COLD HARDINESS AND SURVIVAL OF INTERSPECIFIC VITIS HYBRIDS

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

MASTER OF SCIENCE

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ABSTRACT

Cold hardiness and survival of wine grapes in two locations in North Dakota was determined using differential thermal analysis for five cultivars in 2020 and six cultivars from 2020-2021. Phenological data was collected during the growing season of 2020. Phenological data showed that cultivars broke bud early in the season and matured before the first fall frost. In 2020, cultivars at Red Trail Vineyard were hardier than those at the North Dakota State University Horticulture Research Station and ‘King of the North’ exhibited greatest hardiness, while ‘Frontenac’ and ‘Frontenac gris’ exhibited lowest hardiness. Across both locations, ‘King of the North’ proved to be the most cold hardy cultivar. Unpredictable minimum temperatures during dormancy, subsequent winter injury and herbicide drift all influenced bud cold hardiness, vine recovery, and survival. These results suggest that when growing wine grapes in North Dakota, cultivar selection and vineyard placement are critical factors in sustainable production.

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INTRODUCTION

Cold hardiness is a limiting factor for many perennial fruit crops of North Dakota and freeze injury is one of the greatest issues impacting grape production in northern latitudes (Fennell, 2004; Svyantek et al., 2020). Plants either live or die by the cold and further understanding of *Vitis* cold hardiness is critical for the prolonged survival of wine grape production in North Dakota. Compounding stress on the vine from disease, winter injury and herbicide drift can ultimately reduce or eliminate cold hardiness and lead to vine death. Crop loss due to unpredictable weather, like late spring frosts, and loss of cold hardiness is a hardship that wine grape producers inevitably face (Londo & Martinson, 2016).

The freezing tolerance of grapevine species is variable. *Vitis vinifera* produces grapes with high quality and desirable characteristics but lacks cold hardiness with a reported range of -10°C to -26°C (Mills et al., 2006). *V. riparia* accommodates this shortfall, reported to have the greatest cold tolerance at -40°C (Pierquet & Stushnoff, 1979). *V. labrusca* has been reported to tolerate temperatures from -26°C to -29°C (Dami, 2007). Interspecific hybrids developed from *V. riparia*, *V. vinifera* and *V. labrusca* have been utilized to provide wine grapes with desired cold hardiness for northern latitudes (Londo & Kovaleski, 2019). Cold hardy wine grapes have been reported to tolerate temperatures from -25°C to -38°C but freeze injury can still occur at less severe temperatures depending on vine dormancy status and timing of the freeze events. As temperatures drop in the fall and into the winter, dormant buds survive the colder temperatures and maintain freezing tolerance through low mid-winter temperatures. Vines begin to deacclimate and lose freezing tolerance as chilling requirements are fulfilled and temperatures increase near late winter into early spring (Mills et al., 2006; Ferguson et al., 2014). Temperatures regularly fluctuate throughout the critical periods during the fall and spring, which can cause freezing injury. For these reasons, freezing tolerance is considered a dynamic trait as it is greatly affected by temperature fluctuation and bud dormancy through winter (Londo and Kovaleski, 2019; Londo and Johnson, 2014). Dormancy in buds is separated into three categories, paradormancy, endodormancy and ecodormancy that are all connected to cold acclimation and deacclimation. Paradormancy ensures that latent buds remain dormant in growing vines. Onset of endodormancy in vines is triggered by decrease of daylength during summer and fall, depending upon cultivar (Fennell & Hoover, 1991; Wake & Fennell, 2000). Acclimation to the cold follows the start of endodormancy, with deep acclimation occurring in mid-winter, triggered by the combination of short daylength and low temperatures (Schnabel & Wamples, 1987). Finally, buds enter ecodormancy once chilling requirement is achieved and deacclimation can occur quickly once optimal temperatures are reached. Once budbreak approaches, the deacclimation process is irreversible (Fennell, 2004; Kalberer et al., 2006).

Sustainability of grapevines in northern regions depends upon the interaction of the grapevine's response to temperature during acclimation and deacclimation, along with low temperatures occurring during winter. The objective of this study was to provide measurements for cold hardiness of wine grape cultivars commonly utilized in North Dakota. Additionally, monitoring key growth stages provided insight into how actively growing vines recover from critical low temperatures experienced during dormancy.

LITERATURE REVIEW

The Grapevine

Grapevines (*Vitis* spp.) are perennial plants native to temperate climate zones of the Northern Hemisphere (Mullins et al., 1992). Grapes belong to the *Vitaceae* family and are recorded to be one of the oldest cultivated plants in the world. Archaeological records suggest that cultivation of the domestic grape (*Vitis vinifera* subsp. *vinifera*) began 6,000-8,000 years ago in the South Caucasus between the Caspian and Black Seas from its wild ancestor, *Vitis vinifera* subsp. *sylvestris*, then spread south to the western side of the Fertile Crescent, the Jordan Valley, and Egypt approximately 5,000 years ago (McGoverna, 2003).

The genus *Vitis* contains about 60 inter-fertile species, more than 3,000 taxa and can be divided into two clades, Eurasian and North American species. Domesticated *Vitis vinifera* belongs to the Eurasian clade, originating in West Asia and the Middle East. Traditional *V. vinifera* cultivars primarily make up wine, table and raisin grape production. *V. vinifera* provides good fruit quality, but lacks traits associated with biotic and abiotic stress tolerance. Wild North American species, like *Vitis riparia* and *Vitis labrusca*, are used in breeding to introduce important traits like grape phylloxera (*Daktulosphaira vitifoliae* Fitch) resistance (Riley, 1872) and cold hardiness (Pierquet & Stushnoff, 1979).

Production and Use

The grape is one of the most valuable horticultural crops in the world. In 2018, 7.1 million ha of grapevines were cultivated worldwide, yielding 79.1 million Mg. Top grape producers are China, Italy, US, Spain, France, Turkey, and India (FAO, 2018). In the United States, 6.5 million Mg of grapes were produced in 2020 (USDA, 2020). California dominates grape production, responsible for 335,000 ha. Washington and New York follow, producing

29,500 ha and 14,000 ha, respectively. Vineyards in northern latitudes, like Washington, New York, and the Upper Midwest experience challenging winter conditions for grape production. These areas and temperatures require genotypes that can withstand sub-freezing temperatures for long durations. The grape itself is a versatile fruit and is utilized for various purposes. Most grapes are used for wine, but can also be consumed fresh as table grapes, processed into juice, dried into raisins, made into jam, dietary supplements, grapeseed oil, vinegar and the leaves used for culinary purposes. (Pezzuto, 2008).

Freezing

Climate is a major influencing factor in species distribution on Earth. Low temperatures are the most limiting factor determining plant distribution in higher latitudes, where below freezing temperatures are experienced and plants may be subject to freezing tissues. Early research in cold injury hypothesized that plant death was caused by ice expansion that crushed living cells, or sap coagulation due to freezing (Parker, 1963), while modern literature has shown that alternative forms of damage are caused by the freezing process.

