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Research Article

Cold Hardiness of Cold Climate Interspecific Hybrid Grapevines Grown in a Cold Climate Region

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Abstract: Cold climate interspecific hybrid grapevines (CCIHG) selected for their superior mid-winter cold hardiness have expanded grape production to cold climate regions. However, extreme weather events, such as polar vortexes, and high frequency of fall and spring freezes often result in yield and vine losses. The main objective of this study was to evaluate changes in bud cold hardiness of five CCIHG cultivars grown in the upper Midwest in order to identify relative risk for freeze damage throughout the dormant period, and to adapt a bud cold hardiness prediction model to CCIHG cultivars grown in cold climate regions. Bud cold hardiness was evaluated biweekly throughout the dormant period by measuring lethal temperatures for buds using differential thermal analysis (DTA). CCIHG cultivars in our study had an early acclimation response with increased levels of cold hardiness before the occurrence of freezing temperatures. Maximum levels of hardiness (-28 to -30°C) were observed both years in February, however deeper levels of freezing stress resistance, probably attained by freeze

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dehydration, were not detected using DTA. CCIHG cultivars had a rapid deacclimation response that was accelerated with additional chilling accumulation during spring. The reparametrizing of a discrete-dynamic cold hardiness prediction model by expanding the range of ecodormant threshold temperatures for CCIHG resulted in predictions with an average RMSE = 1.01. Although CCIHG cultivars have superior mid-winter bud cold hardiness, fast deacclimation responses increase the risk of freeze damage during spring, thus this trait should be evaluated for future CCIHG cultivar release. The development of tools, such as the discrete-dynamic cold hardiness prediction model for CCIHG cultivars, will aid growers in decision-making to minimize damage, as well as yield and vine losses.

Key words: differential thermal analysis, explanatory model, freezing stress resistance, hybrid grapevine, prediction model

Introduction

Extreme low temperature is the most significant constraint for grape production in cold climate regions. Freeze injury to buds, canes, cordons, and trunks limits yields and increases production costs due to the additional retraining and replacing of damaged vines (Zabadal et al. 2007). Thus, grapevine genotypes with superior cold hardiness are essential for a successful viticulture industry in cold climate regions.

Cold climate interspecific hybrid grapevines (CCIHG) have genetic backgrounds that include *Vitis aestivalis*, *V. labrusca*, *V. riparia*, and *V. rupestris*, and *V. vinifera* (Smiley and Cochran 2016, Atucha et al. 2018). The development of CCIHG cultivars has combined high fruit quality traits of *V. vinifera* with the superior mid-winter cold hardiness traits found in wild *Vitis* species, which has propelled the development of a $539.2 million viticulture industry in cold climate regions, such as the US Midwest (Dami et al. 2005, Luby and Fennell 2006, Tuck et al. 2017). However, extreme and erratic

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weather events continue to result in substantial freeze damage to CCIHG and, in turn, economic losses. Some recent examples of devastating freezing events that led to unprecedented crop and vine losses include: the Easter freeze of 2007, Mother's Day freeze of 2010, the "killer frost" of 2012, and the polar vortex event of 2014 (Dami and Lewis 2014, Wisniewski et al. 2017). The Easter freeze of 2007 alone was estimated to be nearly $1 billion in economic losses to small fruit crop growers, including grapes, across 21 states (Warmund et al. 2008). Most recently, in 2019, the polar vortex split, in which a portion of the polar vortex separated and traveled southward and resulted in record-setting cold temperatures in many cold climate regions of the United States ("National Centers for Environmental Information" 2020). The economic consequences of these extreme weather events highlight the need for more information on CCIHG cold hardiness dynamics to more specifically identify: 1) periods of high risk, 2) the extent of variability among CCIHG cultivars, and 3) potential risk-mitigation practices.

Previous studies have described a U-shaped pattern of grapevine bud cold hardiness that spans the duration of the dormant period (Mills et al. 2006, Ferguson et al. 2011, 2014, Londo and Kovaleski 2017). This pattern begins with acclimation (gain of cold hardiness) in the fall, continues with maintenance of cold hardiness throughout winter, and ends with deacclimation (loss of cold hardiness) in the spring. While this general pattern of seasonal response has been described extensively, there is substantial variability in grapevine cold hardiness across years, genotypes, climates, and cultural practices (Pierquet and Stushnoff 1980, Williams et al. 1994, Ferguson et al. 2014, Grant and Dami 2015, Londo and Kovaleski 2017). The use of explanatory models, such as the one developed by Londo and Kovaleski (2017), characterizes the relationship between cold hardiness and temperature fluctuations during the dormant season by genotype.

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While characterizing grapevine cold hardiness changes in response to temperature fluctuations across the dormant period is critical to understand how genotypes will behave in different growing conditions, the need for information on short-term changes in cold hardiness is critical for protection decision making by growers. However, routine assessment of bud cold hardiness is a time-intensive process that requires specialized equipment. One approach to this is the use of discrete-dynamic modelling where continual changes to a system are modeled using arbitrary incremental time steps, such as hours, days, etc. A discrete-dynamic model developed by Ferguson et al. (2011, 2014) predicts daily changes of cold hardiness for twenty-one *V. vinifera* cultivars and two *V. labrusca* cultivars using daily maximum and minimum temperatures and cultivar-specific parameters. However, this model was developed using cold hardiness data collected in Washington State from primarily *V. vinifera* genotypes. Therefore, in order to extend its utility to CCIHG and the cold climate regions where they are mostly grown, this model must be reparametrized for these genotypes and evaluated using cold hardiness data collected in these additional relevant growing regions.

The main objective of this study is to evaluate changes in bud cold hardiness of five CCIHG cultivars to identify relative risk for freeze damage throughout the dormant period. The secondary objective is to optimize and evaluate with the same 2-year dataset a bud cold hardiness prediction model for these five CCIHG cultivars grown in a cold climate region. This information will contribute to cultivar selection for particular regions and inform designs for future research into the physiological and mechanistic processes of grapevine cold hardiness. In addition, this work will promote the testing and refinement of predictive models with independent sets of data and will strengthen grapevine bud cold hardiness protection decision making in cold climate regions.

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Materials and Methods

Site description. This study was conducted over two winters, 2017-2018 (Year 1) and 2018-2019 (Year 2), in a vineyard at the West Madison Agricultural Research Station in Verona, WI (lat. 43° 03' 37" N, long. 89° 31' 54" W). The vineyard is in U.S. Department of Agriculture Plant Hardiness Zone 5a (USDA, 2019), and has deep silt Griswold loam soil with 2 to 6% slopes ("Web Soil Survey - USDA NRCS" 2020).

Vineyard design and vine material. The vineyard was established with one-year-old bare root vines. In 2008, Brianna (BR), Frontenac (FR), La Crescent (LC), and Marquette (MQ) were planted and grown on a vertical shoot positioned trellis system with double trunks trained into unilateral cordons one meter above ground. In 2011, Petite Pearl (PP) was planted and trained to a high cordon trellis system with double trunks trained into unilateral cordons 1.5 meters above ground. All vines were spur-pruned to approximately 45 nodes per vine and all cultivars were thinned to 20 shoots per meter-length of trellis. The vineyard is arranged as a randomized complete block design with four replications. Each block includes two rows of vines with six, four-vine panels per row. At the time of the study, seven of the twelve panels within each block contained cultivars that were not included in this study. Rows are oriented north-south with 3.4 meters between rows and 2.1 meters between vines for a total density of 1398 vines/ha (566 vines/acre).

