Ripening Profile of Grape Berry Acids and Sugars in University of Minnesota Wine Grape Cultivars, Select *Vitis vinifera*, and Other Hybrid Cultivars

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ABSTRACT

Recent introduction of new cold-hardy grape (*Vitis* sp.) cultivars has fueled a growing wine industry in non-traditional temperate growing regions. Though widely grown, little is known of the chemical composition of the cold-hardy wine grape cultivars. To develop a profile of the ripening process and quantify key grape berry metabolites, chemical analyses were performed on University of Minnesota wine grape cultivars, select *V. vinifera* cultivars, and other hybrid cultivars throughout fruit ripening. Organic acid and sugar concentrations of the eleven wine grape cultivars were determined using liquid chromatography/ mass spectrometry (LC/MS). All cultivars maintained glucose to fructose ratio of approximately 1:1 ratio and tartaric to malic ratio varied between years and cultivars. Cold-hardy wine grape cultivars 'Frontenac', 'Frontenac gris', and 'La Crescent' retain higher concentrations of organic acids throughout berry development. Year was found to have little or no effect on malic or tartaric acid concentrations. Soluble solids content (SSC), titratable acidity (TA), pH and berry weight measurements were taken for three consecutive years (2010-2012). TA and pH profiles varied substantially among the years. TA, SSC, and pH were compared to growing degree days (GDD) to determine the number of heat units needed to ripen the selected cultivars and investigate if GDD is a valid predictor for grape maturity. Cold-hardy hybrid need approximately 1,400 GDD˚C to ripen fruit in Minnesota. GDD was found to be useful in predicting grape berry maturity especially SSC levels.

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CHAPTER ONE

Literature Review

Grape Production and Germplasm:

The grape is the most valuable fruit crop in the world, and is cultivated mainly for winemaking, bulk juice, fresh fruit (table grapes), and dried fruit (raisins). Since the early 1990's, the introduction of new cold-hardy grape cultivars has fueled a growing wine industry in cold climate regions of the United States. A 2009 -2010 'Cold Climate Viticulture Research Extension Needs Survey' showed that 250 wineries have been established in New England, New York, Nebraska, Iowa, Wisconsin, and Minnesota. These wineries are supported by over 1,300 grape growers cultivating an estimated 3,300 acres of grapes (Martinson et al., 2010). In Minnesota, the wine industry is growing at an annual rate of 28% and is projected to produce 150,000 gallons of wine by the year 2014 (http://mngrapegrowers.com/).

Used in producing 99% of the world's wine and table grapes, *V. vinifera* is the most cultivated *Vitis* species with an estimated 10,000 cultivars (Riaz et al., 2007). Native to Eurasia, *V. vinifera* cultivation is mostly confined to the world's two "grape belts" between 30˚ to 50˚ N latitude in the northern hemisphere and 30˚ to 40˚ S latitude in the southern hemisphere. This geographical confinement is mostly due to the lack of cold tolerance of *V. vinifera* cultivars, which are severely injured or killed at winter temperatures below -20˚C, (Davenport et al., 2008). Due to geographical location, grape growers in north central and eastern United States are limited to a small selection of *V. vinifera* cultivars and hybrid cultivars derived from other species that can be considered marginally to extremely cold-hardy.

In contrast to the geographical limitations of *V. vinifera*, *V. riparia* is one of the most wide ranging *Vitis* species and can be found from the south-central and eastern United States to central Canada (Hedrick, 1908; Hemstad, et al., 2000; NRCS, 2011). *Vitis riparia* is extremely cold hardy and is capable of withstanding temperatures below -40˚C (Pierquet et al., 1980). Due to the extensive cold hardiness and disease resistance of *V. riparia*, it has been used extensively in grape breeding to transfer these traits to domesticated grapes. To gain cold hardiness, *V. riparia* was used by early 20th century French breeders to create moderately cold-hardy hybrids like 'Marèchal Foch' by crossing a selection from (*V. riparia* X *V. rupestris*) with *V. vinifera* cv. Goldriesling. In 1881, an early Minnesota grape breeder Louis Suelter crossed a selected *V. riparia* by 'Concord' a well-known *V. labrusca* cultivar and created 'Beta', one of the first cold hardy juice and jelly grape cultivars. Breeders of cold climate grapes have worked with germplasm that is mostly based on *V. riparia* as a source of hardiness and *V. vinifera* as a source of quality. Although *V. riparia* has provided useful cold-hardiness and disease resistance in breeding, it can also transmit some unwanted traits such as herbaceous flavor, high acidity, high sugar content, and dark pigmentation.

Currently, grape cultivars with cold tolerance are in demand in the upper Midwest and Northeast U.S., Canada, and other temperate locations. To meet this need, interspecific crosses were made between North American *Vitis* species such as *V. riparia* and *V. labrusca* with the European *V. vinifera* in search of cold-hardy wine grapes. The University of Minnesota grape breeding project, which had focused on table and juice grapes in the early 20th century, began breeding cold hardy wine grapes in the late 1970's. In search of commercial cold-hardy wine grapes, over 30,000 interspecific hybrid

seedlings have been planted and evaluated from1985 to 2000 at the University of Minnesota Horticultural Research Center in Excelsior, MN. The mean minimum yearly temperature for Excelsior, MN is -33˚C (Hemstad & Luby, 2000) making it an ideal location for cold-hardy grape breeding. These efforts resulted in the release of four cold hardy wine grape cultivars form 1996 to 2006: 'Frontenac', 'La Crescent', 'Frontenac gris', and 'Marquette'.

'Frontenac' is a red wine grape introduced by the University of Minnesota in 1996 from a cross of 'Landot 1145' and *V. riparia*. This highly productive cultivar is known for producing both high sugar and acid concentrations at harvest. 'Frontenac' can withstand midwinter temperatures dropping below -30˚C (ASHS, 1997). 'Frontenac gris' introduced in 2003 is a gray colored fruit used in making white wine. 'Frontenac gris' was derived as a sport of 'Frontenac' and carries many of the same vine growth and fruit chemistry characteristics as 'Frontenac' (Luby & Hemstad, 2006). 'La Crescent' was introduced in 2002 and was derived from crossing 'St. Pepin' x E.S. 6-8-25 (*V. riparia* x 'Muscat Hamburg') (Luby & Hemstad, 2004). La Crescent is used in making white table and dessert wines. The most recent University of Minnesota release is the red wine grape 'Marquette' introduced in 2006 (Hemstad & Luby, 2008). 'Marquette' is a complex hybrid that has both 'Frontenac' and 'Pinot Noir' in its paternal pedigree. Having a strong resistance to common grape pathogens and ideal wine making chemistry, Marquette has become popular in the cold climate wine industry.

Pioneer grape breeder Elmer Swenson form Osceola, WI began breeding grapes in 1943 when he intercrossed French hybrid grapes with *V. riparia* and *V. lubrusca*. In 1969, Swenson began working at the University of Minnesota Horticultural Research

Center and, with the University, he co-released two cultivars he had bred, 'Swenson Red' and 'Edleweiss'. Of the 24 cultivars developed by Swenson, two of the more popular wine grape cultivars are 'St. Pepin' and 'St. Croix'. These two cultivars share similar characteristics of cold-hardiness, low acidity, and moderate sugar content. 'St. Pepin' is used for white wine or table grape production and was derived from complex interspecific crosses of *V. vinifera*, *V. riparia*, *V. lubrusca, and V. lincecumii* (NGR; Swenson, 1986). 'St. Croix' has a pedigree that also includes *V. riparia* and *V. labrusca* and is used for red wine or table grape production. St. Croix has proven extremely cold hardy and can withstand winter temperatures as low as -39˚F (Swenson, 1982).

Components of the Grape Berry:

Grapes have seeds enclosed in a thick fleshy pericarp classifying them as a berry. Grape berries are organized into clusters and each berry is attached to the cluster by a pedicel containing vascular bundles. After fertilization grape berry growth follows two distinct sigmoid cycles (Coombe, 1987). The first cycle of berry formation begins with rapid pericarp cell division. Cell division slows as the cells enlarge marking the end of the first cycle. The berries in this stage are slow growing, green, and accumulating high amounts of malic acid (Coombe et al., 2000). In the second cycle the berries enlarge as they begin to accumulate sugars and phenolic compounds (Coombe et al., 2000). In this cycle red, black, blue, and gray berries turn color following the biosynthesis of anthocyanins. This color change is known as 'veraison' and denotes the start of the ripening process (Coombe & Bishop, 1980).

Generally, grape berries include both a pericarp and seeds. The pericarp can be divided into three anatomical tissues: exocarp, mesocarp, and endocarp (Galet, 2000).

The physical nature of the exocarp, or "skin", of the grape aids the berry in pathogen defense, UV protection, and transpiration. The exocarp makes up between 5 and 18% of the fresh weight of mature berries. During the onset of ripening when the berries are expanding the softening skin accumulates increased amounts of sugar and potassium (Coombe et al., 2000). Metabolism within endocarp cells dictates physiological and biochemical changes throughout the ripening process. A good example of physiological and biochemical changes is the accumulation of the two most abundant phenolics, anthocyanins, and condensed tannins (Fournand et al., 2006). The natural coloration of grapes due to anthocyanins attracts animals that aid in seed dispersal and protects the berry from UV damage.

The mesocarp commonly known as the "flesh" or "pulp" of the berry acts as the grape's reservoir in storing nutrients, sugars, and organic acids. Mesocarp cells have large vacuoles that make up 99% of the cell volume in ripe berries (Diakou et al., 2001). As the berries mature, sugar content increases and organic acid concentrations decrease. The endocarp is described as the tissue surrounding the seed and is difficult to distinguish from the mesocarp (Keller, 2010). The seeds are rich with phenolic compounds and are a source for tannins in the wine making process. As the seeds mature, the berries become more visually appealing and flavorful, promoting seed distribution by foraging animals (Lund et al., 2006). As seeds mature, they turn from dark green to brown. This color transformation is used by viticulturists as an indicator of berry maturity.

Organic Acids:

From the very beginning through the final stage of berry development, organic acids are produced by glycolysis, the Krebs cycle, and the shikimic acid pathway (Soyer

et al., 2003). During berry development acidity levels are consistently changing as a result of metabolic activities. Typically, tartaric and malic acids account for 90% of the acids found in grapes, with tartaric acid predominating (Lamikanra et al., 1995; Kliewer et al., 1967). Other organic acids such as succinic, acetic, citric, lactic, fumaric, and shikimic acids can be found in various concentrations depending on environmental conditions and cultivar (García et al., 1993). The acidity of grapes is most often expressed in titratable acidity (TA). The TA is an important parameter vintners use to evaluate the quality of juice and wine. Conditions that can influence organic acid composition are cultivar, growing region, or environmental factors such as light, humidity, and temperature (Lamikanra et al., 1995). In general, *V. riparia* grapes contain more malic acid than tartaric acid (García et al., 1967), which may contribute to the high titratable and sensory acidity in *V. riparia* hybrid cultivars.

Malic acid is an intermediary product synthesized in the Krebs cycle, catabolic pathways such as glycolysis, and as a by-product from refixation of CO_2 released during respiration (Ruffner, 1982B). The majority of malic acid found in grapes from cool climates is directly related to the lower temperatures because in hotter climates malic acid is degraded at faster rates. The activity of the malic enzyme responsible for catalyzing conversion of malate to pyruvate is directly related to temperature. As temperatures increase, so does malic enzyme activity (Lakso et al., 1975). Before veraison, the optimal temperature for malic acid accumulation for *V. vinifera* grapes is between 20˚C and 25˚C, but sharply declines at temperatures above 38˚C (Kliewer, 1971; Winkler et al., 1974). After veraison, the degradation of malic and tartaric acids depends on the rate of respiration, which is determined by temperature. The decline of organic acids after

veraison is due to a combination of inhibition of glycolysis, gluconeogenesis from malic acid, and the metabolism of malate and tartrate through respiration (Ruffner, 1982a & b). When there is high TA in juice and wines, malic acid is most often the main contributor (Gallander, 1977).

Grapes that are high in malic acid can cause problems in wines and the vinification process. Malic acid can have a significant influence on the sensory properties of wines, such as overpowering tastes of tartness and astringency, and contributes to herbaceous or 'grassy' flavors (Gallander, 1977; Kallithraka et al., 1997). The chemical properties of malic acid can also have an impact on the winemaking process by affecting the relationship of titratable acidity to pH, which ultimately impacts the buffer capacity in wines (Ribe´reau-Gayon et al., 2000). Buffer capacity of grape musts affects the physicochemical properties, microbiological stability, and flavor balance of the final wine product. The buffer capacity varies depending on what acids are present. To avoid this imbalance, winemakers can add calcium-tartrate and or calcium-bicarbonate to affect the ratio of free acids and their salt form. Another technique used by wine makers to deal with high malic acid in the wine making process it to perform malolactic fermentation which is a secondary fermentation that converts malic acid into less acidic lactic acid using bacteria.

Like malic acid, most tartaric acid is formed pre-veraison, but by different biosynthetic and metabolic pathways. Tartaric acid is a secondary product formed from the metabolism of glucose and ascorbate (Ruffner, 1982A). Grapes are one of the only fruits containing tartaric acid and for most cultivars it is the predominate acid. The concentration of tartaric acid decreases after veraison due to dilution from the influx of

sugar and water to the berry. However, unlike malic acid there is very little degradation and only a trace amount of tartaric acid is metabolized through respiration (Ruffner, 1982A). There is no known relationship between organic acid metabolization and sugar accumulation, so when the sugar content in grapes is increasing, the acid content is not necessarily decreasing. However, comparing the concentrations of both organic acids (titratable acidity) and sugars (soluble solids) can provide viticulturists with a perspective of grape berry development.

Sugars:

After veraison when the berries begin to soften and expand xylem flow slows down and phloem sap rich in sugars becomes the main contributor to the grape. As the berry ripens phloem flow continues until the berry reaches maturity, phloem flow is then suppressed and the water and sugar supply is cut off (Coombe et al., 2000). Sugar accumulation is dependent on photosynthesis in leaves and transported to the berry through the phloem in the form of sucrose (Swanson et al., 1958). After entering the berry it is then cleaved into glucose and fructose (Klierwer, 1967). Glucose and fructose (hexose sugars) account for approximately 99% of the total carbohydrates in grape juice, representing a large portion of the total soluble solids (Klierwer, 1967). Glucose and fructose are very important in determining wine quality as they are accountable for the sweet taste and help balance tastes of sourness, bitterness, and astringency. More importantly the hexose sugars in grape juice are converted into alcohol through anaerobic fermentation conducted by yeasts.

Sugars are the main carbohydrate sources for yeast fermentation. Sugar levels in wine grapes are closely watched as they indicate the potential alcohol and possible residual sugar remaining after fermentation. Sugars typically account for 90% of the soluble solids found in mature grape berries (Keller, 2010). Soluble solids expressed as ^{o}Brix are a proxy for sugar content that is based on refractive index of the juice. Soluble solids represent the relative sugar weight of a juice sample, for example 1^{o}Brix denotes 1% sugar by weight. In mature berries soluble solids give a fairly accurate account of sugar content. Levels of soluble solids are within 1% of actual sugars (glucose and fructose) present (Jackson & Lombard, 1993).

Technologies:

Winemakers and viticulturists rely on new technology to promptly assess grape components using refractometers and titrators. Refractometers measure the refractive index of soluble solids found in juice samples. Field refractometers are used to quickly calculate ^{o}Brix levels and assess grape maturity by sugar content. Acid tirtations are a quantitative chemical analysis that determines the concentration of an acid by neutralizing the acid using a standard base solution to a targeted pH. Automated titrators are bench top devices that measure total or titratable acidity found in juice samples and report the TA in g/L or g/100mL. Advancements in chromatography and mass spectrometry have been essential in identifying and quantifying secondary metabolites found in grapes and wine. Liquid chromatography mass spectrometry (LC-MS) is most often used to analyze non-volatile compounds and gas chromatography mass spectrometry (GC-MS) is used to analyze volatile compounds. However, use of GC-MS

and LC-MS are mostly limited research institutes, university's, and large scale winery operations.

Growing Degree Day:

Growing degree day (GDD) is a method of calculating and tracking heat accumulation units over a period of time. The equation for GDD = $[(T_{MAX} + T_{MIN})/2] - T_{BASE}$ where T_{MAX} is the daily maximum temperature and T_{MIN} is the daily minimum temperature. T_{BASE} is the temperature below the needed heat for plant growth. Normally, the period tracked for grapes starts April 1 and goes to October 31 with a base temperature of $T_{BASE} = 10^{\circ}C$. The calculation of GDD has been effectively used to track and predict many agricultural phenomena including crop development and progression of plant phenological events (McMaster et al., 1997). Average annual accumulation of GDD has been used to predict regions where a grape cultivar can be reliably matured. The rate of heat accumulation throughout a growing season can predict when bud break, flowering, and grape berry maturity will occur (Tait, 2008).

Research Objectives:

Currently, grape cultivars with cold tolerance are in demand in the upper Midwest, Northeast, Canada, and other temperate locations. This demand has led to the interspecific crosses between North American *Vitis* species *V. riparia* and *V. labrusca* and the European *V. vinifera* in search of cold-hardy wine grapes. Though derivatives from initial interspecific hybridization have been intermated for more than five generations, we have little understanding of the berry development of these new cultivars, especially the timing of accumulated sugars and organic acids. Enhanced knowledge of

the range of variation for these compounds in cold hardy wine grape cultivars will guide grape growers in harvest decisions and winemakers in their wine making process. As the cold climate wine industry grows it would be beneficial to identify and quantify these components of wine grape cultivars grown in this newly formed grape growing region.

My research examines the ripening process and quantifies grape berry metabolites in cultivars used for cold climate viticulture. The first objective is to identify and quantify organic acids and sugars in University of Minnesota wine grape cultivars, select *V. vinifera* cultivars, and other hybrid cultivars throughout fruit maturation to develop a dynamic profile of these compounds to better understand grape berry development throughout maturation. Quantifying specific sugars and organic acids will aid vintners in optimizing the wine making process. The second objective is to create a profile for the ripening process with respect to soluble solids, titratable acidity, pH and berry weight for University of Minnesota wine grape cultivars, select *V. vinifera*, and other hybrid cultivars to facilitate the timing of grape maturity. Such a profile would guide harvest decisions leading to production of higher quality wines.

Objective 1:

Identifying and quantifying organic acids and sugars of selected cultivars throughout fruit maturation and developing a dynamic profile of these compounds will outline the chemical composition of grape berry development throughout maturation. The chemical composition of grape berries has generally been accepted as the most important factor when determining the quality of the fruit (King et al. 1988; Lamikanra et al. 1995). Sugars and organic acids in the grape berry play an important role in the wine

making process and contribute to sensory quality of wine. Sugars are the main carbohydrate sources for yeast fermentation. Acidity is important for stability and sensory of wine. The content of organic acids (mainly malic and tartaric acid) in fruit is especially important in wine stability, flavor, and color. Many factors such as maturity can influence the chemical composition of grapes, growing region, and cultivar (Lamikanra et al. 1995). Furthermore, the chemical composition of grape berries constantly changes during berry development as the result of fluctuating rates of synthesis, degradation, and transport processes (Dia et al. 2013). The chemical composition of *V. vinifera* cultivars has been extensively researched, but little is known of the chemical composition of the cold climate wine grape cultivars.

Quantifying the variation of specific organic acids and sugars for the cultivars in this study will give a better understanding of grape berry development and maturation. Better knowledge of the sugar and organic acid composition in cold-climate grape cultivars could lead to more optimal harvest timing and improved wine quality. In previous studies the glucose to fructose ratio and malic to tartaric acid ratio has been used to evaluate *Vitis* germplasm and classify cultivars according to these ratios (Shiraishi, 1995; Shiraishi, 1993; Liu et al., 2006; Kliewer et al., 1967). Though these studies evaluated a large number of cultivars there is still little known of the chemical composition or acid and sugar ratios of the cold climate wine grape cultivars. Malic acid concentration and glucose: fructose ratio are two important harvest considerations. The ratio of glucose to fructose is an important ripening and sensory indicator. Furthermore, *Saccharomyces* spp. (wine yeast) prefer to consume glucose over fructose making the glucose: fructose ratio an important consideration in the wine making process. High

malic acid concentration can contribute to an astringent taste that diminishes wine quality. Malic acid concentrations can also lead to unwanted/uncontrolled malolactic bacterial activity, which can result in secondary fermentation and wine spoilage. For these reasons, knowing the chemical composition in selected wine grape cultivars would be beneficial to wine makers.

Objective 2:

Profiling the ripening process of the selected cultivars consist of tracking the TA, SSC (˚Brix), and pH over several sampling dates in each of three years and locating the approximate time of peak berry maturity or phloem arrest. The ripening profile was compared against accumulated GDD to determine if GDD are appropriate for predicting berry maturity. Harvest dates for wine grapes are selected to optimize the balance between sweetness, acidity, phenolic ripeness, and flavor (Lund et al., 2006). Answering the intricate question of when to harvest can be difficult. The common indicators for grape maturity used by viticulturists and wine makers are the amount of SSC (˚Brix), TA, and pH. Viticulturists want to capture the optimal balance between sugars, acids, and flavor that will contribute to sensory quality, stability, and alcohol potential of wine. Sugar content generally rises and total acidity falls as grapes become more mature, but the rate and magnitude of these changes depends on both genetic and environmental factors. Tracking and comparing the concentration of both organic acids (TA) and sugars (SSC) provides viticulturists with a profile of the ripening process or range of fruit maturity.

Temperature is the most important environmental factor affecting wine or grape quality (Winkler, 1975). Climatic conditions can vary from year to year; using GDD's to track the progression of plant phonological events would be more suitable than date. The rate of heat accumulation through the increase of ambient temperature will determine when bud break, flowering, and grape berry maturity will occur (Tait, 2008). Comparing TA, °Brix, and pH to accumulated GDD, I will be able to estimate the GDD needed to mature grape berries for the selected cultivars. Determining the number of GDD required for these grape cultivars to mature will help identify locations suitable for growing grapes and aid in predicting when cultivars should be harvested. Compared to established wine regions like California and Europe, the upper Midwest is unfamiliar to many wine and grape technologies. A GDD based maturity index would be a vital and easy to use tool for new grape growing regions like Minnesota.

CHAPTER TWO

Introduction:

Grape cultivars with cold tolerance are currently in demand in the upper Midwest and Northeast U.S., Canada, and other temperate locations. In order to develop new cultivars of cold-hardy wine grape vines via classical breeding approaches, interspecific crosses were made between European *Vitis vinifera* and North American *Vitis* species such as *Vitis riparia* and *Vitis labrusca*. *Vitis riparia* is extremely cold hardy and is capable of withstanding temperatures below -40˚C (Pierquet et al., 1980). Due to the extreme cold hardiness and disease resistance of *V. riparia*, it has been used extensively in grape breeding to transfer these traits to domesticated grapes. In contrast *V. vinifera* cultivars offer high quality wine making traits but are severely injured or killed at winter temperatures below -20˚C (Davenport et al., 2008). Due to geographical location, grape growers in the north central and eastern United States are limited to a small selection of *V. vinifera* cultivars and hybrid cultivars derived from other species that can be considered marginally to extremely cold-hardy. Although *V. riparia* has provided useful cold-hardiness and disease resistance in breeding, it may also transmit some unwanted traits such as herbaceous flavor, high acidity, high sugar content, and dark pigmentation.

The chemical composition of grape berries has generally been accepted as the most important factor when determining the quality of the fruit (King et al. 1988; Lamikanra et al. 1995). Sugars and organic acids in the grape berry play an important role in the wine making process and contribute to sensory quality of wine. Many factors can influence the chemical composition of grapes such as maturity, growing region, and

cultivar (Lamikanra et al. 1995). Furthermore, the chemical composition of grape berries constantly changes during berry development as the result of fluctuating rates of synthesis, degradation, and transport processes (Dia et al. 2013).

Grape Berry Organic Acid Content: In *V. vinifera* after fertilization, grape berry growth follows two distinct sigmoidal cycles (Coombe, 1987). The first cycle of berry formation begins with rapid pericarp cell division. During early development berries are slow growing, green, and accumulate high amounts of malic acid. In the second cycle, berries mature, sugar content increases and organic acid concentrations decrease. The acidity of grapes is most often expressed as titratable acidity (TA), given in equivalent units of tartaric acid. The TA is an important parameter vintners use to evaluate the quality of juice and how it will be used in wine production. Throughout berry development, organic acids are produced by glycolysis, the Krebs cycle, and the shikimic acid pathway (Soyer et al., 2003). Tartaric acid is a secondary product formed from the metabolism of glucose and ascorbate (Ruffner, 1982A). Typically, tartaric and malic acids account for 90% of the acids found in grapes, with tartaric acid predominating (Lamikanra et al., 1995; Kliewer et al., 1967). Other organic acids such as succinic, acetic, citric, lactic, fumaric, and shikimic acids can be found in various concentrations depending on environmental conditions and cultivar (García et al., 1993). In general, *V. riparia* grapes contain more malic acid than tartaric acid (García et al., 1967), which may contribute to the high titratable and sensory acidity in interspecific hybrid cultivars.