In freezing conditions, ice may form separately extra- and intracellularly (Molisch, 1897; Parker, 1963; Steponkus, 1984), while the location of ice formation is influenced by cooling rate (Steponkus, 1984). Due to the selective permeability of the plasma membrane, cells suspended in partially frozen solutions must come to a water potential equilibrium with the solution. This equilibrium is successfully reached by intracellular ice formation or cell dehydration. Intracellular ice formation can damage the plasma membrane through rupture or loss of selective permeability (Steponkus, 1984). Within different tissues ice can form preferably. Within buds, ice can form predominately in bud scales and other non-essential portions, thus called extraorgan freezing (Steponkus, 1984; Quamme et al., 1995).

Plants have developed different strategies to withstand seasonal low temperatures (Burke et al., 1976). One survival mechanism deployed is freeze avoidance, which can be separated into two different types: geographical avoidance and plant cycle avoidance. The former being when species that are not cold hardy remain in areas that do not have below freezing temperatures and the latter, when annual plants overwinter as cold hardy dehydrated seeds. Perennial plants that overwinter in areas where temperatures drop below freezing temperatures and have consequently developed specialized freeze avoidance mechanisms.

Some plants in higher latitudes exhibit supercooling abilities, where water freezes extracellularly in the apoplast at high below-freezing temperatures and dehydrates the intracellular space (symplast). The small amount of free water remaining in the cells then supercools, resisting ice formation to temperatures that may reach -40°C. Plants native to areas with harsher winters, like boreal forests, do not supercool. Instead, these plants form extracellular ice at high below-freezing temperatures and then dehydrate intracellular spaces, removing all bound water from the symplast (Burke et al., 1976). Grapevines belong to the group of plants that avoid freezing by deep supercooling.

Supercooling

Freezing of water can occur in two different ways: when foreign substances act as nucleators for ice formation (heterogenous freezing) and when water molecules form aggregates of an ice-like structure (homogenous freezing) (Bigg, 1953). However, water can remain in the liquid state at temperatures below the freezing point in a process called supercooling. This occurs when water molecules fail to form aggregates with an ice-like structure, which would result in ice formation (Zacchariassen et al., 2004). This state is not thermodynamically stable and continued decreasing temperatures lead to a higher probability of aggregate formation as a result of the decreased thermal movement of the molecules. Supercooling ability varies with the volume of water and rate of cooling (Bigg, 1953). The temperature of freezing has a linear relation with the log of volume and water is able to supercool to a lower temperature when the cooling rate is greater. However, the variance of nucleation temperature is independent of volume (Wilson & Haymet, 2012).

Heterogenous freezing requires the presence of an ice-nucleating particle, which aids in orienting the water molecule for nucleation, and all nucleation of supercooled biological solutions is heterogenous (Wilson et al., 2003). Kishimoto et al. (2014) found high ice nucleation activity in the stems of blueberry, localized in the cell wall fraction. This allows ice nucleation in the extracellular space and supercooling of the intracellular fraction. Nucleation agents can be both organic and inorganic in nature. Hiranuma et al. (2015) investigated microcrystalline cellulose as an ice nucleator for ice formation in clouds and found these particles to induce ice nucleation below -20°C. In grapes, cellulose therefore is not likely an ice nucleator in extracellular spaces, since extracellular ice forms around -5°C (Mills et al., 2006). In *Zea mays*, sensitivity to frost increased when *Pseudomonas syringae* was applied to leaf surfaces, demonstrating that bacteria may also act as an ice nucleator (Arny et al., 1976).

Perennial plants must overwinter in areas where temperatures drop below freezing temperatures and therefore, have developed freezing tolerance mechanisms (Xin & Browse, 2000). Grapevines can withstand freezing temperatures through bud supercooling and tolerating extracellular freezing and intracellular desiccation of cane and trunk tissue. Supercooling is the ability of the cell to freeze water extracellularly, in the apoplast, at below freezing temperatures and dehydrate the intracellular space in the symplast. Free water remaining in the cell supercools and resists ice formation to extremely low temperatures (Quamme, 1995). This state is not thermodynamically stable and continued decreasing temperature leads to the likelihood of aggregate ice formation as a result of decreased thermal movement of molecules (Zachariassen et al., 2004). Plants that supercool compartmentalize ice formation in extracellular space, dehydrating adjacent cells, causing them to supercool (Burke et al., 1976). Cold hardiness varies between species, but also among varieties within a given species (Parker, 1963).

Dormancy, Acclimation and Deacclimation

Axillary grapevine buds can be vegetative or mixed buds, and therefore may contain inflorescence primordia (anlage) which will give rise to fruit during the following season (Srinivasan & Mullins, 1976). From a biological standpoint, the survival of these structures during the winter is important for reproduction of the plants, while agriculturally this results in yield the following growing season. The lateral buds can be compound buds depending on the grapevine species (Morrison, 1991), in which primary, secondary, and tertiary buds are present. The shoot within the primary bud is organized as a monopodium (Srinivasan & Mullins, 1976). Six to 10 basal nodes are pre-formed in overwintering primary buds (Morrison, 1991). The leaf primordia are formed in a distichous phyllotaxy, and after approximately five nodes, the first anlage forms opposite to the leaf primordia in each node. Environmental conditions will result in the differentiation of the anlage into inflorescence, tendril, or shoot primordia (Srinivasan & Mullins, 1976).

Temperate perennial plants in cold regions have adapted dormancy mechanisms to allow temporary suspension of visible growth of any structure containing a meristem. Dormancy is initiated in unsuitable growth conditions, like cold temperatures, dehydration, or nutrient deficiency (Lang et al., 1987). In *Vitis*, latent buds are produced in leaf axils of growing vines, which remain dormant due to paradormancy. This inhibition by the shoot growing tip ceases growth of lateral vines. Latent buds may break dormancy depending on environmental cues and continue growing. Reduced photoperiod signals and lower temperatures in the fall stop active growth and initiate necessary changes to induce endodormancy in paradormant buds (Fennell & Hoover, 1991). Cold hardiness is developed following a dynamic sequence of acclimation and deacclimation, giving dormant grapevine tissue the ability to survive freezing temperature stress during fall and winter (Levitt, 1980; Sakai and Larcher, 1987). When temperatures fall below the level of vine cold hardiness, damage can occur to buds, canes, cordons, trunks, or roots, and even cause death of the vine. Sudden temperature drops below cold hardiness thresholds and prolonged low temperature durations that are above these thresholds can often result in vine injury.

There are three classical stages of cold acclimation in grapevines, that are somewhat connected to dormancy: acclimation, mid-winter and deacclimation. The period of acclimation follows endodormancy and deacclimation marks the end of ecodormancy. Mid-winter can be divided into two stages, initially endodormancy and then transitioning to ecodormancy. Onset of endodormancy in buds is triggered by decrease of daylength during summer and fall in some grapevines (Fennell & Hoover, 1991; Wake & Fennell, 2000). Acclimation to cold follows onset of endodormancy, induced by decreasing temperatures (Stergios & Howell, 1977; Xin & Browse, 2000; Ferguson et al., 2011, 2014). During this stage, buds are unable to reach maximum cold hardiness, but can tolerate temperatures below freezing (Howell, 2000; Ferguson, et al., 2014). Deep acclimation is triggered by the combinations of short daylength and low temperatures (Schnabel & Wamples, 1987).

