Stage of maturation, crop load, and shoot density affect the fruit quality of cold-hardy grape cultivars

by

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DEDICATION

Dedicated to my family. Luke 6:43-45

TABLE OF CONTENTS

v

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ABSTRACT

Wine grape production in the Upper Midwest and other cold-climate regions is increasing due to the release of cold-hardy grape cultivars that are interspecific hybrids. Grape production practices for *Vitis vinifera* L. cultivars in regions with long growing seasons, such as California, are standardized more than in other regions due to the extensive amount of research on cultivars of *V. vinifera*. In spite of that, few researchers document changes in grape composition as fruits mature, and those that do primarily only report soluble solids, pH, and titratable acidity.

Two experiments were designed to examine the changes in soluble solids, pH, titratable acidity, acid profile, and sugar profile that occur during the maturation of fruits of commercially important cold-hardy grape cultivars. Fruits from Edelweiss, Frontenac, La Crescent, Marquette, and St. Croix were harvested from vines at the typical commercial harvest time, and one to two weeks before and after. Glucose:fructose ratio ranged from 0.95 to 1.24 and generally decreased with stage of maturation. Malic acid ranged from 5.3 to 15.2 g/L and decreased with stage of maturation. Tartaric:malic acid ratio was smaller than is reported for other interspecific hybrids, and was never larger than 0.55 for Edelweiss and La Crescent. Cold-hardy grape cultivars have unique fruit chemistry as compared to cultivars of *V. vinifera, V. labruscana* Bailey, and French hybrids. Harvesting fruits later in maturation can be effective in reducing malic acid in cold-hardy grape cultivars, however they still have large amounts of malic acid and a small tartaric:malic acid ratios. Soluble solids content is commonly used as an indicator of fruit maturity to determine when grapes should be harvested. However when soluble solids did not change with stage of maturation, there were

changes in other fruit parameters such as pH, titratable acidity, and acid profile, indicating that soluble solids should not be overemphasized as the deciding factor when to harvest grapes.

Yield is often used as a predictor of fruit quality; however grapevine balance is dependent on both fruit yield and the amount of vegetative growth, which is not factored into yield alone. Crop load (grape yield/pruning weight) is increasingly being used as an indicator of vine balance for cultivars of *V. vinifera* and French hybrids. An experiment was designed to determine what changes occur in the fruit chemistry and grapevine canopies of Frontenac and St. Croix, cold-hardy grape cultivars, at crop loads ranging from 2 to 14. Frontenac was less responsive to crop load than St. Croix. Leaf area/grape weight (m^2/kg) and fruit malic acid concentration generally decreased with crop load, while tartaric:malic acid ratio increased. Increasing crop load within the examined ranges can be an effective approach to increase yield and decrease the large amounts of malic acid found in fruits of cold-hardy grape cultivars without negative consequences on other fruit quality parameters and vine growth.

Shoot and cluster quantity are commonly managed on grapevines, however their effects are not separated in most studies. An experiment was designed to impose four shoot levels (15, 30, 45, and 60 shoot/vine) and three cluster levels (15, 30, and 60 clusters/vine) to Marquette grapevines. Grape pH and malic acid concentration increased as the quantity of shoots per vine increased, while the tartaric:malic acid ratio decreased. Cluster quantity did not have an impact on fruit chemistry. Leaf area per vine decreased as shoots per vine decreased, which is the likely mechanism for malic acid decreasing as shoot quantity decreased. Leaf area per kg of fruit decreased as shoot quantity decreased and increased as

cluster quantity decreased. For vigorous vines it is effective to decrease the amount of shoots, within a set cluster quantity, to decrease leaf area per vine and fruit malic acid. Increasing the amount of shoots, within a set cluster quantity, can be useful to increase leaf area per vine and kg of fruit to balance fruit and vegetative growth on grapevines with low vigor.

CHAPTER 1. GENERAL INTRODUCTION

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Dissertation Organization

This dissertation includes six chapters. Chapter one is a general introduction, which includes an introduction to the research and a literature review. Chapters two, three, four, and five are complete manuscripts containing research-based experiments, which address topics pertinent to growing grapes (*Vitis* spp.) in cold-climate regions. General conclusions of all experiments are presented in chapter six.

Introduction

This dissertation is based on research designed to provide new information for extension specialists and growers of interspecific grape hybrids in cold climates. There is a void of information about appropriate production methods for grape cultivars commonly grown in the Upper Midwest and other cold-climate regions. Experiments were designed to address the influence that horticultural practices have on grapevine growth, development, and fruit quality. The impact that the stage of fruit maturation, crop load, and the quantity of shoots and clusters on a vine have on the fruit quality of novel interspecific grape hybrids were investigated.

Grapes grown in cold climates and interspecific hybrid grape cultivars have large amounts of malic in acid in their fruits. Practices which reduce the amount of malic acid are often implemented during the production of wine from these grapes. Managing grapevines to reduce fruit malic acid should also be prioritized in viticultural production. A number of

supplementary vineyard cultural practices, such as leaf removal around fruits on a grapevine, can reduce malic acid in grapes, but they are not always practiced and require additional investments in labor. The purpose of this research was to determine what effect generally applied vineyard practices have on the acid profile of fruits from cold-hardy grape cultivars. The hypothesis is that delaying the stage of maturation when grapes are harvested, increasing grapevine crop load (kg of fruit/kg of pruning weights), decreasing the quantity of shoots, and increasing the quantity of clusters per vine will reduce the amount of malic acid in grapes from recently introduced cold-hardy grape cultivars, without causing excessive negative impacts on the grapevine and other fruit quality parameters.

Impact of stage of grape maturation on fruit quality

The objective of this study was to determine the influence that the stage of maturation has on the fruit quality of recently introduced interspecific hybrid grape cultivars. Soluble solids, pH, and titratable acidity (TA) commonly are reported as fruit quality parameters in grape experiments. These are known to be impacted by stage of fruit maturation; however other factors such as acid and sugar profile of grapes also determine wine production and quality; they are impacted by stage of maturation. Wine grapes are harvested over a wide range of maturation, therefore understanding the changes that occur during maturation will provide both viticulturists and enologists with valuable information for management decisions that need to be made in the vineyard and winery.

Impact of crop load, shoot quantity, and cluster quantity on fruit quality

In spite of the traditional view that fruit quality is negatively correlated with vineyard yield, few reports cite a direct correlation. This is further complicated due the longevity of perennial grapevines. Practices and conditions of one growing season are impacted by

previous growing seasons, and the current season likely impacts future growing seasons. Research suggests that optimal fruit quality is achieved by applying an appropriate ratio of fruit to grapevine pruning weights, known as crop load, rather than fruit weight per unit of land area. To further complicate the matter, shoot density also impacts fruit quality, and to achieve optimal crop loads and yields, vines commonly have differing shoot densities. Grape growers must balance the economics of yield, fruit quality, and long-term vine health when determining appropriate crop levels on their vines. To date, limited information on these factors has been developed for interspecific grape hybrids commonly grown in cold-climate regions. Knowing which viticultural factors impact fruit quality, and which do not will allow viticulturists to optimize both fruit quality and financial return.

Literature Review

Grape production in the Upper Midwest

Grape production has rapidly increased in the Upper Midwest due to the release of several cold-hardy interspecific hybrids. In 2008, there were 1,000 vineyard acres in Iowa, which, in addition to wineries, had a $234 million impact on the state economy (MKF Research LLC Report 2008). In 2012, Iowa had 1,200 acres of vineyards (White 2013).

Other states in the Upper Midwest have seen similar increases in the grape and wine industry. In 2002, there were 222 vineyard acres in Minnesota, and in 2007 the combined vineyard and winery economic impact to the state of Minnesota was $36 million (Tuck and Gartner 2008). By 2012 there were 2,000 estimated vineyard acres in Minnesota, which, when combined with wineries, had an industry impact of $59 million to the state economy (Tuck and Gartner 2013).

There is strong interest in grape production in the Upper Midwest; however in Iowa 47% of the growers had four years or less of experience growing grapes, and only 10% had more than ten years of experience. In 2006, Edelweiss was the second most planted grape cultivar in Iowa followed by Frontenac, La Crosse, St. Croix, and La Crescent as the third, fourth, fifth, and seventh most planted, respectively (USDA NASS 2007). Frontenac was the most planted cultivar in Minnesota, followed by Marquette, La Crescent, and Frontenac Gris, respectively (Tuck and Gartner 2008). In Nebraska, Frontenac was the most planted red wine grape with St. Croix being the third, while La Crosse was the most planted white, followed by Edelweiss (Nebraska Grape Board 2007). Few publications address specific vineyard management practices of any of these cold-hardy interspecific cultivars that are extensively grown and commercially important in the Upper Midwest.

Fruit quality parameters that impact grape and wine production

Grape soluble solids concentration is an indicator of the sugar concentrations in grapes and is used as a major indicator of fruit quality. Fructose and glucose are the common sugars in grapes. Soluble solids concentration increases with stage of fruit maturation and often dictates harvest time. Grape pH increases with stage of fruit maturation. Wine stability is affected by pH; at a low pH, wine is less prone to spoilage organisms. For that reason pH is one of the major factors that impacts time of harvest (Winkler et al. 1974).

Tartaric and malic acids are the most abundant acids in grapes (Jackson and Lombard 1993). Tart wines are caused by large concentrations of titratable acidity (TA) and malic acid (Jackson and Lombard 1993, Main et al. 2007). There is less TA and malic acid in grapes when growing seasons are warm than cold (López-Tamames et al. 1996, Main and

Morris 2004), indicating that regional differences in grapevine and harvest management should exist to manage the differences between grape production regions.

Grape parameters for wine production typically range from a soluble solids of 18 to 24 Brix for white wines and 21 to 25 Brix for red wines, a pH range of 3.0 to 3.5, and a TA between 6 to 10 g/L (Byers et al. 2003). Generally wine grapes are harvested when tartaric acid in grapes is 5 g/L and malic acid stabilizes at 2 to 3 g/L (Bisson 2001). Grapes from predominantly *V. labrusca* L. cultivars have less soluble solids than others when harvested at a similar pH values. Grapes grown specifically for white wine production are harvested typically at a TA of 7.5 g/L for dry wines, a TA of 9.0 g/L for sweet wines, and pH of 3.1 to 3.2. Red wine grapes are harvested typically at a pH of 3.4 to 3.5 and a TA of 6.5 and 8.0 g/L for dry and sweet wines, respectively (Dami et al. 2005).

Organic acid synthesis and degradation in grapes

Tartaric and malic acid account for up to 90% of the acids found in grapes. Both tartaric and malic acids are synthesized within the grape. Tartaric acid is a secondary product from the metabolism of sugars (Ruffner 1982a). After veraison, as potassium concentration in the grapes increases, potassium bitartrate is produced within grapes. In contrast, malic acid is an intermediate in the tricarboxylic acid cycle in the grapevine. Malic acid concentration can decrease rapidly in grapes since late in fruit maturation it is used as an energy source via respiration (Jackson 2000).

Fruit traits of *Vitis* species and interspecific hybrids

Ideal grape soluble solids concentration for wine production varies for cold-hardy interspecific hybrid cultivars (Smiley et al. 2008). *Vitis* species have glucose:fructose ratios

ranging from 0.47 to 1.12 (Kliewer 1967a); these ratios also vary among cultivars within a single *Vitis* species (Kliewer 1967b).

Fruits of interspecific grape hybrids have large amounts of acidity (Main et al. 2007, Main and Morris 2004). Tartaric acid is more prevalent than malic acid in fruit from *V. vinifera* L. (López-Tamames et al. 1996, Nagel et al. 1972, Tardaguila et al. 2010); however this is not the case for every *Vitis* species. The species which comprise the genetic base of many cold-hardy interspecific hybrids are reported to have tartaric:malic acid ratios of 5.85, 2.04, 0.82, and 0.52 for *V. labrusca*, *V. vinifera*, *V. riparia* Michx., and *V. aestivalis* Michx., respectively (Kliewer 1967a). Non-*V. vinifera* grape cultivars often have a high pH, as well as large concentrations of TA and malic acid at harvest (Main et al. 2007, Main and Morris 2004). Cold-hardy interspecific hybrids are distinctly different than *V. vinifera* cultivars and may require cultural practices to alleviate fruit quality parameters that affect wine production.

Impact of grape production practices and fruit quality

Stage of fruit maturation

Research from California confirms there is disconnect between the view of ideal fruit quality parameters for wine production and the parameters that actually lead to the highest quality wine. When presented with fruit from a range of harvest times, winemakers selected the fruit harvested late in maturation as being ideal for wine production. However, when evaluating wines made from that fruit, wines made from fruit harvested mid-way through maturation were selected as superior in quality (Conversano et al. 2008). This study was performed with Cabernet Sauvignon in Napa Valley, California, which is a well-established wine production region. Discrepancies between perceived ideal fruit quality parameters and

the parameters that affect wine quality are likely to exist in more recently developed wine production regions, such as the Upper Midwest, and in areas where temperatures and length of growing season are more variable.

The decrease in TA and increase in pH and soluble solids concentration as grapes mature is well documented (Winkler et al. 1974). Soluble solids concentration commonly is used to determine when to harvest grapes; however other factors need to be taken into consideration. In early berry development, glucose is at greater concentrations than fructose, in contrast to late in fruit maturation (Esteban et al. 1999, Kliewer 1965). In wine production, glucose is preferentially metabolized by yeast, *Saccharomyces cerevisiae* (Meyen) E.C. Hansen, at the expense of fructose (Esti et al. 2003). Yeast strains differ in their capacity to consume fructose (Reynolds et al. 2001, Shütz and Gafner 1995). Soluble solids concentration alone does not predict fermentation hazards that may be encountered from large concentrations of fructose. Large amounts of fructose in grapes can be due to wine grapes being harvested late in maturation or from cultivars with naturally large concentrations of fructose. To date, little is known about the glucose and fructose concentrations of cold-hardy interspecific grape cultivars.

The respiration of malic acid is largely responsible for the reduction of TA during fruit maturation (Coombe 1992, Crippen and Morrison 1986). Fruits of cold-hardy grape cultivars are often harvested with large amounts of acids (University of Minnesota Agriculture Experiment Station 2012), and some are harvested at both a large TA and high pH values (Main and Morris 2004). Due to their large concentrations of TA and malic acid, the trend is to harvest some cold-hardy interspecific grape cultivars when TA drops below 15 g/L rather than harvesting based on soluble solids (University of Minnesota Agriculture

Experiment Station 2012). This delay in harvest is intended to allow malic acid concentration to decrease; however unintended consequences of delaying harvest may also lead to increased fructose concentrations in grapes.

<u>Canopy</u>

Leaves intercepting light are directly responsible for photosynthesis and sugar production in plants. Therefore increasing leaf area per unit of fruit should lead to an increase in soluble solids in grapes; however this is not always the case. A ratio of 0.8 to 1.2 m^2 of leaf area per kg of grapes is required for maximum soluble solids, berry size, and color for undivided canopies of *V. vinifera* grape cultivars in long-growing season regions, such as California. Grapevines with less 0.8 m^2 of leaf area per kg of grapes are overcropped, whereas canopies with a ratio of more than 1.2 m^2 per kg are undercropped (Kliewer and Dokoozlian 2005). Research from a wide range of growing regions indicates 0.7 to 1.4 m^2 of leaf area per kg of fruit is required for optimal ripening (Howell 2001). Regardless, increasing leaf area per unit of grape above the ideal range can lead to slight increases in grape soluble solid concentrations, however gains are minimal.

Excessive leaf area can be induced by undercropping and shaded grapevine canopies can have a negative impact on both fruit and the grapevine. Shaded leaves require carbohydrates to be transported from non-shaded leaves, which has a negative impact on vine carbohydrate status (Vanden Hueval et al. 2002). Soluble solids and secondary metabolites such as anthocyanins, phenolics, and terpenes are at greater concentrations in sun-exposed fruit than shaded fruit, and there is less TA in exposed fruit (Smart and Robinson 1991, Skinkis et al. 2010).

Temperature has a large impact on malic acid concentration in grapes (Ruffner 1982b). Grapes grown in cool climates have more TA and malic acid because the rate of malic acid respiration increases as temperature increases (Jackson and Lombard 1993). Grapevine shading leads to increased malic acid concentrations in grapes at harvest (Morrison and Noble 1990). However, growing season temperature can have a greater impact on acid profile than cultural practices (Main and Morris 2004). Increased shade in grape canopies has been correlated with decreased bud fruitfulness (Vasconcelos et al. 2009) and cold hardiness (Howell and Shaulis 1980).

Increasing shoot density of grapevines of *V. vinifera* cultivars increases leaf area, leaf layers, and shaded clusters (Reynolds et al. 1994). Training systems, shoot orientation, and fruit yield can impact the amount of leaf area per shoot (Zoecklein et al. 2008). Therefore, an increase shoot density may not always increase whole-vine leaf area.

Yield and crop load

The classic view of grapevine yield is that grape and wine quality increases linearly as vineyard yield decreases (Keller, 2005). A survey in California indicated that half of winemakers and viticulturists believe that low vineyard yields produce higher quality wine for cultivars of *V. vinifera* (Chapman et al. 2004). In spite of the fact that few studies have substantiated the relationship, some grape growing regions have regulations in place that limit vineyard yields (Jackson 2000). Many studies include grapevine yield as a measured dependent parameter, few have investigated the main effects of grapevine yield on fruit and wine quality.

Reducing vineyard fruit yield increases juice soluble solids and color (Reynolds et al. 1996a). Large vineyard yields are correlated with reduced malic acid concentrations in wine

(Bravdo et al. 1984, 1985). Researchers cite inconsistent correlations between grapevine yield and wine sensory traits for *V. vinifera* cultivars (Bravdo et al. 1984, 1985; Chapman et al. 2004; Reynolds et al. 1996b).

Crop load is typically defined as ratio of fruit yield to pruning weights. Crop load was determined to be a better indicator of wine quality than fruit yield alone (Bravdo et al. 1984). Optimal crop loads for *V. vinifera* range from 4 to 10 (Kliewer and Dokoozlian 2005) or as high as 10 to 12 (Bravdo et al. 1984; 1985). Crop loads greater than 12 have not substantially decreased fruit quality for appropriately trained French-American hybrid grape cultivars (Reynolds and Vanden Heuvel 2009). Vines with fruit yields greater than the ideal range of crop load are generally described as being overcropped and have less than the ideal 0.7 to 1.4 m^2 of leaf area per kg of fruit, resulting in decreased soluble solids concentrations. Undercropped vines have crop loads less than the ideal range and have fruit and canopy deficiencies associated with excessive shade (Kliewer and Dokoozlian 2005). Hybrid and *V. vinifera* grape cultivars may be able to produce grapes at crop loads greater than 12 without a reduction in wine quality when vine microclimate has been idealized with training systems and other cultural practices (Reynolds and Vanden Heuvel 2009). No studies exist that investigate crop loads in recently introduced cold-hardy interspecific grape cultivars commonly grown in Iowa and the Upper Midwest region.

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CHAPTER 2: STAGE OF MATURATION AFFECTS FRUIT SUGAR AND ORGANIC ACIDS OF COLD-HARDY GRAPE CULTIVARS

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Abstract

Few studies report sugar and organic acid profiles of grapes (*Vitis* spp.) as they mature. Cold-hardy grape cultivars with novel genetic composition recently have been introduced to the grape and wine industry. These cultivars dominate the grape industry in cold-climate regions, but there are few reports about the fruit quality traits of these cultivars. The research objective was to determine soluble solids, pH, titratable acidity, acid profile, and sugar profile of recently introduced cold-hardy grape cultivars as fruits mature. Fruits from Frontenac, La Crescent, Marquette, and St. Croix were harvested from vines at commercial harvest, and one to two weeks before and after. Soluble solids and pH increased as fruits matured, whereas titratable acidity decreased. Glucose:fructose ratio ranged from 0.98 to 1.24 and decreased with stage of maturation. Malic acid ranged from 5.3 to 11.3 g/L and decreased with stage of maturation. Tartaric:malic acid ratio ranged from 0.43 to 0.77 early in maturation and from 0.51 to 1.33 late in maturation. Harvesting fruits late in maturation can be effective in reducing malic acid in cold-hardy grape cultivars, however they still have more malic acid and a smaller tartaric:malic acid ratio than previously reported for other cultivars. Soluble solids was not a good predictor of change in other fruit

parameters and its use to determine the stage at which grapes should be harvested should not be overemphasized.

Introduction

Wine grape production (*Vitis* spp.) in nontraditional regions has expanded due to the introduction of new cold-hardy interspecific hybrids. Frontenac, La Crescent, Marquette, and St. Croix are some of the most commonly planted cultivars in cold-climate regions (Tuck and Gartner 2013). There are few published investigations about the fruit quality traits of these hybrids. The stage of maturation at which fruits are harvested is one of the most important management decisions that impacts fruit and wine quality. However, few studies have investigated the impacts of stage of fruit maturation on fruit components other than soluble solids, pH, and titratable acidity (TA).

Tartaric and malic acids are the most abundant organic acids in grapes (Jackson and Lombard 1993). Malic acid concentration has a large impact on fruit quality (Ruffner 1982), and wines with greater concentrations of TA and malic acid have a tart flavor (Jackson and Lombard 1993, Main et al. 2007). Titratable acidity decreases during maturation, largely due to the use of malic acid during respiration (Coombe 1992, Crippen and Morrison 1986). The rate of malic acid respiration increases with temperature, therefore grapes grown in regions with cooler climates have more TA and malic acid (Jackson and Lombard 1993).

Temperature has a large impact on malic acid concentration in mature grapes (Ruffner 1982). Cultural practices that increase sunlight exposure in the grapevine canopy and to berries decrease TA and/or malic acid concentrations (Morrison and Noble 1990, Skinkis et al. 2010). Practices such as grapevine training systems (Bordelon et al. 2008,

Zoecklein et al. 2008), leaf removal (Di Profio et al. 2011, Main and Morris 2004, Tardaguila et al. 2010), shoot thinning (Sun et al. 2011; 2012), and cluster thinning (Di Profio et al. 2011) decrease TA and/or malic acid concentrations in grapes. However, temperature during the growing season can have a greater impact on TA and malic acid concentration than cultural practices (Main and Morris 2004).

