/wine making regions around the world. Though viticultural management, environmental influences, climate, and grape physiology affect grape acidity, only enological

practices that manage and control heightened acidity will be discussed for the scope of this experiment.

Many enological deacidification treatments affect the acids that make up a wine's acidity differently and these acids help determine the sensory quality of the wine. The major organic acids found are Acetic, Adipic, Ascorbic, Citric, Citramalic, Formic, Fumaric, Galacturonic, Glucuronic, Glutaric, Ketoglutaric, Lactic, Malic, Maleic, Malonic, Oxalic, Propionic, Pyruvic, Shykimic, Succinic, Tannic, and Tartaric acids (Mato et al., 2005). The two acids, tartaric and malic predominate in all stages of grape development that represent the most significant influences on the acidity and pH of grape juice and wine. Tartaric and malic acids account for 69 to 92% of all acids within the grape berries (Lakso and Kliewer, 1975; Morris et al., 1983; Ruffner, 1982) Tartaric acid is a secondary product within grapes formed from the metabolism of glucose and ascorbate and its concentration remains relatively stable as it forms an insoluble salt that is not affected by catabolizable enzymes (Ruffner, 1982; Saito and Kasai, 1968). Similarly, in wine, tartaric acid is not greatly affected by biological acid reduction techniques and is mainly reduced by chemical means. Malic acid, an active intermediate in grape metabolism, is available as an intermediary product synthesized in the Krebs cycle, found in catabolic pathways such as glycolysis, and as a by-product from refixation of CO_2 released during respiration (Ruffner, 1982). Similarly, malic acid remaining in grape juice and wine is affected primarily by biological acid reduction techniques. Other important acids to note in wine are citric, lactic, acetic and succinic. Citric is derived from the grape and lactic, acetic and succinic are present in wine mainly from alcoholic or malolactic fermentations. The combination of these acids along with tartaric, malic, and trace amounts of many other acids determine the overall acid strength.

When ranked by order of potency, tartaric acid has the greatest acid strength followed by lactic, malic, citric, acetic and lastly succinic. This individual acid strength is determined by the

ability of these acids to lose a proton, the dissociation constant (pka). The pka value is the pH at which the acid and anion concentrations are equal. The lower the pka, the stronger the acid and the greater its ability to donate protons (dissociation constants: acetic acid pk 4.8, lactic pk 3.8, succinic pk1 4.2 pk2 5.7, malic pk1 3.5 pk2 5.0, tartaric pk1 3.0 pk2 4.2 citric pk1 3.1 pk2 4.7 pk3 5.0) (Da Conceicao Neta et al., 2007).

Cumulative acid strength is determined by TA and pH. TA is the concentration of free protons and undissociated acids in a solution that react with a strong base and become neutralized (Boulton, 1980). Hence the strength and concentration of each individual acid affects the titratable acid strength and effects the overall cumulative acid strength in the resulting wine. The pH is a concentration term, a negative logarithmic concentration for free dissociated protons in solution, and represents how much acid is in a solution (Boulton, 1980). Here, only free protons determine the strength of the acid at a particular point in time, and pH is a snapshot of the dilution and concentration. The pH of a solution is a concentration affected by buffers, a measure of the degree of relative acidity versus the relative alkalinity on a scale of 0 to 14. As there is no correlation between TA and pH, both are needed to determine a wine's relative acidity.

The recommended ranges of grape juice parameters for optimal red wine quality are juice TA at harvest between 6.0 g/L and 8.0 g/L (Winkler et al., 1974), and pH between 3.4 and 3.5 (Amerine et al., 1972; Dami et al., 2005). Recommended ranges for white table wine quality are juice TA at harvest between 6.0 g/L and 7.5 g/L and a pH between 3.1 to 3.2 (Dami et al., 2005). As our cultivars have higher acidity than traditional grapes, enological deacidification treatments that manage this heightened acidity will be discussed. Acid reduction techniques are divided into three categories physical, chemical and biological.

Physical reduction methods include amelioration, blending, and sugar additions (Gallander, 1977). Amelioration is the blending of water or sugar water to the must to be fermented, diluting the acidity. (Must is the grape juice, seeds, and skins in combination before pressing.) The addition of water is subject to federal regulations and can reduce desired or undesired aromas and flavors (Nagel et al., 1975; Beelman and Gallander, 1979). Blending is the creation of a wine from more than one varietal/cultivar to combine important wine constituents and improving wine quality. Blending is an effective technique in reducing wine acidity given that there is a low acid wine that is available and would benefit from increased acidity (Nagel et al., 1975). Sugar, the addition of small amounts of sugar can reduce the perception of a slight to moderate acidity in wine. Even wines finished in a dry style (0.2-0.3% residual sugar) may benefit in body and mouth feel with the addition of .25 to .45 percent without being noticed on the palate (Steiner, n.d.).

Chemical deacidification methods include calcium carbonate, potassium carbonate or potassium bicarbonate, double salting, ion exchange, and cold stabilization.

Calcium carbonate reduces wine acidity through chemical instability and precipitation. Calcium will react with the grape acids, malic and tartaric to form insoluble salts, preferentially reacting with tartaric. Grape acids can have a negative charge and will react with positively charged calcium forming calcium tartrate and calcium malate, water and carbon dioxide. Calcium tartrate precipitates very slowly and may require months before equilibrium is established. Calcium malate has a higher solubility than calcium tartrate and may not fully precipitate resulting in a salty taste (Nagel et al., 1975; Dharmadhikari, 2001). The solubility of calcium tartrate is much less temperature-dependent than that of potassium bitartrate. Cooling a wine close to its freezing point rarely results in calcium tartrate precipitation (Clark et al., 1988).

Potassium carbonate is generally used for less aggressive deacidification than calcium carbonate and also reduces acidity through precipitation and neutralization. Again, tartaric takes precedence because it dissociates more quickly and is a more available proton donor. The negatively charged grape acids will react with the positively charged potassium and create several salts, (potassium tartrate, potassium bitartrate, potassium malate, and potassium bimalate). Potassium bimalate is soluble in wine and can be difficult or will not precipitate out. Either potassium bicarbonate or potassium carbonate is added, bicarbonate has an ability to remove more acid, carbon dioxide is given off and precipitation slowly occurs and is assisted by a reduction in temperature (Nagel et al., 1975; Dharmadhikari, 2001).

Double salting in theory claims that under certain circumstances calcium carbonate can be used to completely remove both tartaric and malic acids as a calcium tartro-malate salt, completely consuming calcium carbonate in the process so no instabilities and latent precipitations occur (Munz, 1960; Munz, 1961; Steele and Kunkee, 1978). Chemical treatments exploit that fact that adding an acid and base together creates an insoluble solid that can precipitate out. Typically tartaric acid is preferentially reduced. Double salting claims removal of both malic and tartaric acids seeming ideal to winemakers requiring a larger reduction in acid. However, it was found that regardless of ratio of acids not all malic is reduced in the first steps of the process and calcium carbonate is not completely consumed, potentially resulting in latent instabilities and lengthy precipitations. Additionally, the double salt calcium tartro-malate does not form but two separate salts, calcium tartrate and calcium malate (Mansfield and Cook, n.d.). Due to the kinetics of the reaction and the pH of wine, the formation of calcium tartrate is favored over calcium malate and pH manipulations are needed to preferentially form calcium malate. The needed pH conditions for this salt formation may not be possible, and the varying

buffering capacity of individual wines could further influence reactability and success of this treatment, as such double salting is not widely used (Steele and Kunkee, 1978; Nagel et al., 1975; Dharmadhikari, 2001).

Ion exchange requires specific equipment in which the tartrate or malate ions are exchanged with hydroxyl ions, therefore removing them from the wine or must (Beelman and Gallander, 1975).

Cold stabilization is the use of cold temperatures to reduce the solubility of potassium tartrate within a must or wine. Tartaric acid and its salt, potassium bitartrate are normal constituents of wine. Solubility of potassium bitartrate is dependent on alcohol, pH, and temperature, with alcohol and reduced temperatures reducing solubility. Typically, a wine is cooled to -3 to -1^{0}C for one to two weeks until the excess potassium bitartrate precipitates as crystals. Rapid cooling forms smaller crystals, but results in a more complete precipitation and is preferred. Cold stabilization may or may not be seeded with potassium tartrate before chilling commences as this gives a crystalline nuclei and can decrease the amount of time to precipitate (Zoecklein, 1988; Enache and Tofan, 2007).

Biological reduction methods are the most traditional and common methods chosen to reduce acidity and include carbonic maceration, acid metabolism by yeast through maloethanolic fermentation, acid metabolism by lactic acid bacteria through MLF, and genetically engineered yeast (Redzepovic et al., 2003).

Carbonic maceration is a biological method because it is carried out by and within the grape cells themselves. Whole, uncrushed fruit is placed in a carbon dioxide saturated environment, causing the cell to undergo anaerobic metabolism which will respire malic acid

within the grape and reduce the acidity level. Tartaric and citric acids remain unaffected (Beelman and Gallande, 1979; Gadek et al., 1980).