The transition from endodormancy to ecodormancy in grapevines is accelerated by low temperatures through chill accumulation (Lang et al., 1987). The chilling requirement for transition is usually found through linear regression as the number of chill hours or units for 50% budbreak within 21-30 days (Lloyd & Firth, 1990; Ben Mohamed et al., 2010; Londo & Johnson, 2014). Once chilling requirement is achieved and plants become ecodormant, buds can deacclimate at a much faster rate once optimal temperatures are reached. Rates of acclimation and deacclimation vary dynamically and are reversible (Damborska, 1978; Wolf & Cook, 1994; Gu et al., 2008), but as budbreak approaches, the loss of cold hardiness ceases to be reversible (Fennell, 2004; Kalberer et al., 2006). Cultivars differ in rates of acclimation and deacclimation, maximum level of cold hardiness and response to temperature fluctuations (Mills et al., 2006).

Differential Thermal Analysis

Cold hardiness achieved through supercooling in grapevines is typically measured using differential thermal analysis (DTA), or low temperate exotherm (LTE) analysis. This method measures the release of latent heat at the temperature (°C) when symplastic water freezes (Andrews et al., 1983; Tinus et al., 1985; Wolf & Pool, 1987; Burr et al., 1990; Wisniewski et al., 2017; Mills et al., 2006; Ferguson et al., 2011). When supercooled water freezes extracellularly, the heat released is called a high temperature exotherm (HTE). Extracellular freezing is considered nonlethal. Conversely, the freezing of intracellular water creates a similar low temperature exotherm (LTE) and is lethal (Burke et al. 1976).

In single buds, HTE is observed around -7°C, followed by one to three LTEs. The largest, medium, and smallest LTEs are assumed to be the result of freezing of primary, secondary and tertiary buds, respectively (Andrews et al., 1984). Mills et al. (2006) found that buds removed from freezer directly after LTE presented injury of primary bud, while buds removed before LTE presented no injury, demonstrating that LTE coincides with freezing injury. Once the LTEs are identified for a population of buds, the LTE_{50} (temperature required to kill 50% of the bud popuation) can be calculated (Proebsting et al. 1980). DTA is not only used to conduct research on the mechanisms of freeze tolerance, but also to predict lethal temperatures for grapevine buds.

Cold Hardy Grapes and Wines

Grape cultivars are utilized based upon their ability to tolerate specific climates and satisfy a particular purpose. When growing grapes for wine, certain considerations must be considered. The region, winemaking method and harvest timing are all crucial for a reliable crop (Guerrini et al., 2018). Table grape and wine grape cultivars were limited to regions without temperatures exceeding -20°C before the development of cold-hardy interspecific hybrids (Perry et al. 2012). These interspecific hybrids have given the opportunity for wine grape production in northern latitudes.

Common cultivars used in cold climates are divided into four standard groups: northern hybrids, French-American, American and *Vitis* cultivars (Domoto et al., 2016). After grape phylloxera (*Daktulosphaira vitifoliae* Fitch) tore through wine vineyards in Europe, northern hybrids were utilized for disease resistance. They were often used as rootstock in wine producing countries, with an even more positive impact in northern latitudes. Northern hybrids are the core of northern grape growing and are based upon the riverbank grape (*V. riparia)* that is reportedly tolerant to -50°C. French American hybrids are interspecific hybrids of *V. vinifera* with native American wild species (*V. labrusca, V. lincecumii, V. rupestris, V. aestivalis*). Although these hybrids have reported accidental winter hardiness, their growing region extends only to southern Minnesota. American cultivars are the standard of eastern North American grape growing areas, based on *V. labrusca* cultivars and hybrids. These cultivars tend to perform marginally in cold climates, but with some *V. riparia* parentage can be considered Northern hybrids. *Vitis vinifera* cultivars are the most notable wine grapes in Europe and California but are also the most susceptible to low winter temperatures.

The following cultivars are described using the *Vitis* International Variety Catalogue (VIVC) variety number. Frontenac (VIVC 15904; Hemstad et al., 1996) is a cross that included *V. vinifera* (Landot 4511) and *V. riparia* (*V. riparia* 89). This is a very cold hardy and disease resistant vine and has produced a crop after -35°C. Berries are small and black, producing a garnet red to dark red wine with cherry, plum and berry aromas. ‘Frontenac’ is a versatile grape for wine making and lacks the unpleasant herbaceous aroma usually associated with *V. riparia*. (Domoto et al., 2016). ‘Frontenac gris’ (VIVC 23928; Luby & Hemstad, 2006) is a color mutation of ‘Frontenac noir’ (University of Minnesota, Excelsior Research Station) and is culturally identical to ‘Frontenac’. Berries ripen to bronze and produce a white or salmon colored white wine with apricot or peach flavors, preferably finished slightly sweet, but can also be utilized for ice wine.

‘La Crescent’ (VIVC 17632; Okie, 2002) is a cross between ‘St. Pepin’ and ES 6-8-25 and is 45% *V. vinifera*, 28% *V. riparia*, and less than 10% each of *V. rupestris*, *V. labrusca*, and *V. aestivalis*. Bud break is early and vine growth habit is sprawling and drooping. Berries are small and yellow-amber when ripe and cane be made into either a dry or sweet wine. When sweet, ripe melon, citrus, pineapple, tropical fruit and muscat flavors are revealed (Domoto et al., 2016).

‘Marquette’ (VIVC 22714; Hemstad & Luby, 2008) originated from a cross between MN 1094 and the French hybrid, Ravat 262, which has ‘Pinot noir’ as a parent and *V. riparia*, *V. vinifera* and other *Vitis* species. Bud break is early like ‘La Crescent’ and vines bear lightly. ‘Marquette’ can be made into a complex red wine comparable to *V. vinifera*, with moderate tannings and notes of cherry, black currant, raspberry, and black pepper and no hybrid characteristics (Domoto et al., 2016).

‘King of the North’ (VIVC 26642) is an extremely hardy *V. riparia* and *V. labrusca* hybrid grape reported to be hardy to -38°C. The cultivar is very rigorous and productive and produces juicy medium-sized blue berries. This grape is versatile, used for making juice, jelly, wine or simply just eating. ‘King of the North’ wines tend to be rich, aromatic, and grapey labrusca style reds. The precise origins of this cultivar are unknown but appears to be a labrusca-riparia hybrid (Domoto et al., 2016).

‘Valiant’ (VIVC 14500; Peterson, 1982), is a *V. labrusca* and *V. riparia* hybrid known as the one of the cold-hardiest cultivars available, but extremely susceptible to downy mildew (*Plasmopara viticola*) and black rot (*Guignardia bidwellii*). Northern wineries value this cultivar for its vigor and dependability. The berries are black and small and can be used in juice and wine making, commonly produced as a port-style red wine (Domoto et al., 2016).