Grapes from *V. vinifera* L. contain more tartaric acid than malic acid (López-Tamames et al. 1996, Nagel et al. 1972, Tardaguila et al. 2010). Grapes from interspecific hybrids have large concentrations of TA and malic acid (Main et al. 2007, Main and Morris 2004). The single-season means of tartaric:malic acid ratios for white grape musts from Washington were 2.8, 1.3, and 0.9 for *V. labruscana* Bailey, *V. vinifera*, and French hybrid cultivars, respectively (Nagel et al. 1972). Species of *Vitis* have tartaric:malic acid ratios of 5.85, 2.04, 0.82, and 0.52 for *V. labrusca* L., *V. vinifera*, *V. riparia* Michx., and *V. aestivalis* Michx., respectively (Kliewer 1967a). These species represent a large portion of the genetic base for cold-hardy interspecific hybrid cultivars (Reisch et al. 1993). Attention to the stage of maturation at which grapes are harvested must be prioritized for cultivars with potentially large amounts of malic acid.

In spite of the vineyard cultural practices available to reduce malic acid in grapes, enological techniques often are required to reduce malic acid during wine production. Techniques used to mitigate malic acid in wine include additions of carbonate (Mattick et al. 1980), malolactic bacteria (Main et al. 2007), *Schizosaccharomyces pombe* Lindner yeast (Dharmadhikari and Wilker 1998), and genetically enhanced strains of *Saccharomyces cerevisiae* (Meyen) E.C. Hansen yeast (Main et al. 2007, Volschenk et al. 1997). These

techniques often are used in wine production of interspecific hybrids or grapes grown in cold climates.

Fructose and glucose are the predominate sugars in grapes. Glucose:fructose ratios across species of *Vitis* range from 0.47 to 1.12 (Kliewer 1967a). A wide range of glucose:fructose ratios also exist among cultivars within a species (Kliewer 1967b). *Saccharomyces cerevisiae*, the yeast commonly used in wine production, consumes glucose preferentially to fructose (Esti et al. 2003). Greater fructose concentrations can slow or stop fermentation because yeast strains differ in their ability to consume fructose (Reynolds et al. 2001, Shütz and Gafner 1995). The efficacy of complete sugar consumption by yeast strains is often determined by the glucose:fructose ratio in grape musts (Cavazza et al. 2004). Glucose is at a larger concentration early in berry development, whereas late in berry development fructose concentration increases (Esteban et al. 1999, Kliewer 1965). Therefore, the stage of maturation at which wine grapes are harvested may affect fermentation in wine production.

When to harvest grapes, is one of the most important decisions that can affect wine quality. Fruits of cold-hardy grapes are harvested over a wide range of maturation (Smiley et al. 2008). The trend is to harvest grapes later in maturation (Hansen 2006), but the full implication of late harvests is unknown. The few reports on this topic have focused primarily on the effect of stage of maturation on soluble solids, pH, TA (Christensen et al. 1995a; 1995b), and secondary metabolites of *V. vinifera* (Reynolds et al. 1995). Reports of the effect that stage of maturation has on fruit chemistry for interspecific hybrids have focused on Marechal Foch (Johnson and Nagel 1976, Sun et al. 2011). The objective of this research was to provide specific fruit-composition information on cold-hardy interspecific hybrids

used in the commercial grape and wine industry. This study provides viticulturists and enologists with information about how maturation impacts fruit composition, enabling informed decisions in the vineyard and winery.

Materials and Methods

Fruit from seven-year-old Frontenac and St. Croix grapevines, and six-year-old La Crescent and Marquette grapevines at the Iowa State University Horticulture Research Station Ames, IA, (42°06'35.8"N 93°35'27.1"W) was harvested at different stages of fruit maturation in 2009. Spring frost damage in 2010 limited vineyard yield at the Horticulture Research Station and required the study to be moved. In 2010, fruit from seven-year-old Frontenac and St. Croix grapevines, and four-year-old La Crescent and Marquette grapevines, was harvested at different stages of maturation at a commercial vineyard near Oskaloosa, IA (41°19'01.0"N 92°38'56.7"W). All vines were own-rooted. Growing degree days (base 10 °C, maximum 30 °C) at the experiment locations were 1562 and 1983 from 1 Apr. to 15 Oct. in 2009 and 2010, respectively (Iowa Environmental Mesonet 2013).

All vines were planted on a spacing of 2.4 by 3.0 m and trained to a cordon 1.8-m-tall high wire cordon system, with the exception in 2010 when La Crescent and Marquette vines were trained to a mid-wire cordon at 1.0 m and Marquette was planted on a spacing of 1.8 by 3.0 m. Vines were cluster-thinned to limit vineyard yield to 11 t/ha. Standard cultural practices were followed to manage vines (Dami et al. 2005).

Completely randomized designs were used with stage of fruit maturation the treatment within each cultivar. Fruit were harvested at three stages of maturation (Table 1): one to two weeks before commercial harvest (early), at typical commercial harvest (middle),

and one to two weeks after commercial harvest (late). Soluble solids and pH were used to determine commercial harvest date (middle) based on ranges used in the industry for each cultivar (Smiley et al. 2008). Harvest dates were also dictated by impending weather conditions and physical condition of the fruit. Treatments were applied to four single-vine replications.

A 100-berry sample was retained from each vine and treatment. Samples were juiced with a bench-top juicer and pressed through cheesecloth. Juice was stored at -20 °C before chemical analysis. Fruit soluble solids were determined using a temperature-compensating refractometer (ATAGO, Bellevue, WA).   A Thermo Scientific pH meter (Thermo Scientific Orion 2 Star, Waltham, MA) was used to measure juice pH. A 5-ml juice sample was used to quantify TA (expressed as g tartaric acid/L) by titration with 0.1-N NaOH to an endpoint of pH 8.2. All soluble solids, pH, and TA analyses were performed in duplicate and reported as an average value.

Several techniques were examined for organic acid quantification before sample analysis. The appropriate method used for sample analysis was adapted from methods previously described (Castellari et al. 2000, Falqué López and Fernández Gómez 1996). Juice samples were filtered through a 0.45-µm filter, diluted to 10% v/v with deionized water, and analyzed for citric acid, fructose, glucose, malic acid, and tartaric acid by HPLC at the Iowa State University Midwest Grape and Wine Industry Institute (Ames, IA). An Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA) with diode-array and refractive index detectors was used with two Aminex HPX-87H (300 × 7.8 mm) columns (Bio-Rad, Hercules, CA) linked end-to-end with a micro-guard Cation H guard column (Bio-Rad, Hercules, CA) was heated to 65 °C. The isocratic mobile phase was HPLC-grade water

with 0.045-N sulfuric acid and 6% acetonitrile. The refractive index detector was heated to 55 °C. Flow rate was 0.5 ml/min for 35 min. Sample injection volume was 20 µl. Peaks were identified by retention time at 210 nm for acids or by refractive index for sugars. Ratios of tartaric:malic acid, glucose:fructose, and sugar:acid were calculated by dividing tartaric acid concentration by malic acid concentration, glucose by fructose, and Brix by percent TA, respectively, for each individual stage of maturation replication.

All data were analyzed with Statistical Analysis System ver. 9.3 software (SAS Institute 2011). The general linear models procedure was used for analysis of variance to evaluate the significance of stage of fruit maturation, cultivar, growing season, and the impact of interactions on measured fruit quality parameters. Fisher's least significant difference test was used to compare means at a $P \leq 0.05$.

Results

All main effects of cultivar, stage of maturation, and growing season had a significant effect on measured fruit parameters, except growing season did not have an effect on malic acid concentration. There were first-order and/or second-order interactions for all fruit parameters except malic acid (Table 1).

Late-harvested grapes had 14% greater soluble solids than fruit harvested early. Fruit of Marquette had the most soluble solids and St. Croix grapes had the least. Frontenac, La Crescent, and St Croix fruit had 94%, 96%, and 74% of the soluble solids of Marquette, respectively (Table 1). Fruit harvested late in maturation had more soluble solids than fruit harvested early, except in 2010 for La Crescent, Marquette, and St. Croix when the soluble solids did not change with stage of maturation (Table 2).

There was a wide range of glucose:fructose ratio, 0.98 to 1.24 (Table 2). Frontenac had the largest glucose:fructose and La Crescent had the smallest glucose:fructose ratio (Table 1). All cultivars and stages of maturation had a glucose:fructose ratio of greater than 1, with the exception of the late harvest of La Crescent in 2010. Glucose:fructose ratio decreased with stage of maturation for Frontenac in 2010, La Crescent in 2009 and 2010, and Marquette in 2009 (Table 2).

The main effect of pH increased with stage of maturation and St. Croix fruit had a higher pH than all other cultivars. Fruit in the 2010 growing season had a greater pH than fruit in 2009 (Table 1). Fruit pH was lower early in maturation than late, except for Marquette in 2009 and St. Croix in 2010 when pH values did not change with stage of maturation for those cultivars (Table 2).

The TA varied by cultivar, with Frontenac having the largest amount of TA and St. Croix the least. There was more fruit TA in 2009 than in 2010 (Table 1). Titratable acidity decreased with stage of maturation for all cultivars except St Croix in 2010. Early in maturation in 2009, Frontenac, La Crescent, Marquette and St. Croix fruit had 20%, 10%, 24%, and 24% more TA, respectively, than fruit harvested late. In 2010 Frontenac, La Crescent, Marquette fruits harvested early in maturation had 28%, 35%, and 48% more TA, respectively, than fruits harvested late (Table 2).

Each cultivar had a different sugar:acid ratio. Marquette fruit had the largest and Frontenac had the smallest sugar:acid ratio. Sugar:acid ratio was larger in 2010 than in 2009. Sugar:acid ratio increased with stage of maturation for all cultivars and growing seasons except St. Croix in 2010 (Table 1). A sugar:acid ratio greater than 30 only occurred late in

maturation for La Crescent and St Croix cultivars in one growing season, and both seasons for Marquette (Table 2).

Fruit in 2010 had more tartaric acid than in 2009. The tartaric acid concentration of Marquette, St. Croix, and La Crescent grapes was 80%, 68% and 54%, respectively, of Frontenac fruit (Table 1). In 2010, the tartaric acid concentration was 35%, 15%, and 14% greater late in maturation than early in maturation for Frontenac, Marquette, and St. Croix cultivars, respectively (Table 3).

Malic acid concentration for all cultivars ranged from 5.3 to 11.3 g/L and decreased with stage of fruit maturation in at least one growing season for each cultivar (Table 3). Malic acid concentration was the only fruit parameter not affected by growing season or interactions between main effects. Frontenac and La Crescent fruit had more malic acid than Marquette and St. Croix fruit (Table 1). In 2010, Frontenac and St. Croix fruit harvested early had 21% and 26% more malic acid, respectively, than did late-harvested fruit. Marquette fruit harvested early had 62% and 54% more malic acid than late-harvested fruit in 2009 and 2010, respectively (Table 3).

La Crescent fruit had a tartaric:malic acid ratio almost half that of Marquette fruit (Table 1). La Crescent tartaric:malic acid ratio was not affected by stage of fruit maturation and never was greater than 0.55. Frontenac, Marquette, and St. Croix had a 67%, 87%, and 28% greater tartaric:malic acid ratio in late-harvested fruit than early-harvested fruit, respectively, in 2009. In 2010, fruits of Marquette and St. Croix harvested late in maturation had a 68% and 44% greater tartaric:malic acid ratio, respectively, than fruits harvested early (Table 3).

Frontenac fruit had 69% more citric acid than Marquette grapes. There was 58% more citric acid in fruit harvested in 2010 than in 2009. Citric acid comprised 3 to 5% of measured organic acids (Table 1). Fruit citric acid concentration increased with stage of maturation for Frontenac, Marquette, and St. Croix fruit (Table 3).

Discussion

Interactions involving stage of fruit maturation and cultivar were expected due to the differing genetic composition of the cultivars in this study. It was apparent that in 2010 the fruit was more advanced in maturation, likely due the greater amount of growing degree days, as compared to 2009. There were significant differences between growing seasons and interactions involving growing seasons (Table 1), however the stage-of-maturation trends within each cultivar and fruit parameter were consistent between growing seasons (Tables 2 and 3).

Fruit from all cultivars and stage of maturation treatments was suitable for commercial wine production. Soluble solids and pH values increased while TA decreased with stage of fruit maturation, as is typically reported (Coombe 1992); however the values differed among cultivars (Table 1). Glucose:fructose ratio decreased with stage of fruit maturation for Frontenac, La Crescent, and Marquette, as reported in other cultivars (Esteban et al. 1999, Kliewer 1965). However, the magnitude of change is likely not enough to justify altered vinification techniques because Frontenac, Marquette, and St. Croix fruit had greater concentrations of glucose than fructose at all stages of maturation and growing seasons (Table 2). Frontenac grapes, in particular, had a greater glucose:fructose ratio (Table 1) than

previously reported in mature grapes (Kliewer 1967a; 1967b); greater glucose:fructose ratios have only been reported in immature fruit (Esteban et al. 1999, Kliewer 1965).

Ideal sugar:acid ratios for table wine production range from 30 to 35 for *V. vinifera* cultivars (Ough and Singeton 1968). Sugar:acid ratios for the cultivars in this trial ranged from 14.8 to 38.3. Most cultivars and stages of fruit maturation had a sugar:acid ratio less than 30, except Marquette which was greater than 30 late in maturation (Table 2). Interspecific grape hybrids can have optimal wine quality at sugar:acid ratios as low as 15 (Gallander 1983), indicating that sugar:acid ratio likely has limited value as an indicator to determine the optimal stage of maturation to harvest wine grapes for cold-hardy cultivars.

Vitis riparia is in the genetic composition of the cold-hardy cultivars in this study (Smiley et al. 2008), the species with large concentrations of malic acid (Kliewer 1967a). Concentrations of malic acid in the cold-hardy cultivars of this study were greater than for most *V. vinifera* cultivars (Kliewer 1967b, López-Tamames et al. 1996, Tardaguila et al. 2010). The grape cultivar Cynthiana (*V. aestivalis*) is known for having large malic acid concentrations, however, malic acid concentrations of the cultivars in this study were equal to or greater than those reported in Cynthiana (Main and Morris 2004). With the exception of Marquette fruit late in maturation and St. Croix fruit at all stages of maturation, the cultivars in this study had greater malic acid concentrations than most hybrid grape cultivars grown in Washington (Nagel et al. 1972), except Marechal Foch which has malic acid concentrations ranging from 7.4 to 19.1 g/L in similar ranges of pH (Johnson and Nagel 1976). Even though St. Croix grapes have *V. labrusca* in their genetic composition (Smiley et al. 2008), malic acid concentrations of St. Croix fruit were substantially greater than

previously reported in *V. labrusca* (Kliewer 1967b) and *V. labruscana* cultivars (Nagel et al. 1972).

Tartaric:malic acid ratios for all examined cultivars, except La Crescent, increased with stage of maturation (Table 3). The increase in tartaric:malic acid ratios for Frontenac, Marquette, and St. Croix is due both to malic acid decreasing and tartaric acid increasing with stage of maturation, a result which has not been reported previously. Tartaric:malic acid ratios ranged from 0.43 to 0.77 early in maturation and from 0.51 to 1.33 late in maturation. Tartaric:malic acid ratios of musts from mature fruit of *V. vinifera* cultivars typically are greater than 1 (Tardaguila et al. 2010), and often greater than 2 (López-Tamames et al. 1996). Tartaric:malic acid ratios ranged from 0.76 to 1.37 for Marechal Foch fruit (Johnson and Nagel 1976) and 0.9 to 1.5 for Cynthiana grapes (Main and Morris 2004) at a pH values similar to those reported in this study. Cold-hardy cultivars are primarily interspecific hybrids of *V. labrusca*, *V. riparia*, and *V. vinifera* (Reisch et al. 1993). The tartaric:malic acid ratio for *V. riparia* was 0.82 (Kliewer 1967a), similar to the results of this study. Grapes from *V. labrusca* had tartaric:malic acid ratios as high as of 5.85 (Kliewer 1967a); however St. Croix, the cultivar in our study with *V. labrusca* in its genetic composition (Smiley et al. 2008), had tartaric:malic acid ratios less than 1.05. Malic acid has 'green' taste perception (Jackson, 2009), therefore fruit with a larger tartaric:malic acid ratio will taste less 'green' than those with a small tartaric:malic acid ratio. Grapes from Frontenac, Marquette, and St. Croix would also taste less sour late in maturation than early in maturation, since malic acid is more sour than tartaric acid (Amerine et al 1965). In contrast, La Crescent had the same tartaric:malic acid ratio at all stages of maturation (Table 3), therefore harvesting La Crescent

late in maturation will not have the same effect on the taste attributed to changes acid profile at a constant TA.

In contrast to other reported grape cultivars, citric acid concentrations increased with stage of maturation (Esteban et al. 1999). Citric acid concentrations in mature grapes of reported cultivars range from 0.15 to 0.24 g/L (Esteban et al. 1999, López-Tamames et al. 1996), which is considerably less than the cultivars in this study (Table 3). This indicates that the acid profiles of cold-hardy interspecific grape hybrids consistently are different than other cultivars.

Soluble solids measurement is commonly used to determine the stage of maturation at which grapes should be harvested and to determine grape prices (Cooperative Research Centre for Viticulture 2005). Soluble solids were not affected by stage of fruit maturation in 2010 for La Crescent, Marquette, and St. Croix. This may be due to the harvest dates among treatments being closer in 2010 than in 2009, which was dictated by the rapid accumulation of growing degree days in 2010. For every instance where soluble solids within a cultivar did not change with stage of maturation, many other fruit parameters such as pH, TA, sugar:acid ratio, and the organic acids were affected by stage of maturation. This indicates that soluble solids is not a good predictor of other fruit quality parameters and should not be the sole indicator to determine the stage of maturation at which fruits from cold-hardy grape cultivars should be harvested.

The cold-climate grape cultivars in this study differed in their fruit chemistry, indicating that viticulturists and enologists should target different ideal harvest parameters for each cultivar. Of the cold-hardy cultivars we tested, Marquette had fruit composition similar to previously reported cultivars and most *V. vinifera*-like with a larger tartaric:malic

acid ratio, more soluble solids, and less TA than the other cultivars in this study. St Croix

fruit had the most *V. labruscana*-like fruit chemistry with a smaller amount of TA and

soluble solids than the other cultivars in this study. Fruit chemistry of Frontenac and La

Crescent grapes were very similar, except in regard to the smaller amount of tartaric acid in

La Crescent fruit. While grapevine canopies for all cultivars should be managed to provide

fruit exposure to sunlight, fruit exposure should be a high priority for Frontenac and La

Crescent grapes due the large amounts of TA, malic acid, and lack of consistent reduction of

tartaric:malic acid ratio with stage of maturation. Impacts of canopy management practices,

such as leaf removal, can range from no effect to as much as a 27% reduction in malic acid

and an 8% reduction in TA, and as much as a 35% increase in tartaric:malic acid ratio in

grapes (Main and Morris 2004). While increasing cluster exposure for these cultivars may

reduce TA and malic acid, they likely will still have greater concentrations of TA and malic

acid at harvest than other grape cultivars.

Delaying harvest of Frontenac, Marquette, and St. Croix grapes until late in

maturation can be effective at reducing the TA and malic acid without substantial

concomitant increases in fructose. However, TA, tartaric acid, and malic acid in La Crescent

grapes did not decrease from middle to late in maturation, indicating that delaying harvest

until late in maturation will not affect the acid composition of this cultivar. Further research

to investigate the impact that stage of maturation has on secondary metabolites for each

cultivar and to identify the ideal stage of maturation for all fruit parameters important for

wine production is warranted.

Conclusions

While cold-hardy interspecific grape cultivars are often grouped together, results indicate that there are clear cultivar differences among the parameters and stages of fruit maturation investigated in this study. Therefore harvest management practices for each cultivar should differ.   Harvesting fruit late in maturation decreased the glucose:fructose ratio, but the magnitude of change was insufficient to require changes in vinification practices since there was generally more glucose than fructose. The results of this study show that tartaric:malic acid ratios of cold-hardy cultivars were less than ratios of other cultivars, primarily due to greater concentrations of malic acid. Tartaric:malic acid ratios decreased with stage of maturation for all cultivars except La Crescent. However, fruit harvested late in maturation will likely still require vinification to contend with large amounts of malic acid. Previously reported decreases in tartaric:malic acid ratios were due to decreases in malic acid concentrations. Cultivars in this study showed decreases in tartaric:malic acid ratios attributed in part to tartaric acid increasing as fruits matured. Harvesting later in fruit maturation can reduce malic acid concentrations for some cold-hardy grape cultivars, however managing malic acid by other vineyard cultural practices should be a priority. It is evident that a change in soluble solids did not always coincide with changes in other fruit parameters, indicating that soluble solids measurement should be de-emphasized as the main indicator of fruit quality and the primary factor to determine harvest date for grapes.

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Table 1. Three-way analysis of variance of fruit parameters measured from grapes harvested from four cultivars at three stages of fruit maturation during the 2009 and 2010 growing seasons (n = 4).