Factors that can influence organic acid composition are cultivar, growing region, or environmental conditions such as light quality, humidity, and temperature (Lamikanra

et al., 1995). During berry development, acidity levels are consistently changing as a result of metabolic activities. Malic acid is an intermediary product synthesized in different biochemical processes including the Krebs cycle, catabolic pathways such as glycolysis, and recapture of CO_2 released during respiration (Ruffner, 1982B). Temperature is the most important environmental factor affecting wine or grape quality including organic acid content (Winkler, 1975). The activity of the malic enzyme responsible for catalyzing the conversion of malic to pyruvic acid is directly related to temperature *in vitro*. As temperature increases, so does malic enzyme activity (Lakso et al., 1975). Before veraison, the optimal temperature for malic acid accumulation for *V. vinifera* grapes is between 20 ˚C and 25 ˚C, but sharply declines at temperatures above 38 ˚C (Kliewer, 1971; Winkler et al., 1974). After veraison, the degradation of malic and tartaric acids depends on the rate of respiration, which is determined by temperature. The decline of organic acids after veraison is due to a combination of inhibition of glycolysis, gluconeogenesis from malic acid, and the metabolism of malic and tartaric acids through respiration (Ruffner, 1982a, b). Malic acid is degraded at faster rates in hotter climates (Lakso et al., 1975; Kliewer, 1971; 1974) suggesting the majority of malic acid found in grapes from cool climates is directly related to the lower temperature.

When TA is high in juice and wines, malic acid is most often the main contributor (Gallander, 1977). Grapes that are high in malic acid can cause problems in wines and the vinification process. Malic acid can have a significant influence on the sensory properties of wines, such as overpowering tastes of tartness and astringency, and contributes to herbaceous or 'grassy' flavors (Gallander, 1977; Kallithraka et al., 1997). The chemical properties of malic acid can also have an impact on the winemaking

process by affecting the relationship of titratable acidity to pH, which ultimately impacts the buffer capacity in wines (Ribe´reau-Gayon et al., 2000). Buffer capacity of grape musts affects the physicochemical properties, microbiological stability, and flavor balance of the final wine product. To avoid this imbalance, winemakers can add calcium-tartrate and or calcium-bicarbonate to both modulate the ratio of free acids to their conjugate base form and to selectively precipitate the base as a calcium salt. Another technique used by wine makers to compensate for high malic acid content is to perform a secondary bacterial fermentation called malolactic fermentation, which helps by converting malic acid into the less acidic lactic acid.

Like malic acid, most tartaric acid is formed pre-veraison, but by different biosynthetic and metabolic pathways. Grapes are one of the only fruits containing tartaric acid and for most *V. vinifera* cultivars it is the predominate acid (Shiraishi, 1995; Liu et al., 2006; Kliewer et al., 1967). The concentration of tartaric acid decreases after veraison due to dilution from the influx of sugar and water to the berry. However, unlike malic acid there is very little degradation and only a trace amount of tartaric acid is metabolized through respiration (Ruffner, 1982A). There is no known relationship between organic acid metabolism and sugar accumulation, so when the sugar content in grapes is increasing, the acid content is not necessarily decreasing.

Grape Berry Sugar Content: After veraison, when the berries begin to soften and expand, xylem flow slows down and phloem sap rich in sugars and phenolic compounds becomes the main input for the grape berry anabolism (Coombe, et al., 2000). Sugar accumulation is dependent on photosynthesis in leaves and is transported to the berry through the phloem in the form of sucrose (Swanson et al., 1953). After entering

the berry, sucrose is cleaved into glucose and fructose (Klierwer, 1967). Glucose and fructose account for approximately 99% of the total carbohydrate content of grape juice, representing a large portion of the total soluble solids (Klierwer, 1967). Glucose and fructose are very important in determining wine quality as they are primarily responsible for sweetness, which can also help to balance traits such as sourness, bitterness, and astringency. Sugars typically account for 90% of the soluble solids found in mature grape berries (Keller, 2010). For this reason, soluble solids content (SSC, expressed as ˚Brix), which is based on the refractive index of the juice, is a reasonable proxy for sugar content in grapes, unlike in other crop species where other types of compounds contribute significantly to refractive index. Levels of soluble solids within mature grape berries are within 1% of actual sugars (glucose and fructose) present (Jackson & Lombard, 1993).

Enhanced knowledge of the range of variation for acids and sugars throughout berry development will guide grape growers in harvest decisions and wine makers in their wine making process. In previous studies, the glucose to fructose ratio and malic to tartaric acid ratio has been used to evaluate *Vitis* germplasm and classify cultivars according to these ratios (Shiraishi, 1995; Shiraishi, 1993; Liu et al., 2006; Kliewer et al., 1967). Though these studies evaluated a large number of cultivars, little is known of the chemical composition or acid and sugar ratios of the cold climate wine grape cultivars. As the cold climate wine industry grows, identifying the variation and range of organic acids and sugars and their respective ratios of cold hardy wine grape cultivars would be beneficial. This research examines the ripening process and quantifies key grape berry metabolites in cultivars used in cold climate viticulture. The objective of this study is to identify and quantify organic acids and sugars in University of Minnesota wine grape

cultivars, select *V. vinifera* cultivars, and other hybrid cultivars throughout fruit maturation to develop a dynamic profile of these compounds to better understand grape berry development throughout maturation.

Materials and Methods:

Plant Material: The grapevines used in this study were located at the University of Minnesota Horticultural Center in Excelsior, Minnesota. A total of eleven different cultivars were used for this study and listed in Tables 2.1.1 and 2.1.2. The number of vines and trellis systems varied slightly. The number of vines for cold hard hybrid cultivars were 'Frontenac' (4), 'Frontenac gris' (4), 'La Crescent' (4), 'Marquette' (4), 'St. Croix' (4), 'St. Pepin' (4), and 'Maréchal Foch' (8) and all were grown using the High Bilateral Cordon training system (Jackson, 2001). The *V. vinifera* vines were 'Merlot' (4), 'Pinot noir' (4), 'Chardonnay' (8), and 'Riesling' (8) and grown using the mini-J training system (MGGA, Hemstad et al., 1991) to prevent winter injury and permit fruiting.

Fruit, Harvest Dates, and Sampling: Two subsamples of forty berries per cultivar were harvested every eight to ten days from early August to late September or early October of the 2010 and 2011 growing seasons. In total, there were five harvest dates in 2010 and eight harvest dates in 2011. Each forty-berry subsample contained twenty berries from each side of the trellis. To decrease the influence of fruit removal on the chemical composition of the remaining grapes of later samples, no more than two grapes were harvested from an individual cluster per harvest. Not all clusters were harvested on consecutive sample dates.

Berry samples were taken from one vineyard location for each cultivar. Berry sampling was conducted on four or eight vines for a cultivar depending on availability. To avoid bias in sampling, harvesters followed a defined protocol for berry collection. Harvesters first defined four zones on each grape cluster to be sampled: the right and left shoulders, middle section, and the tail. Harvesters alternated from each of these cluster zones for berry selection as they moved from cluster to cluster throughout the vine. To avoid imposing visual selection bias in the designated zone, the harvester manually selected each grape without visually inspecting the grape cluster prior to selection. As the harvesters picked the berries as described above, they also alternated from the front of the cluster to the rear of the cluster. For example, if there were eight clusters the first four berries would be selected from the outer-facing or front side of the first four clusters (one berry from each of the four zones), and the second four berries would come from the rear-facing side of the remaining four clusters (in sequence with the four cluster zones). Berries that were severely shriveled or damaged by insects, birds, or hail were discarded and a new berry was selected.

Juice Extraction and Berry Storage: After each harvest one subsample of forty berries for each harvested cultivar was immediately stored in a -20 ˚C freezer and later transferred to a -80 ˚C freezer for long-term storage. The other subsample of forty berries was divided into four, ten berry replicate portions, each of which was pressed separately with a hand juicer (WEAR-EVER, T.A.C U.C.O).

Chemicals and Reagents: The following analytical grade reagents were purchased: acetronitrile (CH_3CN), D(+) glucose, D(+) fructose, tartaric acid, succinic

acid (Aldrich, Milwaukee, WI), formic acid, L(-) malic acid, L(-) malic acid, L(-) tartaric acid, and ammonium hydroxide (Fluka, Buchs, Switzerland).

Juice Analysis: Soluble solids content (SSC) was measured in ˚Brix using an ATAGO® Pocket Refractometer PAL-1 (Atago Inc., Bellevue, WA). Juice titratable acidity (TA), soluble solids, and pH were measured for four replicates for each cultivar. The TA and pH were tested with the ATI ORION 950 Ross FASTQC Titrator (Orion, Beverly, MA). The titrator was calibrated to pH 4.0 and 7.0 daily, before use. For TA analysis 1 mL of juice was diluted into 50 mL of distilled water and titrated to the pH of 8.0 using a 0.05 M sodium hydroxide solution. The titrator was recalibrated every day before use and 0.05 M sodium hydroxide solution was changed every seven days.

Sugars and organic acids were analyzed by liquid chromatography-mass spectrometry (LC-MS) using ultra-performance liquid chromatography (UPLC) with a binary pump system coupled to a single quadrupole detector (SQD) mass detector and a photo diode array (PDA) detector (ACQUITY UPLC system, Waters, Milford, MA) using an ACQUITY UPLC BEH Amide column (2.1 x 100mm, 1.7 μm; Waters). Organic acids were separated by gradient elution using mobile phases: Phase A was 90% CH_3CN, 9% water, and 1% formic acid; and Phase B was 50% CH_3CN, 49% water, and 1% formic acid. Sample elution was performed using a gradient from 65 to 80% B from 0 to 5 min and 99.9% A for 3 more min. The column temperature was maintained at 40˚C, and the mobile phase flow rate was maintained at 0.4 mL min^{-1}. Injected sample volumes were 1 μL. Sugars were also separated by gradient elution different using mobile phases: Phase A was 90% CH_3CN, 10% water, and 0.01% ammonium hydroxide; and Phase B

was 70% water, 30% CH_3CN, and 0.01% ammonium hydroxide. Sugar elution was performed using a gradient from 95% A for 1min then a linear increase to 40% A over the next 3 min where the % A was maintained for 1 min and then decreased to 95% A, and held for 3 min. The column temperature was maintained at 40 ˚C, and the mobile phase flow rate was maintained at 0.35 mL min^{-1} for the first four min followed by 0.2 mL min^{-1} for one min, and back to 0.35 mL min^{-1} for the final three min. Injected sample volumes were 2 μL. All data were collected in the selected ion recording (SIR) mode and ionization was performed in negative-ion mode. The instrument control software used was MassLynx (Waters).

Juice samples were prepared by dilution; first they were diluted in water and then they were further diluted into UPLC mobile phase components. After each dilution, the samples were mixed by vortex mixer and clarified by centrifugation (8 min, 20,000 g) prior to further dilution or analysis. For organic acids analysis, juice was first diluted 1 part juice to 3 parts water, and was then further diluted 1 part dilute juice to 8 parts CH_3CN to 1 part 10% (v/v) formic acid in water, juice dilute (3:1 water to juice). Juice sample prep for sugar analysis, juice was first diluted 1 part juice to 10 parts water, and was then further diluted 1 part dilute juice to 8 parts CH_3CN to 1 part 1% (w/v) ammonium hydroxide in water. For standardization, six concentrations of calibration mixtures were prepared for the four organic acids (malic, tartaric, citric, and succinic acids) and two sugars (glucose and fructose). QuanLynx (Waters) software was used to calculate standard curves and absolute quantities of each analyte in the samples.

Growing Degree Days: Data from the NOAA National Weather Service station CHASKA, MN US located in Chaska, MN (www.ncdc.noaa.gov) was used to calculate growing degree days (GDD) as follows: GDD = [(TMAX + TMIN)/2] – TBASE where TMAX is the daily maximum temperature and TMIN is the daily minimum temperature. TBASE is the chosen minimum temperature for plant growth. The period tracked for this study started April 1 and went through October 31 with a base temperature of TBASE = 10 ˚C (Winkler, 1974).

Statistical Analysis: Fruit data were subjected to means separation analyses using least significant difference (LSD) and analysis of variance (ANOVA) for the eleven cultivars using the R statistical software 3.0.1 (R Development Team, 2013). The ANOVA and LSD tests were used through the Agricola package (De Mendiburu, 2010). Eight traits (malic, tartaric, citric, and succinic acid concentration, ratio of tartaric to malic acid, glucose and fructose concentrations, and ratio of glucose to fructose) were tested against main effects year and GDD and the GDD and year interaction. Main effects were fixed for all analyses. A separate analysis was performed for each cultivar. Area graphs, heat map, and tables were produced using Excel 2007 (Microsoft Corp., Seattle, WA, USA). Two year means for malic and tartaric acid were subjected to a second order polynomial regression model calculated using Excel 2007 (Microsoft Corp., Seattle, WA, USA) and labeled with and corresponding R^2 value. Line graphs were produced using the R statistical software 3.0.1 (R Development Team, 2013).

Results:

Statistical analysis: The analyses of variance for ratio of tartaric to malic acid (T/M) indicated no significant effects ($P < 0.001$) due to year by GDD interaction effects for all traits in all cultivars except 'Merlot' (Table 2.1.1). Citric acid concentration varied significantly ($P < 0.05$) among years for all cultivars. Year had no effect ($P > 0.05$) on malic acid concentrations for all cultivars except 'St. Pepin'. Cultivars for which year x GDD interaction effects were significant ($P < 0.05$) for malic acid concentrations were 'St. Croix', 'St. Pepin' and 'Riesling'. Cultivars with significant ($P < 0.05$) for year x GDD interaction effects for tartaric acid concentrations were 'Chardonnay' and 'Pinot noir'. Year x GDD interaction effects were not significant ($P > 0.05$) for G/F ratio in all cultivars (Table 2.1.2). Year had no effect ($P > 0.05$) on glucose or fructose concentrations for all cultivars. Year x GDD interaction effects were not significant ($P > 0.05$) for glucose and fructose concentrations in all cultivars except 'Frontenac', 'St. Croix', and 'Maréchal Foch'.

The means separation analyses (LSD) were used to determine when significant changes ($P < 0.05$) were observed for all traits. All cultivars had significant decrease in malic and tartaric acid concentration in both years except 'Frontenac', 'Frontenac gris', and 'La Crescent' (Tables 2.2.1 and2.2.2). In 2010, 'Frontenac' exhibited no change in malic or tartaric acid concentrations. In 2010 'Frontenac gris' exhibited no change in tartaric acid concentration and 'La Crescent' exhibited no change in malic acid concentration.

Sugars: UPLC profiles of soluble sugar composition were obtained for the eleven cultivars. The two major peaks identified correspond to glucose and fructose (Figure 2.2), which were quantified using external standard curves. The standard curve linear range for glucose fructose was 165 g/L and correlation coefficient of .95 or higher. Small amounts of sucrose were detectable prior to juice dilution, but were not quantifiable. The relative glucose and fructose composition varied between cultivars and throughout berry development (Table 2.4.1-2). The first harvest date for 2011 showed the lowest levels of glucose and fructose with only trace amounts in *V. vinifera* cultivars. The highest relative glucose and fructose content was found in 'Marquette' and 'Frontenac' at concentrations between 170 and 180 g/L (Table 2.4.1-2). In cultivars 'La Crescent' and 'St. Croix', glucose was the most abundant sugar during berry ripening. While in 'Frontenac', fructose was the most abundant sugar. The ratio of glucose to fructose (G/F ratio) had little to no variation between cultivars and years (Table 2.4.1-2). The range of G/F ratio after 1,240 GDD was from 0.90 to 1.1 for all cultivars at all harvest points.

Acids: UPLC elution profiles for citric, tartaric, malic, and succinic acids are shown in Figure 1 for a single juice sample. The standard curve linear range for malic and tartaric acid was 11.3 g/L and 1.6 g/L for citric and succinic acid with a correlation coefficient of 0.98 or higher. Mean values for TA, pH, malic, tartaric, citric, and succinic acid for selected cultivars and corresponding GDD is shown in Tables 2.2.1-3. Cultivars with high amounts of citric acid were 'Frontenac', 'Frontenac gris', and 'La Crescent'. 'La Crescent' has the highest concentration of succinic acid.

Malic and tartaric acids decreased throughout the 2011 growing seasons for all samples. Cultivars 'Frontenac' and 'Le Crescent' did not have a significant change in malic acid levels and 'Frontenac' and 'Frontenac gris' did not have a change in tartaric acid levels in 2010. The average high and low malic acid accumulation of the two seasons was between 8.3 and 13.8 g/L. The rate at which malic and tartaric acid concentrations decreased varied between cultivars and year (Figure 2.5). In years 2010 and 2011 at all harvest dates, malic was the most abundant acid for 'Frontenac', 'Frontenac gris', 'La Crescent', 'St. Pepin', and 'Chardonnay'. Tartaric acid was the most abundant acid after 1,330 GDD for 'Marquette', 'Maréchal Foch', 'St. Croix', and 'Riesling'. For cultivars 'Pinot noir' and 'Merlot', tartaric acid was the most abundant acid in 2010, however, in 2011 malic acid was found to be more abundant than tartaric. The trends of malic and tartaric acid compared to GDD are shown in Figures 2.3 and 2.4. Of red grape cultivars 'Frontenac' maintained the highest concentration of malic acid and 'St. Croix' had the lowest. 'Marquette', 'Maréchal Foch', and 'Frontenac' had similar tartaric acid concentrations throughout the two growing seasons. The largest decrease in tartaric acid was in 'Pinot noir', which went from 8.2 to 2.7 g/L. Of white grape cultivars, 'Frontenac gris' and 'La Crescent' maintained the highest concentration trend for malic acid accumulation, and 'St. Pepin', 'Riesling', and 'Chardonnay' were similar.

Average TA and tartaric and malic acid concentrations compared to GDD of harvest date for each cultivar in the 2010 and 2011 growing seasons are shown in Figures 2.5.1 – 2.5.11. Tartaric acid concentrations were lower in 2011 than in 2010 for all cultivars except 'Maréchal Foch', 'Marquette', and 'La Crescent'. GDD differences

accounted for no significant differences in TA or malic acid concentration between years. After 1,450 GDD there was very little change in TA, tartaric or malic acid concentration.

A heat map of tartaric to malic acid ratio (T/M ratio) for selected cultivars and corresponding GDD at each harvest is shown in Table 2.3. Throughout the 2010 harvest 'La Crescent' retained the lowest T/M ratio and 'St. Croix achieved the highest T/M ratio. During the 2011 growing season 'La Crescent', 'St. Pepin', 'Merlot', and 'Frontenac' retained low T/M ratios and 'Marquette', 'Maréchal Foch', 'St. Croix' and 'Riesling' achieved high T/M ratios. An increase in GDD resulted in continuous increase of T/M ratio for 'Frontenac gris', 'Marquette', 'St. Croix', 'Pinot noir', and 'Riesling'. In contrast, an increase in GDD resulted in continuous decrease in T/M ratio for cultivars 'La Crescent', and 'Pinot noir'.

Discussion:

The purpose of this study was to examine the ripening process and quantify grape berry metabolites in cultivars used in cold climate viticulture. The *V. vinifera* cultivars were included for comparison. The results obtained for the 11 cultivars during the two seasons show that sugar and organic acid content and composition varied by cultivar and, in some cases, year. The glucose and fructose composition varied among cultivars; however, the ratios did not, which is consistent with the studies of Shiraishi (1993) and Liu (2006).

Heat units were measured from temperature data and accumulation of heat units or thermal time is expressed in GDD. I found that an increase of heat units (measured in GDD) resulted in a decrease in malic acid, this is consistent with Lakso et al. (1975) and

Kliewer (1967 and1971). Concentrations of malic and tartaric acid decreased throughout the studied harvest seasons. Concentrations of malic acid were fairly consistent between years and decreased at approximately the same GDD for all cultivars. Tartaric acid was found at different concentrations between years and GDD. The difference in tartaric acid between years may be the result of differences in temperature between years. The timing of accumulation varied between years when compared on a basis of GDD. Accumulation of GDD was slower in 2011 than in 2010.

Trends of acid concentrations over the season expressed in terms of GDD varied among cultivars. 'Frontenac', 'Frontenac gris', and 'La Crescent' maintained high levels of malic acid compared to all other cultivars. Of the red grape cultivars, 'Frontenac', 'Maréchal Foch', and 'Marquette' retained higher levels of tartaric acid compared to others. In respect to malic and tartaric acid 'Marquette', 'St. Croix', and 'St. Pepin' were more like *V. vinifera* than 'Frontenac', 'Frontenac gris', and 'La Crescent'. Based on a study of tartaric and malic acid concentrations in 78 grape cultivars grown at the University of California, Davis, Kliewer et al. (1967) proposed classification of all cultivars into four groups based on tartaric to malic ratio (T/M ratio): high-malate (below 1.20), moderately-high malate (1.21 to 1.75), intermediate-malate (1.76 to 2.50), and low-malate (above 2.51). Notably, Kliewer et al. identified 'Pinot noir' high-malate (1.15), 'Chardonnay moderately-high malate (1.71), 'Merlot' intermediate-malate (1.82), and 'Riesling' low-malate (3.12), respectively. Although, M/T ratios reported by Kliewer et al. (1967) were much higher than what was found in the current study, they followed a similar progression with 'Riesling' having the highest M/T ratio. With respect to M/T ratio and selected cultivars, 'St. Pepin' and 'La Crescent' were similar to

‘Chardonnay’; ‘St. Croix’ was similar ‘Riesling’; and ‘Marquette’ and ‘Maréchal Foch’ were similar to ‘Merlot’ and ‘Pinot noir’.

By documenting and examining the ripening profiles for SSC consisting of glucose and fructose and TA consisting mainly of malic and tartaric acid for the cultivars in this study, I have been able to determine 1) the range of variation to be expected in divergent seasons in Minnesota, 2) glucose to fructose ratio is approximately 1:1 throughout ripening 3) that cold hardy hybrid cultivars ‘Frontenac’, ‘Frontenac gris’ and ‘La Crescent’ retain higher concentrations of organic acids throughout harvest.

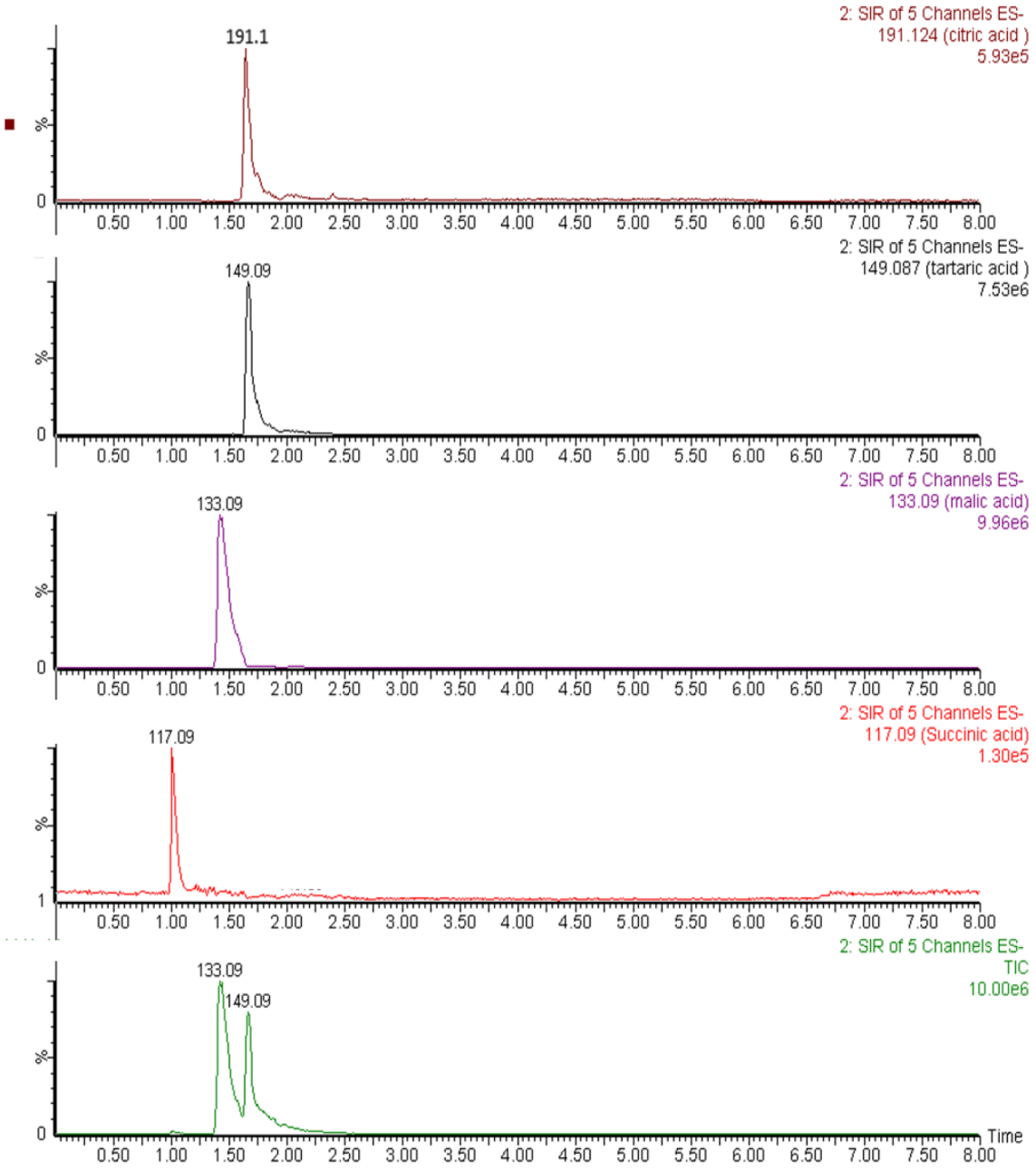

Figure 2.1. UPLC determination of organic acids in 'Marquette'. SIR chromatograms are shown for (from top to bottom) four organic acids: citric (191.12 *m/z*), tartaric (149.09 *m/z*), malic (133.09 *m/z*) and succinic acids (117.09 *m/z*), and the sum of the SIR channels is shown at the bottom.