Yeast metabolism, utilizes the fact that all yeast metabolize a certain percentage of malic acid to ethanol in a process called maloethanolic fermentation, if glucose or another assimilable carbon source is present (Ribéreau-Gayon et al., 2006). The species of domesticated yeast used in winemaking, brewing and baking for hundreds or thousands of years is *S. cerevisiae*, which in Latin means sugar fungus (Richter et al., 2013). These strains are evolutionary adapted to the wine environment stresses; low pH, high osmolarity, anaerobic environment, high ethanol concentrations, low nutrient levels, and the presence of SO_2. These adaptations to the harsh wine environment and consistent desirable sensory traits have made *S. cerevisiae* the prime candidate for wine fermentation. However, *S. cerevisiae* are regarded as the most inefficient metabolizers of extracellular malic acid compared to other nontraditional yeasts. The ability of a yeast strain to degrade extracellular malic acid is dependent on the efficient transport of malic acid and the efficacy of the intracellular enzymes (Ansanay et al., 1996; Volschenk et al., 1997). Yeast *S. cerevisiae* uptake of malic acid is via simple diffusion, and it's malic enzyme has a very low substrate affinity. In contrast, yeasts *S. pombe* and *Zygosaccharomyces bailii* can degrade high concentrations of malic acid and have higher substrate affinity and *S. pombe* has an active transport system for malic acid. However, *S. pombe* and *Z. bailii* have negative sensory impacts on wines due to the production of undesirable metabolites such as acetic acid, hydrogen sulfide, or acetaldehyde and their use in wine is limited (Mylona et al., 2016). Hence *S. cerevisiae* yeasts are primarily used in winemaking, but depending on yeast strain chosen, fermentation profiles and metabolic differences can be quite different. Certain strains have the ability to utilize a larger amount of malic acid compared to others (Richter et al., 2013; Volschenk et al., 2006;

Volschenk et al., 2003; Boles et al., 1998; Saayman and Viljoen-Bloom, 2006; Redzepovic et al., 2003).

Lactic acid bacteria classified as *Oenococcus oeni* have the ability to convert glucose to lactic acid, ethanol and acetic acid and most importantly convert malic acid into lactic acid and carbon dioxide by means of the malolactic enzyme. Due to this conversion *O. oeni* are also call malolactic bacteria as they conduct the malolactic fermentation (MLF) of malic into the weaker lactic acid resulting in a smoother less acidic wine. The malolactic bacteria is more tolerant of high alcohol and low pH of most wines compared to other lactic acid bacteria but still are sensitive to low pH, low temperature, high alcohols and high sulfur concentrations. As such, certain parameters must be maintained if a wine is wished to undergo MLF (Wibowo et al., 1985; Bauer and Dicks, 2004; Kunkee, 1967; Amerine and Kunkee, 1968; Davis et al., 1985; Henick-Kling, 1988; Kunkee, 1991; Henick-Kling, 1993).

Genetic engineering was proposed to transfer the malolactic activity of lactic acid bacteria, and the active malic transport of *S. Pombe* into *S. cerevisiae*, enabling simultaneous alcoholic and malolactic fermentations. This was done in the industrial wine yeast Prise de Mousse and created the ML01 yeast, which has received status from the US FDA as "Generally Regarded As Safe" and has been commercialized in the USA and Moldavia. This malolactic wine yeast has resulted in lower volatile acidity, improved color properties and prevents the formation of biogenic amines (Hunsnik et al., 2006).

The treatments above have their advantages and disadvantages and the cost/benefit analysis for ease of use, sensory effects, expense and potential success determines their utilization. This experiment will evaluate the most commonly used acid reduction treatments in our area; MLF, yeasts known for malic consumption, and cold stabilization. Yeasts utilized will

be 71B and Maurivin B, and the malolactic bacteria Wyeast 4007 blend of cultures ER1A and EY2d.

Lallemand 71B was isolated in INRA (Narbonne at the Institut national de recherche en agriculture) by Jacques Maugenet in 1971. Maugenet characterized this strain as able to produce an aromatic wine from a neutral grape juice in the Narbonne-Montpellier area. Lallemand first tested the active dried form of the Lalvin 71B in Beaujolais in 1980 - 1982, and later introduced it in other regions producing young red wines. Lallemand started offering this yeast commercially in active dried form in North America during the early 1980's. The 71B strain is a rapid starter with a constant and complete fermentation between 15° and 30°C and has the ability to metabolize high amounts (20% to 40%) of malic acid. In addition to producing rounder, smoother, more aromatic wines that tend to mature quickly, it does not extract a great deal of phenols from the must so the maturation time is further decreased. Yeast 71B is used primarily by professional winemakers for young wines and has been found to be very suitable for blush and residual sugar whites. For grapes in regions naturally high in acid, the partial metabolism of malic acid helps to soften the wine (G. Specht, personal communication, November 8, 2015). Yeast 71B is widely used with estimates of 25% of total yeast sold by Northern Brewer, and the leading seller at country cannery in Moorhead MN (R. Stroh, personal communication, October 8, 2015).

Yeast Maurivin B (Mauri Yeast Australia PTY LTD, Toowoomba Queensland Australia) is a popular yeast for red winemaking, recognized for its ability to metabolize malic acid, enhance color and varietal fruit characters as well as produce a lower ethanol yield. Trials undertaken at the Bordeaux Wine Institute showed Maurivin B to consume on average up to 56% of malic acid during fermentation. Maurivin B has the capacity to convert up to 18% (w/v) of

the starting sugar into metabolites other than ethanol. As a result, the ethanol concentration in the final wine is lower when fermenting with this strain. The optimum temperature range for Maurivin B is 25–30°C (J. Mabbett, personal communication, September 24, 2014).

Wyeast 4007 blend (Wyeast laboratories, Hood River, OR) is a blend of ER1A and EY2d *O. oeni* cultures providing rapid and complete malic acid reduction in wine over a broad spectrum of conditions. ER1A was isolated for its low pH tolerance 2.9, and Ey2D was selected for it tolerance to low cellar temperatures of 8°C. These cultures were isolated by Oregon State University from malolactic fermentations occurring at Eyrie Vineyards and Knudsen-Erath Winery in 1978 (Henick-Kling, 1982; Watson and Heatherbell, 1983; Watson et al., 1984)

The treatments of acid reducing yeast, MLF, and cold stabilization will be applied to three cold climate wine grapes grown in our region and known for their high acidity, 'Frontenac', 'La Crescent', and 'King of the North'.

'Frontenac' a 1996 University of Minnesota breeding program release from a cross of Landot 4511 (Landal L.244 X Villard blanc) and *V. riparia* clone #89 found near Nordan, MN. 'Frontenac' is currently the most planted grape cultivar in Minnesota due to its extreme cold hardiness and suitability for wine production. 'Frontenac' has a reported 34,260 vines making up 20% of the total vineyard plantings in Minnesota in 2007 (Mansfield, 2008; Mansfield and Vickers, 2009). 'La Crescent' a University of Minnesota release is an interspecific hybrid containing 45% *V. vinifera*, 28% *V. riparia*, and less than 10% each of *V. rupestris*, *V. labrusca*, and *V. aestivalis*. It was crossed in 1988 and selected for release in 2002 and is reported to produce an excellent quality white wine (Rolfes, 2014). 'King of the North' a cross between *V. labrusca* and *V. Riparia*, is consistently productive and vigorous, producing 5-6 times more growth per year than any other in its climate (MacGregor, 2006). 'King of the North' establishes

quickly, ripens early, fruits at a young age, but its high levels of TA and low pH greatly limit its winemaking styles (MacGregor, 2006; Hatterman-Valenti et al., 2014).

This study will give a snapshot of deacidification treatment effects and resulting acid profiles in the unique chemical matrixes of cold climate cultivars. These results may help local winemakers better predict potential outcomes for traditional practices and determine if grapes can be used for certain winemaking styles. This study and future research hopes to contribute to the optimization of winemaking within our region, and to give insight into the challenges of cold climate vinification.

Materials and Methods

Experimental Design

The experiment was set up as a randomized complete block design (RCBD) with factorial arrangement 3x2x2x2+1 of three cultivars ('La crescent', 'Frontenac', and 'King of the North'), two yeast strains (Lalvin 71B and Maurivin B), two MLF treatments (+ malolactic bacteria), two cold stabilization treatments (+ cold period), and a control juice. Twenty-seven experimental units per replicate and three replications resulted in 81 bottles per run. The experiment was repeated twice for the 2013 & 2014 vintages.

Production of Grapes and Juice

Grapes were harvested in 2013 and 2014 from 'La Crescent', 'Frontenac', and 'King of the North' vines grown at the North Dakota State research vineyard, Absaraka, ND. However, due to harsh winter conditions of 2013, the 2014 'La Crescent' harvest was supplemented with grapes grown near Linton, North Dakota by grower, Bill Baumgartner. Grapes were left to hang until they reached desired fruit characteristics or the threat of impending frost (Table 15). All cultivars of each vintage were crushed, destemmed, and pressed 12-48 hrs post-harvest by an

electric crusher destemmer (Baesso, Curtarolo, Italy) and a 40 L bladder press (Marchisio and Pillan, Italy) around 20psi. All red grapes were processed and vinted as rosés. Pressed must (38L) per cultivar was treated with 40 ppm potassium metabisulfite. The must was mixed sealed and placed in a cooler at 1.8° C for 48 hrs, then transferred to triple lined, 7.5 L, polyethylene bags and frozen at -23°C.