	Soluble solids (Brix)	Glucose: fructose ratio	pH	Titratable acidity (g/L)	Sugar: acid ratio	Tartaric acid (g/L)	Malic acid (g/L)	Tartaric: malic acid ratio	Citric acid (g/L)
Cultivar (C)									
Frontenac	21.4 b[b]	1.21 a	3.39 b	10.4 a	21.2 d	8.1 a	9.6 a	0.87 b	0.93 a
La Crescent	21.8 b	1.03 c	3.36 b	8.9 b	25.1 c	4.4 d	9.0 a	0.50 c	0.61 bc
Marquette	22.7 a	1.09 b	3.39 b	8.2 c	28.7 a	6.5 b	7.0 b	0.98 a	0.55 c
St. Croix	16.8 c	1.08 b	3.73 a	6.3 d	27.0 b	5.5 c	6.8 b	0.82 b	0.64 b
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Maturation (M)									
Early[a]	19.2 c	1.12 a	3.37 c	9.3 a	21.3 c	5.5 b	9.1 a	0.63 c	0.59 b
Middle	20.9 b	1.10 b	3.48 b	8.4 b	25.6 b	6.2 a	8.0 b	0.79 b	0.71 a
Late	21.9 a	1.09 b	3.56 a	7.6 c	29.6 a	6.6 a	7.2 c	0.96 a	0.75 a
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0006**	**<0.0001**	**<0.0001**	**<0.0001**
Growing season (GS)									
2009	20.3 b	1.12 a	3.39 b	9.0 a	23.4 b	5.8 b	8.1 a	0.75 b	0.53 b
2010	21.0 a	1.09 b	3.55 a	7.8 b	27.6 a	6.5 a	8.1 a	0.84 a	0.84 a
P value	**0.0007**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0016**	0.7215	**0.0248**	**<0.0001**
C x M	0.1252	**0.0470**	0.0576	**0.0217**	0.1658	0.0773	0.1309	**0.0065**	**0.0069**
C x GS	0.9833	**<0.0001**	**<0.0001**	**0.0019**	0.0861	**0.0131**	0.1234	**0.0215**	**0.0080**
M x GS	**0.0004**	0.0747	0.3838	0.5398	0.7872	0.7008	0.9244	0.8973	**0.0273**
C x M x GS	0.8462	**0.0034**	**0.0062**	**0.0211**	**0.0053**	0.0700	0.6277	0.7434	**0.0070**

[a] Harvest dates for stage of fruit maturation treatments:
 2009: Frontenac: Early (8 Sept.), Middle (22 Sept.), Late (6 Oct.); La Crescent: Early (1 Sept.), Middle (15 Sept .), Late (29 Sept.); Marquette: Early (31 Aug.), Middle (14 Sept.), Late (25 Sept.); St. Croix: Early (31 Aug.), Middle (10 Sept.), Late (22 Sept.).
 2010: Frontenac: Early (26 Aug.), Middle (30 Aug.), Late (9 Sept.); La Crescent: Early (21 Aug.), Middle (30 Aug.), Late (9 Sept.); Marquette: Early (18 Aug.), Middle (26 Aug.), Late (30 Aug.); St. Croix: Early (18 Aug.), Middle (26 Aug.), Late (30 Sept.).
[b] Treatment means followed by the same letter within columns among main effects are not different at *P* ≤ 0.05 according to Fisher's least significant difference test.
 P values ≤ 0.05 shown in bold.

Table 2. Chemical composition of fruit harvested from Frontenac, La Crescent, Marquette, and St. Croix grapevines as impacted by harvesting at different stages of fruit maturation in 2009 and 2010. Data are means of four single-vine replications.

Stage of fruit maturation	Soluble solids (Brix)		Glucose: fructose ratio		pH		Titratable acidity (g/L tartaric acid)		Sugar:acid ratio	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Frontenac										
Early[a]	18.5 b[b]	20.0 c	1.24 a	1.23 a	3.23 b	3.39 c	12.6 a	10.4 a	14.8 c	19.3 b
Middle	21.5 a	21.8 b	1.22 a	1.17 b	3.33 a	3.46 b	10.9 b	9.9 a	19.8 b	22.1 b
Late	22.9 a	23.5 a	1.24 a	1.18 b	3.36 a	3.58 a	10.5 b	8.1 b	21.9 a	29.0 a
La Crescent										
Early	19.3 b	21.5 a	1.08 a	1.06 a	3.01 b	3.37 b	10.1 a	9.6 a	19.2 b	22.6 b
Middle	22.2 a	22.3 a	1.03 b	1.03 a	3.18 ab	3.56 a	9.5 ab	7.7 b	23.4 a	29.0 a
Late	22.8 a	22.9 a	1.04 ab	0.98 b	3.31 a	3.65 a	9.2 b	7.1 b	24.9 a	31.8 a
Marquette										
Early	20.3 b	22.6 a	1.11 a	1.10 a	3.35 a	3.16 c	9.4 a	9.2 a	21.7 b	25.4 b
Middle	23.0 a	22.9 a	1.06 b	1.09 a	3.43 a	3.35 b	9.0 a	7.8 ab	26.0 ab	29.7 b
Late	23.4 a	23.7 a	1.09 ab	1.08 a	3.47 a	3.59 a	7.6 b	6.2 b	30.9 a	38.3 a
St. Croix										
Early	14.7 b	16.7 a	1.11 a	1.06 a	3.55 b	3.79 a	7.2 a	6.3 a	20.6 c	26.4 a
Middle	16.7 b	17.0 a	1.14 a	1.03 a	3.66 a	3.86 a	6.4 ab	6.0 a	26.0 b	28.9 a
Late	18.5 a	17.7 a	1.11 a	1.05 a	3.73 a	3.78 a	5.8 b	6.3 a	31.9 a	27.9 a

[a] Harvest dates for stage of fruit maturation treatments:
2009: Frontenac: Early (8 Sept.), Middle (22 Sept.), Late (6 Oct.); La Crescent: Early (1 Sept.), Middle (15 Sept .), Late (29 Sept.); Marquette: Early (31 Aug.), Middle (14 Sept.), Late (25 Sept.); St. Croix: Early (31 Aug.), Middle (10 Sept.), Late (22 Sept.).
2010: Frontenac: Early (26 Aug.), Middle (30 Aug.), Late (9 Sept.); La Crescent: Early (21 Aug.), Middle (30 Aug.), Late (9 Sept.); Marquette: Early (18 Aug.), Middle (26 Aug.), Late (30 Aug.); St. Croix: Early (18 Aug.), Middle (26 Aug.), Late (30 Sept.).
[b] Stage of fruit maturation treatment means followed by the same letter within columns among cultivars are not different at $P \leq$ 0.05 according to Fisher's least significant difference test.

Table 3. Organic acid composition of fruit harvested from Frontenac, La Crescent, Marquette, and St. Croix grapevines as impacted by harvesting at different stages of fruit maturation in 2009 and 2010. Data are means of four single-vine replications.

Stage of fruit maturation	Tartaric acid (g/L)		Malic acid (g/L)		Tartaric:malic acid ratio		Citric acid (g/L)	
	2009	2010	2009	2010	2009	2010	2009	2010
Frontenac								
Early[a]	6.4 b[b]	6.9 b	11.3 a	9.9 a	0.57 b	0.72 a	0.57 b	0.85 b
Middle	6.9 ab	10.7 a	9.7 a	9.8 a	0.73 ab	1.11 a	0.74 ab	1.36 a
Late	8.3 a	9.3 ab	8.8 a	8.2 b	0.95 a	1.15 a	0.90 a	1.19 ab
La Crescent								
Early	3.9 a	4.6 a	9.0 a	10.7 a	0.45 a	0.43 a	0.42 a	0.69 a
Middle	4.3 a	4.6 a	8.2 a	8.7 b	0.54 a	0.54 a	0.50 a	0.73 a
Late	4.5 a	4.6 a	8.3 a	9.1 ab	0.55 a	0.51 a	0.54 a	0.75 a
Marquette								
Early	6.1 a	6.1 b	8.6 a	8.3 a	0.71 b	0.77 b	0.48 a	0.51 b
Middle	6.8 a	6.1 b	7.2 ab	7.3 ab	0.97 ab	0.85 b	0.43 a	0.68 a
Late	6.9 a	7.0 a	5.3 b	5.4 b	1.33 a	1.29 a	0.46 a	0.75 a
St. Croix								
Early	4.5 b	5.7 b	6.9 a	7.8 a	0.67 b	0.73 c	0.46 a	0.70 b
Middle	5.1 ab	5.5 b	7.2 a	6.4 b	0.71 ab	0.87 b	0.46 a	0.76 b
Late	5.4 a	6.5 a	6.3 a	6.2 b	0.86 a	1.05 a	0.40 a	1.04 a

[a] Harvest dates for stage of fruit maturation treatments:
2009: Frontenac: Early (8 Sept.), Middle (22 Sept.), Late (6 Oct.); La Crescent: Early (1 Sept.), Middle (15 Sept .), Late (29 Sept.); Marquette: Early (31 Aug.), Middle (14 Sept.), Late (25 Sept.); St. Croix: Early (31 Aug.), Middle (10 Sept.), Late (22 Sept.).
2010: Frontenac: Early (26 Aug.), Middle (30 Aug.), Late (9 Sept.); La Crescent: Early (21 Aug.), Middle (30 Aug.), Late (9 Sept.); Marquette: Early (18 Aug.), Middle (26 Aug.), Late (30 Aug.); St. Croix: Early (18 Aug.), Middle (26 Aug.), Late (30 Sept.).
[b] Stage of fruit maturation treatment means followed by the same letter within columns among cultivars are not different at $P \le 0.05$ according to Fisher's least significant difference test.

CHAPTER 3: STAGE OF MATURATION AFFECTS FRUIT SUGAR AND ORGANIC ACIDS OF EDELWEISS, A COLD-HARDY GRAPE CULTIVAR

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Abstract

Edelweiss (*Vitis* spp.) is an interspecific hybrid grape cultivar used for commercial white wine production in cold-climate grape production regions. There are few reports on Edelweiss fruit during maturation. While many of the organoleptic fruit characteristics of Edelweiss resemble those of *Vitis labruscana* Bailey, its sugar and organic acid profiles are not reported. The objective was to determine soluble solids, pH, titratable acidity, acid profile, and sugar profile of Edelweiss fruit over a range of stages of fruit maturation when grown in a cold-climate. Fruit was harvested from vines at commercial harvest, and one to two weeks before and after. Soluble solids and pH increased, while titratable acidity decreased as fruits matured. Glucose:fructose ratio was not affected by stage of maturation. Tartaric and malic acid ranged from 2.7 to 4.6 and 5.7 to 15.2 g/L, respectively and decreased with stage of maturation. Tartaric:malic acid ratio increased from 0.32 early in maturation to 0.51 late in maturation. While many of the organoleptic fruit traits of Edelweiss are similar to those of *V. labruscana* cultivars, Edelweiss grapes have more malic acid, less tartaric acid, and a smaller tartaric:malic acid ratio than previously reported *V. labruscana* cultivars.

Introduction

Edelweiss (*Vitis* spp.) is a white-fruited interspecific grape hybrid with *V. labrusca* L. and *V. riparia* Michx. in its genetic composition (Smiley et al. 2008). Edelweiss has been used primarily as a wine grape and is known for being adapted to a wide range of climates (Reed and Gamet 2005), resistant to many diseases, and cold hardy to -35 °C (Swenson et al. 1980). Phenotypically Edelweiss most closely resembles *V. labruscana* Bailey due to its slip-skin fruit, strong *V. labruscana* flavor/aroma that develops late in maturation, and canopy structure (Swenson et al. 1980). Edelweiss was the fourth most planted cold-hardy white grape cultivar in the participating states of the Northern Grape Project (Tuck and Gartner 2013b).

Tartaric and malic acids are the most abundant organic acids in grapes (Jackson and Lombard 1993). Large concentrations of malic acid can cause wine to have a tart flavor (Jackson and Lombard 1993, Main et al. 2007). Titratable acidity (TA) decreases as grapes mature due to the respiration of malic acid. Generally grapes grown in cooler regions often have more malic acid since respiration rates increase with temperature (Jackson and Lombard 1993). Shaded grapevine canopies also lead to larger concentrations of malic acid due to reduced temperature within the grapevine microclimate (Morrison and Noble 1990).

More tartaric acid is found than malic acid in cultivars of grapes from *V. vinifera* L. (López-Tamames et al. 1996, Nagel et al. 1972; Tardaguila et al. 2010) and *V. labruscana* (Johnson and Nagel 1976, Mattick et al. 1972, Nagel et al. 1972). Prevalence of tartaric acid or malic acid varies in French hybrids (*Vitis* spp.) (Main and Morris 2004, Nagel et al. 1972) and other cultivars such as Cynthiana (*V. aestivalis* Michx.). *Vitis aestivalis, V. labrusca*, *V. riparia*, and *V. vinifera* are reported to have tartaric:malic acid ratios of 0.52, 5.85, 0.82, and

2.04, respectively (Kliewer 1967). Enological techniques often are required to reduce malic acid in wine made from hybrid grape cultivars (Main et al. 2007).

Sunlight exposure to grapes decreases TA and malic acid (Bledsoe et al. 1988) and increases terpenes in fruits of aromatic white grape cultivars (Skinkis et al. 2010) due to increases in temperature with the grapevine canopy. Shoots on Edelweiss tend to readily break off the vine when manipulated (Smiley et al. 2008), making canopy management difficult to accomplish. Many growers refrain from extensive canopy management practices on Edelweiss vines, which may increase the amount of TA and malic acid in Edelweiss fruits.

Saccharomyces cerevisiae (Meyen) E.C. Hansen preferentially consumes glucose to fructose (Esti et al. 2003), causing slow or incomplete fermentations when large concentrations of fructose are present (Bisson 1999, Shütz and Gafner 1995). Glucose is the predominant sugar early in fruit maturation, while fructose increases late (Esteban et al. 1999, Kliewer 1965).

The stage of maturation at which grapes are harvested is one of the most important management decisions that impacts wine quality. Recommendations are to harvest Edelweiss early in maturation for optimal wine quality to avoid excessive *V. labrusca*-like flavors (Swenson et al. 1980). However, the changes in acid and sugar profile that occur during fruit maturation for Edelweiss grapes are unknown. Cold-hardy grape hybrids with *V. riparia* parentage have more malic acid than tartaric acid (Mansfield and Cook 2014); however Edelweiss fruits exhibits many organoleptic traits of *V. labruscana* cultivars, which are reported to have more tartaric acid than malic acid. This study investigates changes in

the composition of Edelweiss fruits during maturation, providing viticulturists and enologists with a basis for informed decisions in the vineyard and winery.

Materials and Methods

Fruit from six-year-old, own-rooted Edelweiss grapevines located in a commercial vineyard near Oskaloosa, IA (41°20'38.1"N 92°44'44.6"W), were harvested at different stages of fruit maturation in 2008 and 2009. In 2008, fruit also was harvested from Frontenac (*Vitis spp.*) grapevines at different stages of maturation to provide a reference point for Edelweiss maturity. Growing degree days (base 10 °C, maximum 30 °C) for the weather stations nearest the experiment locations were 1588 and 1515 from 1 Apr. to 30 Sept. in 2008 and 2009, respectively (Iowa Environmental Mesonet 2013). Vines were trained to a 1.8-m-high wire cordon system and planted on a 2.4 by 3.0 m spacing. Vines were cluster thinned to limit vineyard yield to 11 t/ha when required. Standard cultural practices were followed for other vine management (Dami et al. 2005).

Fruit was harvested at three stages of maturation (Table 1): one to two weeks before commercial harvest (early), at typical commercial harvest (middle), and one to two weeks after commercial harvest (late). Treatments were applied to five single-vine replications. A completely randomized design was used with stage of fruit maturation as the treatment. Commercial harvest date was determined using pH ranges suggested for white wine grapes (Dami et al. 2005) and suggested cultivar-specific soluble solid concentrations (Smiley et al. 2008). Constraints of impending weather conditions, such as precipitation, dictated actual harvest dates.

A 100-berry sample was retained from each replication. Samples were juiced with a bench-top juicer, pressed through cheesecloth, and stored at -20 °C prior to chemical analysis. Soluble solids content of fruits was determined by using a temperature-compensating refractometer (ATAGO, Bellevue, WA). Fruit pH was measured with a Thermo Scientific pH meter (Thermo Scientific Orion 2 Star, Waltham, MA). A 5-ml juice sample was used to quantify TA (expressed as g tartaric acid/L) by titration with 0.1-N NaOH to an endpoint of pH 8.2. All soluble solids, pH, and TA analyses were performed in duplicate and reported as an average.

Organic acids were quantified by adapting methods previously described (Castellari et al. 2000, Falqué López and Fernández Gómez 1996). Juice samples were filtered through a 0.45-µm filter, diluted to 10% v/v with deionized water, and analyzed for citric acid, fructose, glucose, malic acid, and tartaric acid by HPLC at the Iowa State University Midwest Grape and Wine Industry Institute (Ames, IA). An Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA) with diode-array and refractive index detectors was used with two Aminex HPX-87H (300×7.8 mm) columns (Bio-Rad, Hercules, CA) linked end-to-end with a micro-guard Cation H guard column (Bio-Rad, Hercules, CA), which was heated to 65 °C. The isocratic mobile phase was HPLC-grade water with 0.045-N sulfuric acid and 6% acetonitrile. The refractive index detector was heated to 55 °C. Flow rate was 0.5 ml·min^{-1} for 35 min and sample injection volume was 20 µl. Peaks were identified by retention time at 210 nm for acids or by refractive index for sugars. Ratios of tartaric:malic acid and glucose:fructose were calculated by dividing tartaric acid by malic acid and glucose by fructose, respectively, for each treatment replication.

Data were analyzed with Statistical Analysis System ver. 9.3 software (SAS Institute 2011). The general linear models procedure was used for analysis of variance to evaluate the significance of stage of fruit maturation, growing season, and their interactions on measured fruit quality parameters. Growing seasons were combined when no significant interaction at a 95% confidence level existed. Fisher's least significant difference test compared means at a $P \leq 0.05$.

Results

The soluble solids content, glucose:fructose ratio, pH, TA, tartaric acid, malic acid and tartaric:malic acid ratio of Edelweiss grapes were affected by both stage of maturation and growing season, however there were no significant interactions between growing season and stage of maturation. Because there was not a significant interaction, those parameters for the two growing seasons were combined and analyzed together for presentation (Tables 1 and 2). Citric acid was affected by stage of maturation and there was an interaction between growing season and stage of maturation, for that reason the two growing seasons were presented separately (Table 2). Soluble solids, pH, malic acid, and tartaric:malic acid ratio were greater in 2009 than in 2008, while TA and tartaric acid concentrations were larger in 2008 (data not shown). In 2008, Frontenac fruits had had 56% more soluble solids, 20% greater glucose:fructose ratio, 9% greater pH, 8% more TA, 45% more tartaric acid, 7% more malic acid, and a 31% greater tartaric:malic acid ratio than Edelweiss fruits (Table 3).

Soluble solids concentration of Edelweiss fruit increased 3.4 Brix from early to late maturation (Table 1). Glucose:fructose ratio was not affected by stage of maturation. The pH increased and TA decreased with stage of fruit maturation. Fruit harvested early had 64%

and 148% more TA than fruit harvested in middle and late stages of maturation, respectively (Table 1).

The most abundant organic acid in Edelweiss grapes at all stages of fruit maturation was malic acid, followed by tartaric and citric acid. From early to late fruit maturation, tartaric acid and malic acid concentrations of Edelweiss decreased from 4.7 to 2.7 g/L and 15.2 to 5.7 g/L, respectively (Table 2). Early maturation fruit had 73% and 167% more malic acid than fruit harvested in middle and late stages of maturation, respectively. Fruit harvested late in maturation had a 59% greater tartaric:malic acid ratio than early in maturation. There was 2 to 3 times more malic acid than tartaric acid. Citric acid concentration was larger early in maturation than late in maturation (Table 2).

Discussion

Soluble solids content and pH levels increased with stage of maturation, while TA decreased, as is typically reported. The greater soluble solids and pH in 2009 (data not shown) may indicate that the fruit was harvested at a slightly later stage of maturation than in 2008. In spite of growing degree days and harvest dates varying between growing seasons, the treatment effect for stage of maturation was consistent for all fruit parameters and growing seasons, with the exception of citric acid (Tables 1 and 2).

Edelweiss had less soluble solids content and similar TA (Table 2) to that of *V. vinifera* cultivars, French hybrids, and *V. labruscana* at similar stages of maturation (Nagel et al. 1972). Edelweiss likely has a smaller concentration fruit soluble solids due it being partially *V. labrusca*. Means of soluble solids, pH, and TA for Marechal Foch and Corot noir, interspecific hybrids, were 23.5 Brix, 3.62 and 10.0 g/L and 16.4 Brix, 3.62 and 9.4 g/L,

respectively (Sun et al. 2011; 2012). Marechal Foch can have TA as high as 27.4 g/L at a pH of 3.16 (Johnson and Nagel 1976).

The glucose:fructose ratio of Edelweiss fruit did not decrease with stage of fruit maturation, unlike other reports. This difference may be in part due to a more narrow range of stages of fruit maturation in this study as compared with other reports (Esteban et al. 1999, Kliewer 1965). If harvest would occur both earlier and later in maturation, a decrease in glucose:fructore ratio may also exist for Edelweiss fruit. While Edelweiss fruit always had more fructose than glucose, there was never a glucose:fructose ratio less than 0.95, indicating that yeast stains which metabolize large amounts of fructose may not need to be used for Edelweiss wine production, regardless of the stage of maturation at which fruits are harvested.

Although the TA of Edelweiss fruit was similar to fruit of other cultivars, concentrations of the organic acids were different. Malic acid concentrations in Edelweiss grapes in this study were greater than those in most cultivars of *V. vinifera* (Esteban et al. 1999, López-Tamames et al. 1996, Tardaguila et al. 2010), French hybrids, and *V. labruscana* (Nagel et al. 1972) at similar pH values. Edelweiss fruits had considerably less tartaric acid than other grape cultivars. White-fruited French hybrids, *V. labruscana,* and *V. vinifera* cultivars grown in Washington had mean tartaric acid concentrations of 6.0, 6.5, and 7.0 g/L, respectively, which is almost double that found in Edelweiss fruit (Table 2).

In Edelweiss grapes, the increase of tartaric:malic acid ratio with stage of fruit maturation was due to malic acid decreasing more rapidly than tartaric acid (Table 2), which is consistent with previous reports (Crippen and Morrison 1986, Esteban et al. 1999). At fruit pH levels at which grapes are commonly harvested for wine production, Edelweiss

never had more tartaric acid than malic acid which is contrary to most cultivars. Musts of *V.*

vinifera cultivars have a tartaric:malic acid ratio consistently greater than 1 (Tardaguila et al.

2010), and often greater than 2 (López-Tamames et al. 1996). Tartaric:malic acid ratios of

for Marechal Foch, an interspecific hybrid, were less than 1 early in maturation, but were

never less than 0.76 (Johnson and Nagel 1976), which is still greater than the tartaric:malic

acid ratios observed in Edelweiss fruit at all stages of maturation in this study. The taste

perceptions of tartaric and malic acid are 'hard' and 'green', respectively (Jackson 2009).

Although the tartaric:malic acid ratio of Edelweiss fruits increased with stage of maturation,

the consistently small tartaric:malic acid ratio indicates that Edelweiss grapes will taste more

'green' than previously reported cultivars at the same TA.

  While Edelweiss has the organoleptic fruit traits of *V. labruscana,* its tartaric:malic

acid ratio was more than 10 times smaller than *V. labrusca* (Kliewer 1967a). Compared to

other cultivars, Edelweiss fruit chemistry is more similar to Concord (*V. labruscana*).

Concord grapes are typically harvested at ~16 Brix and a TA of 10 to 14 g/L (Bates 2008).

However, the inverted tartaric:malic acid ratios differentiates Edelweiss from Concord.