MATERIALS AND METHODS

Vineyard Locations

Six *Vitis* cultivars (‘Frontenac’, ‘Frontenac Gris’, ‘Valiant’, ‘Marquette’, ‘La Crescent’ and ‘King of the North’) were used to measure the bud and cane tissue cold hardiness for two years (2020-2021) from two locations, the NDSU Horticulture Research Station (HRS) (46°59'N 97°21'W) near Absaraka, ND and Red Trail Vineyard (46°54'N 97°29'W) near Buffalo, ND. The cold hardy variety trial at the HRS was established with own-rooted genotypes on a Warsing sandy loam, loamy substratum and Swenoda fine sandy loam with 0-2% slopes in 2004. These vines were planted in a randomized complete block design (RCBD) with cultivars planted in four vine experimental units, two cultivars per block (8 plants/block) and replicated four times, clustered by the period of planting. Rows were 83.0 m in length and oriented north to south with 3.0 m between rows and 2.4 m between vines. Vines were grown as a single trunk and trained to form bilateral cordons located approximately 1.8 m above the soil surface on a single high wire. Vines were irrigated as needed only through the establishment year. A 0.5 m weed-free strip was maintained within vine rows using tillage or a pre-emergence herbicide (Flumioxazin, Chateau®, Valent USA, San Ramon, CA, USA) application followed by a post-emergence herbicide (Glufosinate, Rely®, BASF, Florham Park, New Jersey, USA) spot applications for weed misses. Between rows was seeded with creeping red fescue (*Festuca rubra* ssp. rubra) in 2005.

At Red Trail Vineyard, ‘King of the North’ was transplanted in 2003, ‘Frontenac’ and ‘La Crescent’ were transplanted in 2004, while ‘Frontenac gris’ and ‘Marquette’ were transplanted in 2007. ‘Frontenac gris’ and ‘Marquette’ at this location resided in a Barnes-Buse loam, with 3-6% slope, while ‘King of the North’, ‘Frontenac’ and ‘La Crescent’ all resided in a Hamerly-Wyard loam, with 0-3% slope. Vines were maintained as bilateral-cordon-trained to a mid-wire trellis. Vines were spur pruned and supported with three sets of catch wires in a vertical shoot positioning system. Temperature data for 2020 and 2021 was collected from the nearby North Dakota Agricultural Weather Network (NDAWN) station in Prosper, North Dakota.

Phenology

Vine phenology and developmental stages were evaluated using the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) scale (Lorenz et al., 1995) throughout the growing season in 2020. Bud development, leaf development, inflorescence emergence, flowering, fruit development, berry ripening and senescence were all characterized with numerical codes according to BBCH scale. From 11 May to 22 August on each vine, four buds were assessed for phenological ratings once weekly during active developmental stages, and approximately once every two weeks between stages. A total of 16 vines per cultivar at both locations were utilized for each observation date.

Cold Hardiness Determination

Differential thermal analysis (DTA) was performed during dormancy to evaluate dormant bud cold hardiness from November 2019 to May 2020 and from November 2020 to May 2021. Sampling occurred weekly near acclimation and deacclimation at Red Trail Vineyard, due to excess bud availability, and biweekly at the HRS location. ‘Valiant’ was absent at the Red Trail Vineyard location. During mid-winter and deepest acclimation, biweekly bud collection was performed at both locations. Three canes containing six buds were collected of each cultivar, totaling eighteen buds per cultivar at each location per sampling time. Two bud spurs were retained below sampled cane to allow fruit production the following season. Canes were then immediately transported to laboratory.

The DTA procedure followed the same method described by Mills et al. 2006, which enhanced the standard DTA system (Wample et al. 1990). Thermoelectric modules (TEMs) (model CP1.4-127-045L; Melcor Corporation, Trenton, NJ) were used to detect temperature gradients generated by the exotherms and convert thermal signals to voltage (mV) outputs. Thermistors (model 44212; YSI, Dayton, OH) were used for measuring chamber temperature. Buds and cane tissue were excised and placed on moist, pre-cut tissue squares (Kimwipes, Kimberly-Clark, Irving, TX, USA) within individual cells containing TEMs.

LTE peak data was recorded through a Keithley Multimeter Data Acquisition System (DAS) (model 2700, Tektronix, Inc., Beaverton, OR, USA). The DAS scans up to 40 channels of the TEMs and thermistors every 15 sec and runs in conjunction with the program ExcelLINX (Keithley Instruments, Solon, OH, USA). Bud freezing was conducted within a Tenney Model T2C programmable freezer (Thermal Product Solutions, New Columbia, PA, USA). Following stabilization controlled by a Watlow Series 942 temperature regulator (Watlow Electric Manufacturing, St. Louis, MO, USA), the freezer was held at 4°C for one hour prior to experimental cooling, at a rate of -4°C h^{-1}. The freezing cycle completed once the freezer reached a minimum temperature of -50°C. Once the cycle completed, the freezer progressively warmed to 4°C. The LTE values ($LTE_{10, 50, 90}$) were identified manually with Bud Processor 1.8.0 Software (Brock University, St. Catherines, ON, Canada).

Statistical Analyses

Influence of cultivar and location on LTE_{50} values were tested with a one-way analysis of variance (ANOVA) and a subsequent Tukey's honestly significant difference (HSD) test at $\alpha=0.05$ with the stats package in R (version 4.1.1). Models were created for each season and both seasons combined. Model residuals were checked for normality assumptions.

RESULTS AND DISCUSSION

Temperature and Bud Cold Hardiness

The two dormant seasons had different low temperature severity, affecting the vine and bud survival in different ways. In both the 2019-2020 and 2020-2021 dormant seasons, lowest minimum and maximum mean temperatures were experienced from December to February in Prosper, ND (Table 1). The lowest recorded temperatures for both seasons were -33.8°C on 13 February 2020 and -31.6°C on 14 February 2021. Except for October 2020 (3.4°C), the lowest mean monthly temperatures were experienced during the 2019-2020 season.

Table 1: Monthly mean minimum and maximum temperatures during the dormant season of 2019-2020 and 2020-2021.

	2019/20 mean maximum (°C)	2020/21 mean maximum (°C)	2019/20 mean minimum (°C)	2020/21 mean minimum (°C)
October	8.6	9.2	0.7	-2.4
November	0.7	6.7	-7.4	-6.8
December	-6.3	-0.1	-18.9	-12.2
January	-9.2	-3.6	-17.8	-12.2
February	-5.8	-10.2	-19.9	-18.9
March	0.9	8.7	-8.9	-4.5
April	8.8	11.9	-2.7	-1.4

The first fall frost in 2019 occurred on 10 October and the last spring frost on 12 May 2020 (Fig. 1). For 2020-2021, the first fall frost was on 8 September 2020 and last spring frost on 28 May 2021 (Fig 2). Although the fall and spring frosts occurred later in 2019-2020, temperatures dropped below -15°C sooner (-17°C on 10 November 2019, -18.4°C on 14 December 2020) and maintained a lower threshold throughout dormancy compared to 2020-2021 temperature data.