Weather Data. Hourly average, daily maximum, and daily minimum air temperature data were collected from September 1 to April 30, using a Network for Environment and Weather Applications ("NEWA" 2020) participating station (Model MK-III SP running IP-100 software; Rainwise, Trenton, ME) located onsite, 2 m above ground level (lat. 43° 3' 39.6" N, long. 89° 32' 2.4" W, and elevation at 330 m).

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Bud collection. Buds were collected in the morning using similar methods as described in Mills et al. (2006), Ferguson et al. (2014), and Londo and Kovaleski (2017). Buds from node positions four to seven were sampled (4 buds per vine) from canes with green phloem and xylem. Buds were sampled biweekly, across vineyard blocks and vines within blocks (from a total of 16 vines). There were 15 and 14 sampling times in Year 1 and Year 2, respectively. Canes were cut several centimeters away from the bud. Buds were collected in plastics bags, stored on ice during transportation, and processed immediately upon returning to the lab.

Differential Thermal Analysis (DTA). The DTA equipment used in this study was the same as described by Villouta et al. (2020). Thermoelectric modules (TEMs) (model HP-127-1.4-1.5-74 and model SP-254-1.0-1.3, TE Technology, Traverse City, MI) were used to detect freezing exothermic reactions. TEMs were placed in individual hinged tin-plated steel containers lined with 5 mm thick open-cell foam pieces to reduce effects of freezing chamber air turbulence. Eleven TEM units were evenly spaced and attached to each of four 30x30 cm perforated aluminum sheet pieces (hereafter called "trays") with the leads of each tray wired to a single 24-pin D-sub connector. A copper-constantan (Type T) thermocouple (22 AWG) was positioned on each tray to monitor temperature in proximity to the TEM units. Trays were positioned vertically in a Tenney Model T2C programmable freezing chamber (Thermal Product Solutions, New Columbia, PA) and connected to a Keithley 2700-DAQ-40 multimeter data acquisition system (Keithley Instruments, Cleveland, OH). TEM voltage and thermocouple temperature readings were collected at 6-second intervals via a Keithley add-in in Microsoft Excel (Microsoft Corp., Redmond, WA). The effect of freezing chamber fan turbulence on the TEM units was minimized by covering trays with 13 mm open-cell foam sheets and by installing a

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removable piece of perforated, corrugated cardboard across the top of the chamber's interior to function as a damper.

To prepare samples for DTA, nodes were pruned out of the canes. Buds, including the bud cushion, were excised from the node using a razor blade. Five buds were arranged with the cut surface up (i.e., bud on bottom) on a small piece of aluminum foil (Reynolds Consumer Products, Lake Forest, IL). Cut surfaces of the buds were covered with a piece of moistened Kimwipe (Kimberly Clark, Roswell, GA) to provide an extrinsic ice nucleator source and to prevent dehydration prior to bud freezing. The aluminum foil was folded into a packet containing the five buds. Each single aluminum foil packet was randomly assigned to a TEM chamber.

The DTA protocol used a temperature ramp from room temperature to 4 °C over one-hour, a one-hour hold, ramp to 0 °C over one-hour, a one-hour hold, ramp to -44 °C over 11-hours, a 30-minute hold, and then a finishing ramp back to 4 °C over two-hours. The resulting cooling rate was -0.067 °C per minute (or -4 °C per hour). One TEM chamber on each tray was left empty to document baseline background electrical noise. Heat is released with the freezing of each supercooled meristem in the grape compound bud, and this release is referred to as a low temperature exotherm (LTE). A single compound grapevine bud contains primary, secondary, and tertiary meristems. In this experimental setup, a TEM will occasionally record a large peak followed by one or two smaller peaks. The larger peak relates to the freezing of the primary meristem, while the smaller peaks correspond to the freezing of the secondary and tertiary meristems, a reflection of their smaller size and lower water content. Frequently, these peaks are undiscernible. In a given set of DTA run results, up to five bud LTEs were identified and documented for each TEM, corresponding to the freezing of the primary meristems. As

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158 only obvious peaks were identified in this way, occasionally fewer than five peaks were documented for
159 some TEMs.

160 In Year 1, DTA was performed biweekly from November 2 to April 30 (40 buds per sampling
161 date per cultivar) for a total of 15 times. In Year 2, DTA was performed biweekly from September 26 to
162 April 16 (30 buds per sampling date per cultivar**)** for a total 14 times.

163 Additional DTA runs were performed as part of a temperature conditioning experiment
164 following an extreme cold weather event in late January 2019. Buds were sampled from all cultivars (16
165 buds per cultivar) on February 12, 2019, less than two weeks after the extreme low temperatures.
166 Excised and prepared buds were conditioned in the TEM chambers. The protocol used a temperature
167 ramp from room temperature to 4 °C over one hour, a one-hour hold, ramp to 0 °C over one-hour, a one-
168 hour hold, ramp to -33 °C over 33-hours (-1 °C/hour), a 30-minute hold, a ramp to -10 °C over 6 hours,
169 ramp to 0 °C over 5 hours, ramp to 4 °C over 2 hours. A freezing experiment was then started with the
170 standard DTA protocol, which cools to a minimum of -44 °C.

171 **Visual bud injury evaluation.** Following the extreme cold weather event in January 2019, an additional
172 collection of buds was dissected while visually evaluating freeze injury using an Olympus SZX12
173 microscope with a 1x objective (Olympus Optical Company, Tokyo, Japan). Forty buds for each cultivar
174 were collected following the same protocol as for DTA. Before dissection, buds were incubated in
175 sealed plastic bags on ice for 24 hours, then at 4 °C for 24 hours, and finally at room temperature for 24
176 hours. Sequential cross-sections were cut from the buds with a double-edged razor blade until meristems
177 were visible for evaluation. Freezing injury was assessed for primary and secondary meristems in each
178 bud. Oxidative browning (Goffinet 2004) was rated as present (injured) or absent (not injured).

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Statistics. For each DTA run, the temperatures at which 10%, 50%, and 90% of the buds froze were determined and referred to respectively as the LT_{10}, LT_{50}, and LT_{90} temperatures. Two models were developed in R (ver. 3.5.2, R Foundation for Statistical Computing, Vienna, Austria), an explanatory regression model (Londo and Kovaleski 2017) and a predictive discrete-dynamic model (Ferguson et al. 2011, 2014). Symbols and abbreviations used for each model are listed in Table 1. Data from both years of the study were used in the evaluation of each model (more detail below).

Explanatory Model. An explanatory linear regression model was created in R using multiple linear regression and a model selection process based on Londo and Kovaleski (2017). The model was used to determine significant differences among cultivars' seasonal bud cold hardiness and their relative responsiveness to temperature fluctuations and time. The explanatory variables used were Cultivar, Time, $Time^2$, Year, and a temperature index (σ_T). Cultivar was included as a categorical variable. Time and $Time^2$ were measured in units of days from September 6. The temperature index σ_T describes shifts in temperature during a time period preceding cold hardiness measurement by DTA and was calculated using the same formulas described by Londo and Kovaleski:

$$T_E = T - T_{base}$$

$$\sigma_T^2 = \sum_{i=1}^{n} (T_E \times |T_E|)_i$$

$$\sigma_T = sign(\sigma_T^2) \times \sqrt{|\sigma_T^2|}$$

where T_E is the temperature experienced by the plant and T is the hourly average temperature. The base temperature, T_{base}, used was 0 °C. The 'n' used was 168 and specifies that σ_T is a sum of the temperature variations experienced during the 168 hours (or 7 days) preceding cold hardiness measurements by

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DTA. The square of T_E was calculated by multiplying the value of T_E by its absolute value in order to keep the sign. A similar tactic is used to calculate the square-root of σ_T^2 to keep the sign.