Reported malic acid and tartaric acid concentrations for Concord have ranged from 1.5 to 4.4

g/L and 5.0 to 10.5 g/L when harvested at a pH range of 3.08 to 3.45 in New York (Mattick

et al. 1972). Concord grapes in Washington had malic acid ranges of 3.2 to 11.8 g/L, and

11.2 to 13.9 g/L tartaric acid at a pH of 3.14 to 3.51 (Johnson and Nagel 1976). While malic

acid and tartaric acid concentrations of Concord grapes vary per region, unlike Edelweiss

there was only more malic acid than tartaric acid very early in fruit maturation.

Tartaric:malic acid ratios of Concord range from 0.88 to 3.48 at a pH of 2.92 to 3.58

(Johnson and Nagel 1976, Mattick et al. 1972). Both Edelweiss and Concord share *V.*

labrusca as the predominant species in their genetic composition, however Edelweiss also has *V. riparia* (Smiley et al. 2008), while Concord has *V. vinifera* (Sawler et al. 2013). Tartaric:malic acid ratios for *Vitis riparia* are 0.82 (Kliewer 1967), but on average, Edelweiss still had half the tartaric:malic acid ratio of *V. riparia.* Other factors such as climate and fruit exposure to sunlight may also contribute to the large concentrations of malic acid in Edelweiss fruits.

Canopy management practices such as shoot positioning (Patterson and Zoecklein 1990) and leaf removal are commonly used to increase fruit exposure to sunlight in order to reduce malic acid concentrations in grapes (Bledsoe et al. 1988, Main and Morris 2004). Extensive fruiting-zone leaf removal in Cynthiana can reduce grape malic acid concentrations by as much as 27%, while at the same time increasing the tartaric:malic acid ratio by 35% and reducing fruit TA by 8% (Main and Morris 2004). Edelweiss has a much smaller tartaric:malic acid ratio that Cynthiana. If these canopy management practices were extensively applied to Edelweiss grapevines, it may have an even larger impact due to the consistently large amounts of malic acid and small amounts of tartaric acid found in its fruits. Vineyards in the Upper Midwest have relatively small yields (Tuck and Gartner 2013a). Increasing grape yields decreases malic acid in fruits (Bravdo et al. 1985). Increasing yield could reduce malic acid in Edelweiss fruits and should also decrease the shoot vigor commonly associated with Edelweiss. Doing so may also increase sun exposure to fruits and lead to a concomitant reduction in malic acid.

Citric acid concentrations decreased with stage of maturation, as reported for other cultivars (Esteban et al. 1999). However citric acid concentrations for Edelweiss were 2 to 4

times that of other cultivars at similar fruit pH values (Esteban et al. 1999, López-Tamames et al. 1996).

The comparison between Edelweiss and Frontenac indicates that there are also clear differences between the fruit parameters of cold-hardy interspecific grape hybrids; these differences were most apparent for soluble solids, glucose:fructose ratio, tartaric acid, and tartaric:malic acid ratio (Table 3). The genetic composition of cold-climate interspecific grape hybrids varies considerably. Edelweiss is primarily a *V. labrusca* hybrid (Smiley et al. 2008), while *V. riparia* is the predominant species in Frontenac (University of Minnesota Agriculture Experiment Station 2012).

Conclusions

Results indicate that despite TA concentrations of Edelweiss being similar to those of other hybrid grape cultivars, tartaric:malic acid ratios for Edelweiss are smaller due to malic acid concentrations being greater than and tartaric acid concentrations less than other reported cultivars at similar stages of fruit maturation. While Edelweiss tends to have the organoleptic fruit characteristics similar to *V. labruscana*, it has a much smaller tartaric:malic acid ratio, even smaller than *V. riparia.* Edelweiss often is harvested early in maturation to prevent the fruit from developing strong *V. labruscana*-like flavors and aromas. Enologists must be aware that early in maturation Edelweiss has a large amount of malic acid. In spite of the fact that shoot breakage is common when applying canopy management practices to Edelweiss, canopy management practices which increase the sunlight exposure to fruits should be prioritized to reduce malic acid concentrations when fruit is harvested early in maturation.

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Table 1. Chemical composition of Edelweiss grapes at different stages of fruit maturation. Data are means of five single-vine replications in 2008 and 2009.

Stage of maturation	2008-2009			
	Soluble solids (°Brix)	Glucose: fructose ratio	pH	Titratable acidity[c](g/L)
Early[a]	13.0 c[b]	0.99 a	2.92 c	16.1 a
Middle	14.8 b	0.95 a	3.17 b	9.8 b
Late	16.4 a	0.96 a	3.44 a	6.5 c
	P values			
	Soluble solids	Glucose: fructose ratio	pH	Titratable acidity
Maturation (M)	<0.0001	0.1267	<0.0001	<0.0001
Growing season (GS)	<0.0001	0.3534	<0.0001	<0.0001
M x GS	0.6461	0.5728	0.7811	0.4139

[a] Harvest dates for stage of fruit maturation treatments: 2008: Early (15 Aug.), Middle (22 Aug.), Late (30 Aug.); 2009: Early (14 Aug.), Middle (28 Aug.), Late (11 Sept.).
[b] Treatment means within columns followed by the same letter are not different at $P \leq 0.05$ according to Fisher's least significant difference test.
[c] Titratable acidity as g/L tartaric acid

Table 2. Organic acid composition of fruit harvested from Edelweiss grapevines as impacted by harvesting at different stages of fruit maturation.   Data are means of five single-vine replications in 2008 and 2009.

Stage of maturation	2008-2009				
	Tartaric acid (g/L)	Malic acid (g/L)	Tartaric: malic acid ratio	Citric acid (g/L)	Tartaric acid (g/L)
Early[a]	4.7 a[b]	15.2 a	0.32 b	0.46 a	0.62 a
Middle	3.9 a	8.8 b	0.48 ab	0.40 ab	0.37 b
Late	2.7 b	5.7 c	0.51 a	0.36 b	0.33 b
	P values				
	Tartaric acid	Malic acid	Tartaric: malic acid ratio	Citric acid	
Maturation (M)	<0.0001	<0.0001	0.0055	<0.0001	
Growing season (GS)	<0.0001	<0.0001	<0.0001	<0.0601	
M x GS	0.1571	0.5149	0.3180	<0.0001	

[a] Harvest dates for stage of fruit maturation treatments: 2008: Early (15 Aug.), Middle (22 Aug.), Late (30 Aug.); 2009: Early (14 Aug.), Middle (28 Aug.), Late (11 Sept.).
[b] Treatment means within columns followed by the same letter are not different at $P \leq$ 0.05 according to Fisher's least significant difference test.

Table 3. Means of chemical composition of fruit harvested from Edelweiss and Frontenac grapevines at different stages of fruit maturation. Data represent the means of three stages of maturation, each replicated on five single-vine replications in 2008.

Cultivar	Soluble solids (°Brix)	Glucose: fructose ratio	pH	Titratable acidity [c](g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Tartaric: malic acid ratio
Edelweiss[a]	13.8 b[b]	0.96 b	3.08 b	12.3 b	3.1 b	11.2 b	0.29 b
Frontenac	21.5 a	1.15 a	3.36 a	13.3 a	4.5 a	12.0 a	0.38 a
P values	<0.0001	<0.0001	<0.0001	0.0070	<0.0001	0.0381	<0.0001

[a] Harvest dates for stage of fruit maturation treatments: Edelweiss: Early (15 Aug.), Middle (22 Aug.), Late (30 Aug.); Frontenac: Early (25 Aug.), Middle (9 Sept.), Late (25 Sept.)

[b] Treatment means within columns followed by the same letter are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

[c] Titratable acidity as g/L tartaric acid

CHAPTER 4: CROP LOAD AFFECTS FRUIT ORGANIC ACIDS AND VINE GROWTH OF COLD-HARDY INTERSPECIFIC GRAPE HYBRIDS

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Abstract

Fruit quality and grapevine canopy parameters were quantified for Frontenac and St. Croix grapevines (*Vitis* spp.) at crop loads (grape yield/pruning weight) ranging from 2 to 14. As crop load of St. Croix grapevines increased, grape malic acid, titratable acidity, and leaf area per kg of fruit decreased, while grape tartaric:malic acid ratio increased. Pruning weights of St. Croix grapevines were positively correlated to crop load while pruning weights of Frontenac vines were not affected by crop load. The cold-hardy interspecific hybrid grape cultivars should be managed for crop loads from 8 to 14 to maintain yields, reduce malic acid concentration, and increase net returns to vineyard management.

Introduction

Optimal vineyard performance requires a balance between fruit yield and vegetative growth. The traditional view is that grape (*Vitis* spp.) and wine quality increase as vineyard yield decreases (Keller 2005). However, large grapevine yields have not consistently been correlated with reductions in grapes and wine quality (Bravdo et al. 1984; 1985, Chapman et al. 2004, Reynolds et al. 1996b). A possible reason for inconsistent links between vineyard yield and fruit quality may be due to the fact that yield only addresses fruit quantity and not

the grapevine canopy and both are a function of vine balance. Decreasing vineyard cropping levels increases grape soluble solids (Reynolds et al. 1996a); however there is less malic acid in musts from vines with large yields (Bravdo et al. 1985), and overall, fruit quality parameters do not always predict wine quality (Conversano et al. 2008).

Crop load, a ratio of vine fruit yield and dormant cane pruning weights, is one indicator of vine balance. Crop load predicts wine quality better than grape yield (Bravdo et al. 1984). Optimal crop load ratios for cultivars of *V. vinifera* L. range from 4 to 12 (Bravdo et al. 1984; 1985, Kliewer and Dokoozlian 2005). Appropriately balanced vines can have larger crop loads without a concomitant decrease in fruit quality (Reynolds and Vanden Heuvel 2009).

Growers of cold-hardy grape hybrids in participating states (CT, IA, IL, MA, MI, MN, ND, NE, NH, NY, SD, VT, WI) in the Northern Grapes Project had an average vineyard yield of 7.8 t/ha. The three independently reported states (IA, MN, and NE) in the Upper Midwest averaged yields less than 6.5 t/ha (Tuck and Gartner 2013). However, many of the cultivars in this region are reported to grow vigorously (Brooks and Olmo 1997, Swenson et al. 1980, University of Minnesota Agriculture Experiment Station 2012), indicating that cold-climate grape cultivars currently have smaller than ideal crop loads and could have larger fruit yields without a detrimental effect on grape quality due to sufficient vine vigor.

Grapevine balance also can be assessed by examining the ratio of leaf area to grape weight. A leaf area of 0.8 to 1.2 m^2 per kg of grapes is required for optimal fruit quality for *V. vinifera* grape cultivars in regions with long growing seasons, such as California. Canopies with more than 1.2 m^2/kg of fruit are undercropped and have small crop loads,

while those with less than 0.8 m^2 of leaf area per kg are overcropped and have large crop loads (Kliewer and Dokoozlian 2005). A wider range of 0.7 to 1.4 m^2 of leaf area per kg of fruit for optimal ripening has been proposed based on research data from multiple regions (Howell 2001). Leaf area can be increased by increasing shoot densities; however more shoots can lead to more leaf layers and shading of clusters (Reynolds et al. 1994). Grapes grown in shaded vine canopies have large concentrations of malic acid (Morrison and Noble 1990). Grapes grown in cooler temperatures, due to shade or climate, have more malic acid because malic acid respiration decreases with cooler temperatures (Jackson and Lombard 1993).

Grapes from *V. vinifera* and *V. labruscana* Bailey. cultivars have more tartaric acid than malic acid (López-Tamames et al. 1996, Mattick et al. 1972, Nagel et al. 1972, Tardaguila et al. 2010). Fruits of interspecific cold-hardy grape cultivars have large concentrations of titratable acidity (TA) and malic acid (Main et al. 2007; Main and Morris, 2004). Malic acid tastes more sour than tartaric acid (Amerine et al. 1965), thus grapes at the same TA with a small tartaric:malic acid ratio taste more sour than those with a large tartaric:malic acid ratio. Ratios of tartaric:malic acid for Marechal Foch range from 0.75 to 1.08 at pH values of 3.16 to 3.52 (Johnson and Nagel 1976). Cynthiana has tartaric:malic acid ratios ranging from 0.85 to 1.5 depending on growing season and fruit exposure (Main and Morris 2004). Many of the new cold-hardy interspecific grape hybrids are predominately *V. ripiaria* Michx. (Smiley et al. 2008). *V. riparia* has smaller tartaric:malic acid ratios that both *V. labrusca* L. and *V. vinifera* (Kliewer 1967), indicating that the cold-hardy interspecific hybrids are distinctly different than *V. labrusca* and *V. vinifera* cultivars and may require enhanced practices to reduce malic acid in their fruits. Given the relatively

small yields and vigorous growth of these cultivars, attention to crop load ratio may assist in alleviating large malic acid concentrations.

Many studies include grapevine crop load as a measured dependent parameter to determine if vines are in balance. Investigations on the main effects of grapevine crop load are few; of those studies, none have focused on cold-hardy interspecific grape hybrids that are commercially grown in cold-climate grape production regions. This study investigates the impact that crop load has on vine growth and fruit quality parameters that are important for commercial grape and wine production of cold-hardy interspecific grape hybrids.

Materials and Methods

Crop loads of 6, 8, and 10 were imposed on five-year-old own rooted St. Croix grapevines, while crop loads of 7, 10 and 13 for were applied to Frontenac grapevines in 2008 and 2009 at the Iowa State University Horticulture Research Station (Ames, IA 42°06'35.8"N 93°35'27.1"W). A similar range of crop loads was imposed on Frontenac and St. Croix in 2010 at the Armstrong Memorial Research and Demonstration Farm (Lewis, IA, 41°18'44.0"N 95°10'28.5"W) due to a severe freeze event which limited vineyard yield at the Horticulture Research Station.

All vines were planted on a spacing of 2.4 by 3.0 m and trained to a 2.4 m high wire cordon system. Vines were pruned at dormancy to 120 nodes/vine and the pruning weight of canes was recorded for each vine. The average pruning weights and standard deviation for Frontenac and St. Croix when the experiment began in 2008 were 1.5 ± 0.3 kg/vine and 1.4 ± 0.4 kg/vine, and in 2010 were 1.5 ± 0.5 kg/vine and 1.7 ± 0.3 kg/vine, respectively. Vines were thinned to 33 fruitful shoots/m of cordon prior to bloom. The pruning weight for each

vine was multiplied by the crop load to determine the yield required on each vine. Historical

cluster weights for each cultivar for the vineyards were used to calculate the number of

clusters needed to be retained on each vine. Clusters were removed as needed to achieve the

calculated crop load three to four weeks post bloom, and shoots were thinned up to 20

shoots/m when allowed by the quantity of clusters required on the vine. Completely

randomized designs were used. In 2008 and 2009, crop load treatments were applied to

individual vines within a three-vine panel experimental unit and each treatment panel was

replicated three times. In both 2008 and 2009, one panel of three vines was maintained

without cluster thinning to achieve the maximum crop load possible for the vines. In 2010,

crop loads treatments were applied to 27 single-vine experimental units for each cultivar. All

the leaves from two random shoots were removed from each vine at veraison and leaf area

was quantified using a LiCor Area Meter (Model LI-3000, LiCor Inc., Lincoln, Nebraska).

Grapes were harvested when soluble solids and pH reached values typical for each cultivar

(Frontenac: 1 Oct. 2008, 22 Sept. 2009, 7 Sept. 2010; St. Croix: 11 Sept. 2008, 10 Sept.

2009, 28 Aug. 2010). Prior to dormant pruning, the total number of canes was quantified for

each vine. Leaf area per vine was calculated by multiplying the average leaf area per shoot

by the number of canes per vine. Cane dieback was assessed on 10% of the canes for each

vine. Canes with no live internodes, 1 to 3 live internodes, 4 to 5 live internodes, or more

than 6 live internodes with ripened periderm were scored as 0, 1, 2, and 3, respectively. A

weighted mean of the internode scores for each vine was calculated to determine a cane

dieback score. Primary bud damage was evaluated on 2 five-node canes per vine prior to

dormant pruning. Vines were pruned during dormancy and pruning weights were recorded.

Standard cultural practices were followed for all other practices (Dami et al. 2005). Fruit

yield per vine (kg/vine) for each vine was extrapolated to t/ha based on vine spacing. Vineyard profitability was calculated using a model (y = 855.55x - 6918.6) derived from an economic analysis for French hybrid vineyards with t/ha as the independent variable and returns to land, capital, and management ($/ha) as the dependent variable. Grapes were valued at $1100/t (Woods et al. 2010).

At harvest, a 100-berry sample was retained from each vine. In 2008 and 2009, the fruit from each vine in the three-vine panel was combined and analyzed together, while in 2010 fruits from each vine was analyzed separately. Samples were juiced with a bench-top juicer and pressed through cheesecloth and juice was stored at -20 °C before chemical analysis. Soluble solids content of grapes was determined by using a temperature-compensating refractometer (ATAGO, Bellevue, WA). Juice pH was measured with a Thermo Scientific pH meter (Thermo Scientific Orion 2 Star, Waltham, MA). A 5-ml juice sample was used to quantify TA (expressed as g/L tartaric acid) by titration with 0.1-N NaOH to an endpoint of pH 8.2. All soluble solids, pH, and TA analyses were performed in duplicate and reported as an average.

Organic acids were quantified by adapting reported methods (Castellari et al. 2000, Falqué López and Fernández Gómez 1996). Juice samples were filtered through a 0.45-μm filter, diluted to 10% v/v with deionized water, and analyzed for malic and tartaric acid by HPLC at the Iowa State University Midwest Grape and Wine Industry Institute (Ames, IA). An Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA) with diode-array and refractive index detectors was used with two Aminex HPX-87H (300 × 7.8 mm) columns (Bio-Rad, Hercules, CA) linked end-to-end with a micro-guard Cation H guard column (Bio-Rad, Hercules, CA), which was heated to 65 °C. The isocratic mobile phase was HPLC-

grade water with 0.045-N sulfuric acid and 6% acetonitrile. The refractive index detector was heated to 55 °C. Flow rate was 0.5 ml·min^{-1} for 35 min. Sample injection volume was 20 μl. Peaks were identified by retention time at 210 nm for acids or by refractive index for sugars.

All data were analyzed with Statistical Analysis System ver. 9.3 software (SAS Institute, Cary, NC). Regression analysis was used to evaluate the impact crop load had on measured fruit quality and grapevine parameters.

Results and Discussion

Yield for Frontenac ranged from 5.8 to 17.4, 5.6 to 11.6, and 4.5 to 16.3 kg/vine in 2008, 2009, and 2010, respectively, while yields for St. Croix ranged from in 9.4 to 15.1, 6.4 to 13.6, and 2.4 to 10.0 kg/vine. The number of clusters per vine at harvest increased with crop load for Frontenac in 2008 ($P \leq 0.05$) and 2010 ($P \leq 0.001$), and for St. Croix in 2010 ($P < 0.0001$). Crop load was positively correlated with fruit yield (kg/vine) for Frontenac in 2009 ($P \leq 0.01$), 2010 ($P \leq 0.001$), and for St. Croix in 2010 ($P < 0.0001$) (data not shown). The crop loads in this study ranged from 2 to 14 (Figure 1), which is a wider range than those recommended for cultivars of *V. vinifera* (Bravdo et al. 1984; 1985, Kliewer and Dokoozlian, 2005). Fruit quality can be maintained at larger crop loads for well balanced and appropriately trained vines (Reynolds and Vanden Heuvel 2009).

Organic acids

Fruits of St. Croix had malic acid concentrations ranging from 4 to 10 g/L (Figure 1) which is typical for other non-*V. vinifera* cultivars such as Cynthiana and Vignoles (Main and Morris 2004, Main et al. 2007). Frontenac fruits had concentrations of malic acid

ranging from 7 to 14 g/L, which is more than is reported for *V. vinifera* cultivars (López-Tamames et al. 1996, Tardaguila et al. 2010) and many interspecific hybrids (Main and Morris 2004, Main et al. 2007). Malic acid concentrations in St. Croix fruits were negatively correlated with crop load in both 2009 and 2010 (Figure 1). A decrease in grape malic acid concentration as crop load increased previously was reported (Bravdo et al. 1985). The malic acid concentration increased as crop load increased for Frontenac in 2008, however in all other years there was a general trend for malic acid to decrease as crop load decreased, though it was not statistically significant at a 95% confidence level (Figure 1). Wines made from fruits of interspecific grape hybrids have large amounts of malic acid, resulting in tart wines. Enological and viticultural practices which reduce malic acid in wines made from interspecific hybrids are often prioritized (Main and Morris 2004, Main et al. 2007). Increasing crop load is a practice which needs to be considered to reduce malic acid of fruits of cold-hardy grape hybrids.

Tartaric:malic acid ratios of both Frontenac and St. Croix rarely were greater than 1 and were not consistent between growing seasons (Figure 2). The cultivars in this study had tartaric:malic acid ratios smaller than observed in other hybrids and *V. vinifera* cultivars, which regularly have tartaric:malic acid ratios greater than 1 (Main and Morris 2004, Johnson and Nagel 1976, López-Tamames et al. 1996, Tardaguila et al. 2010). The tartaric:malic acid ratios of Frontenac grapes were not affected by crop load, however the tartaric:malic acid ratios for St. Croix were positively correlated with crop load in both 2009 and 2010 (Figure 2). Tartaric acid concentrations for fruits of St Croix were positively correlated only with crop load ($P \leq 0.01$) in 2009 (data not shown). Most of the increase in tartaric:malic acid ratio could be attributed to malic acid decreasing as crop load increased,

which is typical of what was reported in other cultivars (Bravdo et al. 1984; 1985). Since crop load increases tartaric:malic acid ratio, grapes harvested from vines with large crop loads should taste less sour when harvested at a similar TA, because malic acid tastes more sour than tartaric acid (Amerine et al. 1965). Malic acid also has a 'green' taste perception; therefore increasing crop load should result in fruits also having a less 'green' taste perception (Jackson, 2009).

Titratable acidity and pH

Titratable acidity was largely unaffected by changes in crop load, except for St. Croix in 2010 when TA was negatively correlated with crop load (Figure 3), likely due to malic acid decreasing as crop load increased (Figure 1). Fruits from grapevines with small crop loads typically have more TA than those at large crop loads (Bravdo et al. 1984; 1985). Increasing fruit exposure to sunlight can decrease TA in grapes (Main and Morris 2004, Skinkis et al. 2010). Crop load did not affect whole vine leaf area in this study (data not shown); therefore the grape clusters in this study likely had similar fruit microclimate which explains why TA was not correlated with crop load.