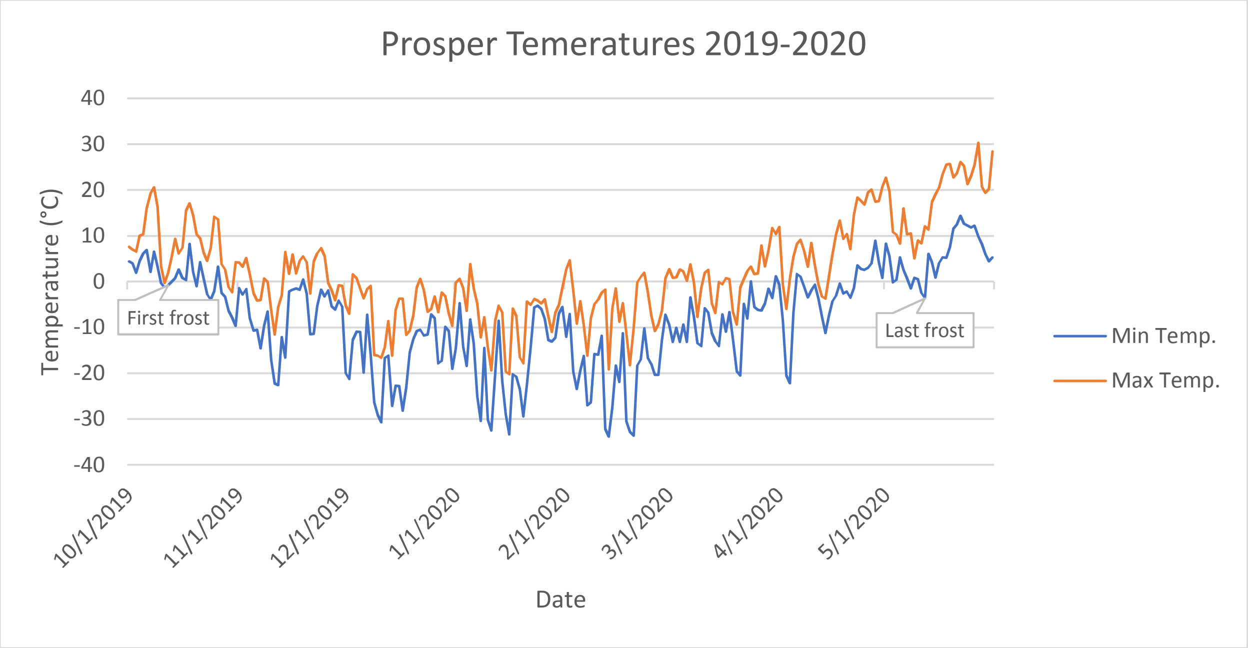

Figure 1: Daily maximum and minimum temperature from October 2019-May 2020 for Prosper, ND.

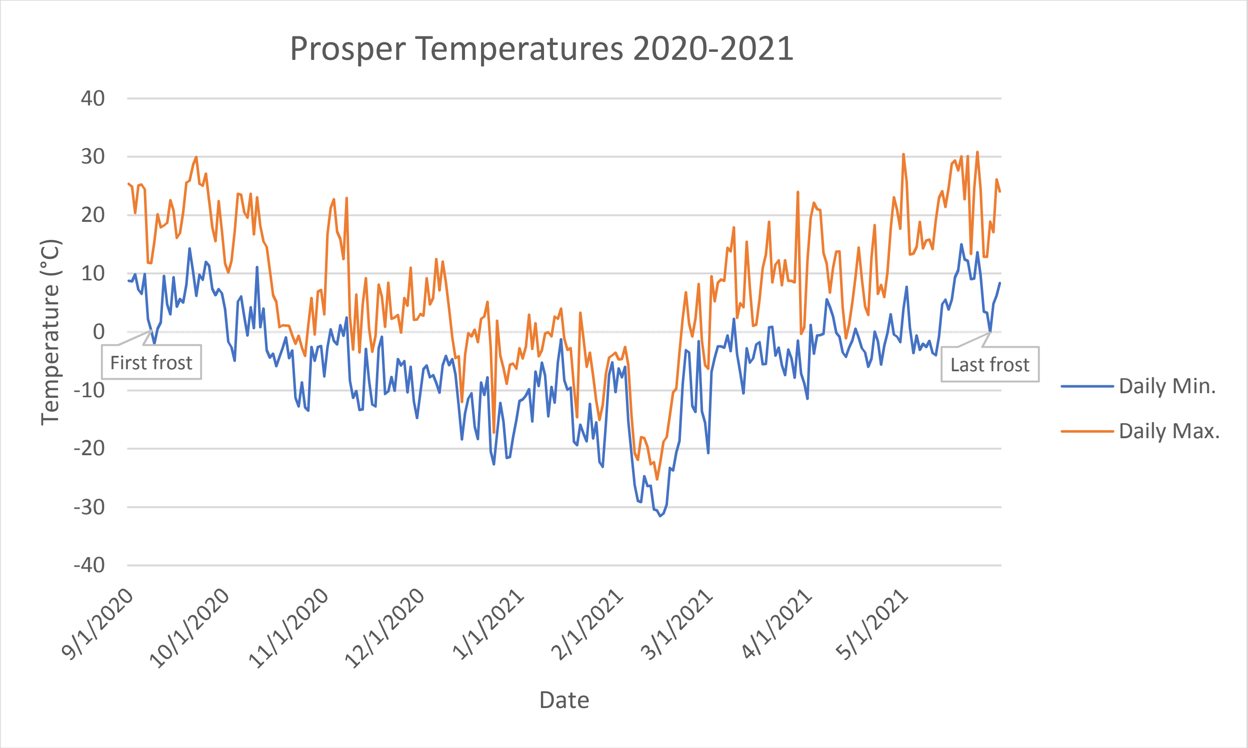

Figure 2: Daily maximum and minimum temperature from September 2020-May 2021 for Prosper, ND.

The influence of winter minimum air temperature on primary bud survival and hardiness was observed throughout both seasons using DTA. After verasion, vines began to pass through acclimation at a rapid rate, which typically concludes around the middle of February. At this point the vines began to deacclimate and defense mechanisms reverse, making vines more sensitive to temperature swings (Londo and Martinson, 2016).

In 2019, extreme low temperatures were experienced early in the acclimation process, reaching -22°C on 11 November and 12 November (Figure 1). December of 2019 introduced the start of an extremely cold stretch lasting until March 2020. During December, temperatures were below -20°C on 10 separate days and below -30°C once (11 December). January provided no relief from this cold stretch, as temperatures were below -30°C on four days and a minimum of -33°C on 16 January. Unfortunately, once the deacclimation period initiated in February, temperatures once again plummeted on 12 February (-32°C), 13 February (-34°C), 18 February (-30°C), 19 February (-33°C) and 20 February (-33°C). Cold temperatures also occurred in March with -20°C on the 19th, 20th and 21st. The last cold snap occurred on 4 April with temperatures reaching -22°C (Figure 1).