Juice Characteristics

Table 15. Juice Characteristics of 'Frontenac', 'La Crescent' and 'King of the North' immediately after press and again after frozen from Absaraka and Linton, ND in 2013 and 2014.

	Cultivar		**pH**	**Soluble solids**	**TA**
			-- -log[H+] --	----%-----	---g/L tartaric----
2013	'Frontenac'[z]	fresh	3.13	25.35	13.5
		Frozen	3.09	24.2	13.25
	'La Crescent'[y]	fresh	2.89	20.25	17.6
		Frozen	2.78	19.6	16.6
	'King of the North'[z]	fresh	3.0	19.5	21.6
		Frozen	2.88	20.5	18
2014	'Frontenac' [x]	fresh	3.0	24.3	19
		Frozen	2.87	24.4	13.25
	'La Crescent'[x]	fresh	3.02	22.6	14
		Frozen	3.07	22.9	15.89
	'King of the North'[x]	fresh	3.02	16.2	18.38
		Frozen	2.82	15.6	21.2

[z] harvested October 9th, Absaraka, ND
[y] harvested September 13th, Linton, ND
[x] harvested October 8th, Absaraka, ND

Climate

Weather data was collected to explain variations in the fruit over the two growing seasons, data was taken from the nearest weather station to the commercial vineyard site, the Prosper NDAWN weather station. Data and graphical information was accessed from NDAWN website (NDAWN, 2015). In 2013, the last spring frost was the 12 May and the first fall frost was the 13 Oct. which resulted in 155 days between frost events, 1428 GDDs (10C)

accumulation and 152 calendar days from the last spring frost until harvest with 1417GDDs accumulated in that time. In 2014, the last spring frost was the 16 May and the first fall frost was the 13 Sep. which resulted in 121 days between frost events, 1156 GDDs accumulated between events, and 146 days from last spring frost till harvest, which accumulated 1294 GDDs.

Winemaking

2013 and 2014 vintages were treated in the same manner. Frozen must was removed from the freezer and thawed in 48-60 hours in sanitized Rubbermaid® containers. Specific gravity, brix, and potential alcohol were determined by hydrometer and adjusted to correct values due to temperature differences. A total of 375mL of 'Frontenac' 'La Crescent' and 'King of the North' must was measured and pumped with a Masterflex Digi-staltic 7527-34 Peristaltic pump (Cole Palmer, Vernon Hills, IL) into 81, 750mL, clear claret/Bordeaux bottles without punt. Nine control bottles were evaluated for final pH, TA, and HPLC analysis, three of each cultivar. Juice TA and pH were determined using standard methods with an Orion star series A111 bench top pH meter (Thermo Scientific, Beverly, MA) (Iowa State University Extension and Outreach, 2013). Soluble solids were determined by a portable pocket refractometer (pal-1, ATAGO, Tokyo, Japan). HPLC methods are discussed below. Yeast *S. cerevisiae*, Lalvin 71B-1122 (Lallemand Inc., Montréal, Canada), and Maurivin B (Mauri Yeast Australia PTY LTD, Toowoomba Queensland Australia) were chosen for malic acid reduction capabilities. Yeast 71B-1122 is a popular acid reduction choice in the upper Midwest and has been shown to reduce 35.7% malic acid (Richter et al., 2013). Yeast Maurivin B is a young strain that may have the potential to consume 56% malic acid. Yeast rehydration nutrient (Go-Ferm; Lallemand Inc., Montréal, Canada) was prepared at a concentration of 0.396g/L in 30mL of 43˚C water. The mixture was allowed to cool to 40˚C, and then yeast was added at the rate of 0.33 g/L to start the

rehydration process. After 15 minutes, 15mL juice (16˚C) was added to the yeast mixture and allowed to sit an additional 15 minutes. This process was repeated until the yeast mixture temperature dropped to within 10˚C of the must temperature, in order to prevent yeast cold shock. When the yeast/juice mixture reached the proper temperature, it was delivered to each bottle. Due to differences between treatments, the remaining vinification protocol will be explained separately (Table 16).

Table 16.Deacidification treatments applied to 'Frontenac', 'La Crescent', and 'King of the North' varietals from Absaraka and Linton, ND in 2013 and 2014.

Treatment	Yeast	MLF[z]	Cold Stabilization[y]
0	None	No	No
1	Maurivin B	No	No
2	Maurivin B	No	Yes
3	Maurivin B	Yes	No
4	Maurivin B	Yes	Yes
5	71B	No	No
6	71B	No	Yes
7	71B	Yes	No
8	71B	Yes	Yes

[z]MLF=Malolactic Fermentation, the secondary fermentation, the conversion of malic acid into lactic, treatments labeled 'yes' were inoculated with malolactic bacteria to initiate this fermentation, treatments labeled 'no' were not inoculated.
[y] Cold stabilization is the chilling of the wine to reduce potassium bitartrate solubility, treatments labeled 'yes' were subjected to chilling temperatures, treatments labeled 'no' were not.

Treatments 1 and 5

After yeast addition of either Maurivin B or 71B, lysozyme was added. Granular lysozyme, Lysovin, (Scott Laboatories, Petaluma, CA) is an enzyme from egg white that has lytic activity against lactic acid bacteria. The granular lysozyme was applied at a rate of 0.60 g/L as a protectant to prevent MLF. After additions, three-piece air locks and labels were added to

bottles and ambient temperature was maintained between 22-23.8 ˚C. Internal temperature and soluble solids were measured daily to monitor fermentation. A pocket refractometer was used to estimate the fermentation progress, as small sample sizes prevented the use of hydrometer. Once all wines had depleted a quarter of their sugars the complete yeast nutrient (Fermaid K; Scott Laboatories, Petaluma, CA), was added at a rate of 0.26g/L. Wines were determined dry by Clinitest tablets (Bayer Helth Care LLC, Mishawaka, IN). White wines were considered dry at 0.1-0.2% residual sugar, while red wines were considered dry at 0.2-0.3% residual sugar. Wine phenolics cause a 0.2-0.3% elevation in clinitest results, so red wines were determined dry at 0.4-0.6 residual sugars by clinitest tablet. Once wines were considered dry, they were racked into sanitized 375mL bottles. To prevent unwanted microbial activity, additions of 0.8 mg/L molecular potassium metabisulfite 'sulfited' was added according to pH. Air locks were removed and bungs were placed. Wines were racked a second time and samples were taken for HPLC analysis, pH, and TA.

Treatments 2 and 6

In treatments 2 and 6, fermentation was completed using the same methods as treatments 1 and 5. Post fermentation wines were subjected to cold stabilization to reduce tartaric acid. Liquids can absorb increasing amounts of gas at lower temperatures so airlocks were exchanged with bugs. Wines were placed in a chilling chamber (Revco, Asheville, NC) at -3˚C for two weeks. Temperature and time was based on alcohol content of the wine, the closer the temperature to freezing at a particular alcohol percentage the less amount of time required to cold stabilize a wine. Once cold stabilized, wines were racked to eliminate bitartrate crystals and samples were taken for HPLC acid analysis, pH and TA.

Treatments 3 and 7

Post yeast addition, three-piece air locks and labels were added and room temperature was maintained between 22-23.8 ˚C as previously explained. Internal temperature and soluble solids by pocket refractometer were similarly measured daily to monitor fermentation. Due to difficult juice conditions, malolactic bacteria co-inoculation was utilized in hopes to reduce bacteria stress. On the second day of fermentation liquid cultures of malolactic bacteria (MLB) *O. oeni* was added, 4007 Blend (Wyeast laboratories, Hood River, OR). The 4007 Blend consisted of two different cultures; ER1A, has a tolerance to low pH conditions, and Ey2D, was suggested for tolerance to low cellar temperatures. Juices from both vintages were highly acidic, and a strain tolerant to low pH was critical. Due to harsh juice conditions, the liquid MLB culture was treated and hydrated, at a rate of 0.34g/L for 15 minutes with a nutrient, Acti-ML (Scott Laboratories, Petaluma, CA) prior to addition. A mixture of 3.5mL MLB/Acti-ML was added to each MLF treatment bottle, resulting in 1.2 x 10^9 viable cells/mL, <1.0 cfu/ml total bacteria, and <1.0 cfu/ml wild yeast & mold (Malo-Lactic Cultures, n.d.). When 25% of sugars were depleted an addition of Fermaid K (Scott Laboatories, Petaluma, CA), was added at a rate of 0.26g/L. Wines were determined dry by Clinitest tablets (Bayer Helth Care LLC, Mishawaka, IN) following the same residual sugar percentages as listed above. Post primary fermentation wines were given a second equal addition of MLB and ActiML as previously described. Wines with MLF treatments took some time to start fermenting, so room temperature was dropped to 18 ˚C for 5 days and fermentation was visibly noticeable at this time. Wines with MLF were monitored with paper chromatography. Once wines were completed with MLF according to paper chromatography they were allowed to sit for three days to metabolize any malic not detectable by paper chromatography. Paper chromatography's lower limit of detection is rather

high at 100mg/L malic acid, and MLF isn't considered safely complete until the malic acid concentration is below 30 mg/L. After three days they were racked sulfited to 0.8 mg/L molecular according to pH and samples were taken for HPLC, pH, and TA.