Crop load had variable effects on grape pH values. Frontenac fruit pH was not affected by crop load (Figure 4). In previous studies, crop load did not affect fruit pH values (Bravdo et al. 1984; 1985). In 2009, there was a negative correlation between St. Croix crop load and fruit pH, while in 2010, there was a positive correlation with increases in crop load (Figure 4). The negative correlation of pH values with crop load in 2009 are likely due to the increase in fruit tartaric acid concentration and tartaric:malic acid ratio as crop load increased (Figure 2), while TA was unaffected by crop load (Figure 3). Since tartaric is a stronger acid than malic, having more tartaric in proportion to malic would lead to a reduction in pH as

was observed as crop load increased. In 2010, the TA of St. Croix fruits decreased with crop load (Figure 3) which likely led to the increase in fruit pH with crop load (Figure 4).

Soluble solids

Grape soluble solids content only were positively correlated with increases in crop load for Frontenac in 2009 (Figure 5). The soluble solids content did not vary with crop load in previous reports (Bravdo et al. 1984; 1985). The amount of soluble solids in grapes is commonly used to determine the stage of maturation at which grapes should be harvested and to determine grape prices (Cooperative Research Centre for Viticulture 2005). Since grape soluble solids were largely unaffected by crop load, fruit from vines at different crop loads would be valued equal quality if soluble solids was used as the main factor to determine grape prices. Typically soluble solids increase with leaf area per kg of fruit up to 0.8 to 1.2 m^2/kg (Kliewer and Dokoozlian 2005).

Leaf area and grapevine parameters

The leaf area per kg of fruit in this study was consistently greater than 0.8 m^2/kg except for a few Frontenac vines in 2010 (Figure 6), which explains why fruit soluble solids were largely unresponsive to crop load. Leaf area per kg of fruit was negatively correlated with increases in crop load in 2010 for both Frontenac and St. Croix, but not in 2008 and 2009. Leaf area per kg of fruit responses to vines of different crop loads are reported to range from having no correlation (Myers et al. 2008) to having a negative correlation with crop load (Kliewer and Dokoozlian 2005), which was observed in this study as well depending on the growing season and cultivar.

A range of 0.7 to 1.4 m^2 of leaf area per kg of fruit is required to ripen grapes (Howell 2001). Grapevines with a larger than then ideal leaf area per kg of fruit are undercropped and

those with less than that are overcropped (Kliewer and Dokoozlian 2005). The leaf

area/grape weight in this study ranged from 0.53 to 12.18 m^2/kg, and for most vines, it was

greater than 1 m^2/kg (Figure 6), indicating that many of the vines in this study had adequate

to excessive leaf area per kg of fruit. Leaf area per kg of fruit was negatively correlated with

crop load for both St. Croix and Frontenac in 2010. Small crop loads consistently had

excessive leaf area per kg of fruit. Frontenac vines in 2008 and 2010 had excessive leaf area

per kg of fruit at crop loads less than 8. St. Croix had excessive leaf area per kg of fruit at

crop loads less than 10 in 2008 and 2009, while in 2010, vines at crop loads less than 6 had

excessive leaf area, indicating they were undercropped at those crop loads and out of balance.

Changes in leaf area per vine and primary bud damage within each cultivar and

growing season were not correlated with crop load at a 95% confidence level (data not

shown). Grapevine primary buds exposed to sunlight are more cold hardy than shaded buds

(Wolpert and Howell 1985). Since leaf area per vine was not affected by crop load, it is

likely that vines at different crop loads had similar bud exposure to sunlight, which explains

the lack of response of primary bud damage to crop load. Cane dieback score was only

negatively correlated to increases in crop load in 2008 for Frontenac grapevines ($P \leq 0.05$)

(data not shown), indicating large crop loads in that growing season alone had negative

effects on cane periderm development. Cane dieback and mature node retention were

unresponsive to yield and pruning differences in other studies (Bates 2008, O'Daniel et al.

2012). A lack of correlation between leaf area per vine in response to changes crop load has

been previously reported (Myers et al. 2008). Negative correlations between leaf area per

vine and the amount of clusters on a vine have also been reported at many phenological

stages; however leaf area per vine was not affected by the amount of clusters on a vine at

veraison (Edson et al. 1993; 1995b), which is when leaf area was quantified in this study. While leaf area per vine at veraison was not correlated with crop load in this study, it may be feasible that it could have been negatively correlated at other grapevine phenological stages and therefore affected the grapevine microclimate.

Pruning weights and vine balance

The average pruning weights from Frontenac vines was 0.63 kg/m of cordon in 2008 and 2010 when treatments were first imposed, while St. Croix vines had 0.57 and 0.73 kg/m of cordon, respectively. Vines with pruning weights larger than 0.60 kg/m of cordon are classified as having a large vine size (Dami et al. 2005), indicating that many of the grapevines in this study previously had been undercropped. Grapevines with large yields and crop loads generally have smaller pruning weights than grapevines with small yields and crop loads (Bravdo et al. 1984; 1985, Kliewer and Dokoozlian 2005). When pruning weights are used to determine appropriate grape yields for a vine, such as with crop load and balance pruning formulas, overcropping can reduce the long-term yield potential of grapevines due to a reduction in pruning weights. Changes in pruning weights of Frontenac grapevines were not correlated with crop load (Figure 7), indicating that the crop loads in this study were not excessive for Frontenac and would not decrease the future fruiting potential. The pruning weights of St. Croix grapevines were positively correlated with crop load in 2008 and 2010 (Figure 7), which is in contrast to previous reports of cluster thinning and crop load experiments (Bravdo et al. 1984; 1985, Kliewer and Dokoozlian 2005). However, the magnitude of difference between the crop loads in this experiment were generally less than that of previous reports (Bravdo et al. 1984, Kliewer and Dokoozlian 2005). Photosynthesis rates for individual grapevine leaves increase as the number of clusters per vine increases

(Edson et al. 1993; 1995a). In previous experiments, whole-vine photosynthesis rates were not affected by the amount of clusters per vine; however, the vines with more clusters per vine also had a smaller leaf area/vine (Edson et al. 1993; 1995a; 1995b) which explains the reason more efficient individual-leaf photosynthesis rates for vines with large numbers of clusters per vine would not increase whole-vine photosynthesis rates. Leaf area per vine was not affected by crop load in this study. Since individual-leaf photosynthesis rates increase with the number of clusters per vine, vines with similar leaf area but higher crop loads, such as the vines in this study, may have accumulated more photosynthates than vines with small crop loads, which lead to increases in growth as measured by pruning weights. This supports the concept that imposing low crop loads on grapevines is disadvantageous and increasing crop load can improve vine efficiency within the crop loads investigated in this study. It is unknown if continued gains in efficiency will continue to occur at crop loads greater than in this study, however appropriately trained vines, particularly on divided canopy training systems, can have crop loads as large as 12 to 22 without decreases in yield and fruit and wine quality (Reynolds and Vanden Heuvel 2009). Increasing crop load on large singe-canopy grapevines can be difficult to achieve without leaving excessive shoot densities, therefore large vines should be trained to divided canopies training systems to allow for increases in crop loads, without excessive shoot densities.

Net returns to vineyard management

Profitability is critical in commercial grape production. Optimal vineyard performance requires managing a balance between fruit yield and vegetative growth. The commonly held view is that perceived grape and wine quality increases as vineyard yield decreases (Keller 2005) even though large crop loads and grapevine yields have not

consistently been correlated with reduced wine quality (Bravdo et al. 1984; 1985, Chapman et al. 2004, Reynolds et al. 1996b). Economic returns to land, capital, and management were positively correlated with crop load for both Frontenac and St. Croix grapevines (Figure 8). Crop loads less than 4 generally produced negative returns to land, capital, and management. Costs of land, capital, and management need to be factored into profit but will vary based on entity and location. As crop loads increased above 8, vineyard fruit yield returns to land, capital, and management were consistently positive. The crop loads in this study ranged from 2 to 14, therefore it is unknown if the increase in returns to land, capital, and management would be positively correlated at crop loads greater than 14. Due to the fact that changes pruning weights were either not correlated or positively correlated with crop load (Figure 6), the future yield, which impacts returns to land, capital, and management, of the vines in this study should not decrease at crop loads from 8 to 14. Malic acid is one of the major fruit quality parameters for cold-hardy grape cultivars and increasing crop load increased fruit quality by reducing the concentration of malic acid (Figure 1). Therefore cropping vines at crop loads of 8 to 14 increases vineyard returns to land, capital, and management at a set price/t. Increasing crop load also increases fruit quality, which may increase the price/t of grapes and further increase vineyard economic returns if grape price/t is based on fruit quality.

Conclusions

St. Croix was more responsive to crop load than Frontenac. Increasing crop load increased grape tartaric:malic acid ratio and reduced malic acid, TA, and leaf area per kg of fruit. Increasing crop load can be an affective practice to mitigate some of the large amounts

of malic acid found in fruits of cold-hardy interspecific grapevines. Pruning weights for St.

Croix grapevines increased with crop load, while Frontenac grapevines were not affected

within the crop loads examined in this study (2 to 14), indicating a lack of negative effects on

future vine productivity when high crop loads were imposed. Cold-hardy interspecific

hybrid grapevines should have crop loads between 8 to 14 imposed, in order to increase net

returns to vineyard management and decrease the large amount of fruit malic acid.

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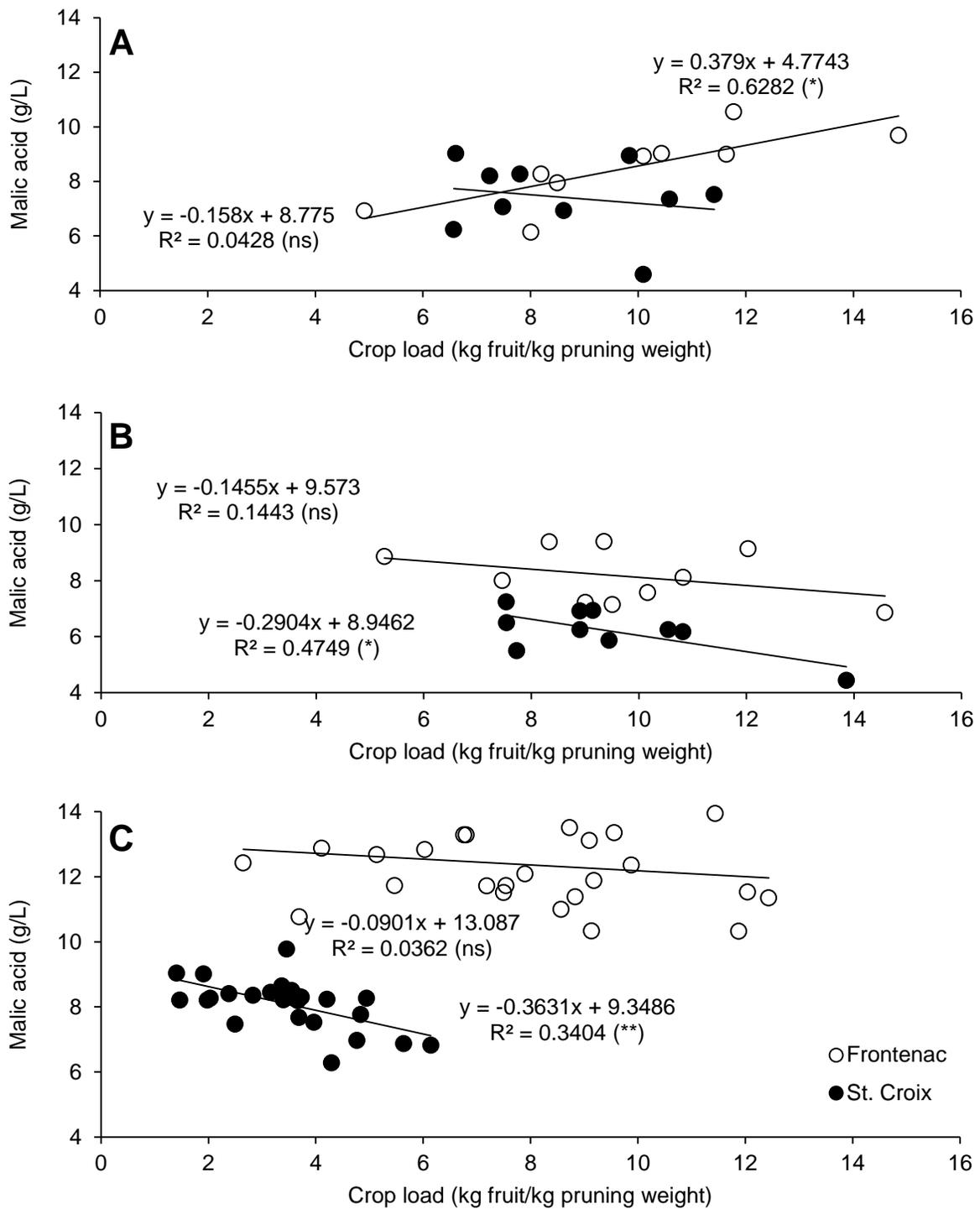
Figure 1. Change in malic acid concentration of Frontenac and St. Croix grapes in response to crop load in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns, *, ** in parenthesis indicate no significance, significance at $P ≤ 0.05$, and 0.01, respectively.
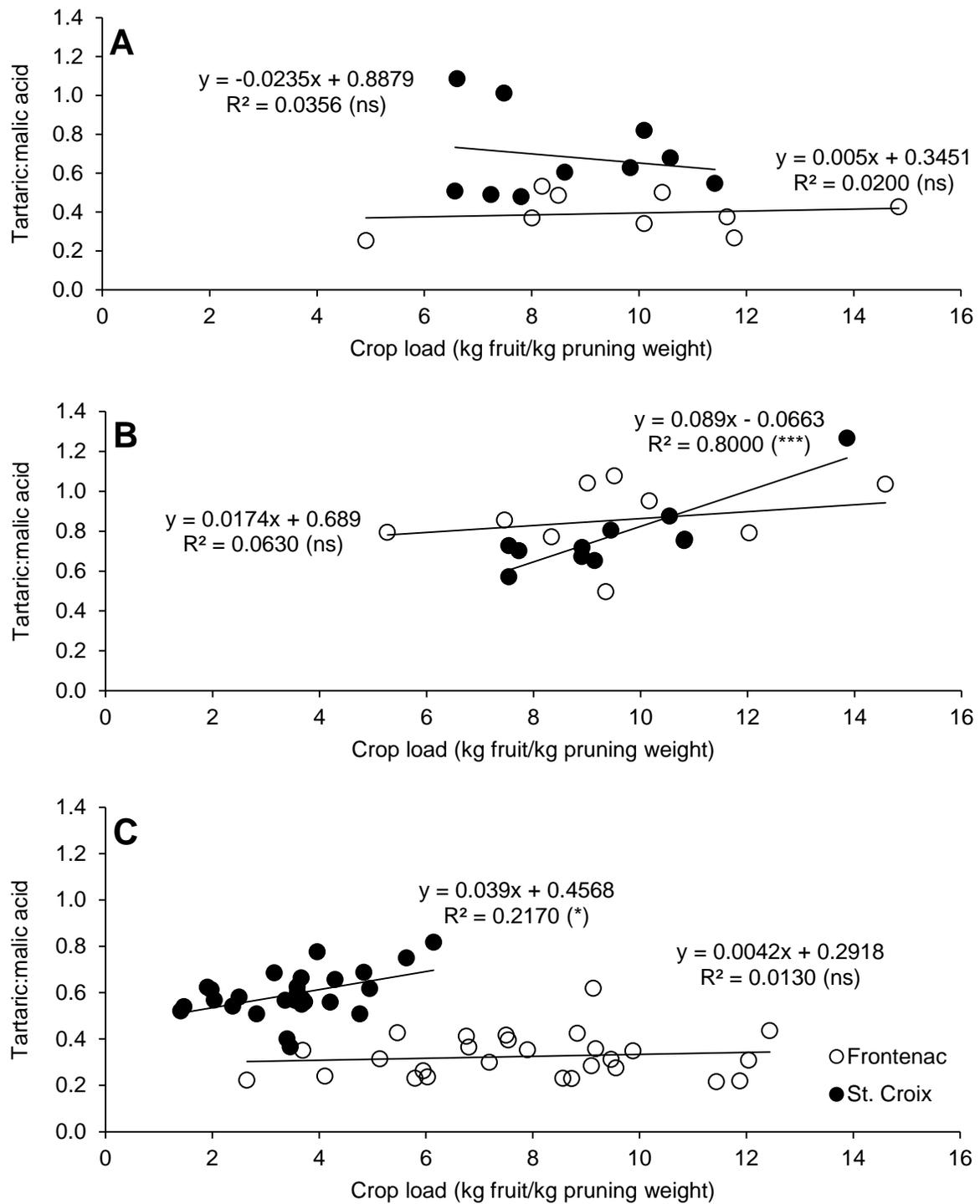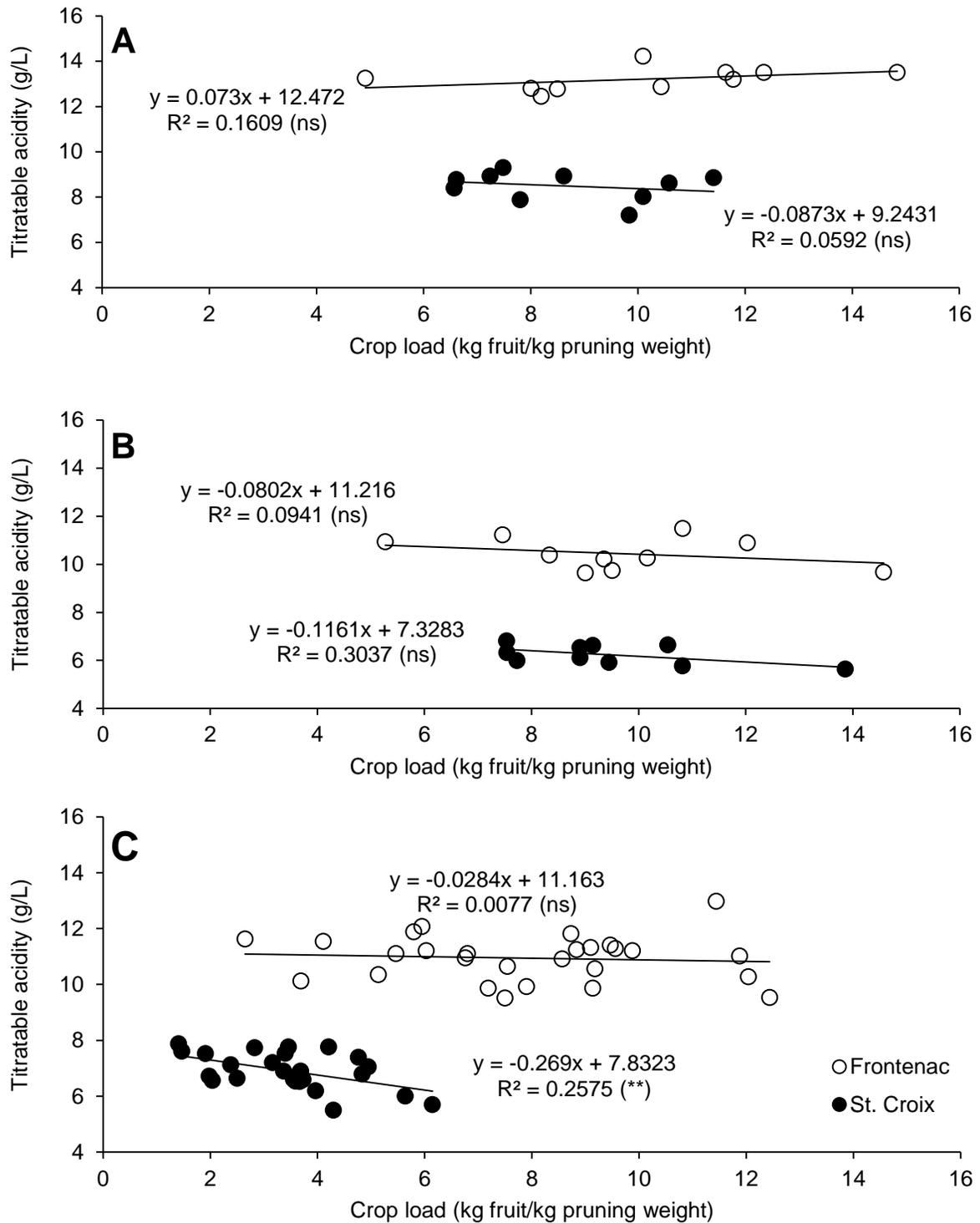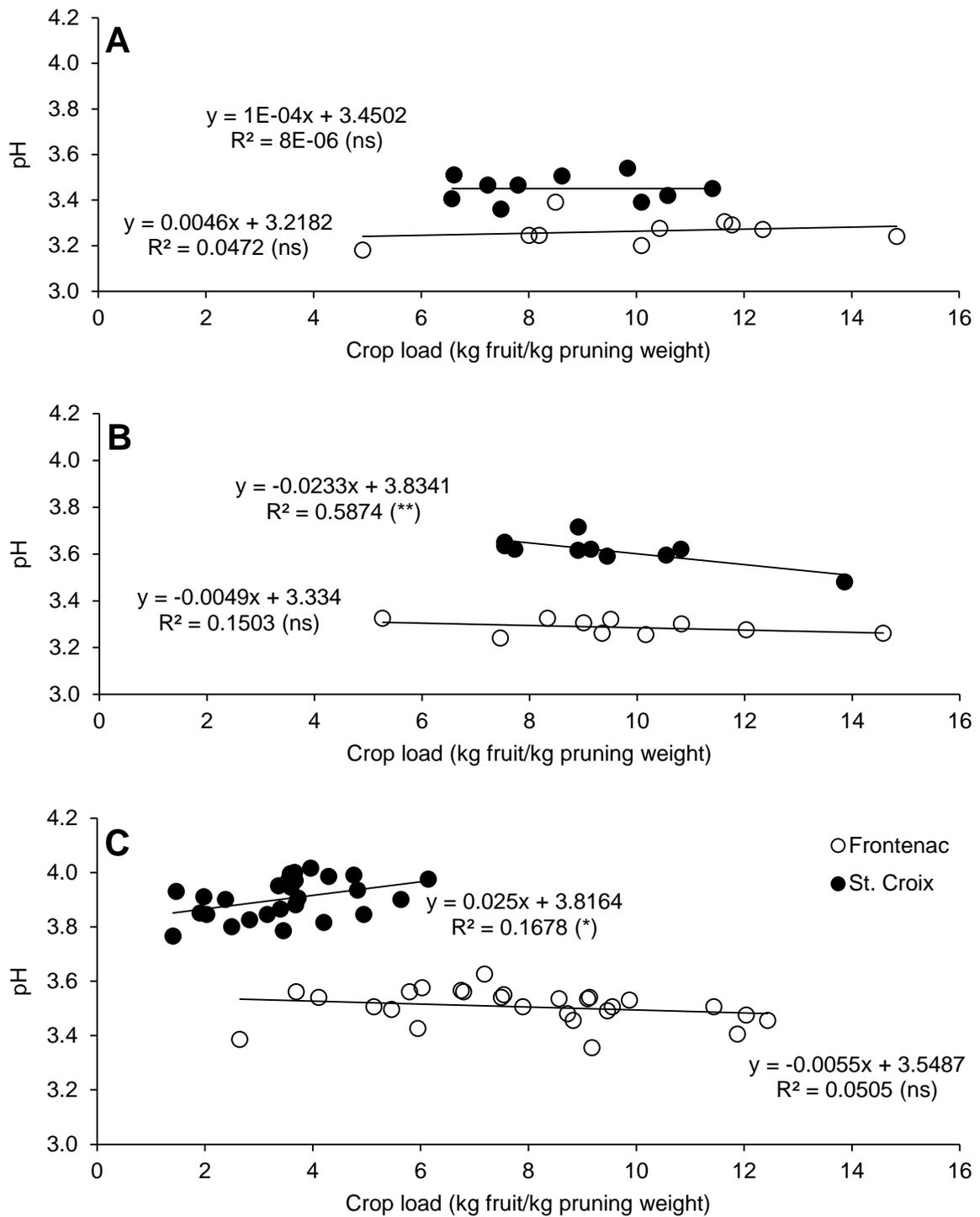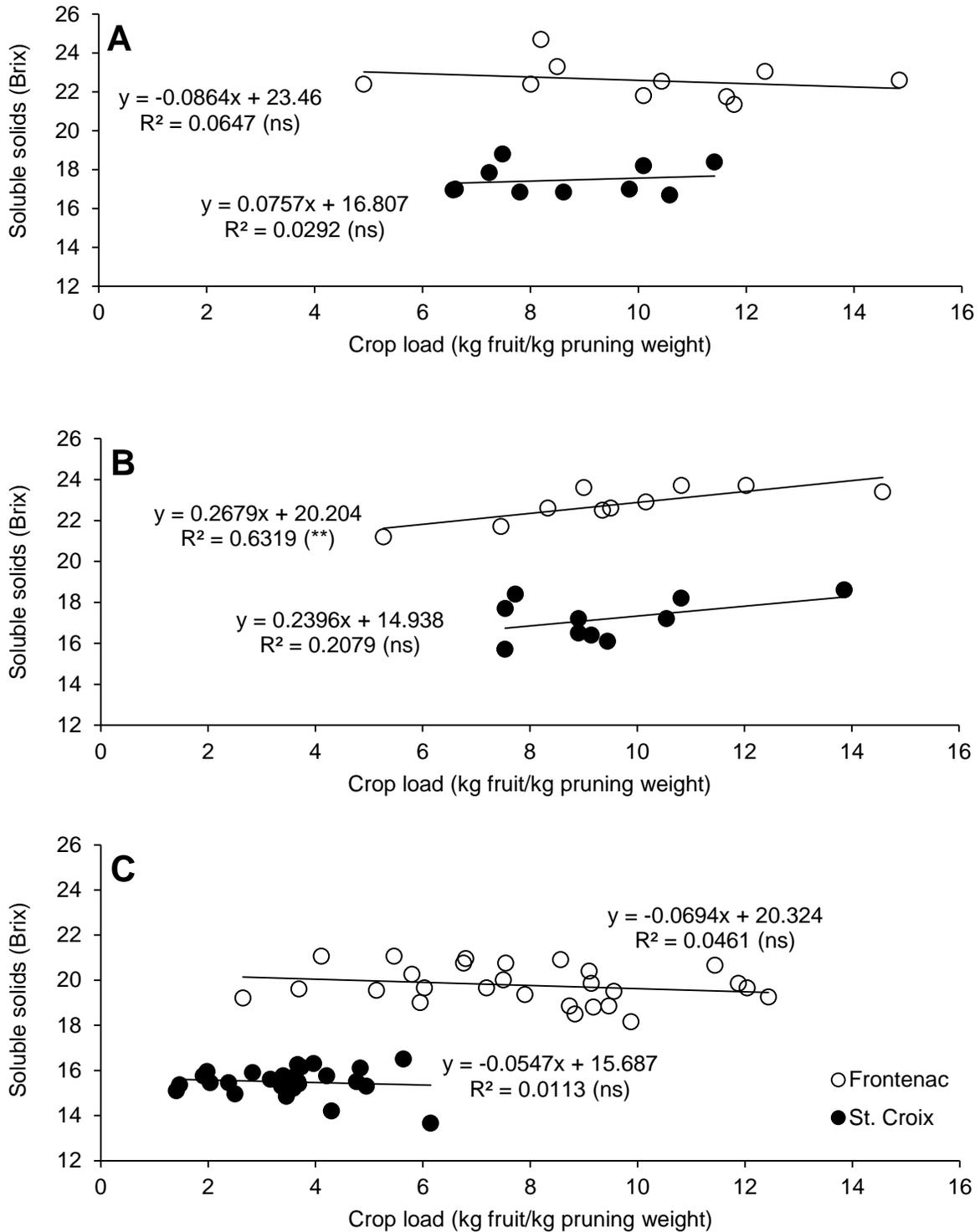
Figure 2. Change in tartaric:malic acid ratio of Frontenac and St. Croix grapes in response to crop load in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns, *, *** in parenthesis indicate no significance, significance at $P \leq 0.05$, and 0.001, respectively.