Model selection included a forward-backward stepwise procedure with a Bayesian Information Criterion (BIC). BIC was chosen to avoid overfitting the model and because there is a high number of sampling points. First, a model was selected using data from a single year (Year 1), precluding the use of a year term. For this process, the null model included only the intercept and the full model included all parameters, as well as interactions. Subsequently, a model was selected with the full two-year dataset. This time the null model was the regression model previously found, and the full model included the interaction between the terms in the null model with year. Using this new regression model, data points with studentized residual ≥ 2 or Cook's distance ≥ 0.002 were ranked as outliers and removed from the dataset. Finally, the regression model was re-fit using the non-outlier subset to obtain the final coefficients. Dominance analysis was performed to evaluate the relative contribution of each parameter.

Prediction Model. A predictive discrete-dynamic model with 1-day time steps described in detail by Ferguson et al. (2011, 2014) was created in R. The model generates daily cold hardiness predictions for grapevine buds from September 7 to May 15. A stepwise iterative method was used to identify eleven pre-defined cultivar-specific parameters. We included four more levels (-1, 0, 1, and 2 °C) for the ecodormant temperature threshold parameter, in addition to the five levels (3, 4, 5, 6, and 7 °C) used by Ferguson et al., resulting in a total of 2,976,750 parameter combinations (1,323,000 additional combinations, Table 2). The model was optimized and evaluated with the same 2-year dataset. The parameter combination that minimized the RMSE between predicted and observed LT_{50} was selected

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and evaluated for each year. Internal model validity was tested by Pearson correlation analysis of predicted versus observed LT_{50}.

Results

Summary of weather conditions. Winter conditions in the two years of this experiment were distinctly different; therefore, the results for each year are described separately. The first day that air temperatures dropped below 0 °C was October 28 and October 12 for Year 1 and Year 2, respectively, and the last day air temperatures dropped below 0 °C was April 29 and April 28 for Year 1 and Year 2, respectively. In Year 1, between September 1 and April 30, on 62 days the maximum temperature was ≤ 0 °C (180 days > 0 °C). During the same time period, in Year 2, on 78 days the maximum temperature was ≤ 0 °C (164 days > 0 °C). The minimum temperature reached during winter of Year 1 was -25.3 °C on January 1. The minimum temperature reached during winter of Year 2 was -32.9 °C on two days, January 30 and January 31, and was part of the 2019 polar vortex split event. These minimum temperatures in Year 2 were extreme for the area. During this event, temperatures in the vineyard dropped below -25 °C for 37 consecutive hours. The minimum temperature during this time, -32.9 °C, was reached twice, on the evening of January 30 and again in the morning of January 31.

Cold hardiness response of CCIHG. All cultivars exhibited the standard U-shaped cold hardiness curve, with acclimation in the fall/early winter and deacclimation in late winter/spring. Figure 1 illustrates the LT_{50} response for each cultivar in both years tested. There were slight differences among cultivars and years in the LT_{50} temperature. The timing of minimal LT_{50} temperatures occurred nearly unanimously in mid-February. The exception to this was Brianna in Year 1, which reached its minimal LT_{50} in late December and had a comparable (+0.3 °C) LT_{50} in mid-February. Frontenac and La Crescent had the

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overall lowest LT_{50} values in both years (respectively, -30.6 and -30.3 °C in Year 1 and -28.4 and -29.7 °C in Year 2).

There were also differences in the range of temperature between LT_{10} and LT_{90} throughout the dormant season. The general trend was a small difference between LT_{10} and LT_{90} in the fall during acclimation, changing to a wide difference in midwinter, and then returning to a small difference in spring during deacclimation. The average LT_{10}-to-LT_{90} range for all cultivars in both years was 3.9 °C. The largest LT_{10}-to-LT_{90} range was 9.5 °C measured in Brianna on December 28 in Year 1. The smallest LT_{10}-to-LT_{90} range was 1.6 °C measured in Petite Pearl on November 15 in Year 1. Across all cultivars, the changes in LT_{90} mimicked changes in LT_{50} and had a similar magnitude of difference (average of 1.5 °C). In contrast, the changes in LT_{10} did not parallel the changes in LT_{50} and were also more distant in magnitude (average of 2.3 °C difference).

Year 1 (2017-18). All cultivars had LT_{50} values lower than -20 °C by November 15. Brianna's maximal hardiness (-29.9 °C) occurred in the end of December, while the maximal hardiness for Frontenac (-30.6 °C), La Crescent (-30.3 °C), Marquette (-29.3 °C), and Petite Pearl (-28.9 °C) occurred in early February. All of the cultivars acclimated through the end of December, followed by a period of fluctuating maximal hardiness until the end of February, before continuously deacclimating for the remainder of the sampling period. The only exception to this pattern was Frontenac, which gained hardiness between February and March. All of the cultivars maintained LT_{50} values lower than the daily minimum air temperatures.

Year 2 (2018-19). All the cultivars had LT_{50} values lower than -20 °C by November 13. The rate of acclimation increased after October 12 (Figure 1). Maximal hardiness was measured in the end of February for all cultivars: Brianna (-27.1 °C), Frontenac (-28.4 °C), La Crescent (-29.7 °C), Marquette (-

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27.8 °C), and Petite Pearl (-27.9 °C). All of the cultivars acclimated rapidly through December, then continued with slow acclimation through the first part of February, before deacclimating rapidly for the remainder of the sampling period.

The minimum temperature of -32.9 °C on January 30 and 31, 2019, was 6.3-7.7 °C colder than the LT_{50} measured most recently (January 16) for all of the cultivars. Buds from all cultivars tested by DTA on February 1 showed no LTEs. Based on these observations, we expected extensive and severe damage in the vineyard. Buds from all cultivars tested by DTA on February 5 showed the resumption of normal peak patterns.

Visual bud injury evaluation:

Following the extreme cold weather event in January 2019, an additional collection of buds was dissected to visually evaluate the extent of damage in the vineyard. While some damage was observed, it was not as widespread as the most recently preceding DTA results had indicated to be likely. A higher rate of injury was visible in primary meristems, as compared to secondary meristems. Specifically, meristem injury was in 12.5% primary and 5.0% secondary for Brianna, 5% primary and 0% secondary for Frontenac, 10% primary and 5.0% secondary for La Crescent, 7.5% primary and 0% secondary for Marquette, and 17.5% primary and 2.5% secondary for Petite Pearl.

Explanatory Model. The final multiple linear regression model selected included four parameters, six interaction terms, and an intercept. The equation selected was:

$$\text{LTE} = Cultivar + \sigma_T + Time^2 + Time + \sigma_T{:}\,Time^2 + \sigma_T{:}\,Time + \sigma_T{:}\,Cultivar + Cultivar{:}\,Time + Cultivar{:}\,Time^2 + Time{:}\,Year$$

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Estimates were calculated separately for each cultivar and year dataset separately (Table 3). Parameters with estimates that vary across cultivar interacted with the Cultivar parameter (σ_T, $Time^2$, Time). The Time parameter (in days) is the only parameter that varies between years, as identified by the Time x Year interaction. The overall model selected had a p-value < 0.0001 ($2.2x10^{-16}$) and adjusted-R^2 = 89.5%. The temperature index parameter, σ_T, had an interaction with cultivar but not with year. Groupings for the significant differences between the estimates for σ_T, Time, and $Time^2$ are listed in Table 3. In addition, overall dominance analysis showed σ_T had the largest relative contribution (50.1%), followed by $Time^2$ (18.0%), and then Time (16.4%) (Table 4).