Treatments 4 and 8

All vinification techniques were the same as treatments 3 and 7 except post MLF. Post MLF, the wines were placed in a chilling chamber (Revco, Asheville, NC) at -3˚C for two weeks. Once cold stabilized, wines were racked to remove bitartrate crystals, sulfited and sampled for HPLC acid analysis, pH and TA.

Analysis of Wines

Samples were taken for pH, TA and HPLC analysis. The TA was determined by titration with electrode to an endpoint of 8.2, and represented as tartaric acid equivalents (Iowa State University Extension and Outreach, 2013). The HPLC was used to analyze organic acids with the assistance of Dr. Narayanaganesh Balasubramanian at the Core Synthesis Lab, North Dakota State University. Wine samples were injected without dilution by filtering through 0.45um PTFE filter, juice samples were diluted before injection (0.5mL sample with 1.0mL water). For standardization, five concentrations of calibration mixtures were prepared for the five organic acids (malic, tartaric, citric, lactic and succinic acids). Tartaric acid and succinic acid were obtained from Aldrich (Milwaukee, WI), formic acid, L(-) malic acid, L(-) lactic acid, and L(-) tartaric acid were obtained from Fluka (Buchs, Switzerland). HPLC was equipped with UV detector and an Autosampler. Analyses were preformed in Shimadzu 2010 HT. Juices were run with a mobile phase consisting of diluted phosphoric acid at a pH of 2.2, a column water Iterra RP 18 (250x4.6) mm 5 um, with a flow rate 1.0 mL/min, ambient column temperature, injection volume of 1 uL, and a wavelength of 210nm. Wine samples were run with a mobile phase

methane diluted sulfonic acid at pH 2.4, the Acclaim organic column (250x4.6)mm 5umm, with a flow rate 0.6mL/min, column temperature at 30^0C, injection volume of 1 uL, and a wave length 210nm. The HPLC chromatographs are shown in Figs. 11 and 12. for a calibration mixture and wine sample.

Statistical Analysis

Statistical analyses of the data were preformed utilizing SAS 9.3 statistical package (SAS Institute Inc., Cary, NC). Data from 2013 and 2014 were combined after the 10-fold f-test method confirmed the homogeneity of variance ratio differed by less than 10 (Tabachnik and Fidell, 2001). The Proc mixed method was used to perform an analysis of variance on the data. Differences were determined by pairwise t-tests, significance of these differences were determined based on a 95% level of confidence on all comparisons.

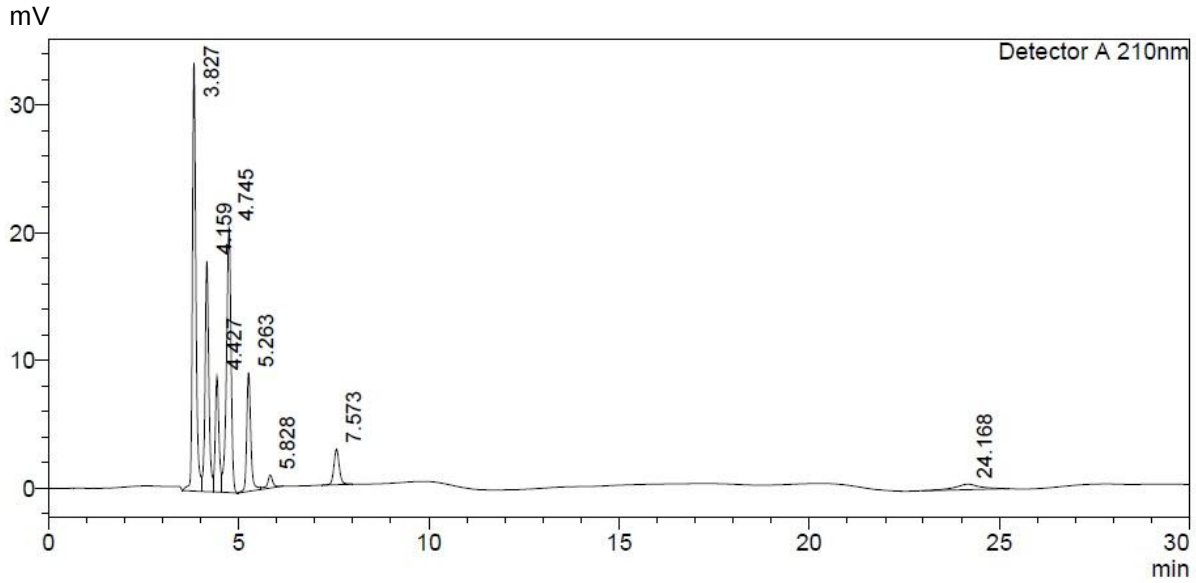

Figure 11. Chromatogram of calibration mixtures used as a standard, showing the separation of tartaric, malic, lactic, citric and succinic acids. Conditions: mobile phase methane diluted sulfonic acid pH 2.4, Column Acclaim Organic (250x4.6)mm 5umm, Flow rate 0.6mL/min, column temperature 30^0C, injection volume 1 uL, wave length 210nm. Peak 1 with a retention time of 3.827, tartaric acid; peak 2 with a retention time of 4.159, malic acid; peak 3 with a retention time of 4.427, lactic acid; peak 4 with a retention time of 4.745, citric acid; peak 5 with a retention time of 5.263, succinic acid, remaining peaks unidentified.

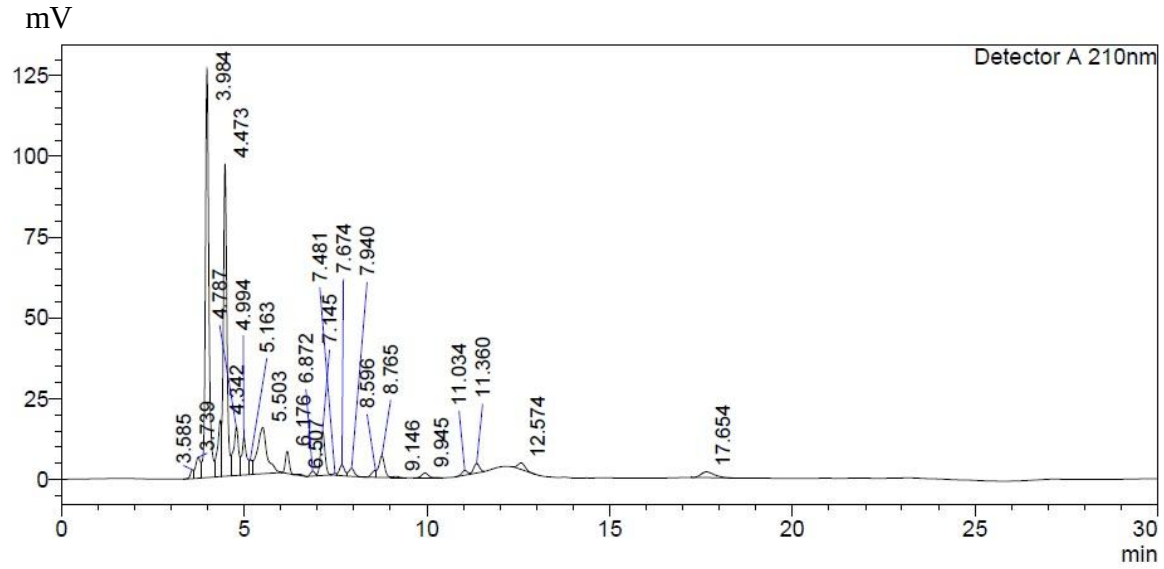

Figure 12. Chromatogram of 2013 vintage 'La Crescent', Rep 1 treatment 5 wine sample (fermented by 71B only), showing the separation of tartaric, malic, lactic, citric and succinic acids with other unidentified substances. Conditions: mobile phase methane diluted sulfonic acid pH 2.4, Column Acclaim Organic (250x4.6)mm 5umm, Flow rate 0.6mL/min, column temperature 30^0C, injection volume 1 uL, wave length 210nm. Peak 3 with a retention time of 3.984, tartaric acid; peak 5 with a retention time of 4.473, malic acid; peak 6 with a retention time of 4.787, lactic acid; peak 7 with a retention time of 4.994, citric acid; peak 8 with a retention time of 5.163, succinic acid, remaining peaks unidentified.

Results

Data Interpretation

Interaction of vintage by deacidification treatment and interaction of cultivar by deacidification treatment were found to be significant for tartaric and malic acids. However, only differences of treatments within a vintage or within a cultivar will be discussed, as the comparison between cultivars and vintages for a particular wine treatment is not of interest as they are not compared to the correct control juice. For all remaining variables (lactic, citric, and succinic acids, pH and TA) the interaction of vintage by cultivar by deacidification treatment was found to be significant. However only significance within a single cultivar within a single vintage will be discussed. Through the test of simple effects, the three way interaction was

analyzed by holding constant vintage and cultivar, enabling us to view variation between deacidification treatments within the three way interaction. This was done, as differences between vintages and between cultivars were not of interest and differences among treatments within a single cultivar within a single vintage were of interest.