Figure 3. Change in titratable acidity of Frontenac and St. Croix grapes in response to crop load in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns and ** in parenthesis indicate no significance and significance at $P \leq 0.01$, respectively.
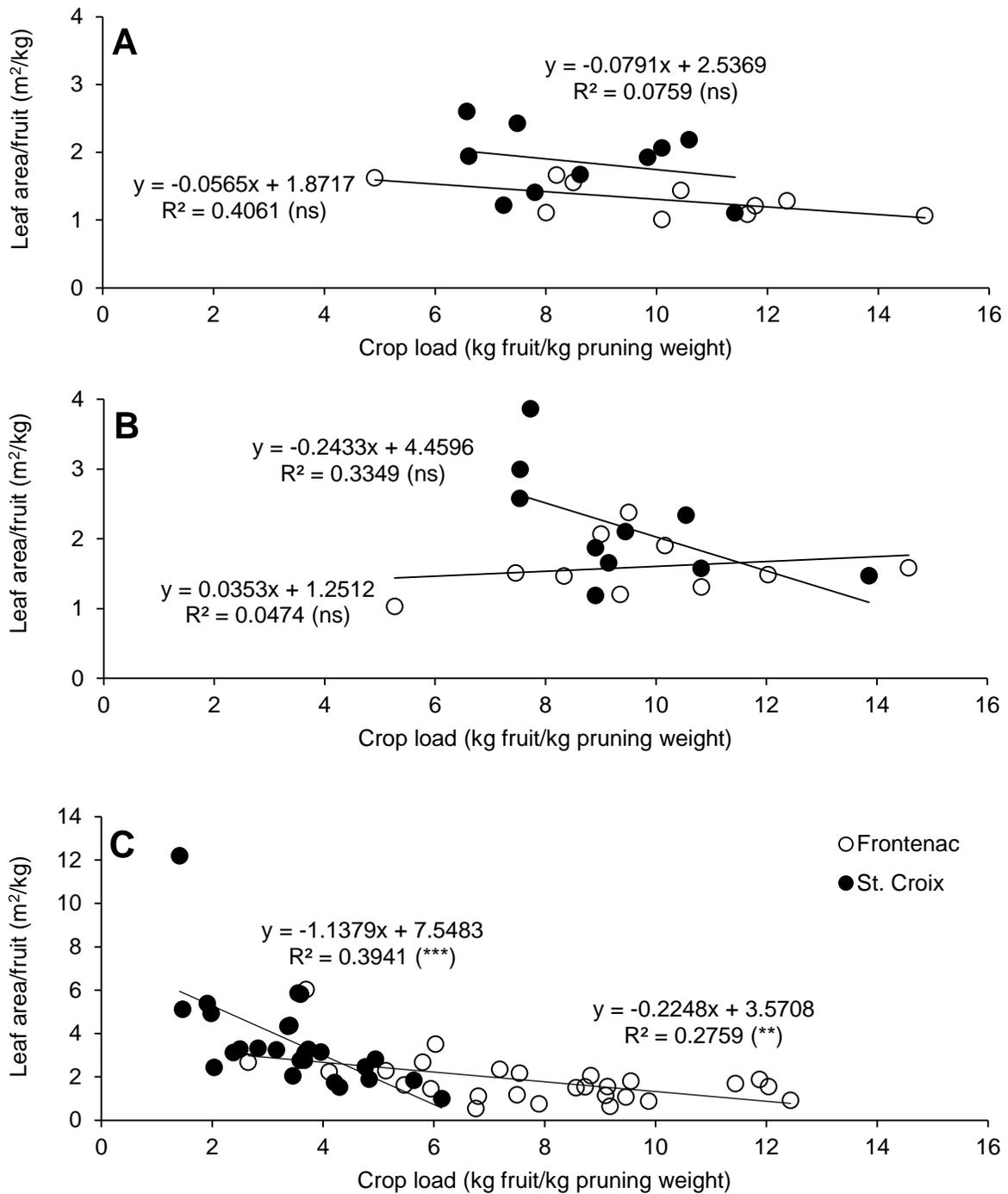
Figure 4. Change in pH of Frontenac and St. Croix grapes in response to crop load in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns, *, ** in parenthesis indicate no significance, significance at $P \leq 0.05$, and 0.01, respectively.
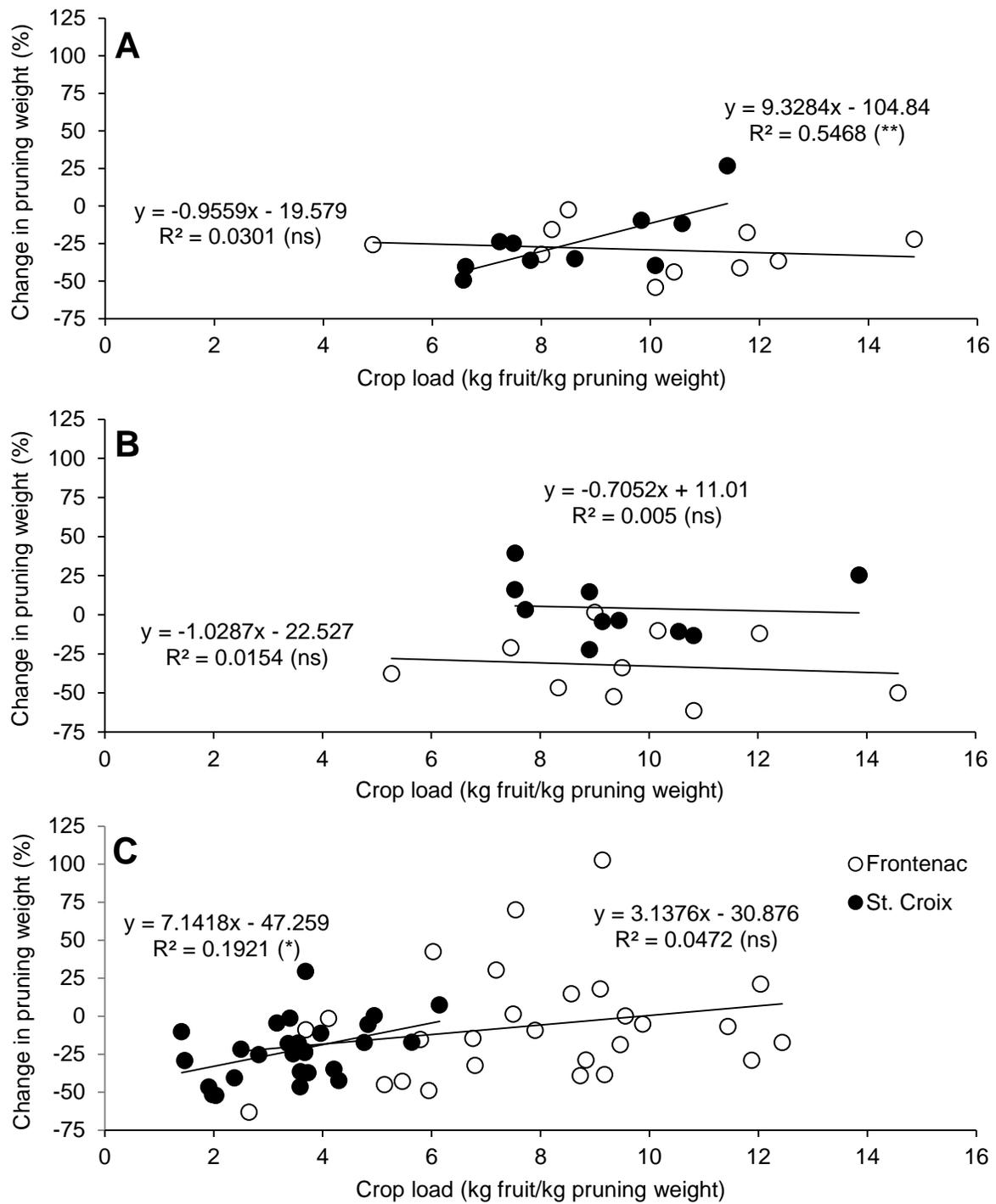
Figure 5. Change in soluble solids of Frontenac and St. Croix grapes in response to crop load in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns and ** in parenthesis indicate no significance and significance at $P \leq 0.01$, respectively.

Figure 6. Change in leaf area per fruit ratio (m^2/kg) of Frontenac and St. Croix grapevines in response to crop load in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns, **, *** in parenthesis indicate no significance, significance at $P \leq$ 0.01, and 0.001, respectively. Note the differences in y axis.

Figure 7. Percent change pruning weight from before and after crop load treatments were applied to Frontenac and St. Croix in (A) 2008 (n=10), (B) 2009 (n=10), and (C) in 2010 (n=27). ns, *, ** in parenthesis indicate no significance, significance at $P \leq$ 0.05, and 0.01, respectively.
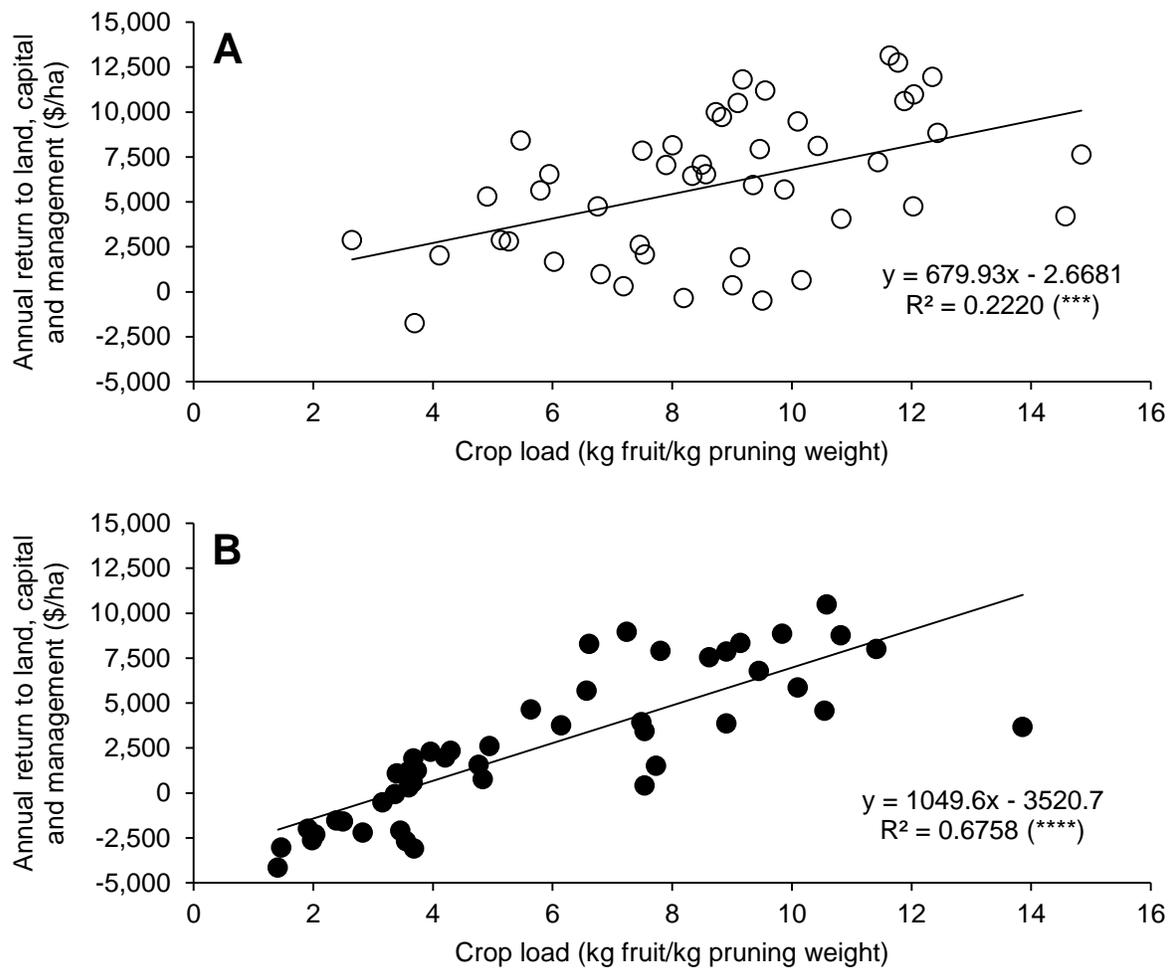
Figure 8. Annual return ($/ha) to land, capital, and management in response to differences in vineyard yield (t/ha) as affected crop load treatments from 2008 to 2010 for (A) Frontenac (n=47) and (B) St. Croix grapevines (n=47). Grapes valued at $1100/t. Profitability was determined using the equation y = 855.55x - 6918.6. *** and **** in parenthesis indicate significance at $P \leq 0.001$, and <0.0001 respectively.

CHAPTER 5: SHOOT AND CLUSTER QUANTITY AFFECT FRUIT ORGANIC ACIDS AND CANOPY OF MARQUETTE GRAPEVINES

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Abstract

The quantity of shoots and clusters on a vine are commonly manipulated in commercial grape production, however few studies have separated their effects on fruit quality and the grapevine canopy. Four shoot quantities (15, 30 45 and 60 shoots/vine) and three cluster quantities (15, 30 and 60 clusters/vine) were imposed on Marquette grapevines. Shoot quantity had an impact on measured fruit quality parameters while cluster quantity did not. Malic acid concentration and pH increased as shoot quantity increased, while tartaric:malic acid ratio decreased. Both shoot and cluster quantity affected grapevine canopy parameters. As shoot quantity increased leaf area/vine increased and leaf area/shoot decreased. Leaf area per kg of fruit increased as shoot quantity increased and decreased as cluster quantity increased. The results of this study indicate that grapevine canopy, affected by shoot density, has a greater effect on fruit quality than does grape yield. However, both shoot and cluster quantity can be manipulated to shift leaf area/vine and leaf area per kg of fruit to provide better balance between fruit and vine vegetative growth.

Introduction

Marquette (*Vitis* spp.), released in 2006, has been rapidly planted by grape growers in Northern regions of the USA. According to a survey of participating states in the Northern Grapes Project (CT, IA, IL, MA, MI, MN, ND, NE, NH, NY, SD, VT, WI), Marquette is the predominate cold-hardy grape cultivar in the region and 39% of the red wine grape vineyard acreage is planted to Marquette (Tuck and Gartner 2013). However few reports exist which examine this commercially important cultivar.

A balance between fruit yield and vegetative growth is essential for optimal grapevine management and fruit quality. The quantity of shoots and clusters on grapevines is commonly managed by viticulture practices. Shaded grapevine canopies lead to large amounts of malic acid in grapes (Morrison and Noble, 1990). Grapevine canopies with a large number of shoots can have more cane dieback, leaf layers, and shaded clusters than canopies with fewer shoots (Reynolds et al. 1994). Wine quality is affected adversely by vineyard grape yields that are either too large and too small (Bravdo et al. 1984).

Separating the effect that shoot density and grape yield have on fruit quality is challenging. Grapevine yield is often manipulated by pruning (Chapman et al. 2004), cluster thinning (Bravdo et al. 1984; 1985, Reynolds et al. 1996b), or shoot thinning (Reynolds et al. 1994). Previous studies that investigated the effects of differing shoot densities did not keep grape yield constant (Reynolds et al. 1994, Myers et al. 2008). As shoot density increases grape yields also increase (Reynolds et al. 1994), which does not allow for the separation of the effects of shoot and cluster quantity. When cluster thinning is used to limit vineyard yield, grapevines that are routinely cluster thinned have the capacity to increase cluster weight to the extent they can have similar yields as non-thinned vines (Prezler et al. 2013).

Vine yield is often manipulated by increasing or decreasing the amount of nodes left on a vine at pruning (Chapman et al. 2004), which affects shoot quantity on grapevines. Therefore yield and shoot densities often increase at the same time, making it difficult to separate the effects of shoot and cluster number on grape quality.

Increasing shoot quantity does not always increase total leaf area per vine, as vines with more shoots typically have shorter shoots with fewer laterals and less leaf area/shoot (Myer et al. 2008). Previous shoot density investigations have contrasting results. Comparisons of grapevines with several training systems and differing shoot densities, leaf area, and yield have not shown consistent differences in grape soluble solids, pH, titratable acidity (TA), and malic acid concentration, however berry skin glycosides were greater in grapes grown in canopies with fewer shoots, less leaf area, and higher yields (Zoecklien et al. 2008). Increasing shoot quantity on the same training system increased in TA and decreased soluble solids, however yield was not kept constant among all shoot quantity treatments (Reynolds et al. 1994). Grapevine yield has inconsistent effects on fruit and wine quality. Fruit from high yielding grapevines can produce wines with more fruity aromas, less vegetal aromas, and less astringency than low yielding vines (Chapman et al 2004), in contrast low yielding vines can have greater currant-like aromas than higher yielding vines (Reynolds et al 1996b).