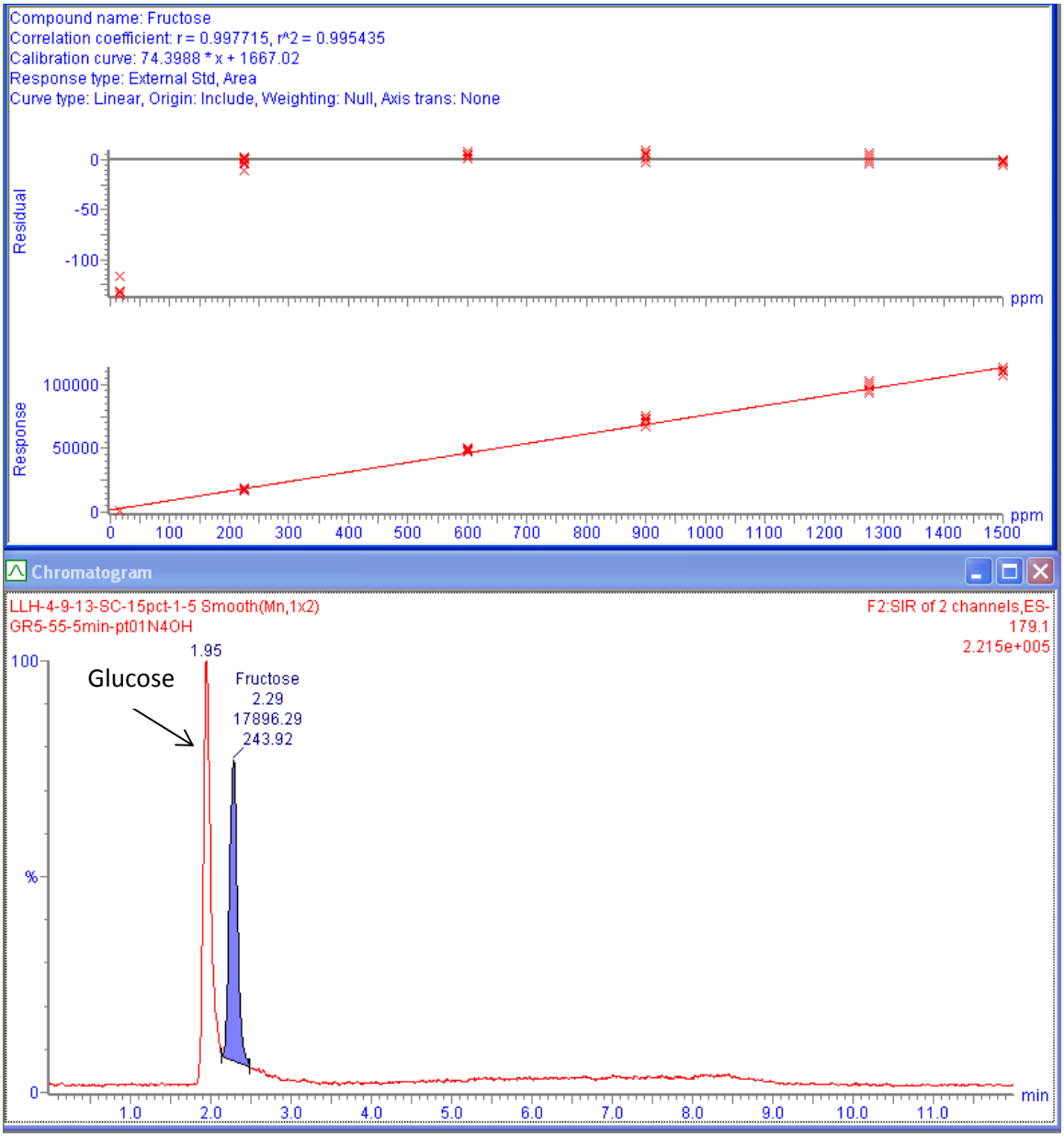

Figure 2.2. Calibration and residual curves (top) and LC-MS SIR chromatogram (bottom) of glucose and fructose (both are 179.1 *m/z*).

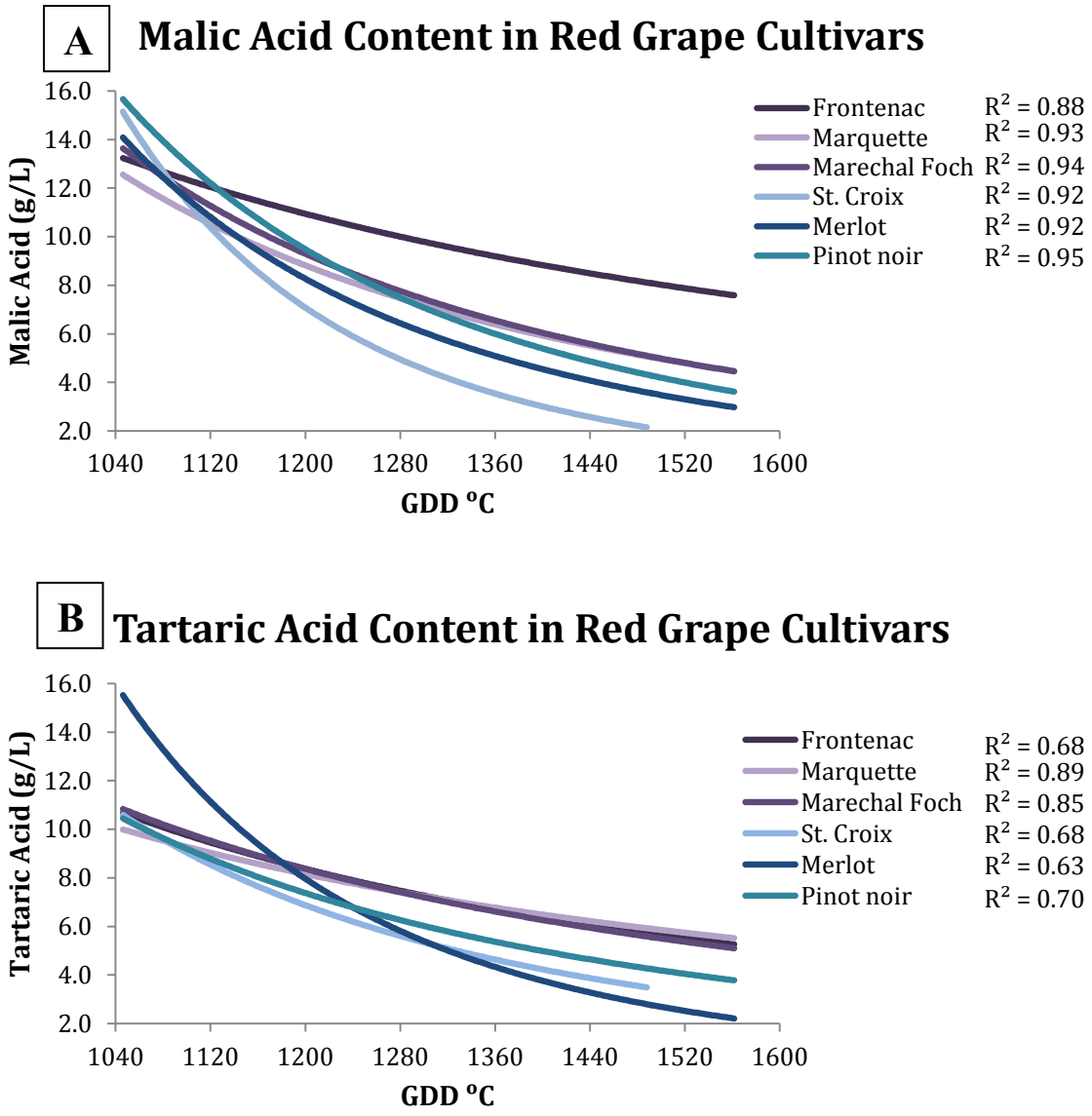

Figure 2.3. Regression lines (second-order polynomial) showing the relationship of Growing Degree Days to malic acid (A) and tartaric acid (B) mean concentrations for red grape cultivars during berry development evaluated years 2010 and 2011.

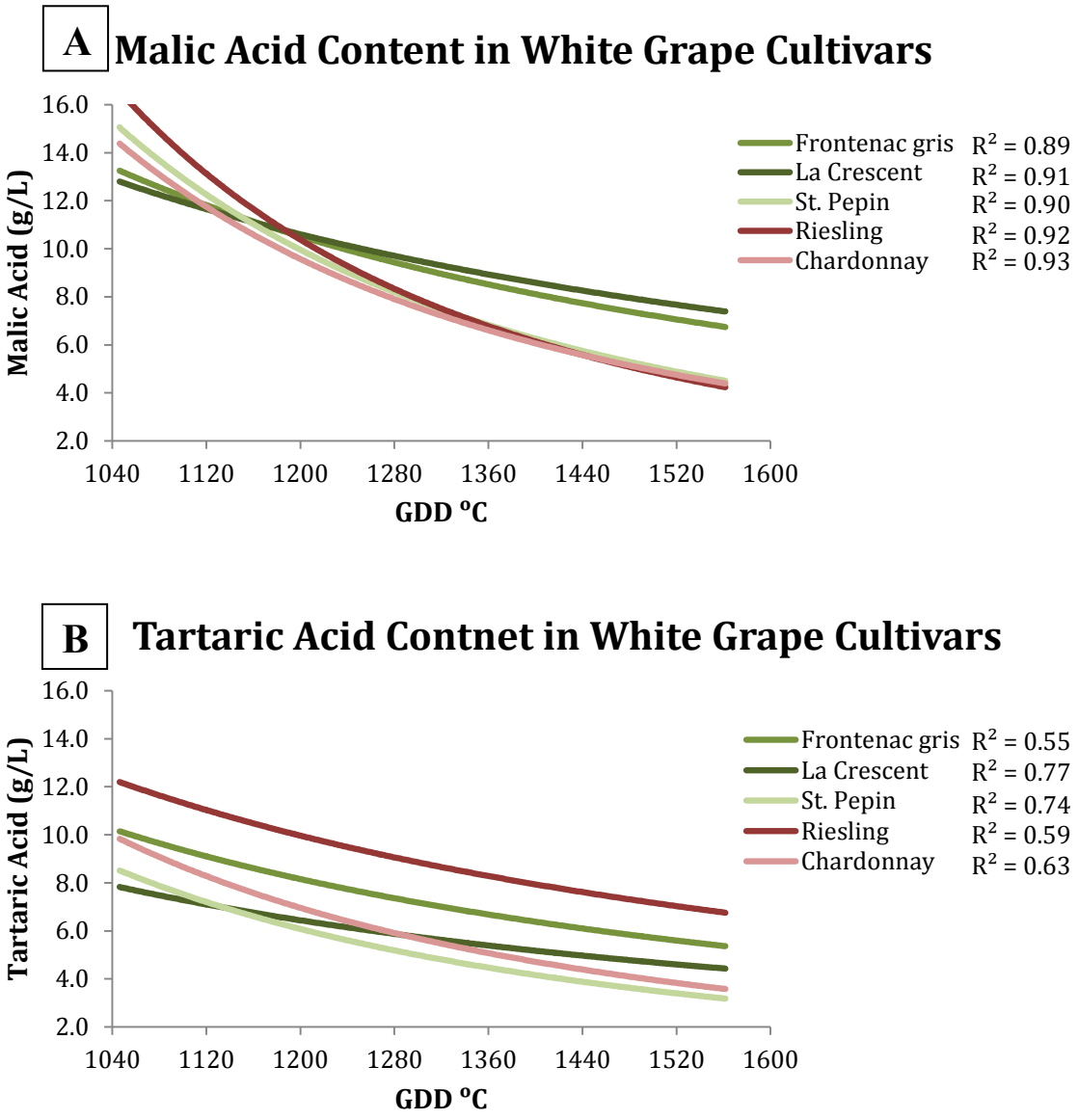

Figure 2.4. Regression lines (second-order polynomial) showing the relationship of Growing Degree Days to malic acid (A) and tartaric acid (B) mean concentrations for white grape cultivars during berry development evaluated years 2010 and 2011.

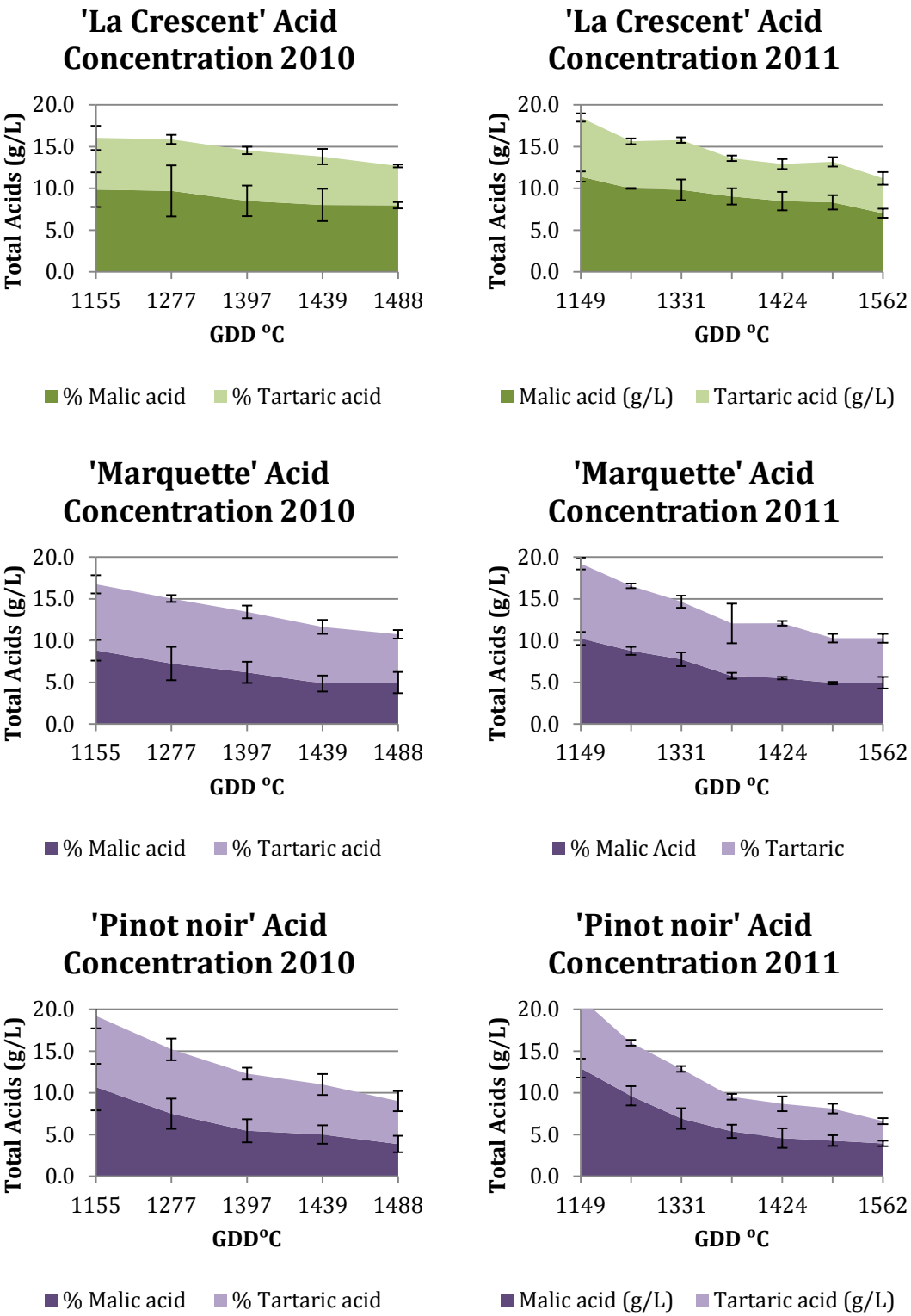

Figure 2.5. Malic and tartaric acid concentrations compared to respective growing degree day (GDD) for 'La Crescent', 'Marquette', and 'Pinot noir' harvested in 2010 and 2011.

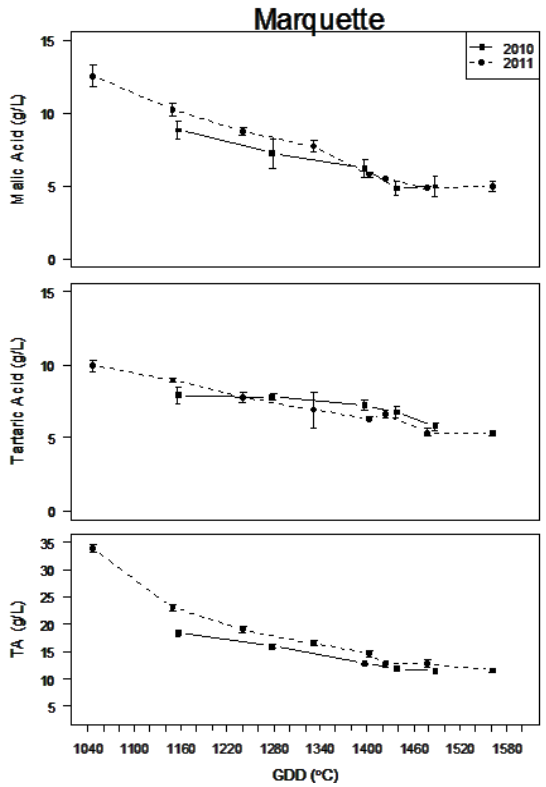

Figure 2.6.1. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Marquette' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

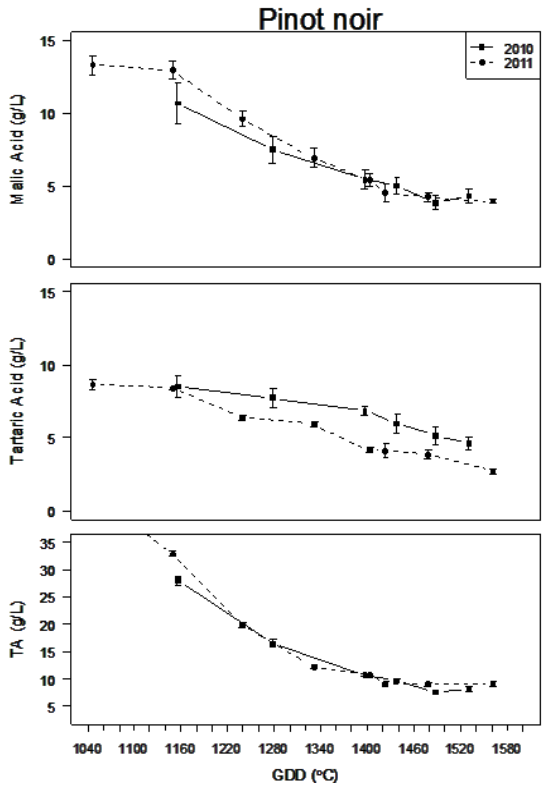

Figure 2.6.2. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Pinot noir' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

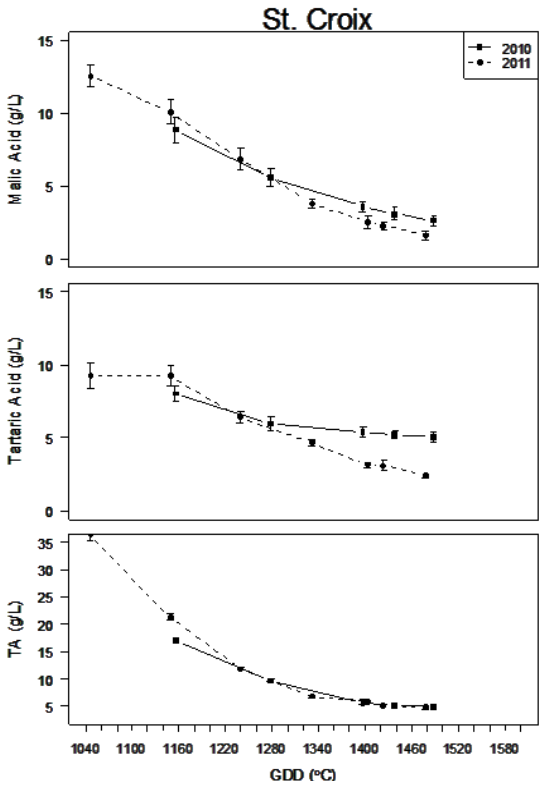

Figure 2.6.3. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'St. Croix' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

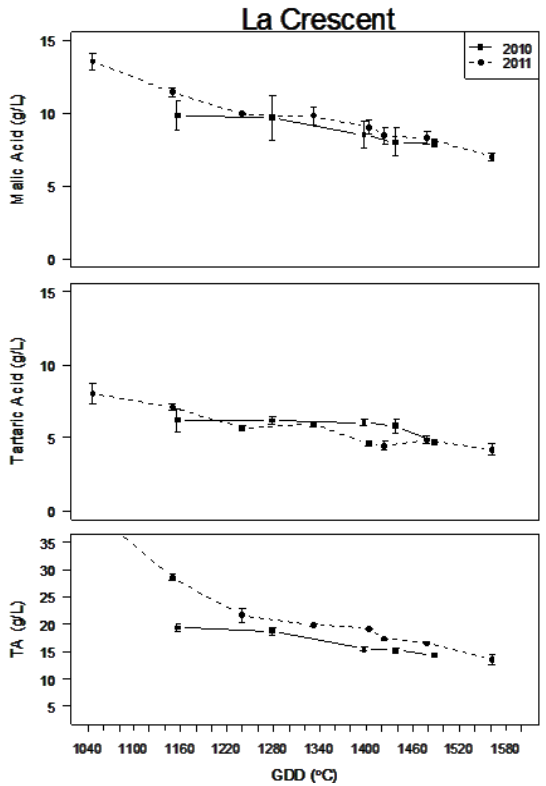

Figure 2.6.4. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'La Crescent' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

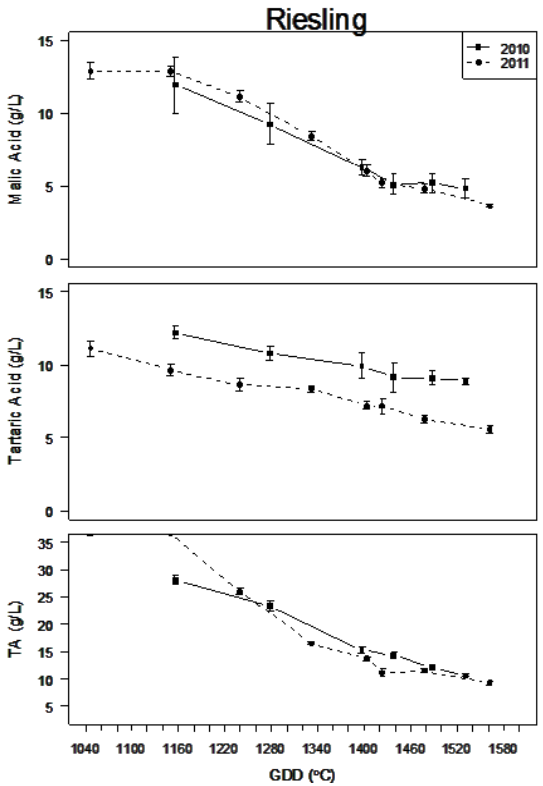

Figure 2.6.5. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Riesling' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

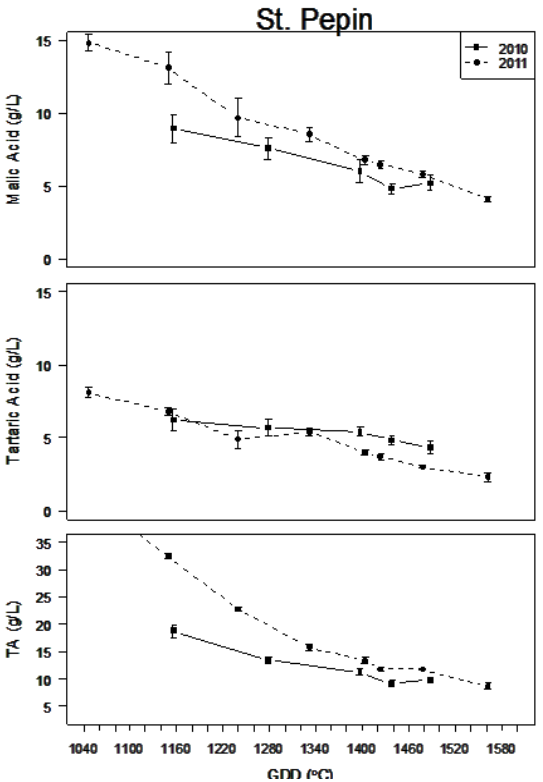

Figure 2.6.6. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'St. Pepin' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

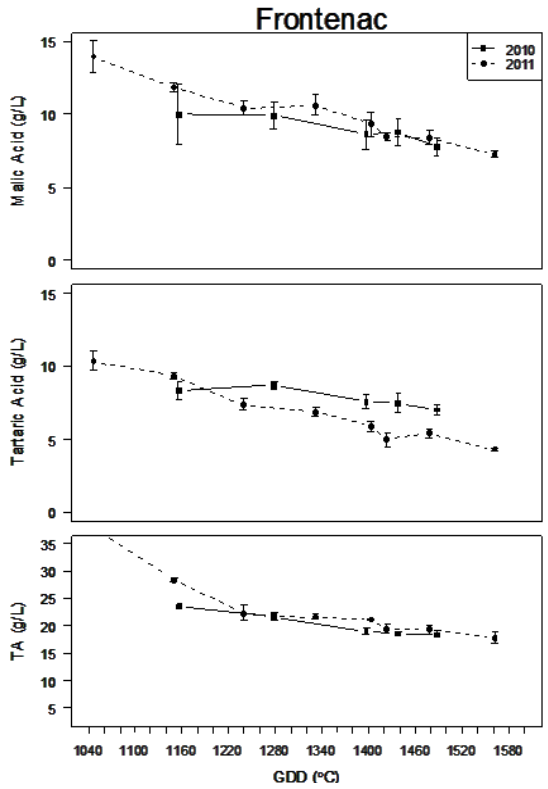

Figure 2.6.7. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Frontenac' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