Tartaric Acid

Deacidification treatments within vintages were found to be different (Table 17). In 2013 the must/control treatment was highest in tartaric acid concentration. All other treatments were vinified and significantly lower in tartaric as compared to the control. Treatments that were only yeast fermented (treatments 1 and 5) had the highest amount of tartaric acid for all vinified treatments. Treatment 1 was Maurivin B fermented, and treatment 5 was 71B fermented. Descending in concentration were treatments of yeast and malolactic bacteria fermented, (treatments 7 and 3). These treatments were different from all other treatments but not each other. Treatment 7 was MLB and 71B fermented and treatment 3 was MLB and Maurivin B fermented. Lowest in tartaric concentration, but not different from each other, were treatments that had undergone cold stabilization (treatments 8, 4, 6, and 2). These treatments resulted in a 79-83% reduction of tartaric acid.

In 2014 the must/control treatment was highest in tartaric acid concentration (Table 17). All other treatments were vinified and significantly lower in tartaric as compared to the control. Treatments that were only yeast fermented (treatments 5 and 1), or yeast and malolactic bacteria fermented (treatments 3 and 7), had the highest amount of tartaric acid for all vinified treatments, but were lower in tartaric concentration from the control. Treatments 5, 1, 3, and 7 were different from all other treatments, but were not different from each other. Descending in tartaric concentration, treatment 4 was fermented by Maurivin B and malolactic bacteria and also cold

stabilized. Treatment 4 differed from all other treatments except treatment 8. Treatment 8 was also cold stabilized and had undergone MLF but was fermented using 71B. Treatment 8 was not different from treatment 2, and treatment 2 was not different from treatment 6. Treatment 6 was lowest in tartaric concentration and both treatments 6 and 2 were only yeast fermented and cold stabilized. Treatment 6 was fermented with 71B and treatment 2 was fermented by Maurivin B. These treatments resulted in a 72-78% reduction of tartaric acid.

Table 17. Effects of deacidification treatments on tartaric acid concentration means within year by HPLC.

Treatment [z]	2013		2014	
	----------g/L tartaric----------			
0	6.28	a[y]	5.93	a
1	4.20	b	5.09	b
2	1.06	d	1.64	de
3	3.58	c	4.80	b
4	1.17	d	2.40	c
5	4.13	b	5.10	b
6	1.08	d	1.59	e
7	3.65	c	4.74	b
8	1.27	d	2.06	cd

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.
[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Deacidification treatments within cultivars were found to be different (Table 18). Within 'Frontenac' and 'La Crescent' the control treatment had the greatest concentration of tartaric acid, while treatments that had undergone cold stabilization were lowest in tartaric acid. In

'King of the North' the control was not different from treatments that had only been yeast fermented (treatments 1 and 5). Treatments that had undergone cold stabilization were again lowest in tartaric concentration and differed from all other treatments.

Table 18. Effects of deacidification treatments on tartaric acid concentration means within cultivar determined by HPLC.

Treatment[z]	Frontenac	La Crescent	King of the North
	----------g/L tartaric----------		
0	7.02 a[y]	5.92 a	5.37 a
1	4.62 b	3.98 b	5.33 a
2	1.69 d	0.95 c	1.40 c
3	4.42 b	3.63 b	4.52 b
4	2.31 c	1.43 c	1.63 c
5	4.68 b	3.92 b	5.25 a
6	1.63 d	0.96 c	1.41 c
7	4.31 b	3.75 b	4.54 b
8	1.91 cd	1.77 c	1.30 c

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.
[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Malic Acid

Treatments within vintages were found to be different in malic acid concentration (Table 19). In 2013, the control was significantly higher in malic acid concentration and differed from all other treatments. Malic concentration decreased in all remaining treatments with treatments 6 and 5 having the second highest malic acid concentration. Treatments 6 and 5 were fermented by the yeast 71B and resulted in a 47% reduction in malic acid concentration compared to the

control and did not differ from each other. Treatments 1 and 2 had the next lowest malic acid concentrations and were fermented by yeast Maurivin B, they reduced malic acid by 62% compared to the control and did not differ from each other. The lowest level of malic acid was in treatments 7, 8, 3, and 4 as they resulted in a 94-97% reduction of malic acid compared to the control. All of these treatments had undergone MLF and were not significantly different from each other.

Table 19. Effects of deacidification treatments on malic acid concentration means within year by HPLC.

Treatment [z]	2013	2014
	----------g/L malic ----------	
0	9.50 a [y]	8.01 a
1	3.69 c	5.07 d
2	3.61 c	4.45 e
3	0.28 d	0.28 f
4	0.26 d	0.29 f
5	5.10 b	6.76 b
6	5.16 b	5.68 c
7	0.53 d	0.37 f
8	0.38 d	0.33 f

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.
[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

In 2014, the malic concentration results were similar to those in 2013 (Table 19). The control had the greatest malic acid concentration and differed from all other treatments. Treatments that were fermented by 71B had a 16-29% reduction in malic acid as compared to the

control, and treatments fermented by Maurivin B had a 37-44% reduction in malic acid as compared to the control. The MLF treatments 7, 8, 3, and 4 were again lowest in malic acid concentration resulting in a 95-96% reduction of malic acid compared to the control and did not differ from each other.

Table 20. Effects of deacidification treatments on malic acid concentration means within cultivar determined by HPLC.

Treatment [z]	Frontenac	La Crescent	King of the North
	----------g/L malic----------		
0	7.88 a [y]	10.40 a	8.00 a
1	3.71 c	4.14 c	5.29 c
2	2.98 d	4.09 c	5.03 c
3	0.41 e	0.26 d	0.18 d
4	0.44 e	0.24 d	0.15 d
5	5.09 b	6.26 b	6.44 b
6	4.26 c	5.80 b	6.20 b
7	0.65 e	0.43 d	0.27 d
8	0.61 e	0.32 d	0.14 d

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.
[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Treatment effects within cultivars were also found to be significant and findings were similar in the prior interaction (Table 20). In all cultivars, the malolactic treatments (treatments 7, 8, 3, and 4), were lowest in malic acid concentration and differed from all other treatments, but not each other. In both 'La Crescent' and 'King of the North' treatments that did not undergo MLF had differences in malic acid concentration by yeast type. Treatments Maurivin B

fermented reduced malic acid concentration to a greater extent than treatments fermented by 71B.

Lactic Acid

In 2013, within 'Frontenac', 'La Crescent' and 'King of the North', lactic acid concentrations were greatest in treatments that underwent MLF (treatments 3, 4, 7, and 8), and these treatments did not differ from each other (Table 21). In 2014, 'Frontenac' lactic concentrations were also greatest in treatments that had underwent MLF treatments 3, 4, 7, and 8. In 2014, 'La Crescent' lactic concentration was greatest in malolactic treatments that had bee fermented by the yeast strain 71B, followed by treatments 3 and 4 that had been fermented by yeast strain Maurivin B. In 2014, the 'King of the North' malolactic treatments had the highest amount of lactic acid except for treatment 8, which was fermented by 71B and cold stabilized. Treatment 8 was significantly different from all other malolactic treatments and had a lower lactic acid concentration.

Citric Acid

In 2013, the 'Frontenac' MLF treatments and control treatment had higher citric acid concentrations than in non-malolactic treatments (Table 22). In 2013, the 'La Crescent' MLF treatments also had higher citric acid concentrations than non-malolactic treatments except treatment 3 and 5 which were not different from each other. Treatment 3 was malolactic fermented and treatment 5 was not. In 2013, the 'King of the North' MLF treatments 3 and 7 did not differ from the control and had the greatest amount of citric acid. However, the MLF treatments 8 and 4 were significantly different from the control and had lower amounts of citric acid. In 2014, the greatest citric acid concentration was in the control treatment for all cultivars. In 'Frontenac' and 'La Crescent' malolactic treatments differed from non-malolactic treatments

and had higher concentration of citric acid. In 2014, the 'King of the North' MLF treatments were not significantly different from non-MLF as a whole.

Succinic Acid

Succinic concentration was low in the control. Succinic acid was also low in MLF treatments in all cultivars for both years in comparison to non-malolactic fermented treatments, except in 'Frontenac' in 2014 where the opposite was observed (Table 23).

Titratable Acidity

In 2013 for both 'La Crescent' and 'King of the North' all treatments were significantly different from every other treatment (Table 24). The control had the highest TA, followed by treatments with only yeast fermentation (5 and1), the next lowest in TA were treatments with yeast fermentation and cold stabilization (6and 2), followed by yeast fermentation and MLF treatments (7&3), and lastly treatments yeast fermented, malolactic fermented, and cold stabilized had the lowest TA (8&4). Treatments fermented by 71B (5,6,7, and 8)had higher TA than their similar treatments with Maurivin B. The strongest treatment was treatment 4, Maurivin B yeast fermented, MLF and cold stabilization it resulted in a 51-57% reduction in TA from the control.

In 'Frontenac' for 2013 the trend was identical to 'La Crescent' and 'King of the North' in 2013 but treatments 7 and 2 did not differ from each other, treatment 2 was Maurivin B fermented and cold stabilized, where treatment 7 was 71B fermented and had undergone MLF. Yet again treatment 4 was strongest at TA reduction with 57% reduction in TA from the control.