Prediction Model. Collectively, the optimized model parameters predicted LT_{50} values with an overall $r^2 = 0.97$ ($r^2_{Year\ 1} = 0.95$ and $r^2_{Year\ 2} = 0.98$) and RMSE = 1.01 °C ($RMSE_{Year\ 1} = 1.11$ and $RMSE_{Year\ 2}$ = 0.91). For all cultivars, $r^2 \geq 0.93$ by internal validity test, while RMSE varied from 0.65 °C for Brianna in Year 2 to 1.30 °C for La Crescent in Year 1. The optimized model parameters for all cultivars and both years predicted LT_{50} values with an overall bias = 3.13×10^{-4} ($bias_{Year\ 1} = 0.21$ and $bias_{Year\ 2} = -0.21$). In general, the model slightly underpredicted LT_{50} values in Year 1 and slightly overpredicted LT_{50} values in Year 2. Individual cultivar parameters are listed in Table 5 and performance is illustrated in Figure 2 and 3.

Discussion

This is the first study to report continuous, time series-based bud cold hardiness measurements for CCIHG cultivars grown in a cold climate region. The main objective of this study was to evaluate changes in bud cold hardiness of CCIHG cultivars during the dormant period, with the goal of identifying periods of higher risk of incurring freeze damage. Bud cold hardiness patterns observed in CCIHG had a similar U-shaped as those previously reported for *V. vinifera* and wild North American

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species (Pool et al. 1990, Wolf and Cook 1992, Kovács et al. 2003, Fennell 2004, Mills et al. 2006, Grant and Dami 2015, Cragin et al. 2017). However, CCIHG cultivars present noteworthy differences within the classic U-shaped pattern.

Acclimation

The CCIHG cultivars in this study acclimated before experiencing freezing temperatures. During Year 1, these cultivars had LT_{50} values ranging from -16.7 to -18.6 °C within 5 days of the first frost. During Year 2, these cultivars had LT_{50} values ranging from -10.7 to -14.0 °C on the day of the first frost (Figure 1). This is consistent with reports that gradually decreasing daylengths and photoperiods less than 13 hours promote cold acclimation in *V. labrusca* and *V. riparia*, while these phenomena do not promote acclimation in *V. vinifera* (Fennell and Hoover 1991, Wake and Fennell 2000, Fennell 2004). It is possible that the synergistic effect of shorter photoperiod and cooler, but not freezing, temperatures during the late summer and early fall in our study area promote faster acclimation rates in CCIHG cultivars than in other areas with warmer falls. In Wisconsin, the fast acclimation rate of CCIHG, plus the low incidence of hard freeze events (<-2.2 °C) before mid-October ("Freeze Maps - MRCC" 2020), result in a relatively low risk of freeze damage during fall for these cultivars.

Midwinter cold hardiness

The lowest LT_{50} values for these CCIHG cultivars ranged from -28.9 to -30.6 °C in Year 1 and -27.1 to -29.7 °C in Year 2 and were recorded in both years during midwinter (Figure 1). This is comparable to bud cold hardiness levels reported for wild *Vitis* species in northern North America, including *V. labrusca*, *V. riparia*, and *V. aestivalis*, that are able to withstand temperatures as low as -35 °C (Fennell 2004, Londo and Kovaleski 2017, Keller 2020). This is not surprising given that CCIHG cultivars include wild *Vitis* species in their complex genetic background (Maul et al. 2020), and that the

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focus of CCIHG breeding programs is to release cultivars to be grown in regions where mid-winter temperatures regularly reach -25 °C and colder.

On January 30 and 31, 2019, minimum temperatures were lower than the LT_{50} measured for all cultivars (most recently tested on January 16), when a polar vortex brought record low temperature across the US Midwest region ("NOAA Online Weather Data" 2020). Temperatures at our study site reached -32.9 °C twice within 48 hours and remained below -25 °C for 37 consecutive hours, including 15 hours below -30 °C. No LTEs were detected from the DTA performed the day after the lowest temperature was recorded (vertical dotted line in Figure 1), which led us to believe buds had been damaged in the field when the temperature was lower than their supercooling capacity. However, there was only 10.5% and 2.5% damage in primary meristems and secondary meristems, respectively found in bud dissections performed on a subset of the buds. It is possible that the long exposure to temperatures below -30 °C resulted in freeze dehydration of the buds, lowering the water content inside the buds, thus leading to an increase in freezing stress resistance. This phenomenon has been described by Kasuga et al. (2020) in interspecific hybrid grapes grown in northern Japan. In that study, buds conditioned to -15 °C for 12 hours experienced partial dehydration of primordial cells, as revealed by cryo-scanning electron microscopy, resulting in a lowering of the buds' median freezing temperature (Kasuga et al. 2020). Similarly, DTA-based studies of *V. riparia* buds did not produce LTEs following prolonged exposure to extreme cold conditions and low relative humidity, which may indicate the loss of all freezable water (Pierquet et al. 1977, Pierquet and Stushnoff 1980). In our conditioning experiments conducted less than two weeks after the extreme low temperature event, no LTEs were detected in the DTA performed after the conditioning protocol and no visual symptoms of freezing damage were observed in bud dissections after allowing expression of damage symptoms (data not shown). DTA is

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widely used in the scientific community to assess cold hardiness of grape buds. However, its exclusive use to monitor changes in bud freezing stress resistance of existing and future releases of CCIHG cultivars adapted to cold climate regions may underestimate their true cold hardiness potential due to their buds' ability to partially dehydrate when exposed to prolonged and extreme cold temperature conditions (e.g., 15 hours below -30 °C). Future studies aiming to quantify the full extent of grapevine midwinter cold hardiness should include controlled freezing tests and visual evaluation of freeze damage in buds (e.g., oxidative browning or water-soaked appearance) to complement DTA (Villouta et al. 2020).

Deacclimation

Grapevines in our study began deacclimating in early February 2018 and March 2019, and from this point, buds lost hardiness (Figure 1). One exception to this general trend was in Year 1 when buds deacclimated in early January but then reacclimated by the time of the first hardiness evaluation in February (Figure 1). Although many factors can affect deacclimation dynamics (Kalberer et al. 2006), the completion of endodormancy appears to be a key factor influencing the onset of deacclimation (Ferguson et al. 2011, 2014). The fulfilment of chilling requirements marks the transition from endo- to ecodormancy (Lang et al. 1987), and is considered to happen in *V. riparia*, *V. vinifera, V. labrusca* and some interspecific hybrids after exposure to 750-1000 chilling hours (Londo and Johnson 2014). In our study, vines experienced 750 chilling hours by February and January in Year 1 and Year 2, respectively. The attainment of 1000 chilling hours by late March in both years coincided with the onset of deacclimation. However, in unpublished data on chilling requirements for these CCIHG cultivars collected by our research group, endodormancy was complete when 400-500 chilling hours had been accumulated, which typically occurs November - mid December in our region. Potential for

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deacclimation could begin as early as December for CCIHG cultivars grown in Wisconsin. However, deacclimation does not occur during this point in ecodormancy until vines are exposed to temperatures above freezing.