Throughout the dormant season of 2020-2021, temperatures were mild compared to 2019-2020. The first extreme low was on 14 December 2020 (-18.4°C), almost an entire month later than in 2019 (Figure 2). Temperatures were below -20°C for four days in December. January 2021 provided two below -20°C days, with a cold snap in early to mid-February. From 5 February to 19 February, minimum temperatures ranged from -20°C to -31°C. This cold snap tapered off, ending on 1 March with -21°C. Subfreezing temperatures were experienced throughout April with the most severe on 1 April (-11°C). Grapevine bud maximum freezing tolerance is generally expected in January, decreasing in February and March as temperatures rise. Interspecific cultivars are reported to respond quickly to temperature fluctuations and have a range of cold hardiness and winter survivability (Bourne and Moore, 1991; Bourne et al., 1991; Wolf and Cook, 1994). Long-term sustainability of cultivars is greatly influenced by their ability to acclimate with changing dormant season temperatures (Yilmaz et al., 2021).

Using analysis of variance and bud LTE_{50} values in 2020, the cultivar by location interaction was not significant, while significance was found in both cultivar and location (Table A1). In 2020-2021, bud LTE_{50} values for cultivar by location interaction and location were not significant while there was significance between cultivars (Table A2). Even though winters differed, data were combined across both years to understand cold hardiness over time. When combined, there was a lack of significance for the cultivar by location interaction and the location main effect, while significance was again found between cultivars (Table A3). Specifically, in 2020, vines at Red Trail Vineyard had buds with lower exotherms than the buds from vines at HRS (Table 2). For cultivars, 'King of the North' buds exhibited lower exotherms than both 'Frontenac' and 'Frontenac gris'. In 2020-2021, 'Valiant', 'Frontenac' and 'Frontenac gris' buds exhibited significantly lower exotherms than 'La Crescent' and 'Marquette'. Combined data from 2020 and 2020-2021 showed that 'King of the North' exhibited significantly lower exotherms than both 'La Crescent' and 'Frontenac gris'.

Low temperature exotherm values for 2020 followed temperature trends, as lowest values were observed during the coldest temperatures in late February (Fig. 1 and Table 3). Additionally, some peaks of 'Marquette', 'Frontenac', 'La Crescent' and 'Frontenac gris' were not detected during February 2020, suggesting that the low temperatures caused bud death in the sampled vines, or bud dehydration was so severe that an exotherm could not be detected. For primary bud hardiness, 'King of the North' at Red Trail Vineyard gave the lowest mean LTE value (-23°C), followed by ‘Marquette’ at Red Trail Vineyard (-22.7°C), ‘King of the North’ at HRS (-22°C), ‘La Crescent’ at Red Trail Vineyard (-20.1°C), ‘Frontenac gris’ (-19.5°C) and ‘Frontenac’ (-18.2°C) at Red Trail Vineyard, ‘La Crescent’ at HRS (-15.5°C), ‘Frontenac gris’ and ‘Marquette’ at HRS (-14.9°C) and ‘Frontenac’ at HRS (-13.9°C).

Table 2: Bud LTE responses for cultivars 'Frontenac', 'Frontenac gris', 'Marquette', 'King of the North', 'La Crescent' and 'Valiant' at Red Trail Vineyard and Horticulture Research Station for 2020, 2020/21 and 2020+2020/21.

		LTE_{50}(°C)	
Treatment	2020	2020/2021	2020+2020/2021
Location:			
Red Trail Vineyard	-19.81 a	-20.41	-20.11
HRS	-17.16 b	-20.29	-18.73
p-value	0.000233	0.904	0.242
Cultivars:			
Frontenac	-16.05 a	-21.68 b	-18.87 ab
Frontenac gris	-14.96 a	-21.52 b	-18.24 a
Marquette	-18.77 ab	-18.80 a	-18.79 ab
King of the North	-22.60 b	-20.12 ab	-21.36 b
La Crescent	-17.78 ab	-18.55 a	-18.17 a
Valiant	N/A	-22.45 b	N/A
p-value	<0.0001	<0.0001	<0.0001
Location*Cultivar:			
RTV*FT[1]	-18.24	-21.89	-20.07
RTV*FG	-19.49	-22.03	-20.76
RTV*MQ	-22.65	-18.66	-20.66
RTV*KN	-23.22	-20.31	-21.77
RTV*LC	-15.46	-19.17	-17.32
HRS*FT	-13.86	-21.47	-17.67
HRS*FG	-14.96	-21.01	-17.99
HRS*MQ	-14.88	-18.94	-16.91
HRS*KN	-21.99	-19.94	-20.97
HRS*LC	-20.11	-17.94	-19.03
HRS*VT	N/A	-22.45	N/A
p-value	0.1801	0.593	0.342

[1] Abbreviations, RTV=Red Trail Vineyard, HRS=Horticulture Research Station, FT=Frontenac, FG=Frontenac gris, MQ=Marquette, KN=King of the North, LC=La Crescent, VT=Valiant

[a,b] Means in a column (year) followed by different letters are significantly different through means separated at P≤0.05 by Tukey’s HSD test.

Comparing mean LTE values for 2020-2021, ‘Marquette’ was the only cultivar where bud peaks were not detected, and this corresponded with the low temperatures experienced in February 2021 (Table 4). Due to the reported hardiness of ‘Valiant’, this cultivar was also investigated for bud hardiness from 2020-2021. ‘Valiant’ buds showed the lowest mean LTE (-22.5°C), followed closely by ‘Frontenac gris’ at Red Trail Vineyard (-22.4°C), then ‘Frontenac’ at Red Trail Vineyard (-21.8°C) and HRS (-21.5°C), ‘Frontenac gris’ at HRS (-21°C), ‘King of the North’ at Red Trail Vineyard (-20.3°C) and HRS (-19.9°C), ‘La Crescent’ and ‘Marquette’ at Red Trail Vineyard (-19°C), ‘Marquette’ at HRS (-18.6°C) and ‘La Crescent’ at HRS (-17.9°C).

Past research has shown that mild winters result in less negative LTE values compared to colder winters (Londo and Kovaleski, 2017; Ferguson et al., 2011). This present study reinforced those results, as less negative LTEs were exhibited during the milder winter, but this may be due to the health of the vines in 2020-2021 compared to 2020. Cold injury has the potential to negatively affect overall vine health, this reducing the ability to acclimate efficiently and reducing the ability to tolerate cold temperatures (Keller and Mills, 20007). Extremely cold temperatures in 2018-2019 and 2019-2020 may have caused extensive cold injury in vines. Notably, HRS vines were detrimentally affected by these temperatures and previous flooding conditions of the vineyard, as many trunks split and/or failed to break bud, flower, and subsequently produce fruit.

Table 3: Mean bud LTEs with same sampling date for 'King of the North', 'Frontenac', 'Frontenac gris', 'La Crescent', 'Marquette' and 'Valiant' at Red Trail Vineyard (RTV) and Horticulture Research Station (HRS) for 2020.