Table 21. Effects of deacidification treatments on lactic acid concentration means within cultivar within year as determined by HPLC.

Treatment[z]	Frontenac				La Crescent				King of the North			
	2013		2014		2013		2014		2013		2014	
	g/L lactic											
0	1.46	a[y]	1.04	a	1.46	a	1.33	a	1.57	a	1.09	a
1	3.55	bc	3.59	c	4.69	b	3.09	b	4.97	c	3.77	b
2	4.11	cd	3.14	bc	4.82	b	2.85	b	4.98	c	3.35	b
3	5.98	e	9.26	d	7.83	c	7.56	c	8.93	d	9.21	d
4	6.16	e	9.42	d	8.04	c	7.92	c	8.56	d	9.00	d
5	2.56	ab	2.90	bc	2.51	a	3.13	b	3.14	b	1.94	a
6	2.32	ab	2.31	b	2.15	a	2.92	b	2.65	ab	1.88	a
7	6.20	e	9.56	d	8.80	c	10.39	d	9.22	d	9.10	d
8	5.10	de	9.86	d	8.58	c	9.90	d	8.62	d	6.20	c

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.

[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 22. Effects of deacidification treatments on citric acid concentration means within cultivar within year as determined by HPLC.

Treatment[z]	Frontenac		La Crescent		King of the North	
	2013	2014	2013	2014	2013	2014
	----------g/L citric----------					
0	1.71 a[y]	1.79 a	1.07 a	1.42 a	1.26 a	1.29 a
1	0.80 de	0.70 d	0.52 d	0.69 c	0.52 cd	0.47 cd
2	0.55 e	0.40 ef	0.41 d	0.48 c	0.39 d	0.21 e
3	1.63 ab	1.11 c	0.79 bc	0.99 b	1.32 a	0.67 bc
4	1.41 bc	1.19 c	0.89 ab	1.13 b	0.62 cd	0.51 cd
5	0.86 d	0.61 de	0.63 cd	0.69 c	0.67 bc	0.54 cd
6	0.55 e	0.34 f	0.27 d	0.49 c	0.42 cd	0.34 de
7	1.65 ab	1.33 bc	0.97 ab	1.16 b	1.21 a	0.82 b
8	1.20 c	1.48 b	0.93 ab	0.98 b	0.90 b	0.54 cd

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.

[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 23. Effects of deacidification treatments on succinic acid concentration means within cultivar within year as determined by HPLC.

Treatment[z]	Frontenac		La Crescent		King of the North	
	2013	2014	2013	2014	2013	2014
	g/L succinic					
0	0.00 a[y]	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
1	0.65 d	0.88 d	0.59 b	0.75 d	0.52 de	0.36 cd
2	0.63 d	0.54 c	0.45 b	0.42 bc	0.48 cde	0.04 a
3	0.50 cd	1.43 e	0.02 a	0.12 a	0.30 b	0.02 a
4	0.34 bc	1.66 f	0.14 a	0.37 b	0.29 b	0.13 ab
5	0.67 d	0.50 c	0.45 b	0.56 c	0.59 e	0.50 d
6	0.54 d	0.27 b	0.08 a	0.29 b	0.34 bc	0.22 bc
7	0.22 b	1.32 e	0.12 a	0.06 a	0.43 bcde	0.03 a
8	0.26 b	1.72 f	0.17 a	0.02 a	0.38 bcd	0.02 a

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.

[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 24. Effects of deacidification treatments on titratable acidity concentration means within cultivar within year as determined by HPLC.

Treatment	Frontenac				La Crescent				King of the North			
	2013		2014		2013		2014		2013		2014	
	g/L tartaric acid											
0	13.25	a	18.40	a	16.60	a	15.87	a	18.00	a	21.20	a
1	9.87	c	13.61	c	12.97	c	10.19	d	13.66	c	15.28	c
2	8.27	e	12.27	d	11.00	e	9.85	e	11.47	e	13.69	e
3	7.50	f	10.00	f	9.39	g	7.57	g	9.00	g	11.83	f
4	6.22	h	8.55	h	7.97	i	6.50	i	7.67	i	9.72	h
5	10.50	b	15.00	b	14.08	b	12.11	b	14.20	b	15.83	b
6	9.22	d	13.39	c	12.37	d	11.52	c	12.87	d	14.55	d
7	8.28	e	11.00	e	9.64	f	8.47	f	9.50	f	11.47	g
8	7.05	g	9.00	g	8.24	h	7.33	h	8.16	h	9.78	h

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.

[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

Table 25. Effects of deacidification treatments on pH concentration means within cultivar within year as determined by HPLC.

Treatment	Frontenac		La Crescent		King of the North	
	2013	2014	2013	2014	2013	2014
	---------- -log[H+] ----------					
0	3.09 a	2.87 a	2.78 a	3.07 a	2.88 a	2.82 c
1	3.37 d	3.09 c	3.17 d	3.42 d	3.19 c	2.94 d
2	3.31 c	2.93 b	3.00 b	3.37 cd	3.13 b	2.65 a
3	3.57 f	3.31 g	3.36 f	3.61 e	3.50 e	3.03 e
4	3.49 e	3.23 ef	3.25 e	3.61 e	3.37 d	2.81 c
5	3.33 cd	3.15 d	3.10 c	3.33 c	3.14 bc	2.93 d
6	3.25 b	2.87 a	3.00 b	3.27 b	3.11 b	2.72 b
7	3.44 e	3.28 fg	3.34 f	3.58 e	3.48 e	3.09 f
8	3.47 e	3.19 de	3.26 e	3.56 e	3.37 d	2.89 d

[z] Treatments applied to 'Frontenac', 'La Crescent', and 'King of the North', 0 is the control, the non-fermented juice; 1 fermented by Maurivin B; 2 fermented by Maurivin B and cold stabilized; 3 fermented by Maurivin B and malolactic fermentation; 4 fermented by Maurivin B, malolactic fermentation and cold stabilization; 5 fermented by 71B; 6 fermented by 71B and cold stabilized; 7 fermented by 71B and malolactic fermentation; 8 fermentation by 71B, malolactic fermentation and cold stabilization.

[y] Means followed by the same letter within each column are not significantly different according to a pairwise t-test ($P \leq 0.05$).

In 2014 the trend was again identical to ‘La Crescent’ and ‘King of the North’ for 2013 with some exceptions. In ‘Frontenac’ treatments 1 and 6 were not different from each other, treatment 1 was Maurivin B fermented and treatment 6 was 71B fermented and cold stabilized. Treatment 4 was again the greatest at TA reduction with a 53% reduction in TA from the control. In ‘La Crescent’ the trend was similar except treatments 6 and 1 were exchanged in order. Treatment 4 was also the largest reduction in TA with a 59% reduction from the control. In ‘King of the North’ the trend was again followed except treatments 8 and 4 did not differ from each other. Treatment 4 was Maurivin B fermented, had underwent MLF, and cold stabilization, Treatment 8 was 71B fermented, had underwent MLF and cold stabilization. These treatments lowered TA by 54% as compared to the control.

pH

In both years and across all cultivars the control juice was lowest in pH compared to all treatments, with the exception of ‘King of the North’ in 2014 (Table 25). In general wine pH increased through fermentation and as acid reduction treatments were applied with the exception of cold stabilization treatments. Wines fermented with Maurivin B tended to have a higher pH than those fermented with 71B. Wines with MLF tended to have a higher pH than wines only fermented with yeast. Cold stabilization primarily caused a depression in wine pH even when all treatments reduced wine acid content. This was noticed in all cultivars but wines in 2014 ‘King of the North’ that were treated with yeast and cold stabilization had a significantly lower pH than the control juice. In both years and across all cultivars wines that received MLF and yeast fermentation (treatments 7 and 3) tended to have the highest pH compared to all other treatments.