We also observed an increase in the deacclimation rate from late March throughout April in both years. In Year 1, deacclimation rates increased from 0.12-0.14 °C/day to 0.26-0.83 °C/day during this time period. In Year 2, deacclimation rates increased from 0.38-0.53 °C/day to 0.46-0.63 °C/day. Kovaleski et al. (2018) established that grapevine bud deacclimation rate increases in a logistic relationship as more chilling is accumulated. During the deacclimation period from late March through April, air temperatures were between 0-7.2 °C about 37% and 55% of the time for Year 1 and Year 2, respectively. This additional chilling accumulated during ecodormancy likely increases deacclimation potential, meaning deacclimation responses happen at cool temperatures above 0 °C.

Cultivar differences

Cultivar differences in cold hardiness responsiveness can be compared using the explanatory model because Cultivar was a significant parameter in the final model (Table 3). Cultivar-specific estimates for a particular parameter quantify differences in aspects of cold hardiness responsiveness across cultivars. Overall, Petite Pearl and Brianna bud cold hardiness had relatively low levels of responsiveness to temperature fluctuations, compared to La Crescent, the cultivar with the highest responsiveness, and to Marquette and Frontenac, which had moderate responses to temperature fluctuation, reflected in their respective σ_T estimates (Table 3). In terms of acclimation and deacclimation responses, La Crescent and Brianna had relatively fast responses, reflected in their high Time and $Time^2$ estimate values, while Petite Pearl and Frontenac had slow acclimation and deacclimation responses, compared to the other cultivars (Table 3).

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Grape bud cold hardiness prediction

The secondary objective of this study was to test and adapt the discrete-dynamic cold hardiness prediction model developed by Ferguson et al. (2011, 2014) for CCIHG cultivars grown in cold climate regions. The ecodormant temperature thresholds above 2 °C in the original version of the model limited the accurate estimation of deacclimation during late winter and early spring for the CCIHG cultivars, and thus over-predicted cold hardiness once deacclimation began. Reparametrizing the model with an expanded range for the ecodormant temperature threshold parameter (including -1, 0, 1, and 2 °C) was critical to improve the performance of the prediction model (Figure 2 and 3). The warmest ecodormant temperature threshold parametrized was for Brianna and Frontenac (1 °C), the coldest was for Marquette (-1 °C), and an intermediate threshold for La Crescent and Petite Pearl (0 °C). Lower ecodormant temperature thresholds than those used by Ferguson et al. allowed the model to accurately calculate deacclimation during cool spring temperatures (Figure 2). Deacclimation at lower temperatures in preparation for bud break could be an important ecological adaptation by CCIHG to maximize their use of shorter growing seasons. However, it also increases the risk of freezing damage for CCIHG buds throughout spring. After reparametrizing with an expanded range of ecodormant temperature thresholds, the overall performance of the predictive model had a RMSE = 1.01 °C, r^2 = 97%, and bias = 3.13×10^{-4}. While these are exceptional model statistics that provide evidence for the possibility to adapt the discrete-dynamic cold hardiness prediction model for CCIHG cultivars, our results are partially a consequence of the limited number of years in our dataset. This model requires further optimization and testing for CCIHG using longer-term cold hardiness datasets collected in the future.

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Conclusion

This is the first study to evaluate continuous changes in bud cold hardiness for CCIHG grown in a cold climate region. These CCIHG cultivars had an early acclimation response during fall, with increased levels of cold hardiness before the occurrence of freezing temperatures, which reflects the decreased risk of freezing damage during the fall in regions with similar fall conditions to southern Wisconsin. Although these CCIHG cultivars maintained deep levels of cold hardiness throughout midwinter, the higher frequency of extreme weather events due to climate change may increase the risk of freezing damage during midwinter. The highest risk of freezing damage to CCIHG is during spring, due to the rapid deacclimation response once air temperatures rise above freezing. This trait should be considered when evaluating future releases of CCHIG cultivars for cold climate regions.

Following an extreme cold weather event during our study, we observed a cold hardiness response that presumably leveraged the mechanisms of both deep supercooling and freeze dehydration, which allowed these hybrids to achieve a deeper level of freeze stress resistance than previously evaluated. Further research to provide direct or further supporting evidence of the physiological mechanism underlying this interplay between deep supercooling and freeze dehydration will provide critical information for the breeding of new CCIHG cultivars.

Finally, our predictive model demonstrates that the discrete-dynamic model can be reparametrized to predict CCIHG cultivars' cold hardiness in a cold climate region. This model and the CCIHG-specific parameters will be a useful tool in the prediction of CCIHG cold hardiness responses to variable climate scenarios and for the evaluation of new sites before planting vineyards, as well as providing assistance to growers in decision-making to minimize yield and vine losses based on freeze damage risk.

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Literature Cited

Atucha A, Hedtcke J, Workmaster BA. 2018. Evaluation of cold-climate interspecific hybrid wine grape cultivars for the upper midwest. J Am Pomol Soc 72:80–93.

Cragin J, Serpe M, Keller M, Shellie K. 2017. Dormancy and cold hardiness transitions in winegrape cultivars chardonnay and cabernet sauvignon. Am J Enol Vitic 68:195–202.

Dami I, Lewis D. 2014. 2014 Grape Winter Damage Survey Report. Ohio State University.

Dami I, Bordelon B, Ferree DC, Brown M, Ellis MA, Williams RN, Doohan D. 2005. Midwest Grape Production Guide. Ohio State University Extension: Bulletin 919.

Fennell A. 2004. Freezing tolerance and injury in grapevines. J Crop Improv 10:201–235.

Fennell A, Hoover E. 1991. Photoperiod influences growth, bud dormancy, and cold acclimation in Vitis labruscana and V. riparia. J Am Soc Hort Sci 116:270–273.

Ferguson JC, Tarara JM, Mills LJ, Grove GG, Keller M. 2011. Dynamic thermal time model of cold hardiness for dormant grapevine buds. Ann Bot 107:389–396.

Ferguson JC, Moyer MM, Mills LJ, Hoogenboom G, Keller M. 2014. Modeling dormant bud cold hardiness and Budbreak in twenty-three Vitis genotypes reveals variation by region of origin. Am J Enol Vitic 65:59–71.

Freeze Maps - MRCC. 2020. (accessed December 29, 2020). as found on the website (https://mrcc.illinois.edu/VIP/frz_maps/area_150.html#frzMaps).

Goffinet MC. 2004. Anatomy of Grapevine Winter Injury and Recovery. Cornell University.

Grant TNL, Dami IE. 2015. Physiological and biochemical seasonal changes in vitis genotypes with contrasting freezing tolerance. Am J Enol Vitic 66:195–203.

Kalberer SR, Wisniewski M, Arora R. 2006. Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts. Plant Sci 171:3–16.

Kasuga J, Tsumura Y, Kondoh D, Jitsuyama Y, Horiuchi R, Arakawa K. 2020. Cryo-scanning electron microscopy reveals that supercooling of overwintering buds of freezing-resistant interspecific hybrid grape ‘Yamasachi’ is accompanied by partial dehydration. J Plant Physiol 253:1–6.

Keller M. 2020. *The Science of Grapevines*. Academic Press, an imprint of Elsevier. London.

Kovács LG, Byers PL, Kaps ML, Saenz J. 2003. Dormancy, cold hardiness, and spring frost hazard in Vitis amurensis hybrids under continental climatic conditions. Am J Enol Vitic 54:8–14.