Studies that have controlled both grapevine yield and shoot quantity indicate that both factors affect fruit quality (Reynolds et al. 1996a). However, in previous research, a small number of shoot and cluster quantity treatment levels have been investigated and those studies focused primarily on the response of soluble solids, pH, TA, and secondary metabolites to changes in shoot and cluster quantity (Reynolds et al 1996a; 1996b, Sun et al.

2012). Grapes grown in cooler climates have more malic acid (Jackson and Lombard 1993) and fruits of interspecific cultivars have large amounts of malic acid (Main et al. 2007, Main and Morris 2004). This study separates the effects that shoot and cluster quantity have on the fruit quality parameters important for wine production from Marquette. Experimental results provide viticulturists with specific information about the impact of manipulation of shoot and cluster quantity on commercial grape production.

Materials and Methods

In 2011, shoot density and cluster quantities treatments were imposed on five-year-old Marquette grapevines in a commercial vineyard near Oskaloosa, IA (41°19'01.0"N 92°38'56.7"W). Vines were planted at a 1.83 by 3.05 m spacing and trained to a 1.0 m high mid-wire cordon with vertical shoot positioning to a trellis height of 1.83 m. Three weeks after bloom (15 June) individual vines were thinned to shoot quantities of 15, 30, 45, and 60 shoots/vine (8, 16, 25, 33 shoots/m). Cluster quantity treatments were imposed on the vines four weeks (22 June) after bloom (Table 1). All treatments were imposed on four single-vine replications. A buffer vine was maintained between all treatment vines within a row. The half of each buffer vine directly adjacent to the treatment vines had the same shoot and cluster quantity imposed as the treated vine to provide a consistent canopy microclimate for the treated vines. All leaves of two randomly selected shoots per vine were collected at the end of veraison (17 Aug.) to quantify leaf area per shoot with a Licor Area Meter (Model LI-3000, LiCor Inc., Lincoln, NE), and leaf area/vine was calculated from the average leaf area/shoot and quantity of shoots/vine. Five random clusters per vine were harvested on 29 Aug., a day prior to commercial harvest for the vineyard. Clusters were weighed to obtain an

average cluster weight, following which they were juiced with a bench-top juicer, pressed

through cheesecloth, and stored at -20 °C prior to chemical analysis. Soluble solids content

of fruit was determined by using a temperature-compensating refractometer (ATAGO,

Bellevue, WA). A Thermo Scientific pH meter (Thermo Scientific Orion 2 Star, Waltham,

MA) was used to measure pH. A 10-ml juice sample was used to quantify TA (expressed as

g tartrate/L) by titration with 0.1-N NaOH to an endpoint of pH 8.2 with Metrohm automatic

titrator (Metrohm 848 Titrino plus, Riverview, FL).

Organic acids were quantified by adapting methods previously described (Castellari

et al., 2000; Falqué López and Fernández Gómez, 1996). Juice samples were filtered through

a 0.45-µm filter, diluted to 10% v/v with deionized water, and analyzed for malic and tartaric

acid by HPLC at the Iowa State University Midwest Grape and Wine Industry Institute

(Ames, IA). An Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA) with

diode-array and refractive index detectors was used with two Aminex HPX-87H (300×7.8

mm) columns (Bio-Rad, Hercules, CA) linked end-to-end with a micro-guard Cation H guard

column (Bio-Rad, Hercules, CA), which was heated to 65 °C. The isocratic mobile phase

was HPLC-grade water with 0.045-N sulfuric acid and 6% acetonitrile. The refractive index

detector was heated to 55 °C. Flow rate was 0.5 ml·min^{-1} for 35 min. Sample injection

volume was 20 µl. Peaks were identified by retention time at 210 nm for acids or by

refractive index for sugars. Ratios of tartaric:malic acid were calculated by dividing tartaric

acid by malic acid for each treatment.

A Type-III test of fixed effects linear model including main effects for shoot and

cluster quantity was performed in Statistical Analysis System ver. 9.3 software (SAS

Institute, Cary, NC) using the Proc GIMMAX function to estimate treatment effects on

measured fruit parameters, because not all cluster quantities were applied to all shoot quantity treatments. Fisher's least significant difference test was used to compare treatment means at a $P \leq 0.05$.

Results and Discussion

None of the measured fruit quality parameters was affected by the main effect of cluster quantity. As the main effect of shoot quantity increased (Table 2), malic acid concentration increased, causing the tartaric:malic acid ratio to decrease. The 60 shoots/vine treatments had 25% more malic acid and a 24% smaller tartaric:malic acid ratio than the 15 shoots/vine treatments. Grapes with a small tartaric:malic acid ratio taste more sour than those with a larger tartaric:malic acid ratio since malic acid tastes more sour than tartaric acid (Amerine et al. 1965), and will also have a more 'green' taste perception (Jackson, 2009). TA was not impacted by the main effects of shoot quantity, however grapes from Marquette vines with 8 shoots/m (15 shoots/vine) will taste less sour than from vines with 16 or more shoots/m because of the larger tartaric:malic acid ratio. Fruit from Marquette vines with 16 to 33 shoots/m should have the same amount of sourness attributed to acidity since there was no change in TA or tartaric:malic acid ratio (Table 2). Fruit pH values increased with increasing shoot quantity (Table 2). Since malic acid is weaker than tartaric acid, the increase in pH values was likely caused by the decrease tartatic:malic acid ratio as shoot quantity increased. Previous reports investigating shoot and cluster manipulations on grapevines found that pH in some years was similar to slightly higher as shoot quantity increased, which is consistent with the results in this study (Reynolds et al. 1996a).

Grapes from vines with the 45 shoots and 15 clusters/vine treatment had more malic acid than any treatment with 15 shoots/vine and the 45 shoots and 30 clusters/vine treatment (Table 3). There were no correlations with shoot quantity and malic acid concentration in previous training systems studies where shoot quantities varied (Zoecklien et al. 2008). Other shoot and cluster quantity studies did not report malic acid concentrations in grapes or wine (Reynolds et al. 1996a; 1996b, Sun et al. 2012). Leaf area/vine (Table 4) and malic acid increased with main effect of shoot quantity, while the tartaric:malic acid ratio decreased (Table 2). Vines with 60 shoots had 63% more leaf area/vine than those with 15 shoots (Table 4). The larger leaf area per vine likely increased the amount of shade in the grapevine canopy, which lead to larger malic acid concentrations in the fruits grown in those canopies (Morrison and Noble 1990). Fruit malic acid concentration was not impacted by the main effect of cluster quantity (Table 2), which conflicts with a previous report (Bravdo et a. 1985). In that study, malic acid concentration decreased as cluster quantity and yield increased, however canopy values were not reported in that study so it is unknown if shoot quantity also varied with treatments

The shoot and cluster main effects did not have an impact on the tartaric acid concentration of grapes. However, tartaric acid concentrations were greater in the 15 shoots 15 clusters/vine treatment than any other treatment including, the 15 shoots 30 clusters/vine treatment (Table 3), indicating there may be an interaction between shoot and cluster quantity. These results contrast with other reports where grapes tartaric acid concentration was not affected by yield or cluster quantity (Bravdo et al. 1984; 1985).

 As expected, leaf area of fruit per kg of grapes decreased as cluster number increased. Vines with 15 clusters and 30 clusters had 6.0 and 3.9 times the leaf area per kg of fruit than

the treatments with 60 clusters/vine. Treatments with 60 shoots/vine had 81% greater leaf

area of fruit per kg of fruit than treatments with 15 shoots/vine (Table 4). Leaf area per kg of

fruit for all treatments ranged from 4.5 to 28.6 m^2/kg (Table 5) which indicates the vines

were undercopped at all cluster quantity treatment levels (Kliewer and Dokoozlian 2005).

The yields in this study (Tables 4 and 5) were smaller than are typically reported for

Marquette (University of Minnesota Agriculture Experiment Station 2012). Soluble solids

typically do not increase then the leaf area per kg of grapes is greater than 1.2 m^2/kg (Kliewer

and Dokoozlian 2005), which could explain the lack of response by soluble solids content to

both the cluster and shoot quantity treatments in this study due to small yields (Table 3).

The treatments with 30 and 60 clusters/vine had 2.1 and 4.7 times the grape yield as

the treatments with 15 clusters/vine (Table 4). Cluster size was less than 60% of those

reported for Marquette, which led to yields lower than is typically reported for Marquette

(University of Minnesota Agriculture Experiment Station 2012). If the yields of the

grapevines in this study had been larger, there could have been a significant cluster effect on

measured fruit parameters. Vines which had the same number of clusters had the same yield

except for the treatments with 60 clusters/vine with 30 shoots/vine and 60 shoots/vine (Table

5).

All vine traits involving leaf area measurements were significantly impacted by the

main effects of shoot quantity; however, cluster quantity only affected leaf area per kg of

fruit (Table 4). As shoot quantity increased, leaf area/shoot decreased, which is consistent

with previous reports (Myers et al. 2008). However, in this study, leaf area/vine also

increased with increasing shoot quantity which contrasts with previous reports. A possible

explanation is that yield also increased with increasing shoot quantity in that investigation

(Myers et al. 2008). The decrease in leaf area/vine as shoot quantity decreased is the likely cause for the concomitant decrease in malic acid due to improved sunlight exposure into the canopy (Morrison and Noble 1990). The main effects of cluster quantity in this study did not affect leaf area/vine (Table 4). In contrast, leaf area/vine had an inverse relationship with cluster quantity in previous investigations where shoot quantity was constant in potted grapevines (Edson et al. 1993; 1995); however lateral shoots were removed in those studies and laterals comprise a large amount of the total leaf area for a vine (Zoecklein et al. 2008), which may have affected canopy response to cluster quantities in that study.

Conclusions

The main effect of shoot quantity impacted fruit quality parameters while cluster quantity did not. This supports the concept that grapevine canopy microclimate has a greater effect on fruit quality than does fruit yield. Minimizing shoot quantity to 8 shoots/m for Marquette vines was effective at decreasing pH values and malic acid concentration of fruits and maximizing tartaric:malic acid ratio, however this also can limit vineyard yield. Vines with 16 to 33 shoots/m had acceptable fruit quality and have larger yield potential. The decision to arrange a grapevine canopy with few shoots or many shoots still depends on the desired outcomes. For vines with a large amount of vigor, fewer shoots should be retained within a given quantity of clusters if the desired goal is to minimize malic acid and pH values in grapes, and to decrease leaf area/vine and leaf area per kg of fruit to better balance vines. However, for vines with small amounts of vigor, it could be beneficial to increase shoot quantity within a given cluster quantity to increase leaf area/vine and leaf area per kg of fruit.

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Table 1. Shoot and cluster treatments imposed on Marquette grapevines (n=4) in 2011.

Treatment	Shoots per vine	Clusters per vine
1	15	15
2	15	30
3	30	15
4	30	30
5	30	60
6	45	15
7	45	30
8	45	60
9	60	30
10	60	60

Table 2. Analysis of estimates of main effects of shoot and cluster quantity per vine on the fruit quality parameters of Marquette grapevines (n=4) in 2011.

	Soluble solids (Brix)	pH	Titratable acidity (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Tartaric: malic acid ratio
Shoots per vine						
15	24.4 a[a]	3.61 c	9.5 a	4.9 a	5.1 b	0.97 a
30	24.2 a	3.67 b	9.5 a	4.2 a	5.4 b	0.78 b
45	23.9 a	3.67 b	10.0 a	4.0 a	6.0 a	0.68 b
60	23.7 a	3.75 a	9.9 a	4.5 a	6.4 a	0.74 b
P value	0.4832	0.0009	0.1754	0.1064	0.0222	0.0105
Clusters per vine						
15	24.0 a	3.69 a	9.73 a	4.6 a	5.9 a	0.81 a
30	24.0 a	3.68 a	9.70 a	4.3 a	5.6 a	0.78 a
60	24.2 a	3.66 a	9.70 a	4.4 a	5.6 a	0.79 a
P value	0.7293	0.3813	0.9904	0.6469	0.4643	0.8994

[a] Treatment means followed by the same letter within columns among main effects are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

Table 3. Chemical composition of fruits harvested from Marquette grapevines (n=4) as affected by shoot and cluster number treatments in 2011.

Shoots per vine	Clusters per vine	Soluble solids (Brix)	pH	Titratable acidity (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Tartaric: malic acid ratio
15	15	24.7 a [a]	3.62 b	9.5 bc	5.6 a	5.1 b	1.11 a
15	30	24.0 a	3.63 b	9.4 bc	4.3 bc	5.2 b	0.83 bc
30	15	23.9 a	3.67 ab	9.6 bc	4.1 bc	5.6 ab	0.75 bc
30	30	24.5 a	3.69 ab	9.8 abc	4.9 ab	5.6 ab	0.87 b
30	60	24.3 a	3.64 b	9.0 c	3.6 c	5.1 b	0.71 bc
45	15	23.8 a	3.69 ab	9.9 abc	3.9 bc	6.5 a	0.62 c
45	30	24.0 a	3.67 ab	9.6 abc	3.9 bc	5.4 b	0.74 bc
45	60	24.0 a	3.64 b	10.4 a	4.1 bc	6.1 ab	0.69 bc
60	30	23.4 a	3.73 a	10.0 ab	3.9 bc	6.0 ab	0.67 bc
60	60	24.1 a	3.75 a	9.9 abc	4.8 ab	6.1 ab	0.80 bc

[a] Treatment means followed by the same letter within columns are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

Table 4. Analysis of estimates of main effects of shoot and cluster quantity on grape yield and vine canopy traits of Marquette grapevines (n=4) in 2011.

	Cluster weight (g)	Yield (kg/vine)	Leaf area (cm^2/shoot)	Leaf area (m^2/vine)	Leaf area ratio (m^2/kg grapes)
Shoots per vine					
15	51.1 a[a]	1.85 a	10100 a	13.9 b	10.1 b
30	54.2 a	1.96 a	4682 b	14.7 b	10.1 b
45	51.7 a	1.85 a	5207 b	23.4 a	17.9 a
60	48.4 a	1.66 a	3626 b	22.6 a	18.3 a
P value	0.5221	0.3238	<0.0001	0.0230	0.0099
Clusters per vine					
15	50.2 a	0.71 c	5506 a	17.86 a	23.3 a
30	48.7 a	1.46 b	6609 a	22.12 a	15.1 b
60	55.2 a	3.32 a	5596 a	15.15 a	3.9 c
P value	0.1519	<0.0001	0.3538	0.1393	<0.0001

[a] Treatment means followed by the same letter within columns among main effects are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

Table 5. Grape yield and vine canopy traits of Marquette grapevines (n=4) as affected by shoot and cluster number treatments in 2011.

Shoots per Vine	Clusters per vine	Cluster weight (g)	Yield (kg/vine)	Leaf area (cm^2/shoot)	Leaf area (m^2/vine)	Leaf area ratio (m^2/kg grapes)
15	15	49.1 ab[a]	0.74 d	9,388 a	14.1 c	19.1 bc
15	30	49.4 ab	1.48 c	11,120 a	16.7 c	11.4 cd
30	15	54.1 ab	0.81 d	4,639 bc	13.9 c	18.0 bc
30	30	47.5 b	1.42 c	3,520 bc	10.6 c	7.4 d
30	60	61.0 a	3.66 a	5,887 b	17.7 bc	4.9 d
45	15	50.3 ab	0.75 d	4,767 bc	21.5 bc	28.6 a
45	30	50.1 ab	1.50 c	6,354 b	28.6 ab	18.9 bc
45	60	54.8 ab	3.29 ab	4,499 bc	20.2 bc	6.2 d
60	30	48.1 b	1.44 c	5,444 b	32.7 a	22.8 ab
60	60	49.9 ab	2.99 b	2,207 c	13.2 c	4.5 d

[a] Treatment means followed by the same letter within columns are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

CHAPTER 6: GENERAL CONCLUSIONS

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General Discussion

Wine grape (*Vitis* spp.) production adds value and diversification to horticultural enterprises and is expanding in the Upper Midwest and other cold-climate regions. Cold-hardy grape cultivars have been introduced from private breeders, such as Elmer Swenson, and public institutions such as Cornell University and the University of Minnesota. These cultivars have provided the basis for grape production in cold-climate regions.

The industry adoption of many of these cultivars has occurred at a rapid pace, and there is a recently expanded commercial wine grape industry. Relatively little research about the impact that viticultural practices have on fruit quality has been performed on the cultivars grown commercially in cold-climate regions. The current enthusiasm for local food and wines needs to be reinforced with science-based and economically viable production practices for the grape and wine industry to succeed in the long term.

Research was designed to provide pertinent information on cold-climate grapes to viticulturists and enologists, and allow better management of grape and wine production. Much of the research presented in this dissertation provides information that is valuable to both viticulturists and enologists working with a wide range of cultivars. The work presented in this document provides information that not only addresses current challenges in grape production in the Upper Midwest and other cold-climate regions, but also provides a basis from which future research can develop.

Impact of stage of grape maturation on fruit quality

The cold-hardy grape cultivars in these studies, Edelweiss, Frontenac, La Crescent, Marquette, and St. Croix differ in their fruit quality parameters. Common assumptions were that these cultivars had similar fruit quality parameters because they are grown in the same regions and originate from the same breeding programs; however we found that not to be the case. Grape soluble solids and pH for the cultivars in these studies were similar to quantified cultivars of *V. labruscana* Bailey*, V. vinifera* L., and French hybrids, with the exception of Edelweiss; which had less soluble solids. The glucose:fructose ratio decreased with stage of maturation for Frontenac, La Crescent, and Marquette, but a major difference observed was that the glucose:fructose ratio was larger, even late in maturation, than is reported for most grape cultivars. Only Edelweiss and La Crescent had more slightly more fructose than glucose, while Frontenac had on average 21% more glucose than fructose. The trend is to harvest cold-hardy grapes late in maturation. Unlike many cultivars, the cultivars in these studies do not accumulate large amounts of fructose late in maturation. Therefore, yeast strains that are able to metabolize large amounts of fructose are unnecessary when harvesting these cultivars late in maturation.

The acid profile of cold-hardy interspecific grapes was the primary trait that distinguishes them from cultivars of *V. labruscana, V. vinifera,* and French hybrids. Acid profile also differed amongst the cold-hardy cultivars in the research. Cold-hardy grape cultivars are a blend of many different grape species, and therefore differences among cultivars were expected. Edelweiss, Frontenac, and La Crescent fruits had larger amounts of malic acid than observed in other cultivars. Malic acid concentration decreased with each stage of maturation for Edelweiss fruits, however malic acid in fruits of Frontenac and La

Crescent did not decrease consistently with every stage of maturation, indicating that delaying harvest may not always lead to decreases in malic acid, as was assumed for these cultivars.

The most prominent difference in the cultivars investigated was the tartartic:malic acid ratios. Generally tartartic:malic acid ratio increased with stage of maturation for all cultivars except La Crescent, for which there were no changes with stage of maturation. Viticulturists and enologists often harvest late in maturation to reduce tart flavors associated with malic acid, which decreases more rapidly later in maturation than tartaric acid and therefore increases the tartartic:malic acid ratio. Based on results from these studies, delaying harvest for La Crescent would be ineffective at changing the fruit tartartic:malic acid ratio. Increases in tartartic:malic acid ratio could be attributed both to decreases in malic acid and also increases in tartaric acid as grapes matured, which is atypical. Most grape cultivars have more tartaric acid than malic acid, however Edelweiss and La Crescent never had a tartartic:malic acid ratio greater than 0.55, indicating that malic acid is the predominant acid in these cultivars.

Soluble solids content and pH generally increased with stage of maturation and titratable acidity (TA) decreased as is typically reported. Soluble solids, pH and TA are the parameters commonly used to determine when to harvest grapes. For some of the cultivars in these studies, there were growing seasons where soluble solids, pH, and TA, individually or in combination, did not change with state of maturation, however, there were still changes in grape tartaric acid, malic acid, citric acid and the tartartic:malic acid ratio. Measuring soluble solids, pH and TA to determine harvest date provides only a partial view of changes in grape quality and has limitations for determining when to harvest wine grapes.

Impact of crop load on fruit quality and the grapevine

The conventional view of grapevine yield is that as grape yield decreases, fruit quality increases, however few studies substantiate this view. Vineyards in the Upper Midwest have small yields and yet grape cultivars grown in this region are described as being very vigorous. Crop load (grape yield/pruning weight) of grapevines is used to determine if grapevines have an appropriate balance of fruit and vegetative growth, while yield by itself can give a limited assessment of grapevine status. Crop loads ranging from 2 to 14 were examined and fruit quality parameters were more highly correlated with crop load for St. Croix grapevines than for Frontenac, indicating that Frontenac may be harvested at a wider range of crop loads without changes in fruit quality parameters. Fruits of St. Croix had less malic acid and TA as crop load increased, while tartartic:malic acid ratio increased. The pH of grapes was both positively and negatively correlated with crop load, which indicates pH is responsive to crop load, however other factors such as the TA and acid profile, as impacted by crop load, may directly determine pH values. As crop load increased, the leaf area per kg of grapes (m^2/kg) decreased. Most of the vines in the study had greater leaf area per kg of grapes than is required for optimal fruit development and likely led to shade within the canopy.

Increasing crop load of vigorous cold-hardy grapevines should be implemented to increase yields and put the large amount of leaf area to productive use. Increases in crop load can not only increase yield and net returns to vineyard management, but also will increase the fruit quality of cold-hardy grape cultivars due to reductions in the large amounts of malic acid commonly found in these cultivars.

Impact of shoot and cluster quantity on fruit quality and grapevines

Many of the cultural practices applied to grapevines involve managing the amount of fruit and shoots on grapevines. In spite of the fact that these practices can be applied alone or in conjunction with each other, we have found few studies that investigate these parameters independent from each other at a wide range of levels. Questions that growers might encounter might be 'If the desired number of clusters/vine is 60, is it preferable to place those the clusters on 30, 45, or 60 shoots/vine?' and 'When a vine ripens 15, 30, or 60 clusters, does the fruit quality differ if each grapevine has 30 shoots?' Knowing which factor has the greatest impact allows growers of grapes to make informed decisions to maximize both quality and quantity of grapes, both of which have an impact on profitability.