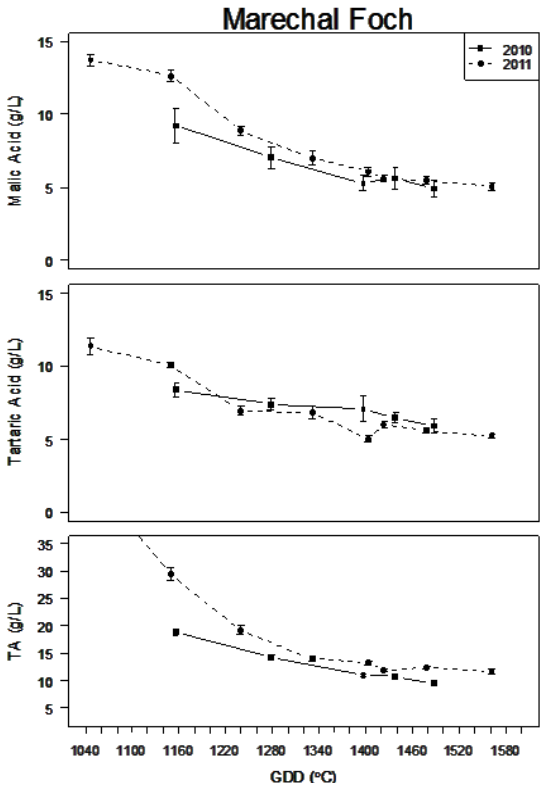

Figure 2.6.8. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Maréchal Foch' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

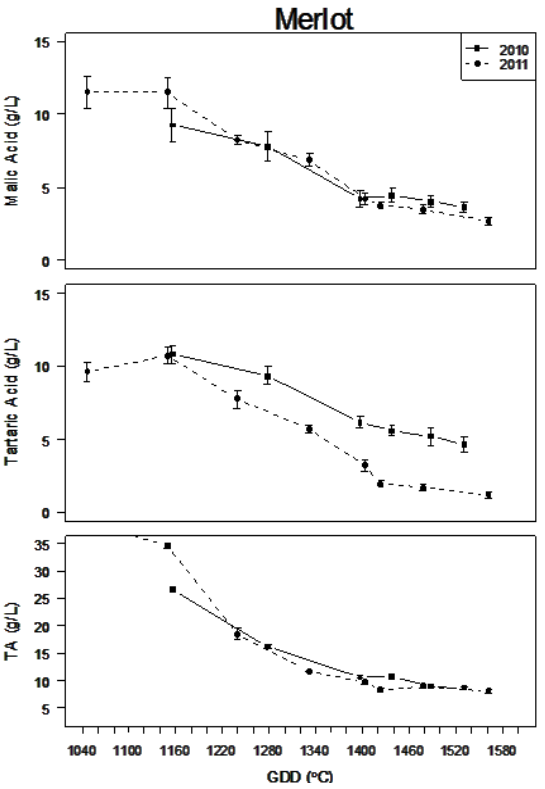

Figure 2.6.9. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Merlot' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

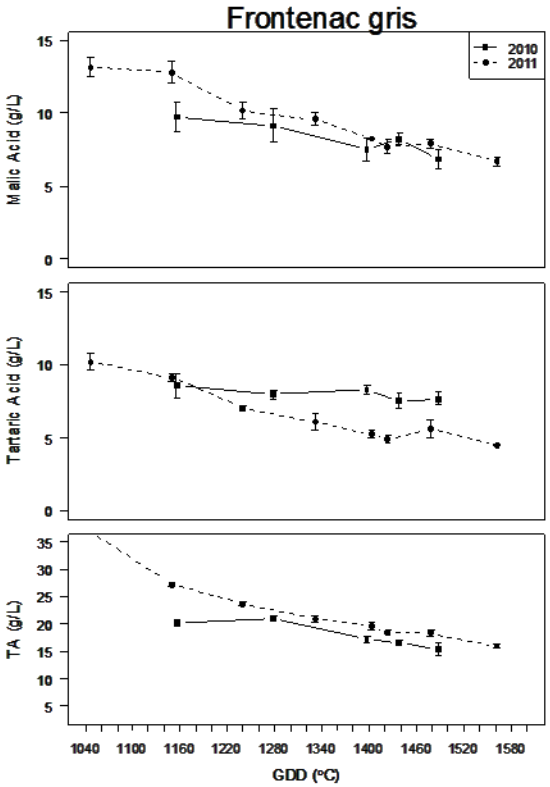

Figure 2.6.10. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Frontenac gris' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

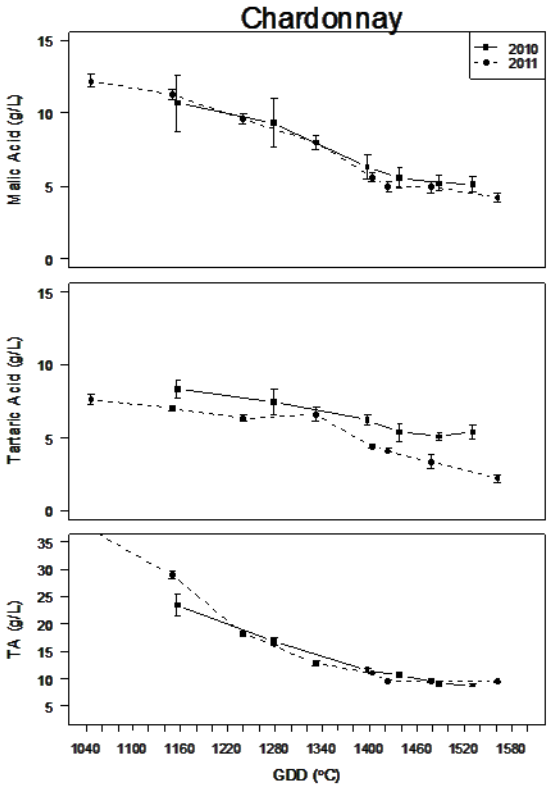

Figure 2.6.11. Relationship of Growing Degree Days (GDD) to titratable acidity (TA), Malic acid, and Tartaric acid for the cultivar 'Chardonnay' during berry development evaluated in 2010 and 2011. Vertical bars indicate the standard error of each mean value (n=4).

Table 2.1.1 Analysis of variance for malic acid, tartaric acid, citric acid, succinic acid and ratio of tartaric to malic acid (T/M ratio) in berries of eleven grape cultivars at multiple harvest dates expressed as accumulated Growing Degree Days (GDD) in two successive years (2010-2011).

Cultivar	Source of Variance	Malic Acid	Tartaric Acid	Citric Acid	Succinic Acid	T/M Ratio
Marquette	Year	NS	NS	***	***	*
	GDD	***	***	***	***	**
	Year*GDD	NS	*	NS	**	NS
La Crescent	Year	NS	NS	**	NS	NS
	GDD	***	***	NS	***	*
	Year*GDD	NS	*	NS	**	NS
Frontenac	Year	NS	*	***	***	**
	GDD	***	***	NS	***	NS
	Year*GDD	NS	***	NS	NS	NS
Frontenac gris	Year	NS	**	*	**	***
	GDD	***	***	NS	***	NS
	Year*GDD	NS	***	NS	**	NS
St. Pepin	Year	*	NS	***	***	***
	GDD	***	***	***	***	NS
	Year*GDD	*	***	*	**	NS
St. Croix	Year	NS	NS	*	***	NS
	GDD	***	***	***	NS	**
	Year*GDD	**	***	**	NS	NS
Maréchal Foch	Year	NS	NS	***	NS	**
	GDD	***	***	***	***	NS
	Year*GDD	NS	**	**	NS	NS
Pinot nior	Year	NS	NS	**	NS	***
	GDD	***	***	***	NS	NS
	Year*GDD	NS	NS	*	NS	NS
Merlot	Year	NS	**	**	NS	***
	GDD	***	***	***	*	*
	Year*GDD	NS	*	NS	NS	*
Riesling	Year	NS	***	**	NS	***
	GDD	***	***	***	***	**
	Year*GDD	*	*	NS	NS	NS
Chardonnay	Year	NS	*	***	NS	***
	GDD	***	***	***	NS	NS
	Year*GDD	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2.1.2 Analysis of variance for glucose, fructose and ratio of glucose to fructose in berries of eleven grape cultivars at multiple harvest dates expressed as accumulated Growing Degree Days (GDD) in two successive years (2010-2011).

Cultivar	Source of Variance	Glucose	Fructose	G/F Ratio
Marquette	Year	NS	NS	NS
	GDD	***	***	NS
	Year*GDD	NS	NS	NS
La Crescent	Year	NS	NS	*
	GDD	***	***	*
	Year*GDD	NS	NS	NS
Frontenac	Year	NS	NS	NS
	GDD	***	***	***
	Year*GDD	*	*	NS
Frontenac gris	Year	NS	NS	NS
	GDD	***	***	NS
	Year*GDD	NS	NS	NS
St. Pepin	Year	NS	NS	NS
	GDD	***	***	**
	Year*GDD	NS	NS	NS
St. Croix	Year	NS	NS	NS
	GDD	**	***	NS
	Year*GDD	*	*	NS
Maréchal Foch	Year	NS	NS	NS
	GDD	***	***	NS
	Year*GDD	**	**	NS
Pinot nior	Year	NS	NS	NS
	GDD	***	***	***
	Year*GDD	NS	NS	NS
Merlot	Year	NS	NS	NS
	GDD	***	***	***
	Year*GDD	NS	NS	NS
Riesling	Year	NS	NS	NS
	GDD	***	***	***
	Year*GDD	NS	NS	NS
Chardonnay	Year	NS	NS	NS
	GDD	***	***	***
	Year*GDD	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2.2.1. Means and LSD for titratable acidity (TA), pH, malic, tartaric, citric, succinic acid, and ratio of tartaric to malic acid for cold hardy cultivars and corresponding date and accumulated growing degree days (GDD) at each harvest in 2010 and 2011.

		2010						2011								
	Date	**8/13**	**8/23**	**9/3**	**9/11**	**9/21**		**8/10**	**8/19**	**8/27**	**9/6**	**9/14**	**9/23**	**10/3**	**10/12**	
	GDD °C	**1,155**	**1,277**	**1,397**	**1,439**	**1,488**	**LSD**	**1,046**	**1,149**	**1,239**	**1,331**	**1,403**	**1,424**	**1,468**	**1,562**	**LSD**
Frontenac	**TA (g/L)**	23.6	21.7	19.0	18.6	18.5	2.4	37.9	28.4	22.4	21.5	21.1	19.5	19.3	17.9	2.4
	pH	2.40	2.72	2.85	2.90	2.86	0.05	2.40	2.69	2.75	2.84	2.92	3.01	3.04	3.12	0.05
	Malic (g/L)	10.0	9.9	8.6	8.7	7.8	2.6	13.9	11.8	10.4	10.6	9.3	8.5	8.4	7.3	1.8
	Tartaric (g/L)	8.3	8.7	7.6	7.5	7.0	1.5	10.4	9.3	7.4	6.9	5.9	5.0	5.4	4.3	1.1
	Citric (g/L)	0.33	0.35	0.30	0.30	0.28	0.09	0.73	0.57	0.54	0.60	0.67	0.63	0.65	0.67	0.15
	Succinic (g/L)	0.01	0.01	0.01	0.01	0.01	0.00	0.06	0.04	0.03	0.04	0.01	0.02	0.02	0.02	0.00
	Tartaric/Malic	0.84	0.88	0.88	0.86	0.90	0.72	0.74	0.79	0.71	0.65	0.63	0.59	0.64	0.59	0.14
Frontenac gris	**TA (g/L)**	20.3	21.1	17.3	16.6	15.5	1.6	36.8	27.3	23.7	21.0	19.6	18.5	18.4	16.1	1.5
	pH	2.40	2.71	2.86	2.88	2.96	0.04	2.35	2.70	2.74	2.82	2.93	2.96	3.01	3.08	0.05
	Malic (g/L)	9.8	9.1	7.5	8.2	6.8	2.3	13.2	12.8	10.2	9.6	8.3	7.7	7.9	6.7	2.0
	Tartaric (g/L)	8.5	8.0	8.3	7.5	7.7	1.5	10.2	9.1	7.0	6.1	5.3	4.9	5.6	4.5	1.6
	Citric (g/L)	0.40	0.46	0.52	0.48	0.52	0.14	0.66	0.62	0.52	0.61	0.63	0.60	0.67	0.63	0.11
	Succinic (g/L)	0.03	0.02	0.01	0.02	0.02	0.00	0.05	0.04	0.03	0.03	0.01	0.02	0.03	0.02	0.00
	Tartaric/Malic	0.88	0.87	1.10	0.92	1.13	0.35	0.78	0.71	0.69	0.64	0.64	0.64	0.71	0.67	0.18
Marquette	**TA (g/L)**	18.8	14.4	11.0	10.7	9.7	1.1	46.7	29.4	19.3	14.0	13.3	12.0	12.5	11.8	1.6
	pH	2.40	2.68	2.80	2.81	2.84	0.03	2.36	2.70	2.74	2.79	2.84	2.92	3.04	3.05	0.04
	Malic (g/L)	8.8	7.2	6.2	4.9	5.0	2.1	12.6	10.2	8.8	7.8	5.8	5.5	4.9	5.0	2.1
	Tartaric (g/L)	7.9	7.8	7.3	6.8	5.8	1.2	9.9	9.0	7.8	6.9	6.3	6.6	5.4	5.3	1.3
	Citric (g/L)	0.30	0.26	0.26	0.23	0.26	0.06	0.60	0.44	0.43	0.35	0.36	0.37	0.35	0.42	0.25
	Succinic (g/L)	0.01	0.01	0.00	0.01	0.01	0.00	0.05	0.03	0.02	0.02	0.01	0.01	0.02	0.02	0.00
	Tartaric/Malic	0.90	1.07	1.17	1.39	1.16	0.67	0.79	0.88	0.89	0.89	1.09	1.19	1.10	1.07	0.12
Maréchal Foch	**TA (g/L)**	18.4	16.0	12.9	12.0	11.5	0.8	33.9	23.1	19.1	16.5	14.7	12.8	12.8	11.6	1.7
	pH	2.40	2.68	2.80	2.81	2.84	0.03	2.36	2.70	2.74	2.79	2.84	2.92	3.04	3.05	0.11
	Malic (g/L)	9.2	7.0	5.3	5.6	4.9	2.4	13.7	12.7	8.9	7.0	6.1	5.6	5.5	5.1	0.9
	Tartaric (g/L)	8.4	7.4	7.1	6.5	5.9	1.7	11.4	10.1	7.0	6.9	5.0	6.0	5.6	5.2	0.9
	Citric (g/L)	0.40	0.32	0.29	0.34	0.32	0.07	0.86	0.71	0.45	0.44	0.42	0.44	0.46	0.50	0.11
	Succinic (g/L)	0.02	0.01	0.01	0.02	0.02	0.00	0.06	0.04	0.03	0.04	0.02	0.03	0.03	0.02	0.00
	Tartaric/Malic	0.91	1.05	1.34	1.15	1.21	0.70	0.83	0.80	0.78	0.98	0.83	1.07	1.03	1.03	0.13

Table 2.2.2. Means and LSD for titratable acidity (TA), pH, malic, tartaric, citric, succinic acid, and ratio of tartaric to malic acid for cold hardy cultivars and corresponding date and accumulated growing degree days (GDD) at each harvest in 2010 and 2011.

		2010						2011								
	Date	**8/13**	**8/23**	**9/3**	**9/11**	**9/21**		**8/10**	**8/19**	**8/27**	**9/6**	**9/14**	**9/23**	**10/3**	**10/12**	
	GDD °C	**1,155**	**1,277**	**1,397**	**1,439**	**1,488**	**LSD**	**1,046**	**1,149**	**1,239**	**1,331**	**1,403**	**1,424**	**1,468**	**1,562**	**LSD**
La Crescent	**TA (g/L)**	19.4	18.7	15.5	15.2	14.4	1.6	40.9	28.6	21.7	19.9	19.2	17.2	16.6	13.6	2.0
	pH	2.59	2.75	2.84	2.88	2.88	0.05	2.43	2.66	2.77	2.79	2.86	2.91	2.94	2.99	0.06
	Malic (g/L)	9.8	9.7	8.5	8.0	8.0	1.9	13.5	11.4	10.0	9.8	9.0	8.5	8.3	7.0	1.3
	Tartaric (g/L)	6.2	6.2	6.0	5.8	4.7	1.5	8.0	7.1	5.7	6.0	4.6	4.4	4.9	4.2	1.0
	Citric (g/L)	0.47	0.49	0.54	0.58	0.49	0.11	0.80	0.57	0.52	0.57	0.62	0.63	0.67	0.56	0.15
	Succinic (g/L)	0.04	0.04	0.02	0.05	0.03	0.00	1.60	0.95	0.83	1.03	0.48	0.78	0.75	0.45	0.00
	Tartaric/Malic	0.63	0.64	0.71	0.72	0.59	0.22	0.59	0.62	0.57	0.61	0.51	0.52	0.58	0.60	0.10
St. Croix	**TA (g/L)**	17.0	9.8	5.7	5.2	5.0	1.0	36.3	21.3	11.8	6.9	5.8	5.1	4.8	NA	1.1
	pH	2.45	2.94	3.13	3.11	3.08	0.05	2.31	2.63	2.86	3.07	3.23	3.20	3.42	NA	0.05
	Malic (g/L)	8.9	5.6	3.6	3.1	2.6	1.7	12.6	10.1	6.8	3.8	2.5	2.3	1.6	NA	1.6
	Tartaric (g/L)	8.0	5.9	5.4	5.2	5.0	1.3	9.3	9.3	6.4	4.6	3.1	3.1	2.4	NA	1.5
	Citric (g/L)	0.51	0.31	0.21	0.19	0.17	0.10	1.09	0.74	0.47	0.30	0.27	0.26	0.22	NA	0.17
	Succinic (g/L)	0.01	0.00	0.00	0.01	0.00	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	NA	0.00
	Tartaric/Malic	0.91	1.07	1.49	1.68	1.92	1.07	0.74	0.92	0.94	1.22	1.23	1.35	1.49		0.57
St. Pepin	**TA (g/L)**	18.8	13.5	11.2	9.2	9.8	2.1	43.0	32.4	22.8	15.7	13.4	11.8	11.8	8.8	1.8
	pH	2.56	2.78	2.93	2.90	2.94	0.05	2.50	2.64	2.71	2.83	2.96	2.96	3.05	3.25	0.05
	Malic (g/L)	8.9	7.6	6.0	4.8	5.2	2.1	14.8	13.1	9.7	8.6	6.8	6.5	5.8	4.1	2.0
	Tartaric (g/L)	6.2	5.7	5.4	4.8	4.4	1.5	8.1	6.8	4.9	5.4	4.0	3.7	3.0	2.3	0.9
	Citric (g/L)	0.37	0.34	0.30	0.25	0.29	0.08	0.70	0.63	0.48	0.42	0.39	0.41	0.38	0.34	0.12
	Succinic (g/L)	0.01	0.01	0.00	0.01	0.01	0.00	0.06	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.00
	Tartaric/Malic	0.69	0.75	0.90	1.00	0.84	0.56	0.55	0.52	0.50	0.63	0.59	0.57	0.52	0.55	0.13

Table 2.2.3. Means and LSD for titratable acidity (TA), pH, malic, tartaric, citric, succinic acid, and ratio of tartaric to malic acid for *Vitis vinifera* cultivars and corresponding date and accumulated growing degree days (GDD) at each harvest in 2010 and 2011.

		2010						2011								
	Date	**8/13**	**8/23**	**9/3**	**9/11**	**9/21**		**8/10**	**8/19**	**8/27**	**9/6**	**9/14**	**9/23**	**10/3**	**10/12**	
	GDD °C	**1,155**	**1,277**	**1,397**	**1,439**	**1,488**	**LSD**	**1,046**	**1,149**	**1,239**	**1,331**	**1,403**	**1,424**	**1,468**	**1,562**	**LSD**
Merlot	**TA (g/L)**	26.7	16.3	10.7	10.8	9.0	1.0	39.1	34.5	18.5	11.8	9.7	8.4	9.1	8.2	1.5
	pH	2.35	2.76	3.05	3.11	3.20	0.05	2.41	2.48	2.74	2.87	3.15	3.27	3.29	3.45	0.05
	Malic (g/L)	9.3	7.6	4.2	4.5	4.0	2.2	11.5	10.5	8.2	6.9	4.2	3.7	3.3	2.7	1.7
	Tartaric (g/L)	10.8	7.7	6.2	5.6	5.2	2.1	9.6	10.5	7.7	5.7	3.2	1.9	1.7	1.2	1.3
	Citric (g/L)	0.36	0.18	0.14	0.16	0.16	0.07	0.47	0.46	0.32	0.29	0.23	0.22	0.24	0.20	0.03
	Succinic (g/L)	0.02	0.01	0.00	0.02	0.02	0.00	0.02	0.03	0.01	0.02	0.01	0.01	0.01	0.01	0.00
	Tartaric/Malic	1.17	1.02	1.47	1.26	1.29	0.81	0.84	1.00	0.94	0.83	0.76	0.52	0.51	0.45	0.23
Pinot Noir	**TA (g/L)**	28.0	16.5	10.8	9.8	7.5	1.4	43.8	32.9	19.9	12.2	10.7	9.1	9.0	9.1	1.4
	pH	2.57	2.74	3.02	3.12	3.20	0.05	2.40	2.59	2.76	2.97	3.08	3.08	3.17	3.29	0.04
	Malic (g/L)	10.7	7.5	5.5	5.0	3.9	2.5	13.3	13.0	9.6	6.9	5.4	4.6	4.3	3.9	1.5
	Tartaric (g/L)	8.5	7.7	6.8	6.0	5.1	1.7	8.6	8.4	6.4	5.9	4.1	4.1	3.8	2.7	0.7
	Citric (g/L)	0.55	0.36	0.26	0.27	0.24	0.08	0.78	0.71	0.50	0.41	0.37	0.35	0.38	0.41	0.07
	Succinic (g/L)	0.02	0.01	0.01	0.03	0.02	0.00	0.04	0.03	0.02	0.02	0.01	0.01	0.02	0.02	0.00
	Tartaric/Malic	0.80	1.03	1.25	1.20	1.34	0.80	0.65	0.65	0.66	0.86	0.77	0.90	0.90	0.68	0.22
Riesling	**TA (g/L)**	28.1	23.4	15.3	14.4	12.2	1.9	36.9	36.6	25.9	16.5	13.8	11.1	11.5	9.3	1.4
	pH	2.54	2.58	2.77	2.82	2.86	0.05	2.54	2.56	2.64	2.86	2.77	2.97	3.07	2.99	0.04
	Malic (g/L)	11.9	9.3	6.3	5.8	5.2	1.5	12.9	12.9	11.1	8.4	6.1	5.2	4.8	3.6	1.0
	Tartaric (g/L)	12.2	10.8	9.9	8.2	9.1	1.0	11.1	9.7	8.6	8.3	7.2	7.2	6.3	5.6	1.1
	Citric (g/L)	0.53	0.39	0.27	0.22	0.26	0.16	0.62	0.58	0.47	0.40	0.34	0.34	0.33	0.30	0.07
	Succinic (g/L)	0.04	0.02	0.01	0.02	0.02	0.01	0.04	0.05	0.02	0.02	0.01	0.02	0.02	0.02	0.00
	Tartaric/Malic	1.02	1.17	1.58	1.40	1.74	0.78	0.86	0.75	0.77	0.99	1.19	1.38	1.29	1.54	0.21
Chardonnay	**TA (g/L)**	23.5	16.8	11.6	10.8	9.2	2.6	37.3	29.0	18.3	12.9	11.1	9.6	9.7	9.5	1.3
	pH	2.56	2.86	3.06	3.16	3.22	0.05	2.57	2.76	2.89	3.00	3.09	3.14	3.12	3.34	0.04
	Malic (g/L)	10.7	9.4	6.3	5.6	5.2	3.5	12.2	11.3	9.6	8.0	5.6	5.0	4.9	4.2	1.1
	Tartaric (g/L)	8.3	7.5	6.2	5.4	5.1	1.7	7.6	7.0	6.4	6.6	4.4	4.1	3.3	2.2	0.9
	Citric (g/L)	0.42	0.36	0.23	0.22	0.25	0.14	0.56	0.46	0.41	0.38	0.32	0.32	0.34	0.34	0.08
	Succinic (g/L)	0.02	0.02	0.01	0.04	0.05	0.00	0.03	0.03	0.02	0.03	0.01	0.03	0.05	0.03	0.00
	Tartaric/Malic	0.78	0.80	0.99	0.96	0.98	0.60	0.62	0.62	0.66	0.83	0.80	0.82	0.68	0.52	0.21

Table 2.3. Heat map comparing tartaric to malic acid ratio for selected cultivars within corresponding date and accumulated growing degree days (GDD) at each harvest in 2010 and 2011. Red color represents low tartaric to malic acid ration and green represents high tartaric to malic acid ratio.