Kovaleski AP, Reisch BI, Londo JP. 2018. Deacclimation kinetics as a quantitative phenotype for

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2021.21001
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

delineating the dormancy transition and thermal efficiency for budbreak in Vitis species. AoB Plants 10:1–12.

Lang GA, Early JD, Martin GC, Darnell RL. 1987. Endo-, Para-, and Ecodormancy: Physiological Terminology and Classification for Dormancy Research. HortScience 22:371–377.

Londo JP, Johnson LM. 2014. Variation in the chilling requirement and budburst rate of wild Vitis species. Environ Exp Bot 106:138–147.

Londo JP, Kovaleski AP. 2017. Characterization of Wild North American Grapevine Cold Hardiness Using Differential Thermal Analysis. Am J Enol Vitic 68:203–212.

Luby J, Fennell A. 2006. Fruit breeding for the northern great plains at the University of Minnesota and South Dakota State University. HortScience 41:25–26.

Maul E, Töpfer R, Röckel F, Brühl U, Hundemer M, Mahler-Ries A, Walk M, Kecke S, Wolck A, Ganesch A. 2020. Vitis International Variety Catalogue. as found on the website (www.vivc.de).

Mills LJ, Ferguson JC, Keller M. 2006. Cold-hardiness evaluation of grapevine buds and cane tissues. Am J Enol Vitic 57:194–200.

National Centers for Environmental Information. 2020. (accessed December 29, 2020). as found on the website (https://www.ncdc.noaa.gov/).

NEWA. 2020. (accessed December 29, 2020). as found on the website (http://www.newa.cornell.edu/).

NOAA Online Weather Data. 2020. (accessed December 29, 2020):Applied Climate Information System. as found on the website (https://w2.weather.gov/climate/xmacis.php?wfo=mkx).

Pierquet P, Stushnoff C. 1980. Relationship of Low Temperature Exotherms to Cold Injury in Vitis Riaria Michx. Am J Enol Vitic 31:1–6.

Pierquet P, Stushnoff C, Burke M. 1977. Low Temperature Exotherms in Stem and Bud Tissues of Vitis riparia Michx. J Am Soc Hortic Sci 102:54–55.

Pool R, Reisch B, Welser M. 1990. Use of differential thermal analysis to quantify bud cold hardiness of grape selections and clones. Vitis 29:318–329.

Smiley L, Cochran D. 2016. A Review of Cold Climate Grape Cultivars. Iowa State University Extension and Outreach.

Tuck B, Gartner W, Appiah G. 2017. Economic Contribution of Vineyards and Wineries of the North, 2015. University of Minnesota Extension.

Villouta C, Workmaster BA, Bolivar-Medina J, Sinclair S, Atucha A. 2020. Freezing stress survival mechanisms in Vaccinium macrocarpon Ait. terminal buds. Tree Physiol 40:841–855.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2021.21001
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

498 Wake CMF, Fennell A. 2000. Morphological, physiological and dormancy responses of three Vitis
499 genotypes to short photoperiod. Physiol Plant 109:203–210.

500 Warmund MR, Guinan P, Fernandez G. 2008. Temperatures and cold damage to small fruit crops across
501 the eastern United States associated with the April 2007 freeze. HortScience 43:1643–1647.

502 Web Soil Survey - USDA NRCS. 2020. (accessed December 29, 2020). as found on the website
503 (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx).

504 Williams L, Dokoozlian N, Wample R. 1994. Grape. *In* Handbook of Environmental Physiology of Fruit
505 Crops, vol. I Temperate Crops. B Schaffer and P Andersen (eds.), pp. 85–133. CRC Press.

506 Wisniewski M, Willick I, Gusta LV. 2017. Freeze Tolerance and Avoidance in Plants. In *Plant Stress*
507 *Physiology*. S Shabala (ed.), pp. 279–299. CAB International. Oxfordshire.

508 Wolf TK, Cook MK. 1992. Seasonal Deacclimation Patterns of Three Grape Cultivars at Constant,
509 Warm Temperature. Am J Enol Vitic 43:171–179.

510 Zabadal TJ, Dami IE, Goffinet MC, Martinson TE, Chien ML. 2007. Winter Injury to Grapevines and
511 Methods of Protection. Michigan State University Extension: Bulletin E2930.

512

513

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Table 1 Symbols, abbreviations, and units of measurement used in explanatory and predictive models.

Abbreviation	Definition	Unit
B	Bias or mean error	°C
EDB	Ecodormancy boundary, accumulation of chilling degree days required to transition from endo- to ecodormancy	°C
$H_{c,\ initial}$	Initial cold hardiness	°C
$H_{c,\ max}$	Maximum hardiness (most hardy condition)	°C
$H_{c,\ min}$	Minimum hardiness (least hardy condition)	°C
$k_{a,\ eco}$	Acclimation rate during ecodormancy	°C/°C
$k_{a,\ endo}$	Acclimation rate during endodormancy	°C/°C
$k_{d,\ eco}$	Deacclimation rate during ecodormancy	°C/°C
$k_{d,\ endo}$	Deacclimation rate during endodormancy	°C/°C
LT_{10}	Temperature lethal to 10% of buds sampled	°C
LT_{50}	Temperature lethal to 50% of buds sampled	°C
LT_{90}	Temperature lethal to 90% of buds sampled	°C
RMSE	Root mean square error	°C
$T_{th,\ eco}$	Threshold temperature to calculate degree days during ecodormancy relevant to changes in hardiness	°C
$T_{th,\ endo}$	Threshold temperature to calculate degree days during endodormancy relevant to changes in hardiness	°C
θ	Theta exponent in deacclimation logistic equation	dimensionless
σ_T	Sigma-T, temperature index in the explanatory model	°C

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Table 2 Parameter levels tested in all combinations stepwise for five cold climate interspecific hybrid grapevine cultivars to minimize root mean square error (RMSE): endodormant temperature threshold ($T_{th,endo}$), ecodormant temperature threshold ($T_{th,eco}$), endodormant acclimation rate ($k_{a,endo}$), ecodormant acclimation rate ($k_{a,eco}$), endodormant deacclimation rate ($k_{d,endo}$), ecodormant deacclimation rate ($k_{d,eco}$), theta exponent in deacclimation logistic equation (θ), and ecodormancy boundary (EDB). A total of 2,976,750 combinations were tested per cultivar and for two years (2017-18 and 2018-19).

	$T_{th,endo}$ (°C)	$T_{th,eco}$ (°C)	$k_{a,endo}$ (°C/°C)	$k_{a,eco}$ (°C/°C)	$k_{d,endo}$ (°C/°C)	$k_{d,eco}$ (°C/°C)	θ	EDB (°C)
Start	9.0	-1.0	0.04	0.02	0.02	0.04	1.0	-300
Stop	15.0	7.0	0.16	0.10	0.10	0.20	7.0	-800
Step	1.0	1.0	0.02	0.02	0.02	0.02	2.0	100
n	7	9[a]	7	5	5	9	5[b]	6

[a] Levels for $T_{th,eco}$ started at -1.0 as compared to 3.0 in Ferguson et al. (2014).
[b] $\theta = 1.5$ was also tested.

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Table 3 Estimates for parameters and interactions for the explanatory linear regression model describing low temperature exotherms for five cold climate interspecific hybrid grapevine cultivars during two years using the temperature index (σ_T), Time (days), and Time^2 (days^2) as parameters. Parameters separated by a colon represent interactions between two parameters. Different letters within a column denote statistical differences between cultivars using a t-test with $\alpha = 0.05$.