Shoot and cluster quantity on Marquette grapevines were manipulated and cluster quantity did not have an effect on the measured fruit parameters. Grape pH and malic acid increased as shoot quantity increased while tartaric:malic acid ratio decreased. The increase in leaf area per kg of grapes and leaf area/vine as shoot quantity increased likely created more shade in the grapevine canopy, which increased fruit malic acid. The vines in this study were vigorous and had indications of being undercropped and excessive leaf canopy. The results of this study indicate that retaining a smaller number of shoots can provide positive benefits for vigorous vines by reducing leaf area/vine and fruit malic acid. This approach is not warranted on less vigorous vines as it could reduce leaf area per kg of grapes (m^2/kg) below optimal and reduce fruit quality. If vines are not vigorous and have less than optimal leaf area per kg of grapes, increasing the number of shoots/vine and decreasing clusters/vine can increase leaf area per kg of grapes to balance fruit and vegetative growth of grapevines.

Primary Conclusions

- Fruit quality traits differed among cold-hardy grape cultivars, and therefore fruits of these cultivars should be harvested at cultivar-specific parameters.

- Soluble solids content should be de-emphasized as the primary predictor of fruit quality. For some cultivars and growing seasons fruit organic acid profiles changed with stage of maturation when soluble solids content did not.

- Large concentrations of fructose do not accumulate late in maturation in fruits of Edelweiss, Frontenac, La Crescent, Marquette, and St. Croix.

- Harvesting grapes late in maturation can lead to a reduction of malic acid, but does not always do so.

- As crop load increased from 2 to 14, fruit quality of St. Croix grapes increased, there was little effect on fruits of Frontenac, and there were minimal negative effects on the grapevines of either cultivar.

- Canopy microclimate has a greater impact on Marquette fruit quality than cluster quantity.

- The following are methods which can decrease that amount of malic acid in grapes: harvesting late in fruit maturation, increasing crop load, and reducing the quantity of shoots on a grapevine.

Recommendations for Future Research

The experiments presented in this dissertation elucidate some of the basic information necessary to produce quality fruit from cold-hardy grape cultivars commercially grown in the Upper Midwest and other cold-climate grape growing regions. Factors that separate high

quality grapes and wine from typical grape and wine quality include soluble solids, pH, titratable acidity, acid profile, and sugar profile. However, flavor and aroma compounds also have a large role in dictating quality. Identifying flavor and aroma compounds in cold-climate grape cultivars is a key component needed in future research. Vineyard practices that affect these compounds need to be identified to allow management to enhance or reduce the abundance of these important flavor and aroma compounds. Recent surveys from the North Grapes Project indicate that grape yields are relatively small in the Upper Midwest, however growers commonly report vines with high vigor. Economic feasibility should have a large role in all future applied research because for growers to be sustained they must be profitable. Many of the treatments investigated in the research presented in this document do not require extra practices that increase costs of production, however some practices do increase the cost of production such as leaf pulling, shoot positioning, training systems, etc. As more is discovered about the growth and management of cold-hardy grape cultivars, we must also learn what we can and cannot afford to do in terms of vineyard management for economic sustainability.

APPENDIX

Additional figures based on data in Chapter 4.
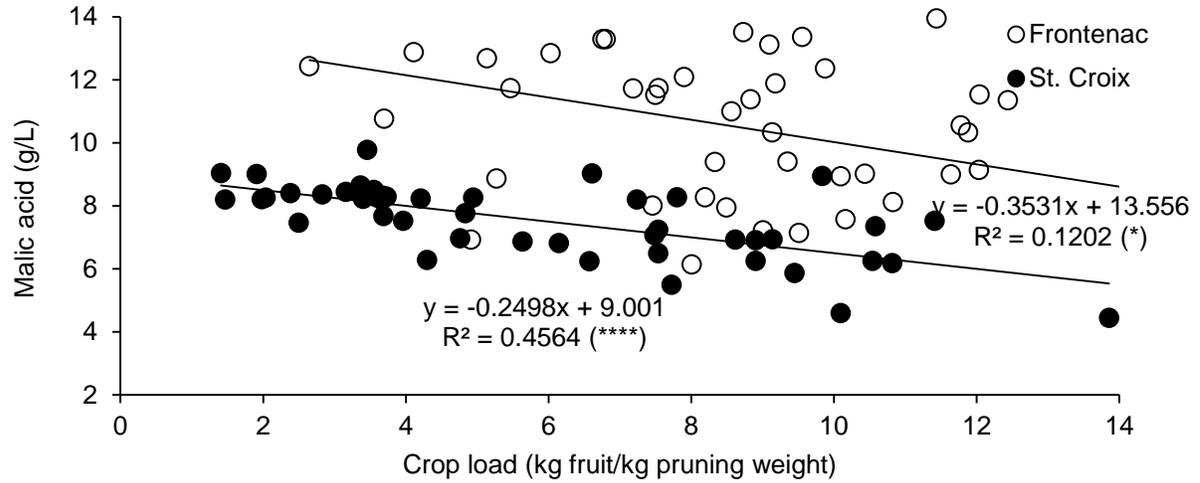
Figure 1. Change in malic acid concentration of Frontenac and St. Croix grapes in response to crop load from 2008 to 2010 (n=47). * and **** in parenthesis indicate no significance and significance at $P \leq$ 0.05 and 0.0001, respectively.
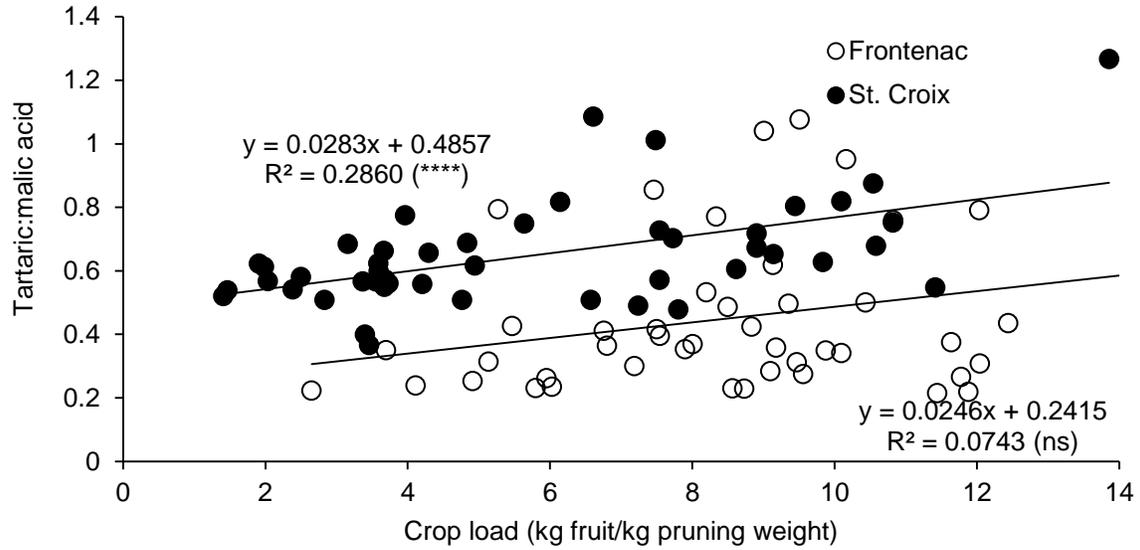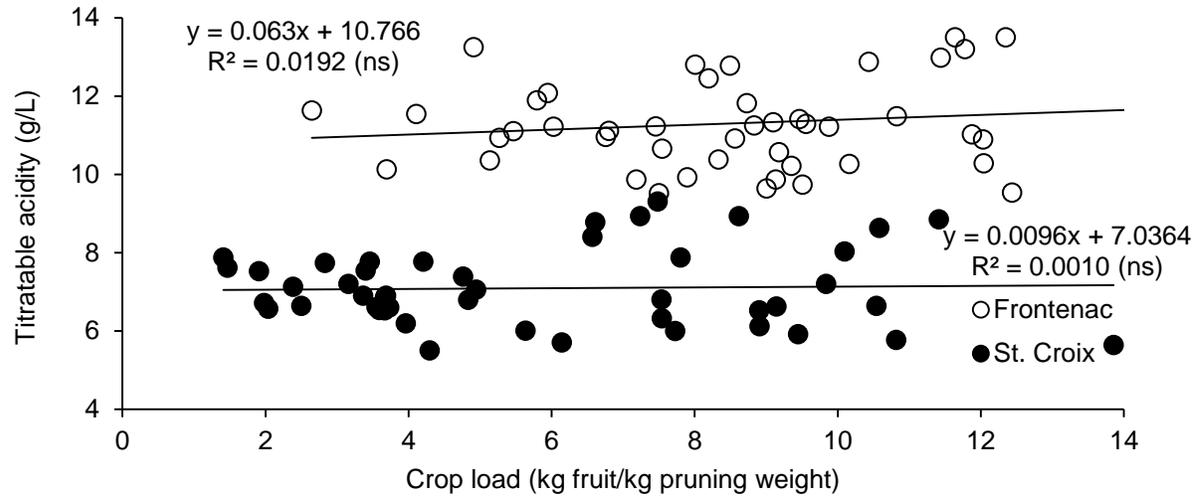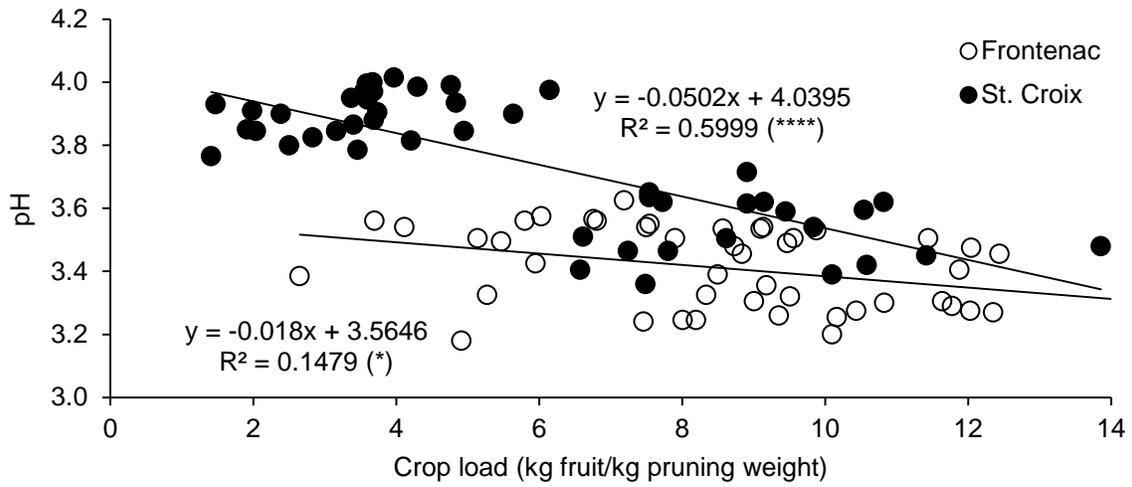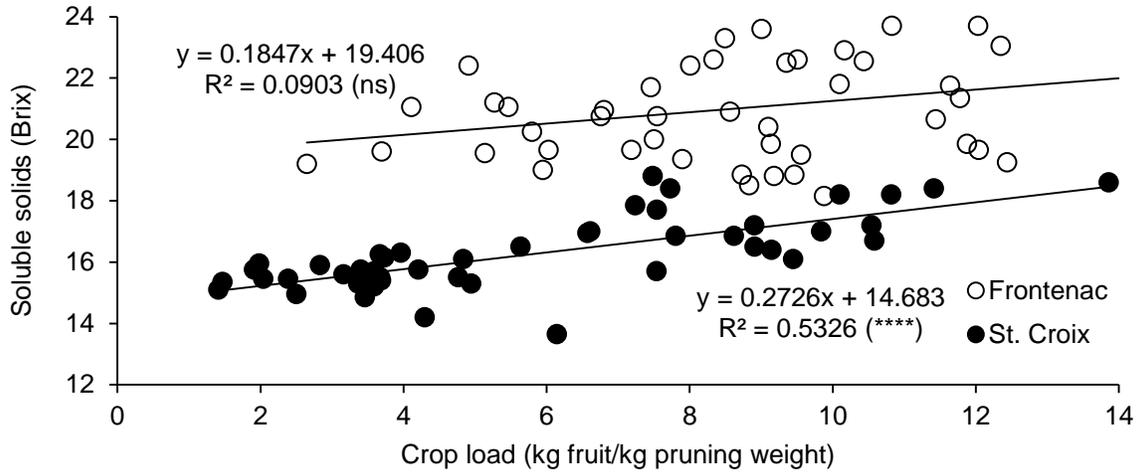
Figure 2. Change in tartaric:malic acid ratio of Frontenac and St. Croix grapes in response to crop load from 2008 to 2010 (n=47). ns, and **** in parenthesis indicate no significance and significance at $P < 0.0001$, respectively.
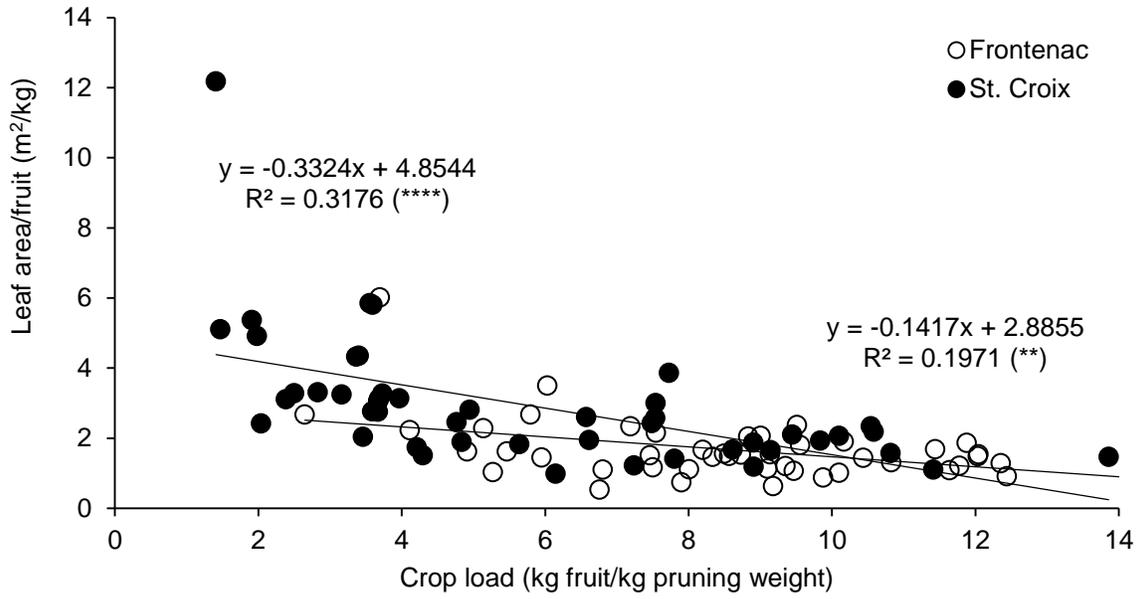
Figure 3. Change in titratable acidity of Frontenac and St. Croix grapes in response to crop load from 2008 to 2010 (n=47). ns in parenthesis indicates no significance.

Figure 4. Change in pH values of Frontenac and St. Croix grapes in response to crop load from 2008 to 2010 (n=47). * and **** in parenthesis indicate significance at $P \leq 0.05$ and 0.0001, respectively.
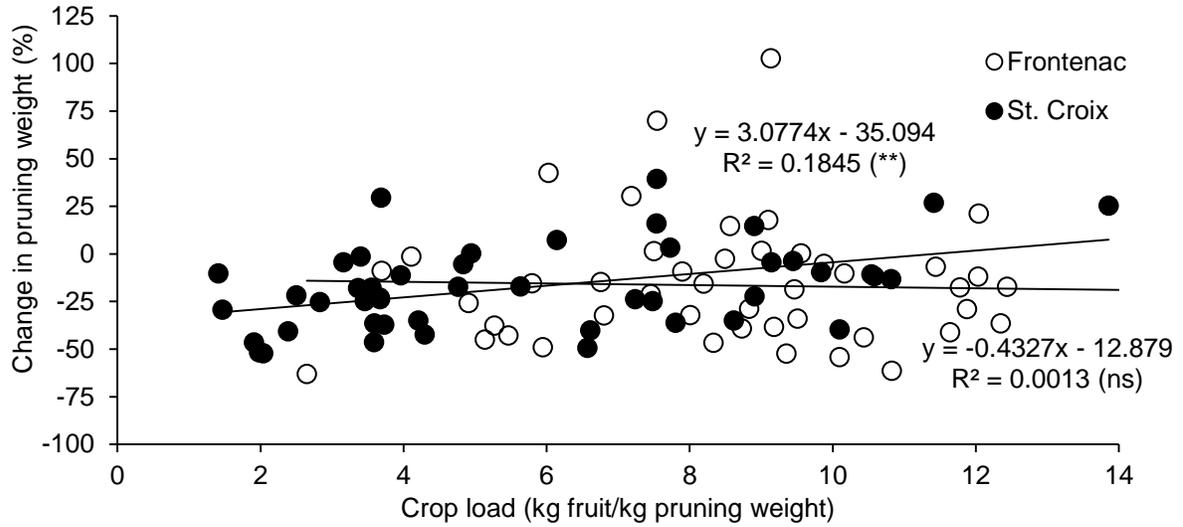
Figure 5. Change in soluble solids of Frontenac and St. Croix grapes in response to crop load from 2008 to 2010 (n=47). ns and **** in parenthesis indicate no significance and significance at $P < 0.0001$, respectively.

Figure 6. Change in leaf area per fruit ratio (m^2/kg) of Frontenac and St. Croix grapevines in response to crop load from 2008 to 2010 (n=47). ** and **** in parenthesis indicate significance at $P \leq 0.01$ and 0.0001, respectively.

Figure 7. Change in pruning weights (kg) of Frontenac and St. Croix grapevines in response to crop load from 2008 to 2010 (n=47). ns and ** in parenthesis indicate no significance and significance at P ≤ 0.01, respectively.
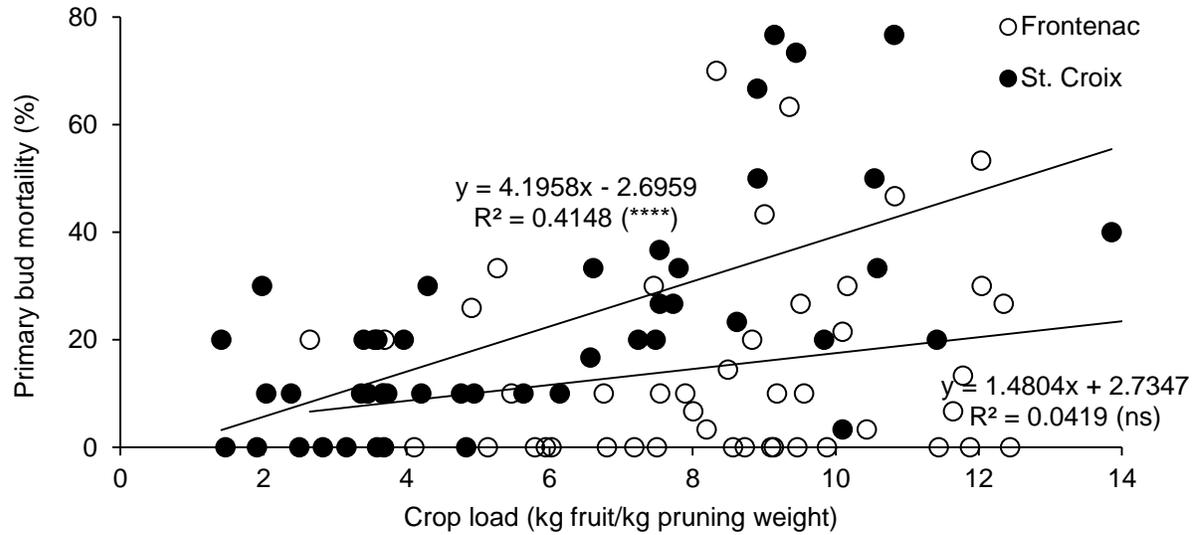
Figure 8. Change in primary bud mortality (%) of Frontenac and St. Croix grapevines in response to crop load from 2008 to 2010 (n=47). ns and **** in parenthesis indicate no significance and significance at $P < 0.0001$, respectively.
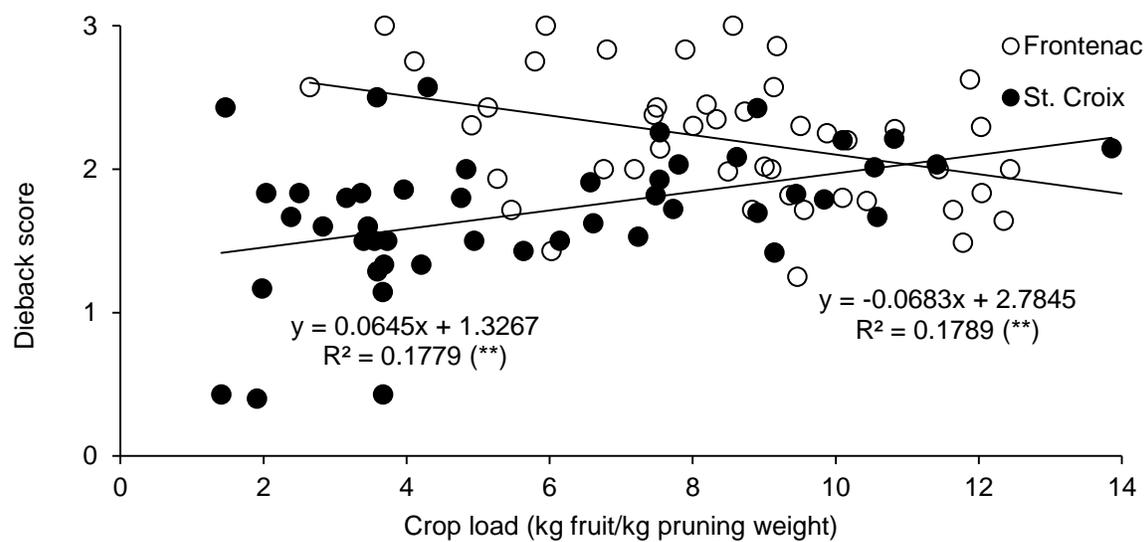
Figure 9. Change in dieback score (0-3) of Frontenac and St. Croix grapevines in response to crop load from 2008 to 2010 (n=47). ** in parenthesis indicates significance at $P < 0.01$.