	2010					2011							
Date	**8/13**	**8/23**	**9/3**	**9/11**	**9/21**	**8/10**	**8/19**	**8/27**	**9/6**	**9/14**	**9/23**	**10/3**	**10/12**
GDD °C	**1155**	**1277**	**1397**	**1439**	**1488**	**1046**	**1149**	**1239**	**1331**	**1403**	**1424**	**1468**	**1562**
Cultivar	**Ratio of Tartaric to Malic Acid**					**Ratio of Tartaric to Malic Acid**							
Frontenac	0.84	0.88	0.88	0.86	0.90	0.74	0.79	0.71	0.65	0.63	0.59	0.64	0.59
Frontenac gris	0.88	0.87	1.10	0.92	1.13	0.78	0.71	0.69	0.64	0.64	0.64	0.71	0.67
Marquette	0.90	1.07	1.17	1.39	1.16	0.79	0.88	0.89	0.89	1.09	1.19	1.10	1.07
Maréchal Foch	0.91	1.05	1.34	1.15	1.21	0.83	0.80	0.78	0.98	0.83	1.07	1.03	1.03
La Crescent	0.63	0.64	0.71	0.72	0.59	0.59	0.62	0.57	0.61	0.51	0.52	0.58	0.60
St. Croix	0.91	1.07	1.49	1.68	1.92	0.74	0.92	0.94	1.22	1.23	1.35	1.49	NA
St. Pepin	0.69	0.75	0.90	1.00	0.84	0.55	0.52	0.50	0.63	0.59	0.57	0.52	0.55
Merlot	1.17	1.02	1.47	1.26	1.29	0.84	1.00	0.94	0.83	0.76	0.52	0.51	0.45
Pinot noir	0.80	1.03	1.25	1.20	1.34	0.65	0.65	0.66	0.86	0.77	0.90	0.90	0.68
Riesling	1.02	1.17	1.58	1.40	1.74	0.86	0.75	0.77	0.99	1.19	1.38	1.29	1.54
Chardonnay	0.78	0.80	0.99	0.96	0.98	0.62	0.62	0.66	0.83	0.80	0.82	0.68	0.52
LSD	0.45	0.52	0.89	0.89	1.22	0.34	0.46	0.89	0.60	0.77	1.04	1.04	1.07

Table 2.4.1 Means and LSD for soluble solids content (SSC), glucose (g/L), fructose (g/L) and ratio of glucose to fructose for cold hardy cultivars and corresponding date and accumulated growing degree days (GDD) at each harvest in 2010 and 2011.

		2010						2011								
	Date	8/13	8/23	9/3	9/11	9/21		8/10	8/19	8/27	9/6	9/14	9/23	10/3	10/12	
	GDD	1,155	1,277	1,397	1,439	1,488	LSD	1,046	1,149	1,239	1,331	1,403	1,424	1,468	1,562	LSD
Frontenac	SSC (°Brix)	14.5	17.0	20.3	21.3	22.9	1.2	9.0	14.8	15.7	18.2	22.3	21.7	24.6	24.9	2.0
	Glucose (g/L)	69.4	95.6	106.3	105.0	125.5	9.8	27.9	64.0	87.1	85.6	138.6	128.6	147.7	140.7	6.9
	Fructose (g/L)	83.2	100.4	114.0	111.6	127.8	10.8	39.2	77.0	98.2	100.2	148.7	140.9	157.1	147.9	9.2
	G/F Ratio	0.8	1.0	0.9	0.9	1.0	0.0	0.7	0.8	0.9	0.9	0.9	0.9	0.9	1.0	0.0
Frontenac gris	SSC (°Brix)	15.4	19.3	23.6	24.5	23.4	0.9	9.9	15.9	18.9	20.6	23.4	24.4	25.7	27.2	1.6
	Glucose (g/L)	78.4	93.8	133.5	150.4	134.7	15.4	51.4	62.8	114.4	114.0	141.3	156.0	148.7	177.4	8.2
	Fructose (g/L)	80.7	102.7	140.8	155.8	131.8	13.6	59.4	75.3	114.7	133.7	147.9	153.1	159.8	174.9	6.3
	G/F Ratio	1.0	0.9	0.9	1.0	1.0	0.0	0.9	0.8	1.0	0.9	1.0	1.0	0.9	1.0	0.0
Maréchal Foch	SSC (°Brix)	15.4	18.9	21.9	22.9	24.6	0.5	5.1	14.5	18.0	20.6	22.4	22.7	25.1	26.1	1.0
	Glucose (g/L)	89.5	117.2	137.9	110.0	122.0	11.5	3.0	74.0	90.2	121.7	112.2	138.8	137.0	149.0	9.6
	Fructose (g/L)	92.9	112.9	129.6	101.2	113.3	12.3	7.5	72.6	93.0	120.8	113.0	130.4	137.1	143.8	7.2
	G/F Ratio	1.0	1.0	1.1	1.1	1.1	0.0	0.4	1.0	1.0	1.0	1.0	1.1	1.0	1.0	0.0
Marquette	SSC (°Brix)	17.6	21.6	24.1	25.5	26.2	0.9	11.6	16.9	19.7	22.0	25.1	24.9	26.4	27.6	1.4
	Glucose (g/L)	87.8	123.9	140.4	167.5	179.7	11.8	64.8	90.0	114.6	124.9	143.9	175.3	174.8	163.1	11.3
	Fructose (g/L)	82.9	116.3	158.2	172.9	170.3	10.3	64.7	84.6	114.8	123.4	147.7	159.8	155.2	153.8	11.6
	G/F Ratio	1.1	1.1	0.9	1.0	1.1	0.0	1.0	1.1	1.0	1.0	1.0	1.1	1.1	1.1	0.0
La Crescent	SSC (°Brix)	17.6	21.6	23.2	23.8	23.7	1.5	9.3	15.5	19.2	21.1	23.6	22.3	23.3	22.7	1.6
	Glucose (g/L)	96.8	115.7	133.5	139.8	138.8	8.5	32.9	90.1	103.2	108.1	127.1	127.8	147.3	136.6	9.3
	Fructose (g/L)	90.8	97.7	121.3	128.6	119.2	6.3	35.6	87.2	98.4	105.1	117.6	113.5	134.5	131.5	8.7
	G/F Ratio	1.1	1.2	1.1	1.1	1.2	0.0	0.9	1.0	1.0	1.0	1.1	1.1	1.1	1.0	0.0
St. Croix	SSC (°Brix)	13.0	16.4	17.2	19.9	20.9	1.1	4.4	10.9	14.3	17.1	19.8	19.6	22.1	NA	2.4
	Glucose (g/L)	74.2	86.8	111.0	147.1	133.3	8.3	1.5	53.1	83.0	113.8	125.3	135.6	160.3	NA	11.8
	Fructose (g/L)	72.1	99.4	105.1	142.6	127.8	9.2	4.9	47.4	83.4	108.1	117.5	125.4	147.2	NA	12.1
	G/F Ratio	1.0	0.9	1.1	1.0	1.0	0.0	0.3	1.1	1.0	1.1	1.1	1.1	1.1	NA	0.0
St. Pepin	SSC (°Brix)	15.2	18.7	20.3	21.2	22.0	1.4	6.4	12.3	16.6	17.0	19.5	19.7	21.9	23.0	1.0
	Glucose (g/L)	71.9	86.2	104.9	104.4	135.8	6.8	11.3	58.4	86.7	96.8	127.5	114.1	117.1	130.3	7.7
	Fructose (g/L)	71.2	84.2	100.0	104.6	127.6	6.2	15.3	60.9	83.3	90.3	120.1	120.5	116.7	124.1	8.8
	G/F Ratio	1.0	1.0	1.0	1.0	1.1	0.0	0.7	1.0	1.0	1.1	1.1	0.9	1.0	1.1	0.0

Table 2.4.2 Means and LSD for soluble solids content (SSC), glucose (g/L), fructose (g/L) and ratio of glucose to fructose for *Vitis vinifera* cultivars and corresponding date and accumulated growing degree days (GDD) at each harvest in 2010 and 2011.

		2010						2011								
	Date	**8/13**	**8/23**	**9/3**	**9/11**	**9/21**		**8/10**	**8/19**	**8/27**	**9/6**	**9/14**	**9/23**	**10/3**	**10/12**	
	GDD	**1,155**	**1,277**	**1,397**	**1,439**	**1,488**	**LSD**	**1,046**	**1,149**	**1,239**	**1,331**	**1,403**	**1,424**	**1,468**	**1,562**	**LSD**
Merlot	**SSC (°Brix)**	5.5	13.5	18.4	19.0	20.0	1.1	4.0	7.3	13.3	17.6	20.0	21.1	23.0	23.6	0.7
	Glucose (g/L)	9.2	60.7	92.4	107.4	114.5	11.9	NA	20.8	66.3	106.6	135.4	112.0	148.9	137.1	7.4
	Fructose (g/L)	17.3	61.5	93.3	104.8	109.5	11.4	5.5	29.4	68.8	97.1	133.8	119.2	143.4	142.9	9.2
	G/F Ratio	0.5	1.0	1.0	1.0	1.0	0.0	NA	0.7	1.0	1.1	1.0	0.9	1.0	1.0	0.0
Pinot noir	**SSC (°Brix)**	7.1	13.8	18.8	19.9	22.2	0.9	3.0	9.8	13.8	17.9	20.5	21.8	23.9	24.9	1.1
	Glucose (g/L)	16.0	67.0	110.1	106.5	136.5	6.3	NA	29.2	57.4	102.4	104.2	140.9	145.2	133.7	8.3
	Fructose (g/L)	22.6	70.3	106.2	103.3	129.6	5.6	NA	34.3	65.2	102.6	101.6	130.5	152.2	144.9	9.4
	G/F Ratio	0.7	1.0	1.0	1.0	1.1	0.0	NA	0.8	0.9	1.0	1.0	1.1	1.0	0.9	0.0
Riesling	**SSC (°Brix)**	6.2	11.2	15.9	16.9	18.7	1.2	3.8	7.8	12.3	16.2	17.1	17.7	18.9	18.2	0.7
	Glucose (g/L)	18.8	49.6	78.7	105.4	107.3	9.0	0.5	17.8	52.1	80.5	109.6	111.2	116.9	118.0	8.0
	Fructose (g/L)	24.6	52.8	77.9	106.6	103.9	9.2	4.8	26.7	58.8	78.3	105.2	105.1	119.5	123.9	8.2
	G/F Ratio	0.8	0.9	1.0	1.0	1.0	0.0	0.1	0.7	0.9	1.0	1.0	1.1	1.0	1.0	0.0
Chardonnay	**SSC (°Brix)**	5.7	13.3	17.3	19.3	20.4	1.5	3.9	8.6	14.6	18.3	20.3	21.0	22.3	23.1	0.8
	Glucose (g/L)	13.6	55.3	74.2	84.2	141.5	9.5	NA	29.5	65.7	98.4	107.5	127.5	137.0	127.9	7.0
	Fructose (g/L)	17.3	62.5	69.4	78.6	135.6	8.8	6.0	39.2	72.7	98.5	99.7	142.4	119.6	129.6	7.3
	G/F Ratio	0.8	0.9	1.1	1.1	1.0	0.0	NA	0.8	0.9	1.0	1.1	0.9	1.1	1.0	0.0

CHAPTER THREE

Introduction:

Recent introduction of new cold-hardy grape (*Vitis* sp.) cultivars has fueled a growing wine industry in non-traditional temperate growing regions. As of 2010, 250 wineries have been established in New England, New York, Nebraska, Iowa, Wisconsin, and Minnesota. These wineries are supported by over 1,300 grape growers cultivating an estimated 3,300 acres of grapes (Martinson et al., 2010). In Minnesota, the wine industry is growing at an annual rate of 28% and is projected to produce 150,000 gallons of wine by the year 2014 (MGGA, 2011). As the cold climate wine industry grows, knowledge of berry development and the ripening process of cold hardy wine grape cultivars grown in this newly formed grape growing region will be useful to guide vineyard management, harvest, and winemaking decisions.

The chemical composition of grape berries has generally been accepted as the most important factor when determining the quality of the fruit (King et al. 1988; Lamikanra et al. 1995). Concentrations of sugars and acids contribute to sensory and quality of wine and dictate when grapes are harvested. Harvest dates for wine grapes are selected to optimize the balance between sweetness, acidity, phenolic ripeness, and flavor (Lund et al., 2006). The common indicators for grape maturity used by viticulturists and wine makers are pH, soluble solids content (SSC; measured in ˚Brix) based on refractive index of the juice, as a proxy for sugar content, and titratable acidity (TA), expressed as equivalent units of tartaric acid. Extensive research has been conducted to determine the range and progression during ripening of these parameters in *V. vinifera* cultivars, but

grape maturity and range of chemical composition for University of Minnesota cold hardy and other hybrid wine grape cultivars has not been systematically profiled.

Berry development follows a double sigmoid pattern and can be divided into three stages (Mullins et al., 1992). Stage I begins after fruit set and the newly formed berries begin to increase in size due to rapid cell division. During this stage the berries are hard, green, and are accumulating large amounts of organic acids. Stage II, known as the lag phase, is characterized by very little berry growth, and is also when the berries reach maximum titratable acidy. Stage III starts with the softening of berry and the appearance of pigmented anthocyanins, also referred to as veraison. Veraison denotes the beginning of the ripening process where the berry goes through significant change in chemical composition, especially accumulation of sugars and degradation of organic acid (Coombe & Bishop, 1980). After veraison and berry softening, the xylem flow slows down and phloem sap rich in sugars becomes the main contributor to the grape berry. As the berry reaches maturity in the final interval of stage III, the phloem transport is suppressed and the water and sugar supply is cut off (Coombe et al., 2000). Peak berry maturity can be defined by the arrest of phloem transport or the period when the berry no longer accumulates water or sugars. Phloem arrest can sometimes be identified at the end of ripening by a plateau of berry weight and/or soluble solids concentration. Assessing the change in berry weight and locating the point at which average berry weight begins decreasing indicates the onset of dehydration. Dehydration increases the concentration of soluble solids in the berry. The relative timing of the events of veraison, ripening, peak maturity, and dehydration, or overripening, in Stage III is depicted in Figure 3.1 using data from ‘Marquette’ collected in this study.

Throughout berry development, organic acids are produced by glycolysis, the Krebs cycle, and the shikimic acid pathway (Soyer et al., 2003). Acidity levels are consistently changing during berry development as a result of metabolic activities. Typically, tartaric and malic acids account for 90% of the acids found in grapes, with tartaric acid predominating (Lamikanra et al., 1995; Kliewer et al., 1967). The acidity of grapes is most often expressed as titratable acidity (TA). The TA is an important parameter that wine makers and viticulturists use to evaluate berry maturity and quality of juice and wine. Factors that can influence organic acid composition are cultivar, growing region, and environmental factors such as light, humidity, and temperature (Lamikanra et al., 1995). The decline of organic acids after veraison is due to a combination of inhibition of glycolysis, gluconeogenesis from malic acid and the metabolism of malate and tartrate through respiration (Ruffner, 1982a & b).

Sugar accumulation is dependent on photosynthesis in leaves and transport to the berry through the phloem in the form of sucrose (Swanson et al., 1953). After entering the berry, sucrose is cleaved into glucose and fructose (Kliewer, 1967). Glucose and fructose (hexose sugars) account for approximately 99% of the total sugars in grape berries, representing a large portion of the total soluble solids (Kliewer, 1967). In mature grape berries, sugars typically account for 90% of the soluble solids found in mature grape berries (Keller, 2010) and soluble solids content estimates sugar content within 1% of actual sugars (glucose and fructose) present (Jackson & Lombard, 1993). Tracking the rates and ranges of sugar accumulation is necessary to understand the ripening profile of a cultivar.

Temperature is the most important environmental factor affecting wine or grape quality and development (Winkler, 1962). Growing degree days (GDD) is a method of calculating and tracking heat accumulation units over a period of time. Climatic conditions can vary from year to year, so using accumulated GDD to track the progression of plant phenological events should be more suitable than calendar date. The rate of heat accumulation through the increase of ambient temperature will determine when bud break, flowering, and grape berry maturity will occur (Tait, 2008). The adoption of GDD has improved prediction of the progression of plant phenological events (McMaster et al., 1997). Determining the number of GDD required for the selected grape cultivars to mature will help define locations best suited to their culture and aid in predicting when cultivars should be harvested.

In this study, I survey the range of organic acid and sugar concentrations observed over three years during grape berry ripening. The objective is to describe a profile for grape berry ripening with respect to SSC, TA, pH and berry weight for University of Minnesota wine grape cultivars, select *V. vinifera*, and other hybrid cultivars to facilitate estimation of grape maturity. In addition, I investigate the validity of GDD to predict levels of organic acid and sugar concentrations in eleven different wine grape cultivars. Comparing TA, SSC, and pH to accumulated GDD, I will estimate the GDD needed to mature grape berries for the selected cultivars. Determining the number of GDD required for these grape cultivars to produce mature fruit will help identify locations suitable for their culture and aid in predicting when these cultivars should be harvested.

Materials and Methods:

Plant Material: The grapevines used in this study were located at the University of Minnesota Horticultural Center in Excelsior, Minnesota. A total of eleven different cultivars were used for this study and are listed in tables 3.2.1 and 3.2.2. The number of vines and trellis systems varied slightly among the cultivars. The number of vines (in parentheses) for the cold hardy hybrid cultivars vines were Frontenac (4), Frontenac gris (4), La Crescent (4), Marquette (4), St. Croix (4), St. Pepin (4), and Maréchal Foch (8) and these cultivars were grown using the High Bilateral Cordon training system (Jackson, 2001). The number of vines for the *V. vinifera* cultivars were Merlot (4), Pinot noir (4), Chardonnay (8), and Riesling (8) and these cultivars were grown using the mini-J training system (MGGA, Hemstad et al., 1991) to prevent winter injury and permit fruiting.

Fruit and Harvest Dates: Two subsamples of forty berries were harvested every eight to ten days from early August to late September or early October during the 2010, 2011, and 2012 growing seasons. In total, there were five harvest dates in 2010, eight harvest dates in 2011, and six harvest dates in 2012. Each forty-berry subsample contained twenty berries from each side of the trellis. To decrease the influence of fruit removal on the chemical composition of the remaining grapes of later samples, no more than two grapes were harvested from an individual cluster per harvest. Not all clusters were harvested on consecutive sample dates.

Sampling: Berry samples were taken from one vineyard location for each cultivar. Berry sampling was conducted on four to eight vines for a cultivar depending on availability. To avoid bias in sampling, harvesters followed a defined protocol for

berry collection. Harvesters first approximately defined four zones on each grape cluster to be sampled: the right and left shoulders, middle section, and the tail. Harvesters alternated from each of these cluster zones in picking berries as they moved from cluster to cluster throughout the vine. To avoid picking the most desirable berry versus a random berry in the designated zone, it was important that the harvester did not visually analyze the grape cluster before choosing a grape to harvest. As the harvesters picked the berries as described above, they also alternated from the font of the cluster to the rear of the cluster. For example, if there were eight clusters the first four berries would come from the outer facing or front side of the first four clusters one berry from each of the four zones. The second four berries would come from the rear facing side of the remaining four clusters again in sequence with the four cluster zones. Berries that were severely shriveled or damaged by insects, birds, or early season hail were discarded by the harvester and a new berry was selected.

Juice Extraction and Berry Storage: After each harvest, one subsample of forty berries for each harvested cultivar was immediately stored in a -20˚C freezer and later transferred into -80˚C freezer for long term storage. This subsample was used to determine weight and moisture content. The other subsample of forty berries was divided into four, ten berry replicates and each replicate was pressed separately with a hand juicer.

Juice analysis: Titratable acidity, soluble solids content, and pH were measured on four juice replicates for each cultivar. The TA and pH were tested with the ATI ORION 950 Ross FASTQC Titrator (Orion, Beverly, MA). The titrator was calibrated to

pH 4.0 and 7.0 before daily use. For TA analysis, 1 mL of juice was diluted into 50 mL of distilled water and titrated to pH of 8.0 using 0.05M sodium hydroxide solution. Soluble solids contents were measured in ˚Brix using an ATAGO® Pocket Refractometer PAL-1 using the manufacturer's instructions (Atago Inc., Bellevue, WA).

Growing Degree Days: Data from the NOAA National Weather Service station CHASKA, MN US located in Chaska, MN (www.ncdc.noaa.gov) were used to calculate growing degree days (GDD) as follows :GDD = $[(T_{MAX} + T_{MIN})/2] - T_{BASE}$ where T_{MAX} is the daily maximum temperature and TMIN is the daily minimum temperature. T_{BASE} is the chosen minimum temperature for plant growth. The period tracked for this study started April 1 and went through October 31 with a base temperature of $T_{BASE} = 10^{\circ}C$ (Winkler, 1974). The selected weather station is approximately four miles from the University of Minnesota Horticultural Research Center in Excelsior, Minnesota.

Statistical analysis: Fruit data were subjected to analysis of variance (ANOVA) for the eleven cultivars using the R statistical software 3.0.1 (R Development Team, 2013). The ANOVA tests were conducted using the Agricola package (De Mendiburu, 2010). The three traits TA, SSC, and pH were tested against main effects year and GDD and the GDD and year interaction. Main effects were fixed for all analysis. A separate analysis was performed for each cultivar. Means separation analyses using least significant difference (LSD) were used as statistical analysis procedures to estimate the level of GDD beyond which no significant change in grape maturity occurred for each cultivar as indicated by changes in SSC, TA, and pH.

Results:

The variation in berry traits over the three years of the study is depicted in Figure 3.1 for 'Marquette' to illustrate relative timing of the ripening stages of veraison, ripening, peak maturity and overripening observed with respect to GDD in this study. Total accumulated GDD for the three years of this study varied with GDD accumulation in 2012 being highest and 2011 being lowest (Figure 3.2). Few GDD were accumulated during the months April and October. The month of September had the greatest variance of GDD accumulation among the three years. The timing by date in which GDD were accumulated by date varied for all three years. Accumulated GDD by September 15 was 1,464 GGD in 2010, 1,404 GDD in 2011 and 1,581 GGD in 2012.

The analyses of variance for SSC, TA, and pH (Table 3.1) indicated significant effects ($P < 0.001$) due to GDD for all three traits in all cultivars. TA varied significantly ($P < 0.05$) among years for all cultivars except 'St. Croix' and 'Merlot'. Cultivars for which year x GDD interaction effects were not significant ($P > 0.05$) for ˚Brix were 'Marquette', 'La Crescent', 'Frontenac', 'St. Pepin', 'St. Croix', 'Pinot noir', 'Merlot', and 'Chardonnay'. Cultivars that did not exhibit significant ($P > 0.05$) year x GDD interaction effects for TA were 'Frontenac', 'Frontenac gris, 'Pinot noir', and 'Merlot'. Year x GDD interaction effects were not significant ($P > 0.05$) for all three berry traits for 'Frontenac'.

The means separation analyses (LSD) were used to determine the GDD when no subsequent significant changes ($P > 0.05$) were observed for SSC, pH, and TA in each year. This was termed the "plateau point" and is shown in Figure 3.3 for each cultivar.

'Marquette', 'Frontenac', 'Frontenac gris', and 'St. Pepin' had no significant changes in SCC and TA based on LSD over a similar GDD range. 'La Crescent', 'Maréchal Foch', 'St. Croix', 'Pinot noir', 'Merlot', and 'Chardonnay' achieved a plateau point for TA before SSC relative to GDD. 'Maréchal Foch' and 'Pinot noir' did not reach a plateau for SSC in 2010. Peak berry weight and plateau point for SSC occurred within the same relative GDD range for all cultivars with exception of 'Frontenac gris' and 'St. Croix'.

The relationship between accumulated GDD and berry traits for each cultivar is depicted in Figures 3.4.1- 3.4.11. During veraison, which occurred from 1,000 – 1,175 GDD for hybrid cultivars and 1,200-1,275 *V. vinifera* cultivars the SSC, pH, and berry weight increased and TA decreased. The post-veraison ripening stage started at 1,200 GDD for hybrid cultivars and 1,300 GDD for *V. vinifera* cultivars. During the ripening stage, SSC accumulation was similar among years. The rate at which TA decreased varied between years for 'La Crescent', 'St. Pepin', 'Chardonnay', and 'Pinot noir', and for these cultivars TA was significantly lower in 2012. Peak maturity occurred between 1,375- 1,450 GDD for hybrid cultivars and 1,450 and 1,550 GDD for *V. vinifera* cultivars (Figures 3.4.1- 3.4.11).

Discussion:

The profiles of SSC, titratable acidity, and pH generated for the selected cultivars demonstrated how these components change throughout berry development in three growing seasons in the upper Midwest US. The three growing seasons in this study were quite dissimilar in GDD accumulation, which is ideal for evaluating the degree of variation that can be observed in a cultivar for berry traits and for determining whether

accumulated GDD is a viable predictor for berry traits as this study covered very contrasting years.

Non-significant interactions between year and GDD support use of GDD to reliably predict berry ripening traits. Eight of the eleven cultivars did not exhibit a significant interaction between year and GDD for SSC (˚Brix) suggesting that GDD is a viable predictor for SSC for most cultivars and could be used to forecast harvest timing. The cultivars that showed significant interactions each had a different basis for interaction. The early accumulation of SSC in 2012 compared to other years accounted for the interaction for 'Maréchal Foch' (Figure 3.4.2). A drop in SSC late in the 2010 season accounted for the interaction for 'Frontenac gris' (Figure 3.4.5). The interaction for 'Riesling' was due to variability among years over a large part of the season from 1,200 to 1,500 GDD (Figure 3.4.10). In respect to the significant interactions for SSC in 'Maréchal Foch' and 'Frontenac gris' an early or late interaction may not matter to a winemaker as the SSC were too low during the interaction for 'Maréchal Foch' and too high at the interaction point for 'Frontenac gris' for optimal winemaking.

In contrast to SSC, TA and pH profiles varied substantially among the years suggesting that timing of GDD accumulation is critical for these traits. Only four and two of the eleven cultivars showed no significant interaction between year and GDD for TA and pH, respectively indicating that a GDD model may not be as widely useful in predicting acidity in the berry.