	Intercept	σ_T	Time^2	Time	Interaction (σ_T:Time^2)	Interaction (σ_T:Time)
Year 1 (2017-18)						
Brianna	-3.4968 ± 0.9976 a	0.0154 ± 0.0032 c	0.0013 ± 0.0001 a	-0.3523 ± 0.0169 a	$1.03\times10^{-6} \pm 1.60\times10^{-7}$	$-1.90\times10^{-4} \pm 4.06\times10^{-5}$
Frontenac	-5.9346 ± 0.9943 b	0.0169 ± 0.0032 bc	0.0010 ± 0.0001 c	-0.2964 ± 0.0170 bc		
La Crescent	-5.7240 ± 1.0104 b	0.0218 ± 0.0032 a	0.0011 ± 0.0001 b	-0.3127 ± 0.0173 b		
Marquette	-5.8606 ± 1.0065 b	0.0184 ± 0.0032 b	0.0011 ± 0.0001 b	-0.3054 ± 0.0171 bc		
Petite Pearl	-6.6509 ± 1.0174 b	0.0144 ± 0.0032 c	0.0010 ± 0.0001 c	-0.2838 ± 0.0174 c		
Year 2 (2018-19)						
Brianna	-4.6544 ± 0.9601 a	0.0154 ± 0.0032 c	0.0013 ± 0.0001 a	-0.3369 ± 0.0168 a	$1.03\times10^{-6} \pm 1.60\times10^{-7}$	$-1.90\times10^{-4} \pm 4.06\times10^{-5}$
Frontenac	-7.5035 ± 0.9555 bc	0.0169 ± 0.0032 bc	0.0010 ± 0.0001 c	-0.2810 ± 0.0168 bc		
La Crescent	-6.7422 ± 0.9679 b	0.0218 ± 0.0032 a	0.0011 ± 0.0001 b	-0.2973 ± 0.0171 b		
Marquette	-8.0404 ± 0.9654 c	0.0184 ± 0.0032 b	0.0011 ± 0.0001 b	-0.2899 ± 0.0169 bc		
Petite Pearl	-7.6518 ± 0.9822 bc	0.0144 ± 0.0032 c	0.0010 ± 0.0001 c	-0.2683 ± 0.0172 c		

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Table 4 Relative contribution of parameters in the explanatory linear regression model calculated using dominance analysis. The temperature index is represented by σ_T. Parameters separated by a colon represent interactions between two parameters.

Relative importance:					
σ_T	50.1%	$Time^2$:Year	0.7%	Cultivar	0.3%
$Time^2$	18.0%	$Time^2$:Cultivar	0.6%	σ_T:Time	0.3%
Time	16.4%	σ_T:Cultivar	0.5%	Cultivar:Year	0.2%
Year	1.8%	σ_T:$Time^2$	0.4%		

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Table 5 Parameter value combinations that minimize root mean square error (RMSE) after reparametrizing the predictive discrete-dynamic model to simulate bud cold hardiness for five cold climate interspecific hybrid grapevine cultivars. Cold hardiness (H_c) predictions begin at $H_{c,\ initial}$, which is the earliest median low temperature exotherm (LT_{50}) observed. Predictions are bound by lower ($H_{c,\ max}$) and upper ($H_{c,\ min}$) limits. $H_{c,\ max}$ is the lowest LT_{50} observed, while $H_{c,\ min}$ is highest LT_{50} observed. Other parameters include: endodormant temperature threshold ($T_{th,endo}$), ecodormant temperature threshold ($T_{th,eco}$), endodormant acclimation rate ($k_{a,endo}$), ecodormant acclimation rate ($k_{a,eco}$), endodormant deacclimation rate ($k_{d,endo}$), ecodormant deacclimation rate ($k_{d,eco}$), theta exponent in deacclimation logistic equation (θ), and ecodormancy boundary (EDB).

Cultivar	$T_{th,\ endo}$ (°C)	$T_{th,\ eco}$ (°C)	$k_{a,\ endo}$ (°C/°C)	$k_{d,\ endo}$ (°C/°C)	$k_{a,\ eco}$ (°C/°C)	$k_{d,\ eco}$ (°C/°C)	θ	EDB (°C)	$H_{c,\ initial}$ (°C)	$H_{c,\ max}$ (°C)	$H_{c,\ min}$ (°C)
Brianna	9	1	0.10	0.02	0.10	0.16	7	-600	-11.0	-29.9	-9.0
Frontenac	12	1	0.08	0.06	0.10	0.20	1.5	-300	-10.5	-30.6	-10.5
La Crescent	11	0	0.08	0.06	0.10	0.14	3	-600	-9.6	-30.3	-9.6
Marquette	13	-1	0.06	0.06	0.04	0.10	7	-600	-11.5	-29.3	-10.2
Petite Pearl	15	0	0.04	0.08	0.10	0.18	1	-600	-11.4	-28.9	-11.4

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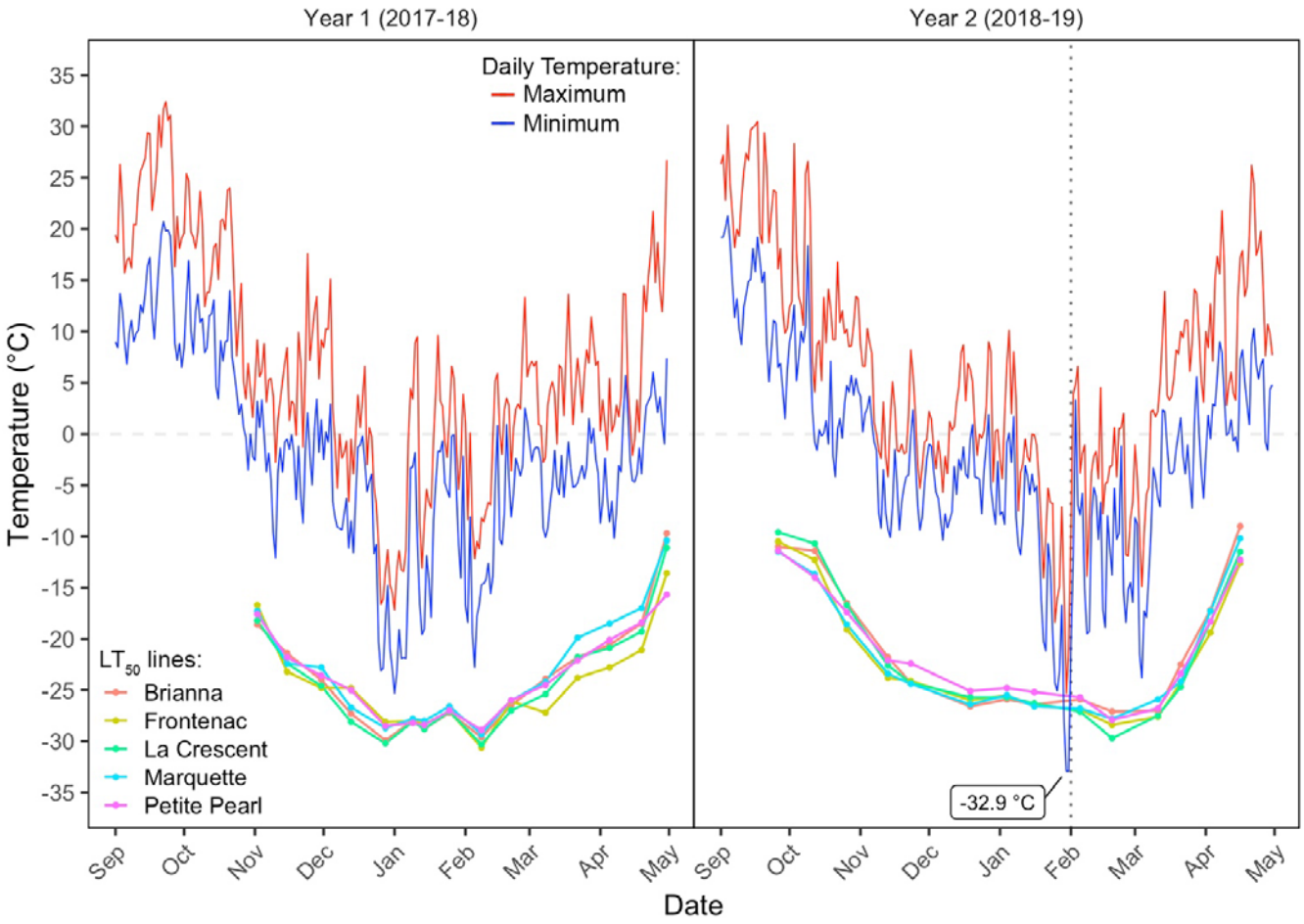