	King of the North		Frontenac		Frontenac gris		La Crescent		Marquette	
Date	RTV	HRS	RTV	HRS	RTV	HRS	RTV	HRS	RTV	HRS
1/16/20	-19.1	-14.7	-15.9	-15.4	-15.2	-14.1	-15.4	-12.9	-21.1	-13.6
1/31/20	-25.7	-29.2	-16.2	-13.8	-25.1	*NP	-23.9	-22.9	-25	-20
2/14/20	-23.9	-22.9	-16.3	*NP	-17.8	-14.1	-26.8	*NP	-20.5	*NP
2/27/20	-28.6	-25.3	-20.1	*NP	-28.6	*NP	-25.7	*NP	*NP	*NP
3/12/20	-20	-20.7	-26.1	-12.4	-12.1	-14.9	-23.8	-29.1	-23.7	-11.6
3/27/20	-21.9	-19.1	-14.9	*NP	-18.1	-16.7	-24.9	-15.5	-23	-14.3
Mean LTE	**-23**	**-22**	**-18.2**	**-13.9**	**-19.5**	**-14.9**	**-15.5**	**-20.1**	**-22.7**	**-14.9**

*NP, no peaks detected

Table 4: Mean bud LTEs with same sampling date for 'King of the North', 'Frontenac', 'Frontenac gris', 'La Crescent', 'Marquette' and 'Valiant' at Red Trail Vineyard (RTV) and Horticulture Research Station (HRS) for 2020-2021.

	King of the North		Frontenac		Frontenac gris		La Crescent		Marquette		Valiant
Date	RTV	HRS	RTV	HRS	RTV	HRS	RTV	HRS	RTV	HRS	HRS
10/20/20	-14.3	-15.4	-17.8	-14.7	-16.4	-13.6	-15.4	-12.9	-15.2	-14.1	-15.3
11/6/20	-16.1	-13.5	-21.2	-13.2	-21.7	-17.1	-12.2	-13.8	-17.2	-17.7	-21.8
11/19/20	-21.3	-15.1	-22.2	-17.4	-24.8	-18.3	-17.8	-16.4	-20.2	-17.4	-22.2
12/5/20	-15.9	-21.3	-22	-20.7	-22.8	-21.5	-16.6	-20.8	-20.5	-20.7	-22.8
12/18/20	-22.4	-20.8	-24.2	-25.9	-27.5	-21.7	-23.3	-17.6	-26.4	-23.4	-27.5
1/12/21	-29.2	-26.9	-25.2	-26.2	-25.3	-25.6	-23.3	-21.8	-19.4	-24.6	-27.9
1/29/21	-25	-25	-19.5	-24.6	-23.6	-23.7	-18.2	-18.1	-21.6	-22	-26.8
2/10/21	-24.7	-27.6	-27.1	-28.3	-26.3	-27.3	-25.1	-20.7	*NP	-24	-28.5
2/26/21	-27.6	-26.9	-25.3	-25.9	-25.1	-24.7	-26.3	-23.5	-24.8	*NP	-29
3/18/21	-26.4	-23	-20.4	-27.3	-24.3	-24.1	-21.5	-21.6	-21.3	-17.5	-21.2
3/31/21	-16	-16	-21.3	-19.7	-20.5	-22.5	-16.2	-18.3	-16.5	-18.4	-20.5
4/10/21	-11.4	-13	-18.4	-18.4	-16.4	-13.5	-14.5	-13.2	-11.7	-10	-14
4/16/21	-13.1	-14.6	-18.9	-16.9	-16	-19.6	-16.9	-14.6	-14.7	-13.3	-14.5
Mean LTE	**-20.3**	**-19.9**	**-21.8**	**-21.5**	**-22.4**	**-21**	**-19**	**-17.9**	**-19**	**-18.6**	**-22.5**

*NP, no peaks detected

Phenology

Phenological development was recorded for all cultivars throughout the entire growing season at Red Trail Vineyard but only 'Valiant' and 'King of the North' at the HRS had all vines that survived through bud break and continued developing throughout the 2020 season (Table 5). As previously mentioned, freeze injury can greatly reduce yield and vine development when critical low temperatures are reached (Keller and Mills, 2007). Cold damage to cane and trunks occurred in both the phloem and xylem. Severe phloem damage can take time to repair, but it has been shown that vines can recover from phloem damage. If severe damage occurs in the xylem, vines are consequentially more susceptible to trunk and cordon death. Vines can no longer transport water to the canopy, causing collapse. Cane specific damage resulted in stunted shoots and/or shoot collapse, while damage to trunks can induce excessive suckering, crown gall development, trunk splitting, or vine death (Keller and Mills, 2007). Due to low, sustained cold periods during the 2019-2020 dormancy, it was assumed that this factor potentially contributed to shoot collapse and vine trunk/cordon death observed in 'Frontenac', 'Frontenac gris', 'Marquette' and 'La Crescent' at the HRS. Additionally, trunk splitting from cold injury was observed immediately and throughout the growing season in some trunks of these cultivars.

At the HRS vineyard, both 'King of the North' and 'Valiant' first bud break was observed on 9 May, with 'La Crescent' and 'Marquette' breaking bud on 11 May and 'Frontenac' and 'Frontenac gris' on 15 May (Table 5). 'King of the North' at Red Trail Vineyard first broke bud on 11 May (Table 6). The remaining cultivars at Red Trail Vineyard, 'Frontenac', 'Frontenac gris', 'La Crescent' and 'Marquette' all broke bud on 13 May, directly after the last spring frost event on 12 May. Frost events are significant events for vine development, as late spring frost can damage primary buds and early fall frost can reduce berry quality (Pedneault et al., 2013; Manns et al., 2013; Frioni et al., 2019). In this study, the overlapping spring freeze event with ‘King of the North’ and ‘Valiant’ had no observed negative effects on subsequent bud development. This may be attributed to either bud stage of development when experiencing these temperatures, or the duration of the freezing event.

At Red Trail Vineyard, ‘Frontenac’ bud break was observed to last 10 days, inflorescence emergence for 6 days, flowering for 10 days, fruit set over a period of 41 days and veraison for 21 days. ‘Frontenac gris’ bud break lasted for 10 days, inflorescence emergence for 6 days, flowering for 10 days, fruit set for 22 days and veraison for 19 days. ‘La Crescent’ bud break lasted 12 days, inflorescence emergence for 8 days, flowering for 8 days, fruit set over a period of 23 days and veraison for 17 days. ‘Marquette’ bud break lasted 10 days, inflorescence emergence for 8 days, flowering for 5 days and 17 days for both fruit set and veraison. For ‘King of the North’, bud break lasted 12 days, inflorescence emergence for 6 days, flowering for 12 days, fruit set for 22 days and veraison for 21 days.

At the HRS, ‘King of the North’ bud break was observed over a period of 13 days, inflorescence emergence for 6 days, flowering for 7 days, fruit set for 24 days and veraison for 24 days (Table 5). ‘Valiant’ bud break was also 13 days, inflorescence emergence for 6 days, flowering for 7 days, fruit set for 20 days and veraison for 24 days. The geographical and environmental differences between the two vineyards may contribute to the disparities in phenological stages of ‘King of the North’, as there is a strong correlation between grape maturation stages and climate (Meier et al. 2007; Sun et al. 2018; Carlo et al. 2019). Alternatively, damage to primary buds frequently delays bud break, as the secondary bud emerges more slowly than the primary bud of a healthy vine (Yilmaz et al., 2021).

Table 5: Phenological development of 'Frontenac', 'Frontenac gris', 'La Crescent', 'Marquette', 'King of the North' and 'Valiant' cultivars grown at the North Dakota State University Horticulture Research Station near Absaraka, ND in 2020.