The means separation analyses (LSD) identified the plateau point where GDD no longer had an effect on SSC, TA and berry weight for each year. Grape maturity and

metabolite plateau points have been discussed in many studies (McCarthy & Coombe, 1999; Johnson, & Carroll, 1973; Saito & Kasai 1968; Du Plessis, 1984). Plateau points for SSC, TA, and berry weight are each important factors in their own respect. Growers who are paid by the SCC and/or weight can estimate the GDD when each cultivar is at its peak berry weight or the targeted SCC. The GDD needed to fully mature berries in the selected cultivars are shown in Figure 3.3. Identifying the plateau point for TA informs both grape growers and winemakers the GDD at which there is no longer a significant drop in TA. Winemakers need manageable acid levels that are not too high or too low. Cultivars that are known for having a high TA should be harvested at the respective plateau point as the respective GDD where the TA is the lowest. In contrast, cultivars that are known for low TA should be harvest before their respective plateau point at which the TA is higher and ideal for winemakers.

The timing of plateau points for SSC, TA, and berry weight indicate that cold hardy hybrid cultivars are at peak maturity between 1,400 and 1,450 GDD. This finding suggests that geographic locations that do not accumulate 1,400 to 1,450 GDD may not be suitable for cultivating cold hardy hybrids. The selected *V. vinifera* cultivars reached their respective plateau points from 1,450 to 1,550 GDD. The plateau points for 2010 and 2011 were most often grouped together with less than 50 GDD of separation for SSC and TA. In all cultivars, the 2012 plateau point for SSC occurred later (higher SSC) than 2010 and 2011 suggesting that warmer years have a prolonged increase in SSC. Most cultivars showed an earlier plateau point for TA in 2011 than in 2010 or 2012, which suggests that cool temperatures slowed acid degradation as observed in other studies (Lakso et al., 1975; Kliewer, 1971; 1974).

Peak maturity may not be the ideal time to harvest every cultivar as SSC and or TA may be too high or low and not ideal for wine making. For example 'St. Croix' reaches peak berry maturity at or around 1,425 GDD with a mean SCC of 20 ˚Brix, but only a mean TA of 5.5 g/L, which is too low for most types of wine (Conde et al., 2007). Heat accumulation is only one factor in grape ripening when determining characteristics during maturity. When compared to GDD, the ripening profile is expected to be different in Minnesota than it is in New York where high quality grapes are grown in areas that harvest before 1,400 to 1,450 GDD (Martinson, 2005).

Cultivars that had the highest average TA after 1,400 GDD were 'Frontenac'(18.2 g/L), 'Frontenac gris'(16.8 g/L), 'La Crescent'(15.2 g/L), and 'Marquette'(12.3 g/L). Of the cold hardy hybrid cultivars, 'St. Pepin', 'Maréchal Foch', and 'Marquette' were most like *V. vinifera* cultivars in respect to TA. Cultivars that had the highest average SSC after 1,400 GDD were 'Marquette' (26.9 ˚Brix), 'Frontenac gris' (25.0 ˚Brix), 'Maréchal Foch' (24.4 ˚Brix), and 'La Crescent' (23.86 ˚Brix). A two year study conducted by Liu et al. (2006) looked at sugar and acid concentrations of 89 different grape cultivars and found that the mean TA for *V. vinifera* cultivars was between 6.29 and 7.54 g/L. Of the selected *V. vinifera* cultivars in my current study had a TA between 8.12 and 11.16 g/L after 1,400 GDD. Cultivars 'Marquette', 'Maréchal Foch', 'St. Pepin', and 'St. Croix' have organic acid and sugar concentrations that are most like the selected *V. vinifera* cultivars. 'Frontenac', 'Frontenac gris', and 'La Crescent' had higher sugar and organic acid concentrations than the selected *V. vinifera* cultivars.

By documenting and examining the ripening profiles for SSC, TA, pH and berry weight for the cultivars in this study, I have been able to determine 1) the range of variation to be expected in divergent seasons in Minnesota, 2) that GDD can be useful to predict peak maturity and, especially, SSC levels in cold-hardy hybrid cultivars and 3) that cold-hardy hybrid cultivars will most likely produce well ripened fruit in Minnesota sites receiving at least 1,400 GDD.

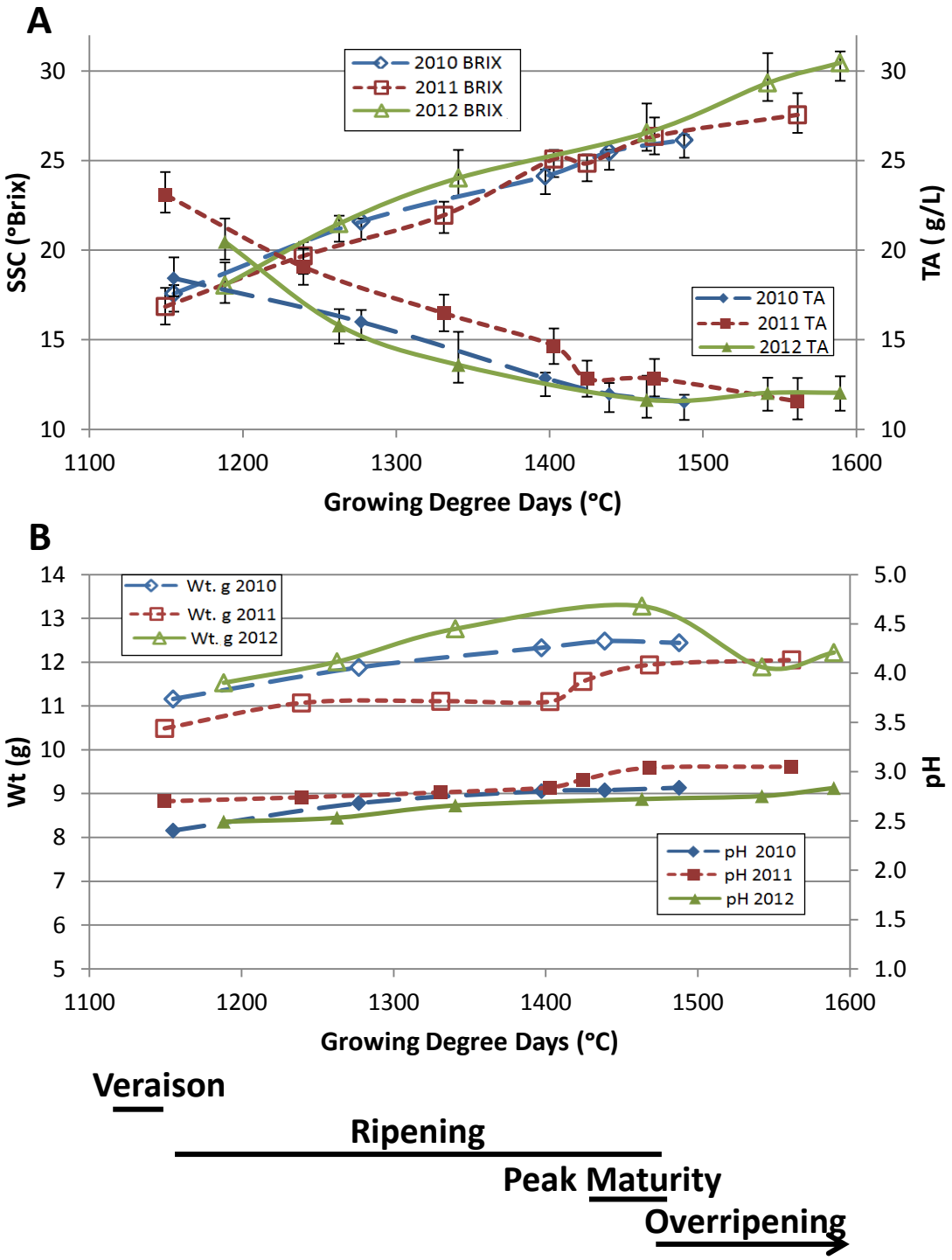

Figure 3.1. Relationship of Growing Degree Days to soluble solids content (SSC) and titratable acidity (TA) (A), and pH and berry fresh weight (Wt) (B) for the cultivar 'Marquette' during berry development in 2010, 2011, and 2012.

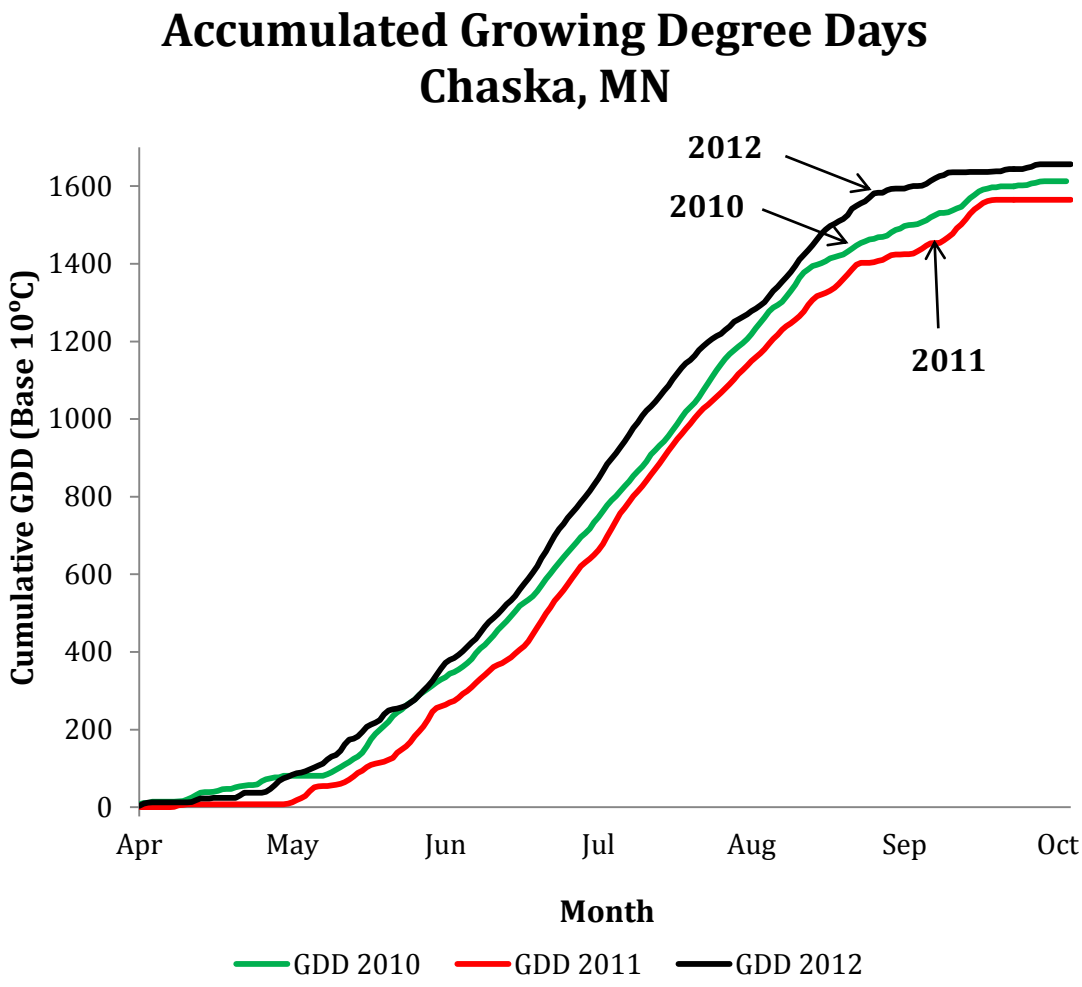

Figure 3.2. Growing Degree Day (GDD) accumulation for Chaska, MN in 2010, 2011 and 2012 based on temperature data from US National Weather Service weather station CHASKA, MN US (www.ncdc.noaa.gov).

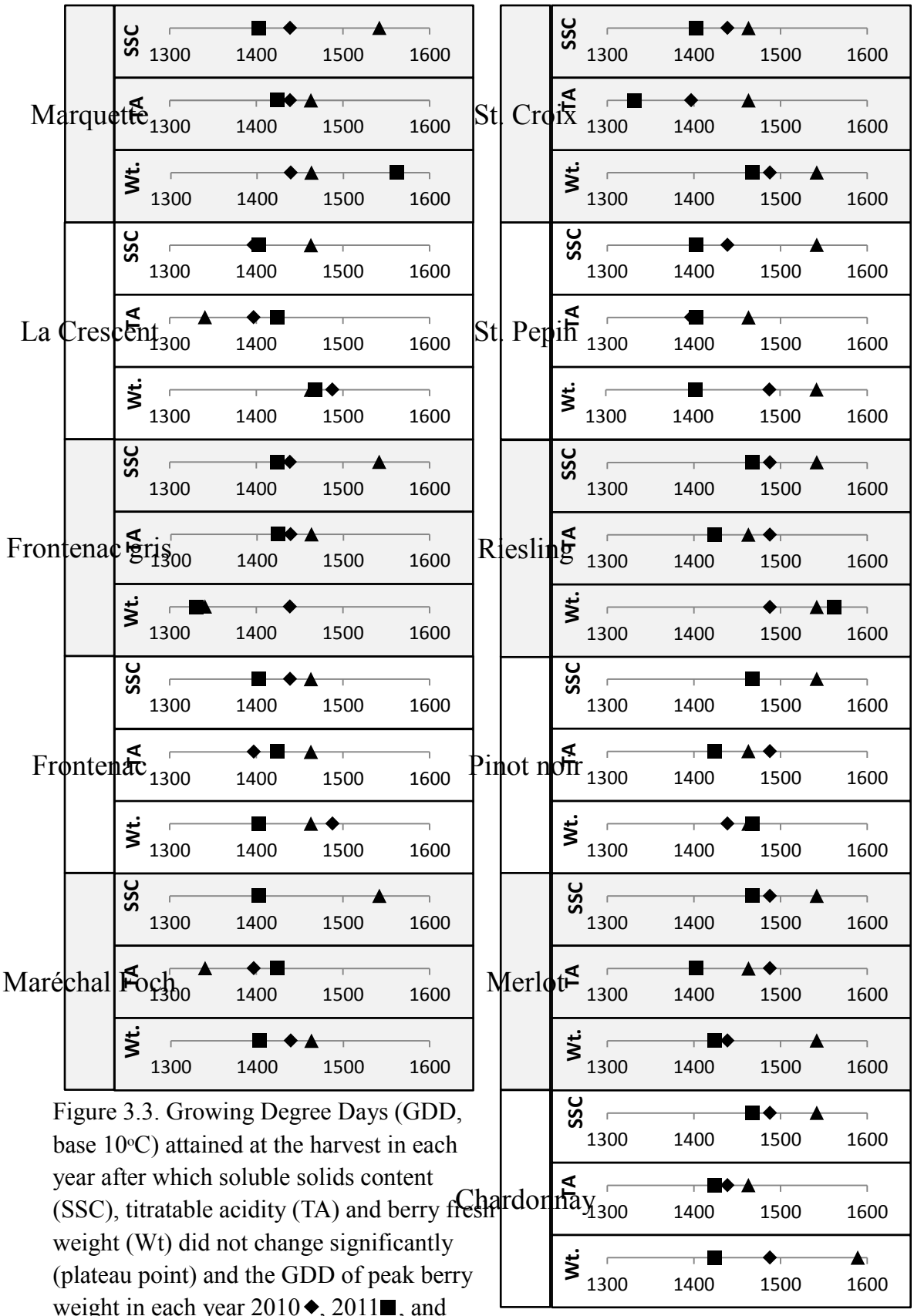

Figure 3.3. Growing Degree Days (GDD, base 10°C) attained at the harvest in each year after which soluble solids content (SSC), titratable acidity (TA) and berry fresh weight (Wt) did not change significantly (plateau point) and the GDD of peak berry weight in each year 2010◆, 2011■, and 2012▲.

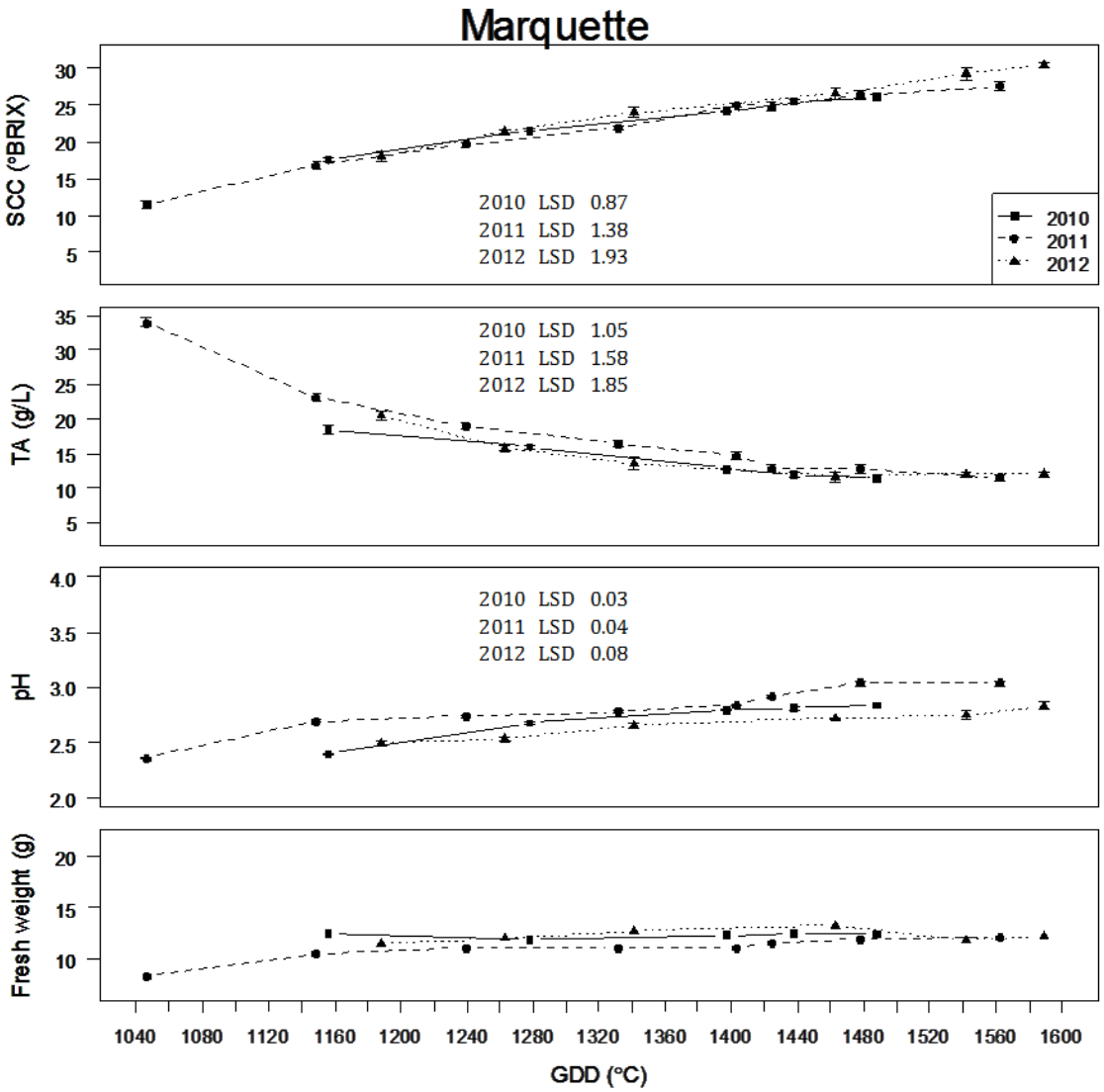

Figure 3.4.1. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Marquette' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

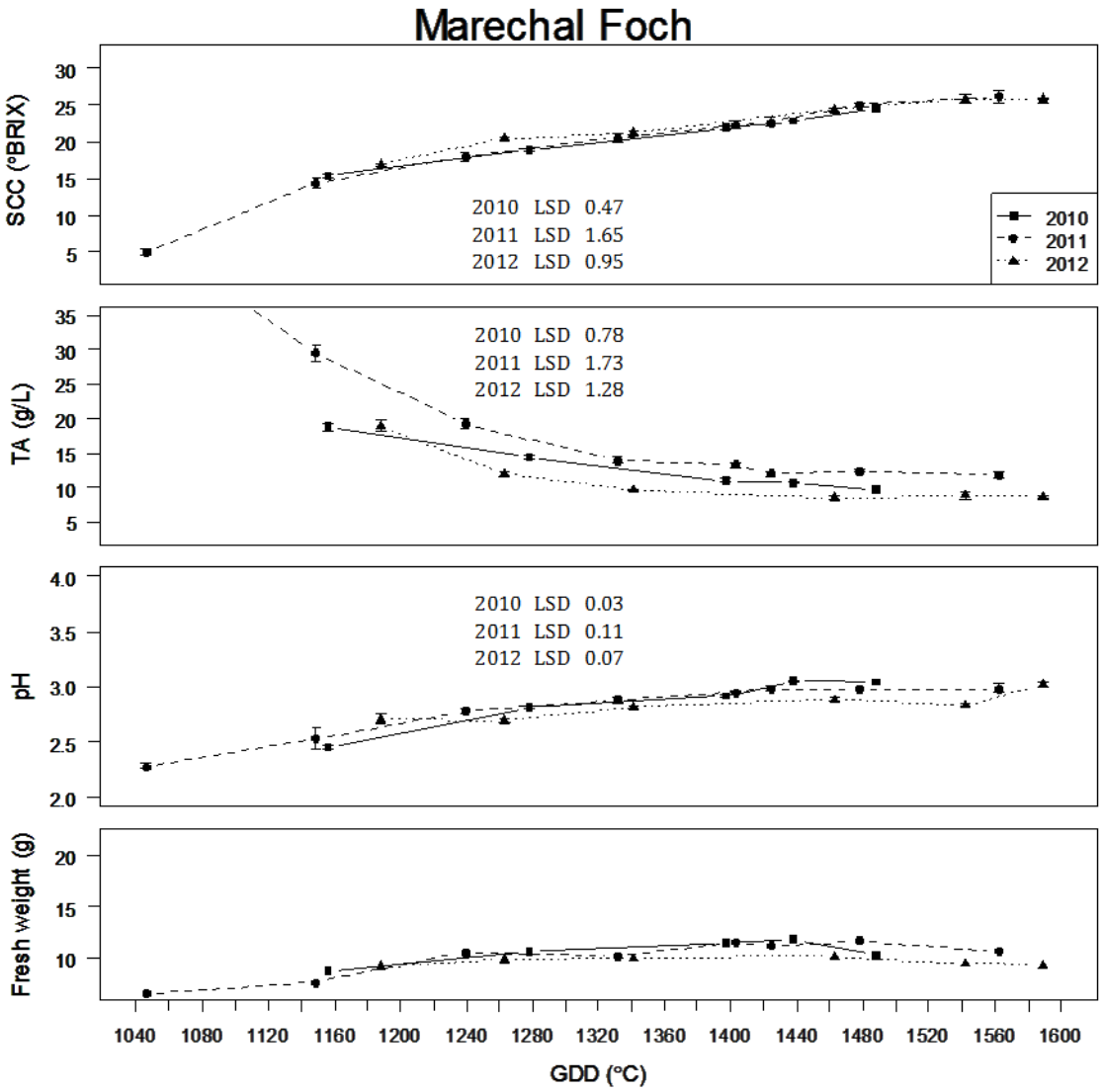

Figure 3.4.2. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Maréchal Foch' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

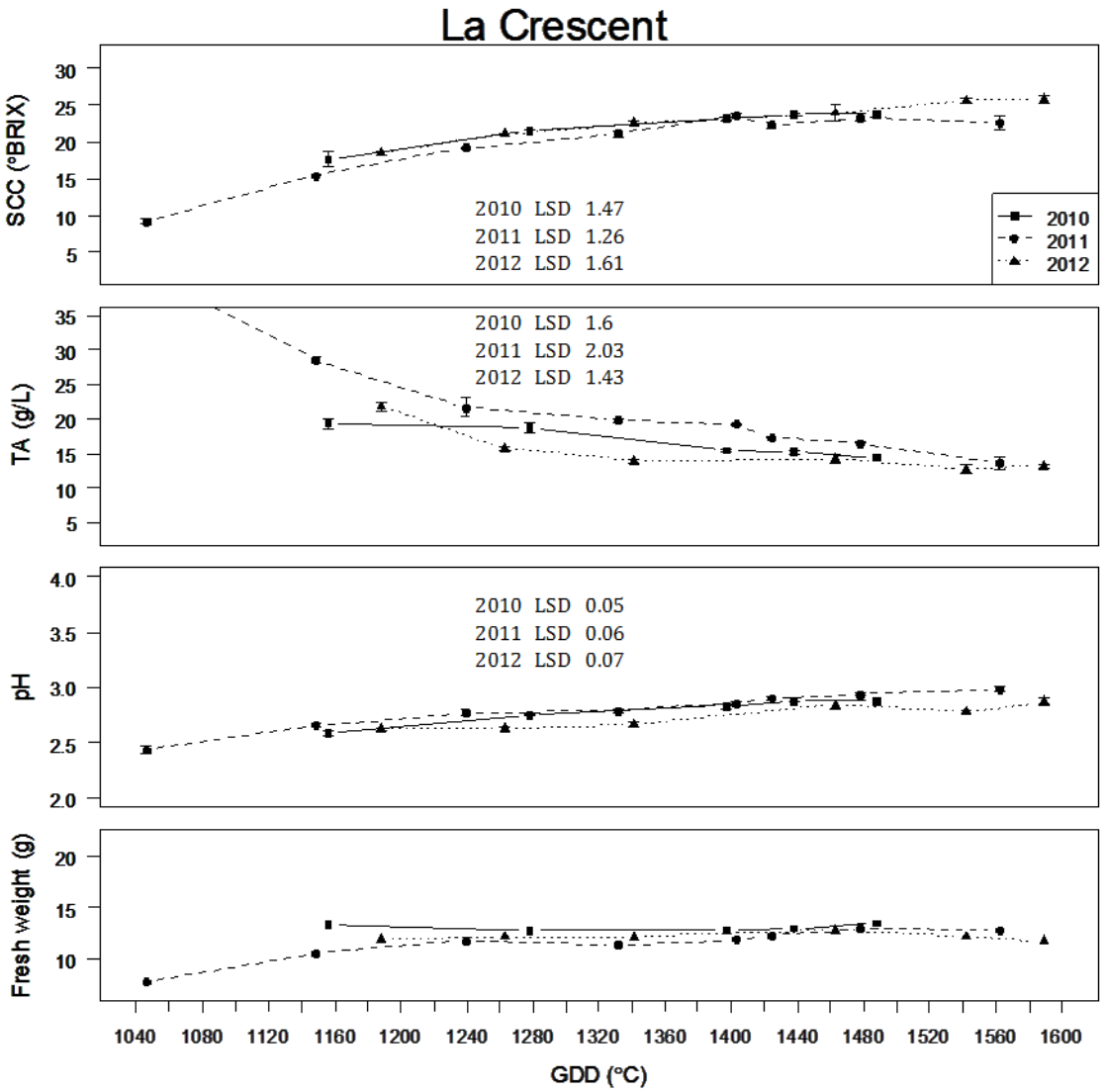

Figure 3.4.3. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'La Crescent' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