Discussion

Tartaric Acid

Tartaric acid concentration was greatest in the control juice regardless of cultivar. Wines that had been cold stabilized had the largest reduction of tartaric acid. Tartaric acid is more soluble in water than in wine, so when the juice ferments and the alcohol level increases, the solubility decreases. In addition, solubility of tartaric acid and its salt, potassium bitartrate, decreases as the temperature decreases. Thus, the greatest reduction of tartaric acid in all cultivars was in treatments that underwent cold stabilization. Significant differences in tartaric concentrations were found between yeast fermented and yeast and malolactic bacteria fermented in 2013 (in the interaction of vintage and treatment) and in King of the North (in the interaction of cultivar and treatment). This is interesting as treatments with malolactic bacteria had significantly less tartaric acid than treatments only fermented. Traditionally it has been reported that biological treatments do not have an effect on tartaric acid concentration therefore this reduction in tartaric acid in malolactic bacteria treatments is not easily explained. It has been stated that malolactic bacteria, of the genus species *O. oeni* (formerly *Leuconostoc oenos*) lack the biochemical capacity for the metabolism of tartaric acid(beelman and gallander 1979, Radler 1993), even though other lactic acid bacteria from the genus lactobacillus can metabolize tartaric acid and other compounds. Interestingly, MLF was often accompanied by small decreases (3% to 30%) in the concentration of tartaric acid. Two differing hypotheses attempt to explain this decrease, in that *O. oeni* may metabolize the tartaric acid similar to its relative lactobacillus (Krumperman and Vaughn, 1996; Radler, 1975; Pilone et al., 1966; Rice and Mattick, 1970) or that the concentration may reflect a solubility change rather than a metabolic effect (Kunkee, 1967; Rice and Mattick, 1970). The lactic acid bacteria used for MLF, *O. oeni* (formerly

Leuconostoc oenos), are heterofermentative (Radler, 1963; Henick-kling, 1993; Vila-Crespo et al., 2010). This means these bacteria metabolize various compounds, which includes fermenting hexoses by the hexose-monophosphate pathway to lactate, ethanol and carbon dioxide along with erythritol, acetate and glycerol (Veiga Da Cunha et al., 1993; Stolz et al., 1995; Richter et al., 2001). The potential increase in ethanol by MLF could be enough to decrease the solubility of tartaric acid in those treatments. In our experiment, all bacteria treatments were co-inoculated explaining bacteria access to hexose before being largely fermented by yeast treatments. Additionally, tartaric acid concentration can be affected by pH, and tartrate present as potassium bitartrate is maximized at a pH of 3.7 and thus, precipitation is greatest at this point. Hence, MLF treatments may have caused an increase in pH change resulting in more potassium bitartrate available to fall out of solution than treatments without MLF. However, further research is necessary to firmly establish whether such small decreases in concentration are due to biochemical utilization or to physical losses through decreased solubility.

Malic Acid

Malic acid concentration was greatest in the control juices regardless of cultivar. Wines fermented with Maurivin B had less malic acid than wines fermented with 71B in 2013 and 2014 (interaction of vintage by treatment), and yeast effects were also found to be different for 'La Crescent' and 'King of the North' (cultivar by treatment interaction). Yeast (*S.cerevisiae),* utilized for wine making began to be isolated from various geographical regions and sold for commercial use around 50-60 years ago (Redzepovic et al., 2003). These strains are likely to be derived from strains domesticated hundreds of thousands of years earlier and were isolated from fermentations with desirable characteristics, such as specific fermentation kinetics and sensory qualities that result in unique fermentation behaviors (Richter et al., 2013). Therefore, each yeast

strain that is commercially available, is unique in its effect on vinification. These differences extend into maloethanolic fermentation. Maloethanolic fermentation is the conversion of malic acid into pyruvate, by means of an intracellular malic enzyme, then decarboxylated to acetaldehyde and reduced to ethanol (Redzepovia et al., 2003; Ribéreau-Gayon et al., 2006). Saccharomyces spp. express remarkable differences with regard of their ability to decompose malic acid during alcoholic fermentation, and the ability of a yeast strain to degrade extracellular malic acids dependent on the efficient transport of the dicarboxylic acid as well as the efficacy of the intracellular enzyme (Ansanay et al., 1996; Volschenk et al., 1997) As such our two yeasts strains behaved differently in their ability to decompose malic acid. Yeast 71B was found to reduce malic acid by 35.7% in a comparison of many yeasts in Chardonnay by Richter (2013), while, Maurivin B has been stated to degrade malic acid by 56% according to studies done at the Bordeaux Wine Institute. When comparing the control juice to only yeast fermented wines we found that in only 71B fermented (treatment 5), malic acid was reduced by 47.4% in 2013 and 16% in 2014. In only Maurivin B fermented wines (treatment 1), malic acid was reduced by 62% in 2013 and 37% in 2014.

Wines that underwent MLF had the lowest amount of malic acid, and were not different from each other regardless of yeast used, but did differ from all other acid reduction treatments. This suggests that the bacteria fermented all remaining malic acid to near completion regardless of original yeast malic degradation level. As a result, if utilizing malolactic bacteria in a wine deacidification program, the yeast strain may have little effect on residual malic content. However, a general trend was observationally observed, 71B MLF treatments had higher malic acid concentration than Maurivin B MLF treatments, even though they were not statically different. Additionally, in all cultivars complete conversion of malic to lactic did not occur, with

detectable amounts of malic acid found in the chemical analysis by HPLC. This differs from other studies using *O. oeni* and HPLC detection were no detection of malic was found and complete conversion into lactic was reported (Herjavec et al., 2003). All experimental units of 'Frontenac', 'La Crescent' and 'King of the North' started MLF but not all completed. This could be due to a myriad of difficult environmental conditions such as: high ethanol concentration, high acidity, low pH, yeast competition, phenolic compounds, sulphur dioxide (SO_2), unmet nutrient needs, temperature, pesticide residues, and fatty acids (Vila-crespo et al., 2010; Ribéreau-Gayon et al., 2006; Vidal et al., 2001; Ruediger et al., 2005; Cabras et al., 1999; Lasik 2013). Traditionally, pH, alcohol, SO_2, and temperature are the first factors discussed when determining the success and potential of MLF treatments.

Wine pH plays an important role in determining which lactic acid bacteria species will survive and have sufficient growth rates, wines of a pH at 3.3 and above generally exhibit few problems whereas wines with lower pH's may expressed difficulty starting, sustaining or fully completing MLF (Kunkee, 1967). A majority of the wines within this study are well below a pH of 3.3 and were inherently difficult for MLF treatments (Table 25).

Wine pH all so affects SO_2, as SO_2 is commonly added to must at the beginning of vinification process to restrict the growth of indigenous yeast and bacteria (Fleet and Heard, 1993). Some yeast strains also produce relatively large quantities of SO_2. At low pH more SO_2 predominates as free SO_2, which is composed of bisulfite anion, a small proportion of molecular SO_2, and sulfite anion. Molecular SO_2 is the only form of SO_2 that can cross cell walls of yeast and bacteria, entering by diffusion and interacting to detrimentally affect the growth of the yeast and lactic acid bacteria by disrupting microbial enzymatic activity. Therefore, the lower pH increases free SO_2 and molecular SO_2 and thus, increases the bacterial stress (Henick-Kling,

1993). Our original sulfur addition was 40 ppm, a level safe for malolactic bacteria and when the wine was checked post primary fermentation and levels were again low and safe for malolactic growth.

Malolactic bacteria can also be negatively affected by alcohol and temperature. A high concentration of ethanol strongly interferes with bacterial growth and metabolic activity, and decreases the temperature of optimal growth (Henick-Kling, 1993). Temperature affects the growth rate and length of the lag phase, and temperature also induces stress proteins and membrane fluidity. Temperature was lowered to start MLF to reduce ethanol stress as wines would not start MLF at traditional temperatures. High ethanol concentrations decrease the optimal growth temperature of lactic acid bacteria and ethanol tolerance is decreased at elevated temperatures. Optimum growth of lactic acid bacteria in the presence of 10-14% ethanol by volume is at 18 to 20^0C compared to 30^0C when ethanol volume is 0-4% (Henick-Kling, 1993). This is due to increased membrane fluidity in the presence of ethanol in *O. oeni,* while decreases in temperature decreases membrane fluidity allowing normal bacterial function (Tourdot-Marcechal et al., 2000; Teixeira et al., 2002).

Any of these factors alone or combined could have caused the incomplete conversion of malic to lactic acid within our cultivars but the low pH and high alcohol concentration are the most likely causes.

Lactic Acid

Lactic acid is primarily formed from MLF where a dicarboxylic acid, malic acid- more acidic in taste, is converted into a moncarboxylic acid, lactic acid –milder in taste, and carbon dioxide (Ribéreau-Gayon, 2006; Volschenk, 2006; Bauer and Dicks, 2004). Hence malolactic treatments have the highest amount of lactic acid and were not different from each other in all

cultivars for both years except in ‘King of the North’ and ‘La Crescent’ in 2014. Interestingly, treatments that did not undergo MLF still had higher lactic concentration than the control. This may be due to the small amount of lactic acid that was actually produced from yeast instead of the lactic acid bacteria. Lactic acid is a secondary product of yeast fermentation. It is derived from pyruvic acid, and directly reduced by yeast lacticodehydrogenase (Ribéreau-Gayon et al., 2006). This small amount of lactic acid also may be due to malolactic bacteria contamination, were a small amount of bacteria was present in these samples.

Citric Acid

Citric acid was greatest in the control juice in 2014 and was different from all other treatments. In 2013, the control was not different from malolactic treatments in all cultivars. In ‘La Crescent’ and ‘Frontenac’ in 2014 and in ‘Frontenac’ in 2013 wines that did not undergo MLF had the lowest amount of citric acid and these treatments were different from all others. The remaining cultivars seemed to follow a similar trend in that wines without malolactic bacteria, had less citric acid, but these treatments did not significantly differ from wines with malolactic bacteria.