	Frontenac	Frontenac gris	La Crescent	Marquette	King of the North	Valiant
Phenological Stages	-----------	-----------	-----Date-----	-----------	-----------	-----------
Bud break start	15 May*	15 May*	11 May*	11 May*	9 May	9 May
Bud break end	--	--	--	--	22 May	22 May
Inflorescence emergence start	--	--	--	--	28 May	28 May
Inflorescence emergence end	--	--	--	--	3 June	3 June
Flowering start	--	--	--	--	12 June	8 June
Flowering end	--	--	--	--	19 June	15 June
Fruit set start	--	--	--	--	15 June	12 June
Fruit set end	--	--	--	--	9 July	2 July
Veraison start	--	--	--	--	3 August	29 July
Veraison end	--	--	--	--	27 August	22 August

*bud break was observed, but vines collapsed before bud break concluded

Table 6: Phenological development of 'Frontenac', 'Frontenac gris', 'La Crescent', 'Marquette' and 'King of the North' cultivars grown at Red Trail Vineyard near Buffalo, ND in 2020.

	Frontenac	Frontenac gris	La Crescent	Marquette	King of the North
Phenological Stages	-----------	-----------	-----Date-----	-----------	-----------
Bud break start	13 May	13 May	13 May	13 May	11 May
Bud break end	23 May	23 May	25 May	23 May	23 May
Inflorescence emergence start	28 May	28 May	28 May	28 May	28 May
Inflorescence emergence end	3 June	3 June	1 June	1 June	3 June
Flowering start	15 June	10 June	17 June	17 June	5 June
Flowering end	25 June	17 June	25 June	22 June	17 June
Fruit set start	17 June	17 June	22 June	22 June	17 June
Fruit set end	28 July	9 July	15 July	9 July	9 July
Veraison start	6 August	6 August	6 August	6 August	6 August
Veraison end	27 August	25 August	23 August	23 August	27 August

CONCLUSION

Wine grape producers in North Dakota and the Upper Midwest need to consider minimum winter temperatures in their specific area before selecting cultivars. Temperature swings in dormancy can induce primary bud damage, negatively affecting crop load in the following seasons and causing irreversible winter injury. Although many cultivars have been deemed suitable for North Dakota, it is important to understand how cold hardiness of grapevines may be impacted by unexpected weather events. Additionally, when selecting vineyard sites, growers should consider the surrounding crops in the area and the potential for herbicide drift causing crop loss and vine damage.

This study evaluated primary bud cold hardiness of six cultivars, ‘Frontenac’, ‘Frontenac gris’, ‘Marquette’, ‘La Crescent’, ‘King of the North’ and ‘Valiant’ through low temperature exotherm analysis. Cold hardiness assays were conducted over two dormancy season, January 2020 to March 2020 and October 2020 to April 2021 at two vineyard locations, Red Trail Vineyard and the North Dakota State Horticulture Research Station near Absaraka, ND (HRS). ‘Valiant’ was excluded from January-March 2020 samplings and was not present at Red Trail Vineyard. Phenological development was monitored for all cultivars at both locations for the growing season of 2020.

Results showed that low temperature exotherms (LTEs) were significantly different between cultivars throughout dormancy both years and locations only the first year. In the first year, ‘Frontenac’ and ‘Frontenac Gris’ primary buds were more sensitive to cold temperature damage than ‘King of the North’ buds (LTEs were less negative) and vine buds at the Red Trail Vineyard were less sensitive to cold temperature damage compared to the same vine buds from the HRS vineyard. During the second season of dormancy, ‘Marquette’ and ‘La Crescent’ buds were more sensitive to cold temperature damage than ‘Frontenac’, ‘Frontenac Gris’ and ‘Valiant’ buds due to more negative LTEs for the buds. Combining both seasons, ‘La Crescent’ and ‘Frontenac gris’ buds were more sensitive to cold temperature than ‘King of the North’.

Considering phenological data, ‘King of the North’, ‘Valiant’, ‘La Crescent’ and ‘Marquette’ vines at the HRS vineyard broke bud before the same cultivars at the Red Trail Vineyard. ‘Frontenac’ and ‘Frontenac gris’ broke bud earlier at Red Trail Vineyard than the same cultivars at the HRS. Bud break of these cultivars overlapped with the last spring frost, which can be detrimental depending upon severity and duration of the cold but visible damage to vines was not observed. Early bud break can be an advantageous trait, yet risks putting buds in danger of cold damage in cases of late spring frost. ‘Valiant’ was the first cultivar to fully reach veraison, followed by ‘Marquette’, ‘La Crescent’, ‘Frontenac Gris’, ‘Frontenac’ and ‘King of the North’ at the Red Trail Vineyard since most of the cultivars at the HRS experienced cold temperature damage. All of the cultivars that did not experience cold temperature damage had matured clusters well before the risk of the first fall frost. ‘King of the North’ was the only cultivar that had phenology collected at both locations. ‘King of the North’ vines at the Red Trail Vineyard were a couple of days later for all phenological stages except the end of veraison when compared to ‘King of the North’ vines at the HRS vineyard.

Overall, this study aligned with previous findings that ‘King of the North’ is the hardiest wine grape suitable for North Dakota’s growing conditions. ‘Frontenac gris’ and ‘La Crescent’ were least cold hardy, which differs from previous findings (Domoto et al., 2016). The other cultivars did not differ in cold hardiness. These results speak to the differences vineyards may experience across the state due to variability in environmental conditions, and/or quality of vines. In order to promote the sustainability of wine grape production in North Dakota, it will be necessary in the near future to develop wine grape cultivars with similar LTEs to ‘King of the North’ or identify and adopt cultural practices that promote consistent cold hardiness beyond the potential cold temperatures associated with late fall, winter, and early springs in North Dakota.

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APPENDIX

Table A1: ANOVA for location, cultivar, and interaction effects on LTEs for 2020.

Terms in the model	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	1	274	274.04	14.323	0.000233*
Cultivar	4	706.9	176.73	9.237	1.32e-06*
Location: Cultivar	4	121.9	30.46	1.592	0.180130
Residuals	131	2506.4	19.13		

Df, degrees of freedom; Sum Sq, sums of squares; Mean Sq, mean sums of squares
*significant at the <0.05 probability level

Table A2: ANOVA for location, cultivar, and interaction effects on LTEs for 2020-2021.

Terms in the model	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	1	0	0.36	0.014	0.904
Cultivar	5	1221	244.20	9.869	5.39e-09*
Location: Cultivar	4	69	17.29	0.699	0.593
Residuals	480	11877	24.74		

Df, degrees of freedom; Sum Sq, sums of squares; Mean Sq, mean sums of squares
*significant at the <0.05 probability level

Table A3: ANOVA for location, cultivar, and interaction effects on LTEs for all seasons.

Terms in the model	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	1	35	34.97	1.370	0.242
Cultivar	5	1028	205.53	8.050	2.28e-07*
Location: Cultivar	4	115	28.80	1.128	0.342
Residuals	621	15856	25.53		

Df, degrees of freedom; Sum Sq, sums of squares; Mean Sq, mean sums of squares
*significant at the <0.05 probability level