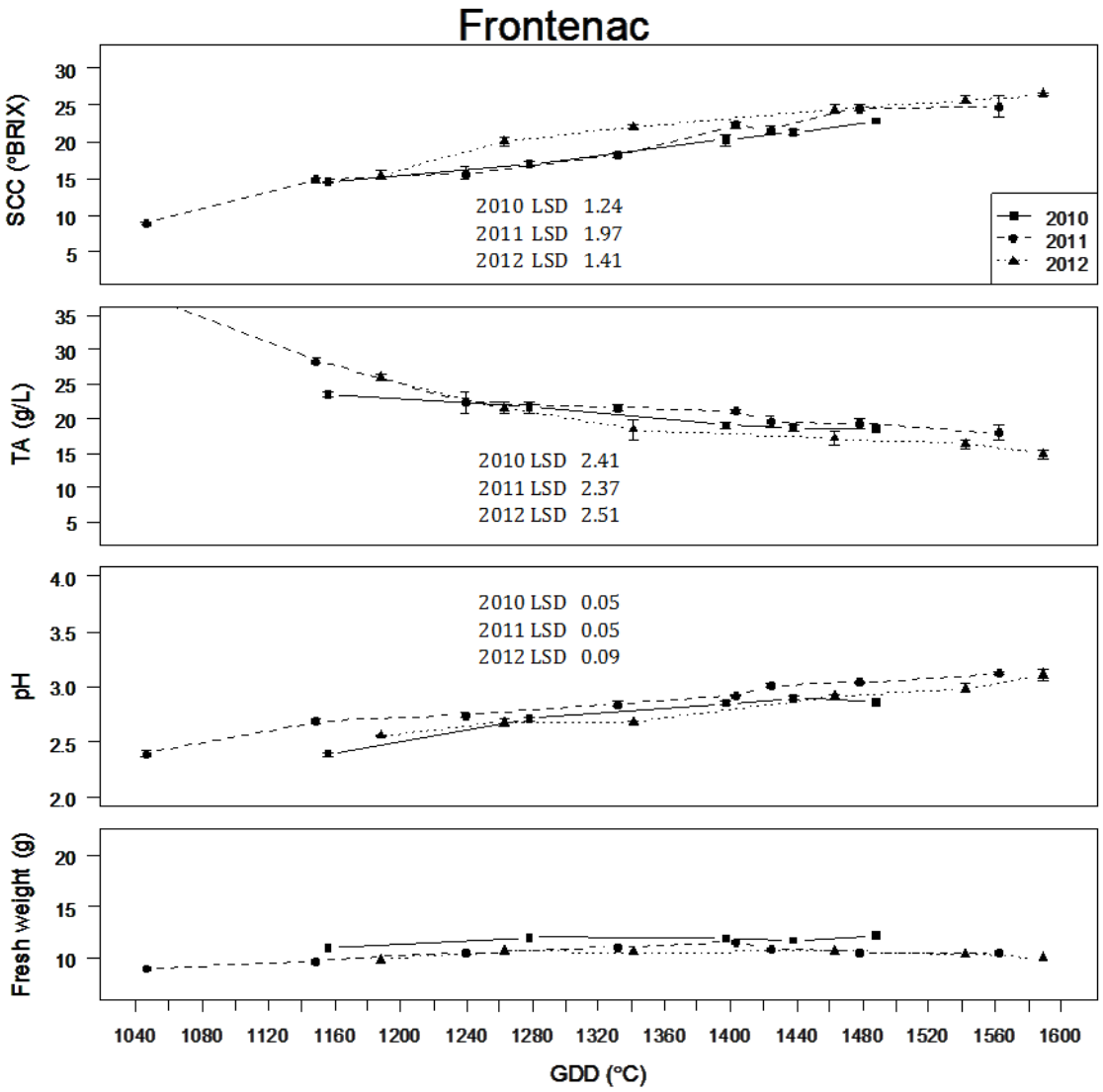

Figure 3.4.4. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Frontenac' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

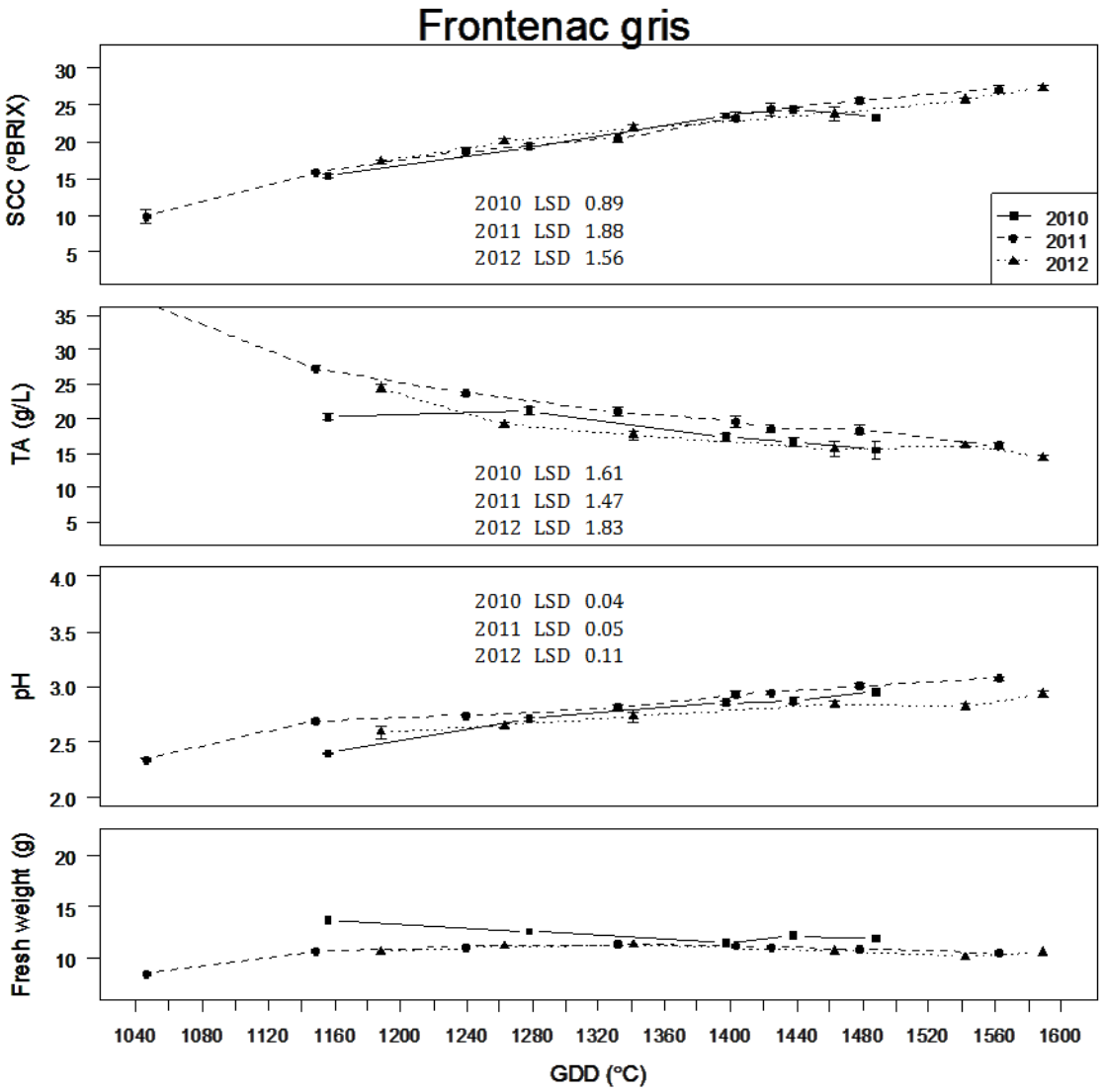

Figure 3.4.5. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Frontenac gris' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

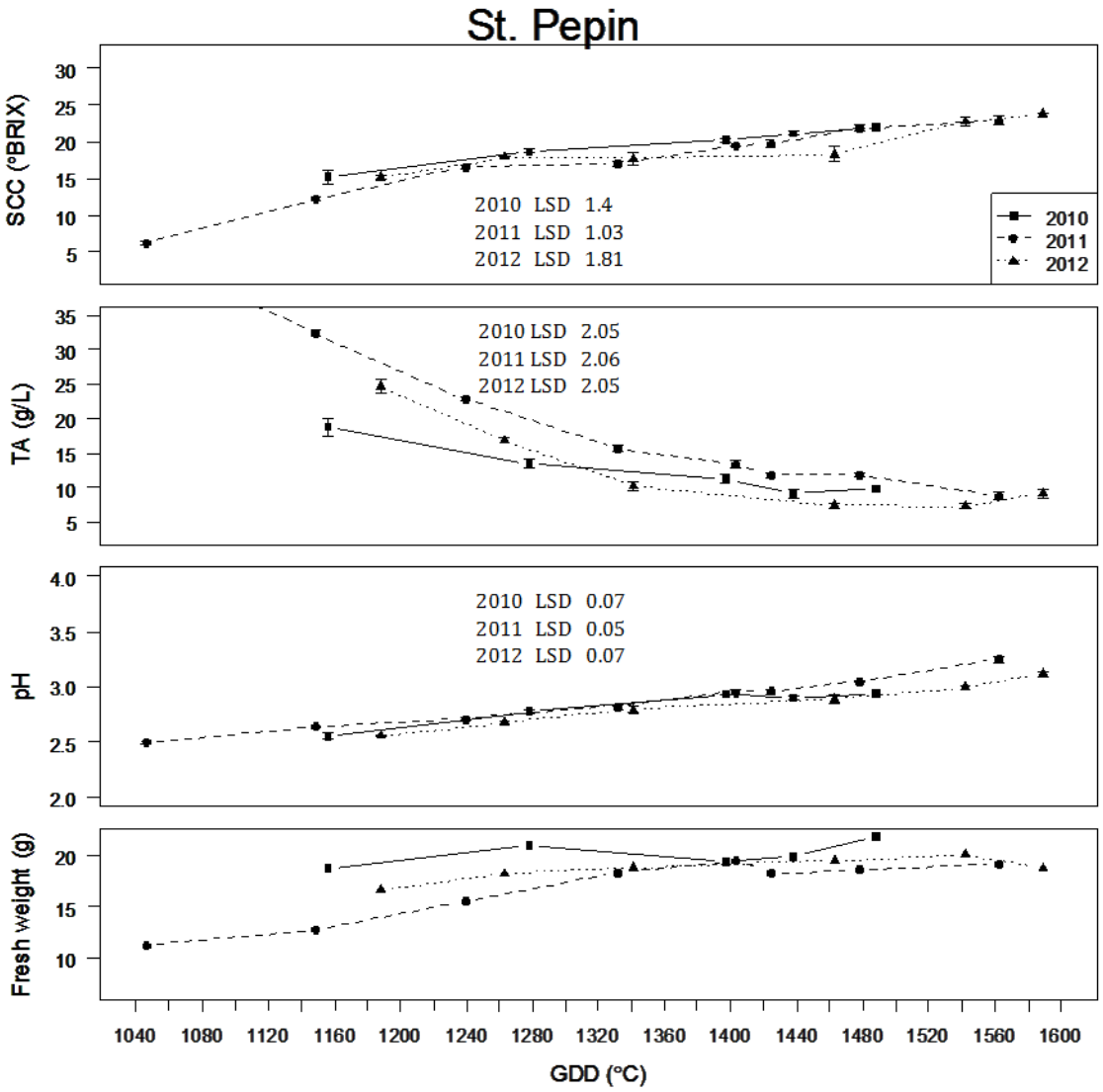

Figure 3.4.6. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'St. Pepin' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

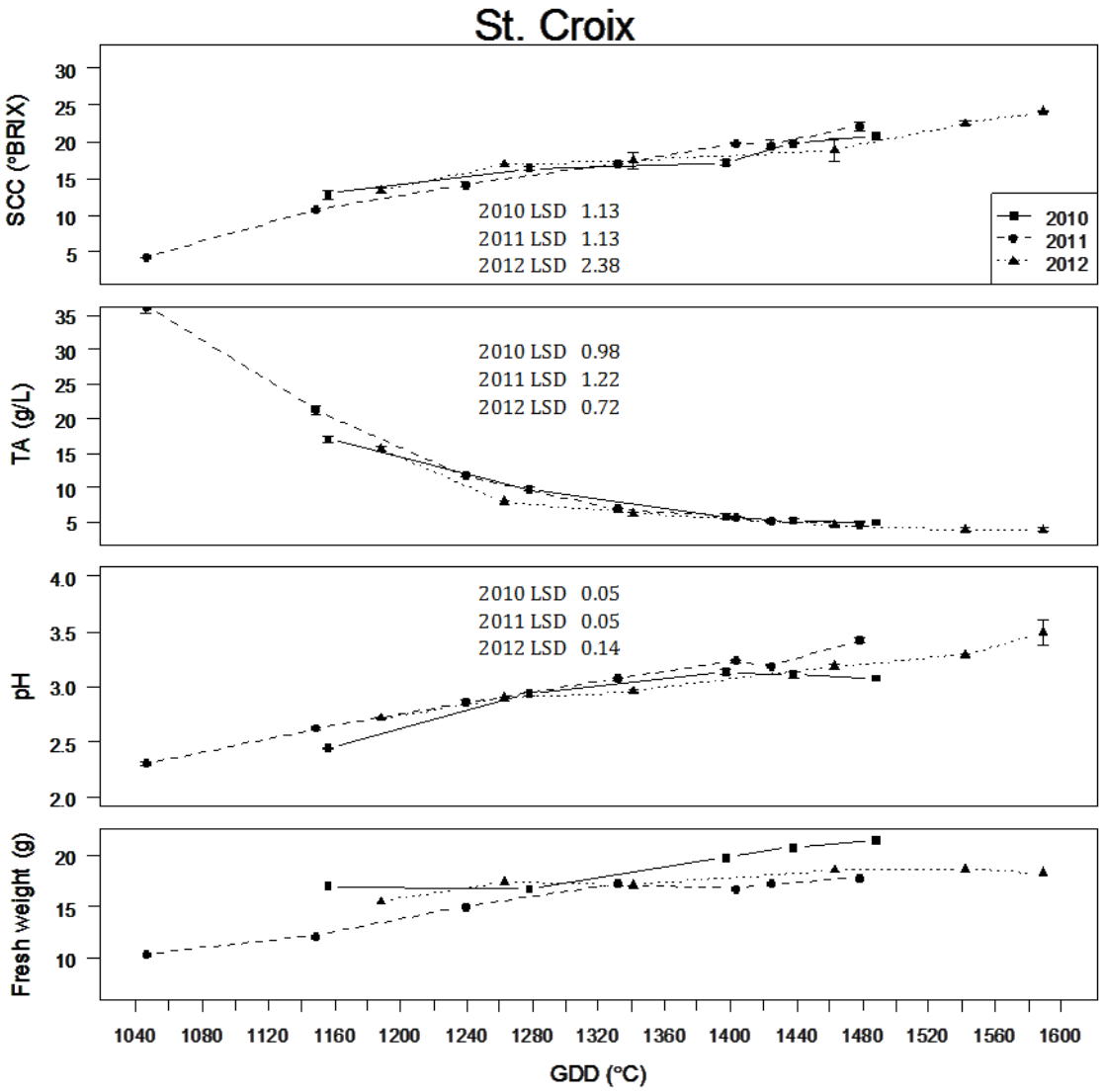

Figure 3.4.7. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'St. Croix' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

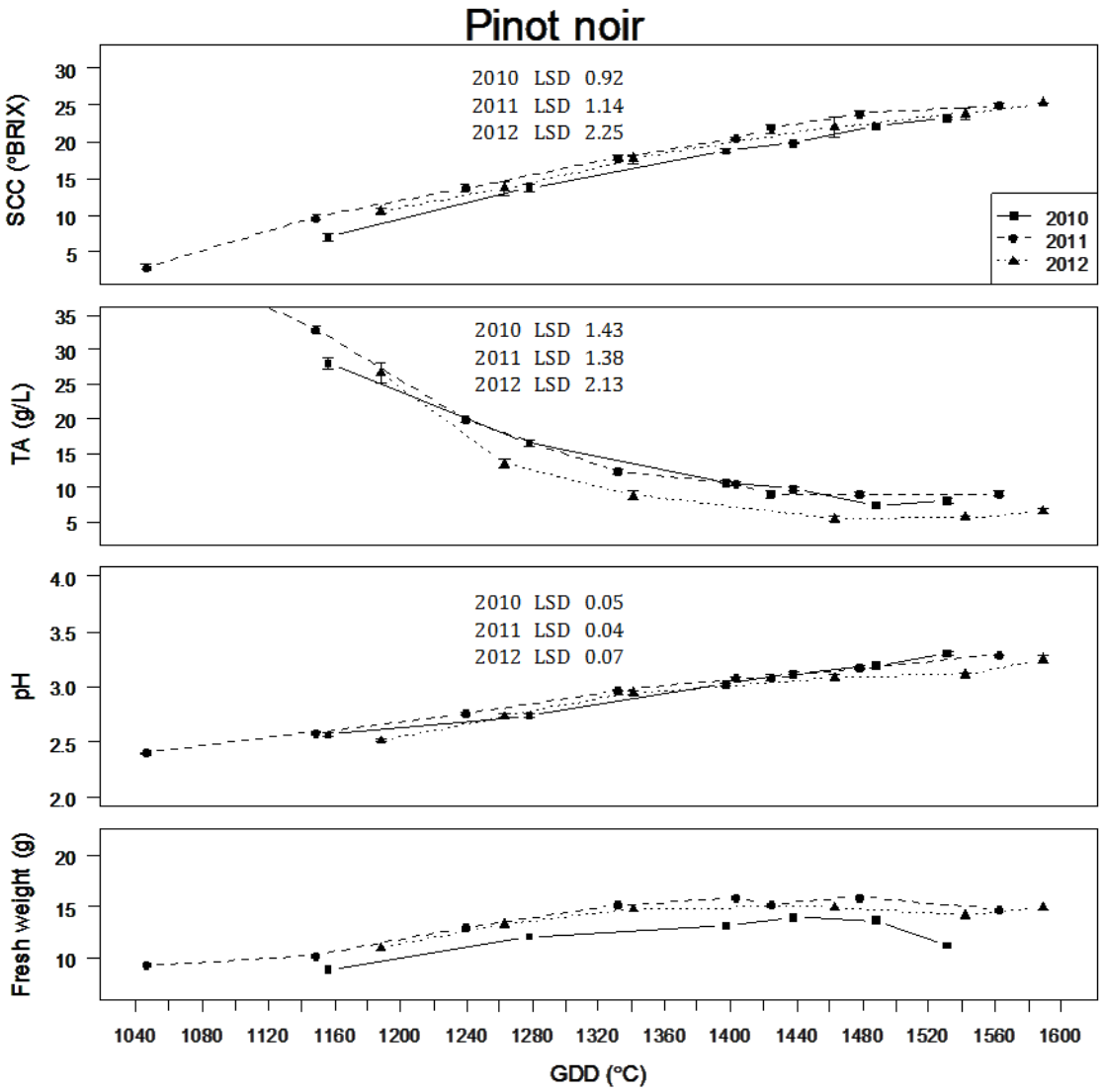

Figure 3.4.8. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Pinot noir' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

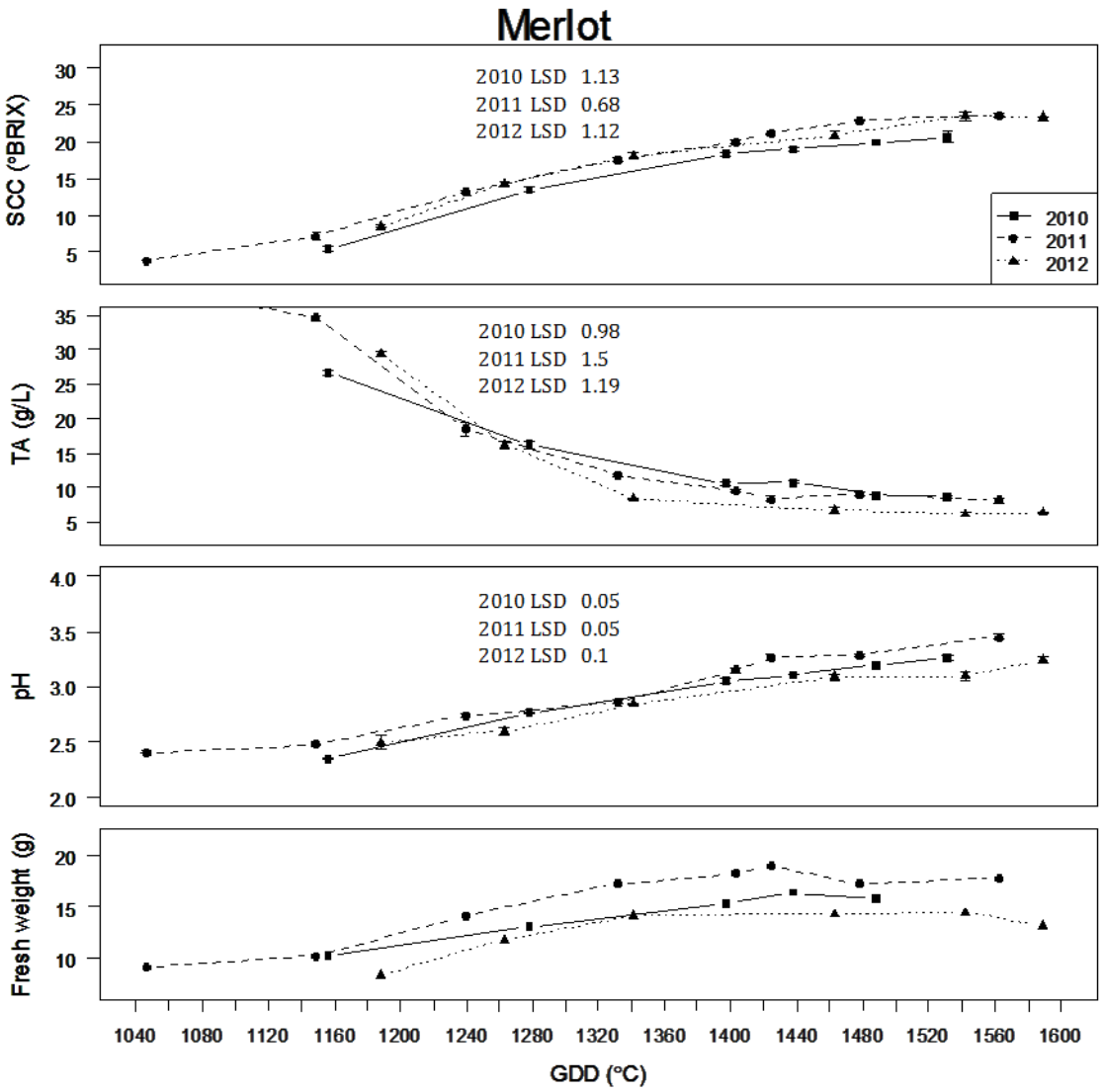

Figure 3.4.9. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Merlot' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