Figure 1 Median low temperature exotherm (LT_{50}) trends plotted for five cold climate interspecific hybrid grapevine cultivars, with daily maximum (red line) and minimum (blue line) temperatures for 2017-18 and 2018-19. Vertical dotted line identifies the date that buds tested with differential thermal analysis showed no low temperature exotherms (February 1, 2019).

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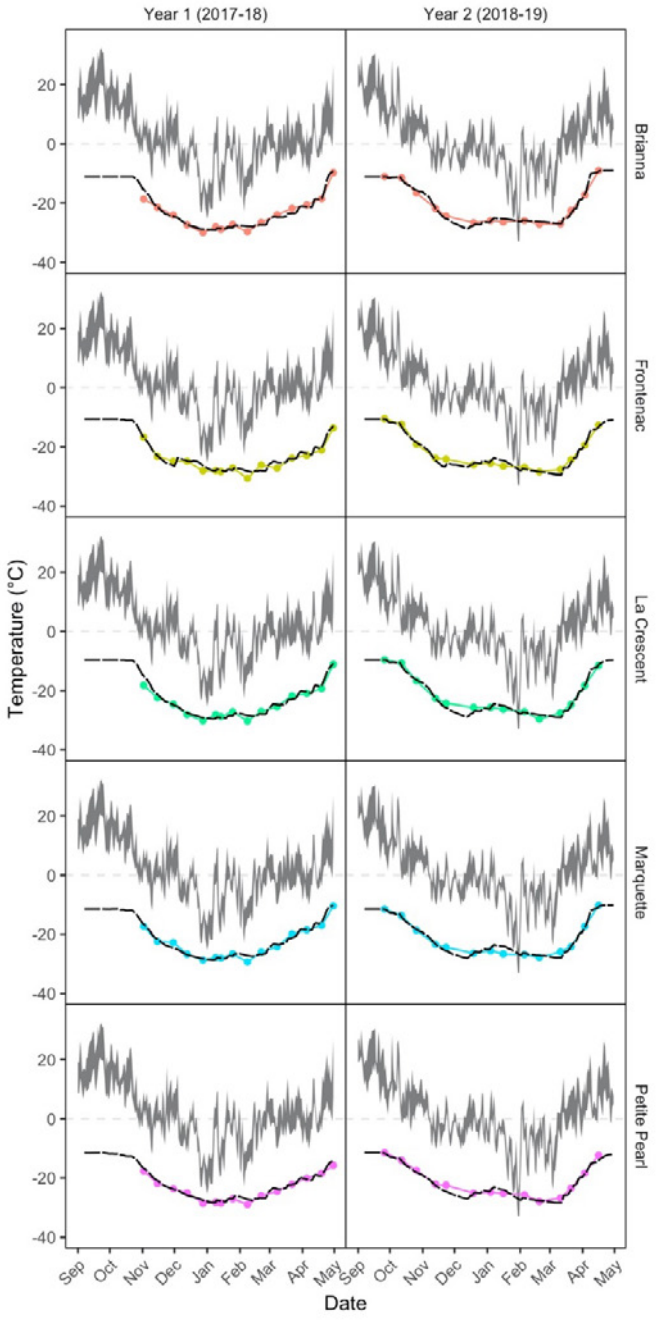

Figure 2 Predicted bud cold hardiness (black, dashed lines) and observed median low temperature exotherm (LT_{50}) values (colored lines and circles) plotted for five cold climate interspecific hybrid grapevine cultivars, with daily maximum and minimum temperature ranges for 2017-18 and 2018-19 (gray shaded area). Predictions were generated using the Ferguson et al. (2011, 2014) model reparametrized for each cultivar.

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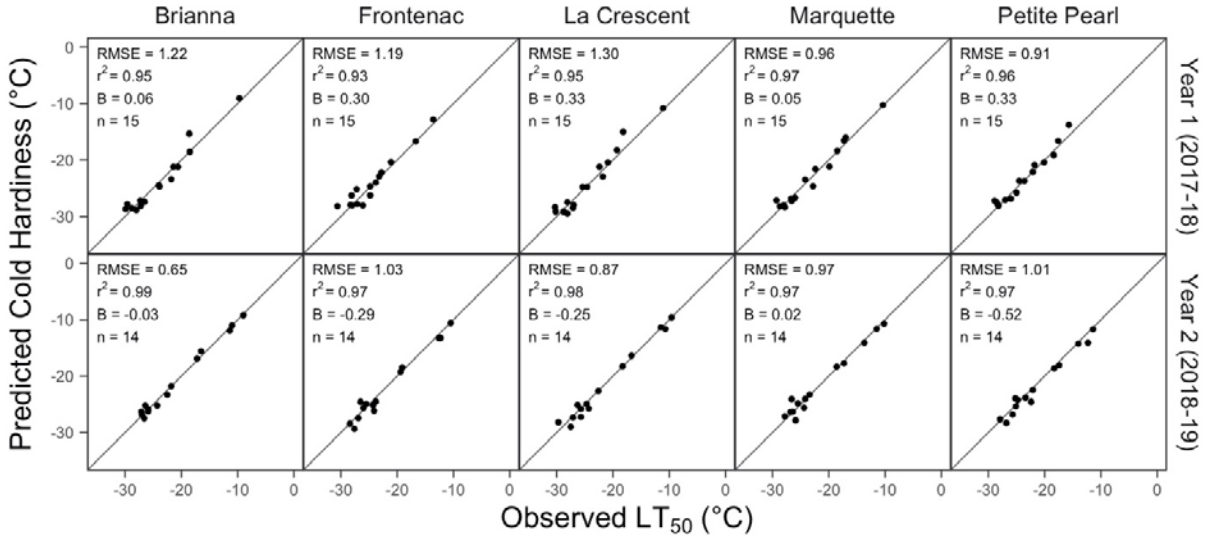

Figure 3 Comparison between predicted and observed bud cold hardiness, expressed as median low temperature exotherm (LT_{50}) for five cold climate interspecific hybrid grapevine cultivars, including r^2, bias (B), and sample size (n) for each cultivar and year. Predictions were generated using the Ferguson et al. (2011, 2014) model reparametrized for each cultivar.