The concentration of citric acid in wine can decrease during MLF. In some wines, citric acid was completely metabolized (Cogan et al., 1981; Webb and Ingrahm, 1960) while in others up to 50% (Shimazu and Watanabe, 1979). The utilization of citric acid by LAB during MLF has in some instances been correlated with the production of diacetyl and acetoin and acetic acid, but this depended on the species involved and wine pH (Zeeman et al., 1982, Pilone et al., 1966; Fornachon, 1957). Unfortunately, this was not what was found in the current study, and instead of MLF decreasing citric acid, the treatments with MLF had higher citric than those without MLF. These findings are unique to this experiment, as no other research has reported these

findings, and future research may be needed to explore this outcome. However, these findings may be misleading. HPLC analysis and interpretation are subject to human error and it is possible that an unknown compound was not filtered out and could have had the same retention time. The peak picked also may not have been the desired acid but an unknown compound. In this experiment, HPLC conditions were modified from prior tests and better separation was achieved through sulfonic acid buffered with sodium sulfate and a silica column. However, future studies should involve modifying the current conditions to achieve better resolution resulting in greater separation of peaks. Additionally, wines are very complex matrixes and different filtration and/or wine samples injected with known additions of citric acid may help reduce complication and determine if these findings are repeatable.

Succinic Acid

Succinic acid is known as one of the major organic acids produced by yeast during fermentation for the production of alcoholic beverages. It can be formed in the glyoxylate cycle by oxidation of isocitrate, as well as in the reductive citric acid cycle (Raab & Lang, 2011). Succinic acid was only found within fermented treatments and was not detectable in the control juice. Treatments were significantly differently within each cultivar and each year. These differences between treatments though significant are very small in volume, as the control had 0 g/L Succinic acid and fermented treatments had 0.5-1.7 g/L Succinic acid. A trend was observed in all cultivars and in both years for malolactic treatments to have low concentrations of succinic acid, while yeast fermented wines to have a greater concentrations of succinic acid expect for 'Frontenac' in 2014. This trend is not yet understood and further research is needed to discover if these results are repeatable and what biological processes are causing the difference in succinic acid concentrations between treatments, or if HPLC interpretation was incorrect.

Titratable Acidity

Unlike all other previously mentioned variables, TA is a measurement that is inclusive of all acids but is represented as a single concentration of tartaric acid. TA is a measurement of free protons and undissociated acids in solution that can react with a strong base and be neutralized, hence the concentrations of each individual acid affects this value. As the objective of this study was to determine potential acid reduction within northern grape cultivars, an important results was that the control or base juice was highest in TA and differed from all other treatments. Descending in acidity from the control were treatments with singular acid reduction components, and treatments most significant in acid reduction were a combination of all acid reduction components. Cold stabilization treatments had greater TA than MLF treatments and were significantly different from each other except for 'Frontenac' in 2013 where cold stabilization and MLF treatments were different from all others but not each other. This greater reduction in TA by MLF could be due to a higher inherent amount of malic acid within the cultivars or due to the fact that cold stabilization left a higher residual acid content than MLF did, as solubility reduction acts differently than microbial activity. Combinations of acid reduction components (cold stabilization and MLF) had a much greater TA reduction than singular components. Since these components affect different acids, the combination resulted in the greatest reduction.

Additionally yeasts were found to have significantly different effects on wine TA with or without MLF. This yeast effect on wine TA was in a direct contradiction to lack of difference found in malic concentration in malolactic treatments by HPLC. This could be due to TA sampling error as TA testing was done in triplicate due to the natural high error of the test, and it may be possible that testing error resulted in a false positive for significance. HPLC has higher accuracy than the typical TA test, and its results may be more reliable. Another hypothesis is

that the difference found between yeast could be explained by the nature of TA itself. For wines receiving MLF treatment and fermented with 71B yeast malic acid concentration was higher, but not statistically different from wines fermented with Maurivin B and MLB. Those malic acid concentration means were expressed as grams per liter malic acid. TA is the summation of all acids titrated within a wine but is expresses as tartaric acid. Could it be possible the malic acid concentration when represented as tartaric inflated the TA numeric value enough to change means and result in treatment significance. If so, differences found between yeasts within malolactic treatments may be due to expression of malic concentration as tartaric. However, this hypothesis could be flawed as TA calculations assume the titration was done on pure tartaric acid with no acid conversion known, thus, TA testing error is more probable.

The greatest reduction in TA for all treatments and cultivars was with Maurivin B, malolactic bacteria fermented, and cold stabilized (treatment 4), except for 'King of the North' in 2014 were Maurivin B yeast was not different from 71B, so both treatments 8 and 4 had the greatest reduction in TA. Wines subjected to Maurivin B, MLF, and cold stabilization (treatment 4) caused a 51-59% TA reduction compared to the control amount. This reduction lowers wine parameters into a more acceptable range and shows that high acid levels with cold-hardy interspecific hybrid grapes may could be greatly reduced if treated correctly. Wines subjected to Maurivin B, MLF, and cold stabilization (treatment 4) in 'Frontenac' in 2013 and 'La Crescent' in 2014, and wines subjected to MLF, cold stabilization and either Maurivin B or 71B (treatments 4 and 8) in 'King of the North' in 2013 were within the recommended pH and TA ranges for optimal wine quality according to the standards set by Winkler et al., 1974; Amerine et al., 1972 and Dami et al., 2005. However, it is important to note that treatments applied to

small sample sizes may not be representative of treatment effects on a larger scale, thus, results found in our experiment may be inflated due to the size of the test.

pH

As stated before, both TA and pH are necessary to show acidity, and that the trends which appeared with TA values may not be the same for pH. The pH can be buffered and affected by many things. Additionally, cold stabilization has an interesting effect to pH in that if the original pH was below 3.6, cold stabilization will depress the pH, but if the original pH was at or above 3.6, cold stabilization will increase the pH, moving the solution to be more basic. Therefore, both the acid concentration and pH can be reduced.

The pH of a wine or juice is a measure of the concentration of free hydrogen ions in solution, while the TA is a measure of the total amount of hydrogen ions titrated at to a pH of 8.2. Based on these definitions, one might be tempted to think there is a relationship between the pH and the TA in juices and wines. Unfortunately, there is no direct or predictable relationship between pH and TA, and the same TA value can be measured in different juices with either low pH or high pH. The pH is not correlated with the concentration of acids present, but is influenced by their ability to dissociate.

The difference in TA and pH was very noticeable in our treatments. A greater drop in acidity and TA was found in wines fermented and cold stabilized (treatments 6 and 2) than wines only fermented (treatments 1 and 5). However the pH in wines fermented and cold stabilized (treatments 6 and 2) was lower than wines only fermented (treatments 1 and 5), this is opposite of what would be thought, the pH didn't reflect the drop in acidity. This was due to the nature of cold stabilization. Cold stabilization is the precipitation of tartaric acid as potassium bitartrate. Potassium bitartrate(KHT) is amphoteric and can act as both an acid and as a base, and

potassium bitartrate is both influenced by, and has an influence on, the pH and TA of a wine. When wines with pH values below 3.65 are cold stabilized, the pH lowers as potassium bitartrate drops out and the TA decreases. This occurs because for every molecule of potassium bitartrate that forms and precipitates, one free hydrogen ion is formed (that had been attached to the tartrate in KHT). Alternatively, when KHT precipitation occurs in wines with pH values above 3.65, the pH will increase (while the TA still decreases), as one free hydrogen ion is removed from solution (due to its incorporation into KHT). The magnitude of the pH shift will vary depending on the amount of KHT that is removed during both fermentation and cold stabilization (Waterhouse et al., 2016).

The pH values were different between yeast treatments that had not undergone MLF, and this might be due to the differing malic acid metabolism. However, when comparing pH to malolactic treatments, comparisons of yeasts were not significant, except for 'King of the North' in 2013 and 'Frontenac' in 2013. This lack of significant difference in pH between yeasts in malolactic treatments indicates that malic concentration was not significantly different, and difference found in TA was due to tartaric representation, or incorrect TA values. Wines highest in pH were those receiving MLF.

Conclusion

In summary, tartaric acid concentration was most greatly reduced in cold stabilization treatments, resulting in a 72-83% reduction from the control juice. Wines receiving MLF resulted in the lowest malic acid concentration (treatments 7, 8, 3, and 4), with yeast utilized and cold stabilization having no effect on malic acid content. Malic acid content was decreased by 94-97% in these treatments as compared to the control. In treatments not undergoing MLF, Maurivin B metabolized 15-29% more malic acid than 71B. Lactic acid concentration was

greatest in wines receiving MLF. Citric acid concentration was reduced in all treatments as compared to the control, but the reasons are unknown. The HPLC protocol was modified for better separation, but future studies should involve modifying the current conditions to achieve better resolution and especially to determine if citric acid results are valid. The pH values were greatest in wines receiving MLF as expected. TA reduction was greatest when combining cold stabilization, yeast and MLF, and resulted in a 51-59% reduction. TA values in treatment 4 for 'Frontenac' in 2013 and 'La Crescent' in 2014, and treatments 4 and 8 for 'King of the North' in 2013 were within the recommended ranges for optimal wine quality according to the standards set by Winkler et al., (1974), Amerine et al., (1972), and Dami et al., (2005). TA's were significantly different between yeasts in malolactic treatments, which was contradictory to HPLC malic acid findings. This is believed to be due to the representation of malic acid concentration as tartaric acid or test error, as pH and malic concentration did not differ between these treatments. This research gives further insight into the challenges of cold climate vinification and shows the importance of deacidification techniques. However further research and cultivar improvement will be needed to optimize wine quality within our region.

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