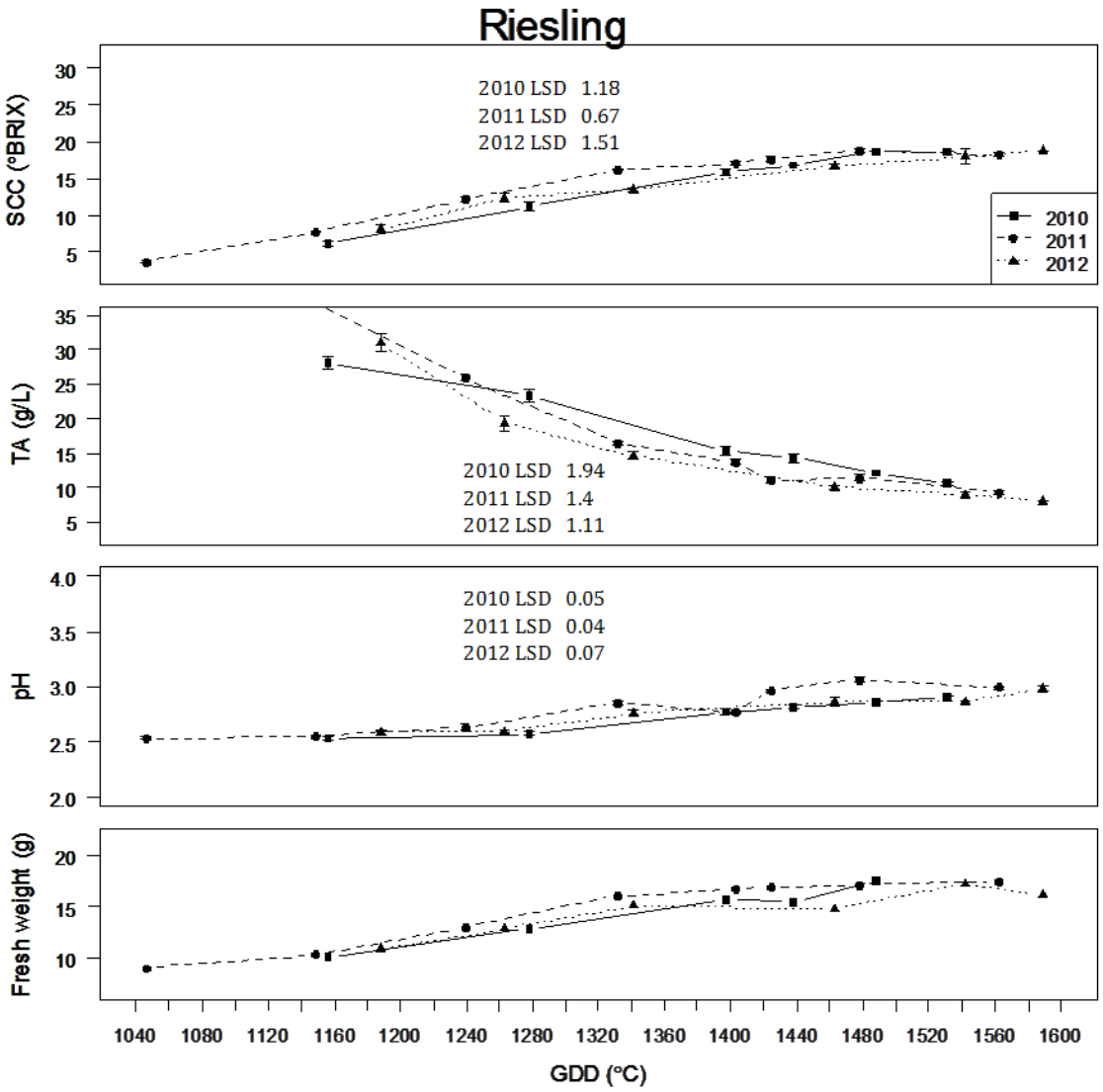

Figure 3.4.10. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Riesling' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

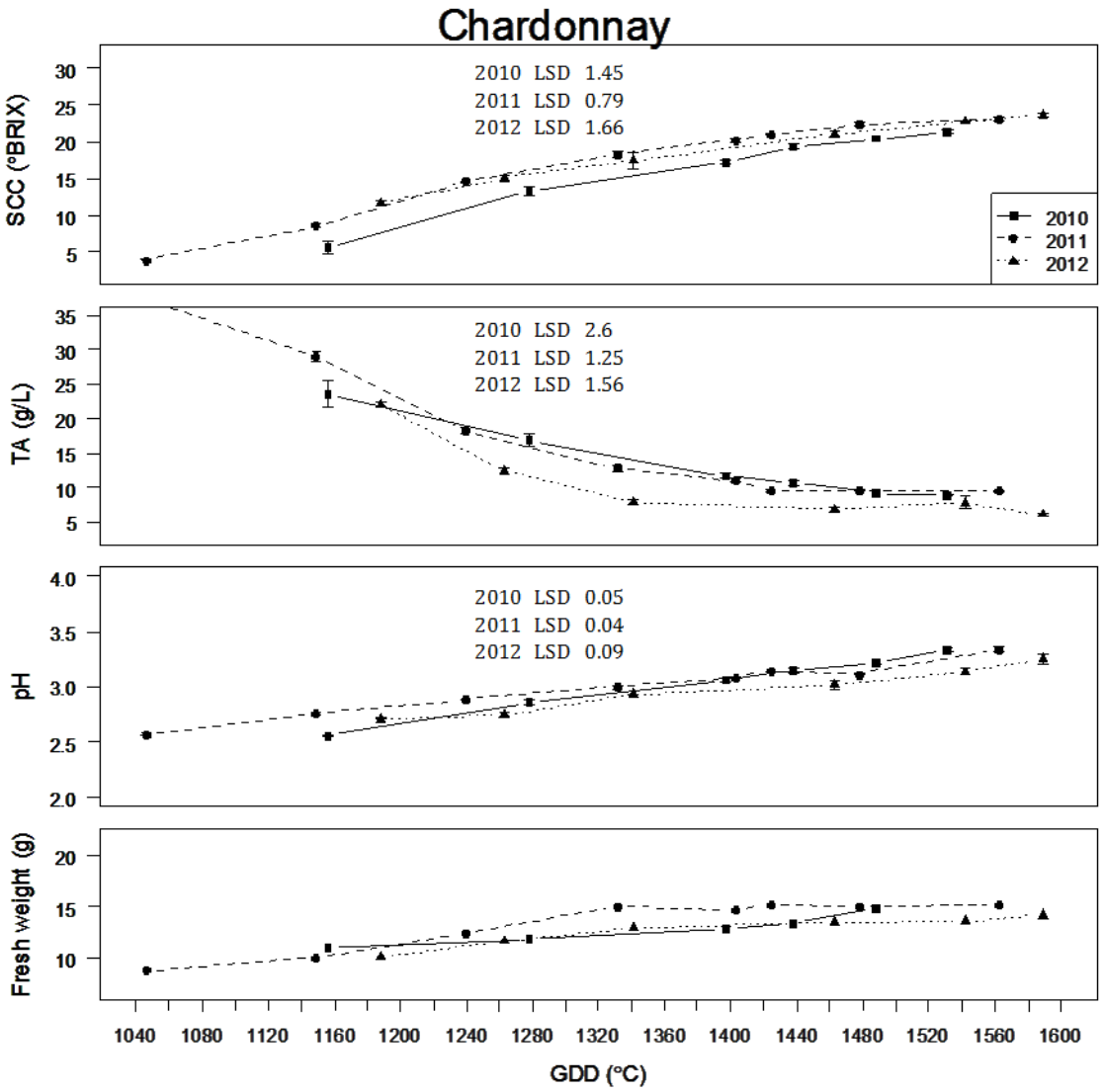

Figure 3.4.8. Relationship of Growing Degree Days (GDD) to soluble solids content (SSC), titratable acidity (TA), pH, and berry fresh weight for the cultivar 'Chardonnay' during berry development evaluated in 2010, 2011, and 2012. Vertical bars indicate the standard error of each mean value (n=4).

Table 3.1. Analysis of variance for soluble solids content (SSC), titratable acidity (TA), and pH in berries of eleven grape cultivars at multiple harvest dates expressed as accumulated Growing Degree Days (GDD) in three successive years (2010-2012).

Cultivar	Source of Variance	SSC (°Brix)	TA	pH
Marquette	Year	***	***	***
	GDD	***	***	***
	Year*GDD	NS	**	**
La Crescent	Year	*	***	***
	GDD	***	***	***
	Year*GDD	NS	***	*
Frontenac	Year	***	***	***
	GDD	***	***	***
	Year*GDD	NS	NS	NS
Frontenac gris	Year	***	***	***
	GDD	***	***	***
	Year*GDD	*	NS	***
St. Pepin	Year	*	***	***
	GDD	***	***	***
	Year*GDD	NS	***	**
St. Croix	Year	***	NS	***
	GDD	***	***	***
	Year*GDD	NS	*	*
Maréchal Foch	Year	***	***	**
	GDD	***	***	***
	Year*GDD	**	***	***
Pinot noir	Year	*	***	**
	GDD	***	***	***
	Year*GDD	NS	NS	**
Merlot	Year	*	***	**
	GDD	***	***	***
	Year*GDD	NS	NS	**
Riesling	Year	*	***	***
	GDD	***	***	***
	Year*GDD	*	**	NS
Chardonnay	Year	*	**	***
	GDD	***	***	***
	Year*GDD	NS	**	***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3.2.1. Means for soluble solids content (SSC), titratable acidity (TA), and pH for grape berries for selected cultivars and corresponding date and accumulated growing degree days (GDD) at each harvest in 2010, 2011, and 2012.

Cultivar		Frontenac			Frontenac Gris			Marquette			La Crescent			Maréchal Foch		
Date	GDD °C	SSC (°Brix)	TA (g/L)	pH	SSC (°Brix)	TA (g/L)	pH	SSC (°Brix)	TA (g/L)	pH	SSC (°Brix)	TA (g/L)	pH	SSC (°Brix)	TA (g/L)	pH
8/13/10	**1,155**	14.53	23.56	2.40	15.40	20.26	2.40	17.58	18.43	2.40	17.60	19.37	2.59	15.40	18.84	2.46
8/23/10	**1,277**	17.03	21.71	2.72	19.33	21.12	2.71	21.60	15.99	2.68	21.60	18.72	2.75	18.88	14.42	2.82
9/3/10	**1,397**	20.30	19.02	2.85	23.55	17.30	2.86	24.13	12.86	2.80	23.15	15.46	2.84	21.93	11.01	2.92
9/11/10	**1,439**	21.30	18.62	2.90	24.48	16.61	2.88	25.48	11.97	2.81	23.78	15.24	2.88	22.85	10.73	3.06
9/21/10	**1,488**	22.88	18.53	2.86	23.35	15.46	2.96	26.15	11.53	2.84	23.70	14.43	2.88	24.60	9.66	3.05
8/10/11	**1,046**	8.98	37.91	2.40	9.93	36.83	2.35	11.55	33.89	2.36	9.28	40.88	2.43	5.13	46.71	2.28
8/19/11	**1,149**	14.83	28.37	2.69	15.90	27.27	2.70	16.85	23.09	2.70	15.53	28.59	2.66	14.48	29.44	2.28
8/27/11	**1,239**	15.70	22.39	2.75	18.86	23.74	2.74	19.68	19.07	2.74	19.20	21.65	2.77	18.03	19.28	2.78
9/6/11	**1,331**	18.23	21.45	2.84	20.55	21.01	2.82	21.95	16.48	2.79	21.13	19.87	2.79	20.55	14.02	2.88
9/14/11	**1,403**	22.33	21.14	2.92	23.38	19.58	2.93	25.08	14.65	2.84	23.58	19.21	2.86	22.38	13.33	2.96
9/23/11	**1,424**	21.70	19.45	3.01	24.41	18.50	2.96	24.85	12.82	2.92	22.33	17.23	2.91	22.73	12.04	2.99
10/3/11	**1,468**	24.55	19.34	3.04	25.65	18.39	3.01	26.35	12.83	3.04	23.33	16.58	2.94	25.13	12.50	2.98
10/12/11	**1,562**	24.85	17.93	3.12	27.23	16.11	3.08	27.55	11.56	3.05	22.65	13.56	2.99	26.10	11.79	2.99
8/8/12	**1,188**	15.45	25.94	2.56	17.38	24.39	2.59	18.05	20.47	2.49	18.58	21.73	2.63	16.93	18.95	2.71
8/17/12	**1,263**	20.15	21.49	2.68	20.08	19.20	2.65	21.48	15.80	2.53	21.15	15.79	2.63	20.50	12.03	2.69
8/25/12	**1,341**	22.08	18.42	2.68	21.90	17.71	2.73	24.03	13.60	2.66	22.60	13.94	2.67	21.25	9.69	2.82
9/3/12	**1,463**	24.33	17.18	2.92	23.80	15.64	2.84	26.55	11.65	2.72	23.95	14.21	2.84	24.35	8.50	2.88
9/11/12	**1,542**	25.65	16.38	2.99	25.73	16.13	2.82	29.33	12.04	2.75	25.63	12.71	2.78	25.78	8.92	2.84
9/19/12	**1,589**	26.43	14.86	3.11	27.28	14.32	2.93	30.45	12.04	2.84	25.78	13.17	2.88	25.78	8.74	3.02

Table 3.2.2. Means for soluble solids content (SSC), titratable acidity (TA), and pH for grape berries for selected cultivars and corresponding date and GDD at each harvest in 2010, 2011, and 2012.

Cultivar		St Croix			St Pepin			Merlot			Pinot Noir			Riesling			Chardonnay		
Date	GDD ˚C	SSC (˚Brix)	TA (g/L)	pH	SSC (˚Brix)	TA (g/L)	pH	SSC (˚Brix)	TA (g/L)	pH	SSC (˚Brix)	TA (g/L)	pH	SSC (˚Brix)	TA (g/L)	pH	SSC (˚Brix)	TA (g/L)	pH
8/13/10	**1,155**	12.98	17.01	2.45	15.23	18.77	2.56	5.45	26.66	2.35	7.08	27.97	2.57	6.15	28.06	2.54	5.65	23.46	2.56
8/23/10	**1,277**	16.43	9.77	2.94	18.73	13.49	2.78	13.53	16.28	2.76	13.80	16.51	2.74	11.23	23.35	2.58	13.33	16.83	2.86
9/3/10	**1,397**	17.23	5.74	3.13	20.30	11.21	2.93	18.35	10.66	3.05	18.75	10.77	3.02	15.88	15.27	2.77	17.30	11.63	3.06
9/11/10	**1,439**	19.88	5.21	3.11	21.18	9.17	2.90	19.03	10.77	3.11	19.90	9.79	3.12	16.85	14.37	2.82	19.28	10.80	3.16
9/21/10	**1,488**	20.90	4.96	3.08	21.95	9.83	2.94	19.95	8.95	3.20	22.15	7.53	3.20	18.73	12.15	2.86	20.43	9.24	3.22
8/10/11	**1,046**	4.35	36.29	2.31	6.35	43.01	2.50	3.98	39.08	2.41	3.00	43.78	2.40	3.78	36.86	2.54	3.89	37.29	2.57
8/19/11	**1,149**	10.85	21.27	2.63	12.28	32.35	2.64	7.28	34.52	2.48	9.75	32.89	2.59	7.83	36.64	2.56	8.60	29.04	2.76
8/27/11	**1,239**	14.30	11.84	2.86	16.60	22.82	2.71	13.30	18.52	2.74	13.80	19.89	2.76	12.25	25.94	2.64	14.60	18.27	2.89
9/6/11	**1,331**	17.13	6.92	3.07	17.01	15.73	2.83	17.60	11.83	2.87	17.88	12.23	2.97	16.23	16.50	2.86	18.30	12.88	3.00
9/14/11	**1,403**	19.80	5.77	3.23	19.50	13.44	2.96	20.00	9.69	3.15	20.45	10.65	3.08	17.05	13.75	2.77	20.30	11.05	3.09
9/23/11	**1,424**	19.58	5.10	3.20	19.68	11.81	2.96	21.08	8.38	3.27	21.80	9.13	3.08	17.68	11.13	2.97	21.03	9.58	3.14
10/3/11	**1,468**	22.10	4.82	3.42	21.90	11.78	3.05	22.95	9.06	3.29	23.85	9.03	3.17	18.85	11.49	3.07	22.33	9.68	3.12
10/12/11	**1,562**	NA	NA	NA	22.95	8.78	3.25	23.60	8.20	3.45	24.93	9.13	3.29	18.23	9.31	2.99	23.10	9.47	3.34
8/8/12	**1,188**	13.38	15.61	2.72	15.15	24.65	2.56	8.53	29.43	2.50	10.70	26.55	2.52	8.18	30.97	2.59	11.70	22.03	2.72
8/17/12	**1,263**	16.95	8.03	2.90	17.93	16.84	2.68	14.38	16.21	2.60	13.73	13.42	2.74	12.38	19.34	2.60	15.10	12.41	2.75
8/25/12	**1,341**	17.50	6.23	2.96	17.70	10.27	2.80	18.10	8.46	2.85	17.78	8.86	2.95	13.50	14.62	2.76	17.45	7.88	2.94
9/3/12	**1,463**	18.95	4.59	3.19	18.35	7.50	2.89	20.88	6.72	3.09	22.03	5.50	3.09	16.78	10.06	2.86	21.08	6.87	3.02
9/11/12	**1,542**	22.55	4.00	3.29	22.75	7.37	3.00	23.55	6.19	3.10	23.88	5.69	3.11	18.03	8.98	2.87	22.73	7.80	3.14
9/19/12	**1,589**	24.13	3.87	3.50	23.83	9.23	3.12	23.33	6.37	3.25	25.28	6.60	3.25	18.78	8.09	2.99	23.63	6.11	3.26

LITERATURE CITED

American Society of Horticultural Science. 1997. The Brooks and Olmo Register of Fruit & Nut Varieties, 3rd Ed. Frontenac, p 265. ASHS, Alexandria, VA.

Conde, C., P. Silva, N. Fontes, A.C. Dias, R.M. Tavares, M.J. Sousa, and H. Gerós. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food (Global Science Books). 1:1-22.

Coombe, B.G. 1976. The development of fleshy fruits. Annual Review of Plant Physiology. 27(1):207-228.

Coombe, B.G., and G.R. Bishop. 1980. Development of the grape berry. II. Changes in diameter and deformability during veraison. Crop and Pasture Sci. 31:499-509.

Coombe, B.G. 1987. Distribution of solutes within the developing grape berry in relation to its morphology. Am. J. Enol. Vitic. 38:120–127.

Coombe, B.G., and M.G. McCarthy. 2000. Dynamics of grape berry growth and physiology of ripening, Aust. J. of Grape Wine Res. 6:131-135.

Dai, Z. W., C. Léon, R. Feil, J.E. Lunn, S. Delrot, and E. Gomès. 2013. Metabolic profiling reveals coordinated switches in primary carbohydrate metabolism in grape berry (Vitis vinifera L.), a non-climacteric fleshy fruit. J. Exp. Bot. 64:1345-1355.

Davenport, J.R., M. Keller, and L.J. Mills. 2008. How Cold can you go? Frost and winter protection for grape. HortScience, 43:1966-1969.

Diakou P., and J.P. Carde. 2001. In situ fixation of grape berries. Protoplasma. 218:225-235.

Du Plessis, C.S. 1984. Optimum maturity and quality parameters in grapes: A review. S. Afr. J. Enol. Vit. 5:35.

Fournand, D., A. Vicens, L. Sidhoum, J. M. Souquet, M. Moutounet, and V. Cheynier. 2006. Accumulation of extractability of grape skin tannins and anthocyanins at different advancd physiological stages. J. Agric. Food Chem. 54:7331-7338.

Frontenac Grape. University of Minnesota Cold hardy grapes. http://www.grapes.umn.edu/frontenac/viticulture.html (Prepared by: Jim Luby and Peter Hemstad).

Galet, P. 2000. General Viticulture. Oenoloplurimédia, Chaintré France.

Gallander, J. F. 1977. Deacidification of eastern table wines with *Schizaccharomyces pombe*. J. Enol. Vit. 28:65-68.

García, W.M., L. Howarth, and M. Omori. 1967. Concentrations of Tartaric and Malic acids and their Salts in *Vitis* Grapes. Am. J. Enol. Vit. 18:42-54.

García Romero, E., G. Sánchez Muñoz, P.J. Martín Alvarez, and M.D. Cabezudo Ibáñez. 1993. Determination of organic acids in grape musts, wines and vinegars by high-performance liquid chromatography. J. Chrom. A. 655:111-117.

Hedrick, U.P. 1908. The Grapes of New York. Report of the New York Agricultural Experiment Station for the year 1907. J.B. Lyon, Albany. P.117.

Hemstad, P., and J.J. Luby. 2008. U.S. Patent No. PP19,579. Washington, DC: U.S. Patent and Trademark Office.

Hemstad, P.R. and J.J. Luby 2000. Utilization of *Vitis riparia* for the development of new wine varieties with resistance to disease and extreme cold. 487-490.

Jackson, D.I., and P.B. Lombard. 1993. Environmental and management practices affecting grape composition and wine quality-a review. Am. J. Enol. Vit. 44:409-430.

Jackson, D.I. 2001. Pruning and training (Vol. 1). Lincoln University Press.

Johnson, L.A., O.E. Carroll. 1973. Organic acid and sugar contents of Scuppernong grapes during ripening. J. Food Sci. 38(1):21-24.

Kallithraka S., J. Bakker, and M.N. Clifford. 1997. Red wine and model wine astringency as affected by malic and lactic acid. J. Food Sci. Technol. 62:416-20.

Keller, M. 2010. The science of grapevines: anatomy and physiology. Academic Press/Elsevier.

King, R.C., C.A. Sims, L.F. Moore, and R.P. Bates. 1988. Effects of maturity, skin contact and carbonation on the quality of sterile filtered white Muscadine grape juice. J. Food Sci. 53:1474-1485.

Kliewer, W.M. 1967. The glucose-fructose ratio of V. vinifera grapes. Am. J. Enol. Vit. 18:33-41.

Kliewer, W.M., L. Howarth, and M. Omori. 1967. Concentrations of tartaric acid and malic acids and their salts in Vitis vinifera grapes. Am. J. Enol. Vit. 18(1):42-54.

Kliewer, W.M. 1971. Effects of day tempature and light intensity on concentration of malic and tartaric acids in Vitis vinifera L. grapes. Journal Am. Soc. Hortic. Sci. 9:372-7.

Kliewer, W.M. 1973. Berry composition of Vitis vinifera cultivars as influenced by photo and nycto-temperatures during maturation. J. Am. Soc. Hortic. Sci. 98:153-9.

Lakso, A.N., and W.M. Kliewer. 1975. The influence of temperature on malic acid metabolism in grape berries I. Enzyme responses. Plant Physiology, 56(3):370-372.

Lamikanra, O., I. Inyang, and S. Leong. 1995. Distribution and effect of grape maturity on organic acid content of red Muscadine grapes. J. Agric. Food Chem. 43:3026-3028.

Liu, H.F., B.H. Wu, P.G. Fan, S.H. Li, and L.S. Li. 2006. Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. J. Sci. Food Agric. 86(10):1526-1536.

Luby, J and P. Hemstad. 2004. A grape plant named 'La Crescent'. U.S. Plant Patent PP14,617.

Luby J. and P. Hemstad. 2006. A grapevine named 'Frontenac gris'. U.S. Plant Patent No. PP16,478.

Lund S.T., and J. Bohlmann. 2006. The molecular basis for wine grape quality-- a volatile subject. Science. 311:804-805.

Martinson T.M. 2005. Macroclimate in New York and Site Suitability. http://grapesandwine.cals.cornell.edu

Martinson, T. E., J.J. Luby, and A. Garris. 2010. Addressing Research and Extension Needs of the Cold Climate Wine industry. http://blogs.cce.cornell.edu/grapes/files/2011/06/AppendixA_StakeholderMeetingsPriorities.pdf

McCarthy, M.G., and B.G. Coombe. 1999. Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport?. Aust. J. of Grape Wine Res. 5(1):17-21.

McMaster, G.S., and W.W. Wilhelm. 1997. Growing degree-days: one equation, two interpretations. Agr. Forest Meteorol. 87(4):291-300.

Midwest. Progress Report for Viticulture Consortium-East. Geneva Research Station, Cornell University, Geneva, NY.

Minnesota Grape Growers Association (MGGA), Hemstad, P., and T. Plocher. 1991. Growing Grapes in Minnesota. Minnesota Grape Growers Association.

Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. Biology of the grapevine. Cambridge University Press. p. 124-139

National Grape Registry (NGR) website: http://ngr.ucdavis.edu/. Supported by University of California Agriculture and Natural Resources, Foundation Plant Services, and National Clonal Germplasm Repository of the USDA Agricultural Research Service.

NRCS, 2008. Vitis riparia species profile. Available online at: http://plants.usda.gov/java/profile?symbol=VIRI

Pierquet, P.L., and C. Stushnoff. 1980. Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. Am. J. Enol. Vit. 31(1):1-6.

Riaz, S., A. Doligez, R.J. Hernry, and M.A. Walker. 2007. Mango. In; C. Kole (Ed.). Genome Mapping and Molecular Breeding in Plants, Volume 4. Fruits and Nuts. Springer-Verlag Berlin Heidelberg. 63-94.

Ribe´reau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. 2000. Handbook of Enology. Vol. 2. The Chemistry of Wine Stabilisation and Treatments; Wiley: Chichester, U.K. 8-25.

Ruffner, H.P. 1982. Metabolism of tartaric and malic acid in Vitis: a review–Part A. Vitis 21:247-259.

Ruffner, H.P. 1982. Metabolism of tartaric and malic acid in Vitis: a review–Part B. Vitis 21:346-358.

Saito, K., and Z. Kasai. 1968. Accumulation of tartaric acid in the ripening process of grapes. Plant and Cell Physiology. 9:529-537.

Shiraishi, M. 1995. Proposed descriptors for organic acids to evaluate grape germplasm. Euphytica, 81(1):13-20.

Shiraishi, M. 1993. Three descriptors for sugars to evaluate grape germplasm. Euphytica, 71(1-2):99-106.

Soyer, Y., N. Koca, and F. Karadeniz. 2003. Organic acid profile of Turkish white grapes and grape juices. J. Food Comp. Anal. 16(5):629-636.

Swanson, C.A., and E.D.H. El-Shishiny. 1958. Translocation of Sugars in the Concord Grape. Plant Physiology, 33(1):33.

Swenson, E. 1982. A grapevine named ‘St. Croix’. U.S. Plant Patent No. PP4,928. (assigned to Swenson-Smith Vines, Inc.).

Swenson, E. 1986. A grapevine named ‘St. Pepin’. U.S. Plant Patent No. PP5,771. (assigned to Swenson-Smith Vines, Inc.).

Tait, A.B. 2008. Future projections of growing degree days and frost in New Zealand and some implications for grape growing. Weather and Climate. *28*:17-36.

Winkler, A.J. 1962. General viticulture. Univ of California Press.

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. 1974. General Viticulture, (4th ed.): University of California Press, Berkeley, p. 740.

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider 1975. General viticulture. Soil Science, 120(6):462.