

Southern Region Small Fruit Consortium

Final Report Research

Title: Assessing the cold hardiness of muscadine grapes with differential thermal analysis, electrolyte leakage, and field observations

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Public Abstract

Cold hardiness is the primary barrier to expanding muscadine grape production beyond the southeastern United States. Most muscadine cultivars sustain major injury below 10 °F, whereas some other *Vitis* species tolerate temperatures as low as -40 °F. Earlier differential thermal analysis (DTA) work suggested that buds of certain muscadine cultivars can equal the cold hardiness of notably hardy bunch grapes, raising the question of whether bud tissue is truly the limiting factor for the species' northern range. Although cane hardiness assessments using electrolyte leakage are common in grapevine cold hardiness research, such measurements have never been reported for muscadines. Over two winters, we collected bud and cane samples from muscadine and bunch grape cultivars in Arkansas, with parallel bunch grape cane samples collected in New York. DTA results indicated that midwinter bud hardiness of muscadines such as Mighty Fine™ and 'Carlos' was comparable to, and sometimes exceeded, that of hardy bunch cultivars 'Reliance' and 'Jupiter'. In contrast, electrolyte leakage data showed that 'Reliance' canes in Arkansas were consistently hardier than muscadine canes, and that 'Reliance' often achieved even greater cane hardiness in New York. This work provides the first electrolyte leakage-based characterization of muscadine cane hardiness and supports the conclusion that insufficient cane hardiness is the most likely physiological constraint preventing muscadine grapes from withstanding severe winter conditions.

Introduction

The areas where muscadine grapes can be successfully grown is limited by their lack of cold hardiness. Dearing (1938) suggested that vines should not be grown commercially in areas where winter low temperatures frequently dip below $-12\text{ }^{\circ}\text{C}$ ($10\text{ }^{\circ}\text{F}$), and definitely not in areas where temperatures reach below $-18\text{ }^{\circ}\text{C}$ ($0\text{ }^{\circ}\text{F}$). These recommendations have been cited frequently since then, with many researchers claiming that vine damage begins at $-12\text{ }^{\circ}\text{C}$ and mortality occurs at $-18\text{ }^{\circ}\text{C}$ (Ahmedullah and Himelrick 1990; Basiouny 2001; Hegwood and Himelrick 2001). However, other researchers have noted that muscadines have survived far lower temperatures, including $-23\text{ }^{\circ}\text{C}$ ($-9\text{ }^{\circ}\text{F}$) in Central North Carolina during the winter of 1984-1985. Muscadine cultivars vary in their cold hardiness. Poling et al. (1989) listed ‘Carlos’, ‘Doreen’, ‘Magnolia’, ‘Nesbitt’, and ‘Sterling’ the most hardy cultivars, while Clark and Moore (1990) found ‘Carlos’, ‘Dixiered’, ‘Magnolia’, and ‘Sterling’ were the hardiest cultivars, and Hoffman et al. (2020) listed ‘Black Beauty’, ‘Black Fry’, ‘Fry’, ‘Late Fry’, and ‘Noble’ as the cultivars with the most cold hardiness.

The primary site of University of Arkansas System Division of Agriculture (UA) Fruit Breeding program is at the Fruit Research Station (FRS) in Clarksville, AR. This site is located in the foothills of the Ozark Mountains (lat. $35^{\circ}31'5''\text{ N}$, long. $93^{\circ}24'12''\text{ W}$, USDA plant hardiness zone 7b) where mid-winter low temperatures are almost always below $-12\text{ }^{\circ}\text{C}$ ($10\text{ }^{\circ}\text{F}$), and often below $-18\text{ }^{\circ}\text{C}$ ($0\text{ }^{\circ}\text{F}$). Clark and Moore (1990) found that many vines planted in Clarksville, AR survived a winter low of $-21.6\text{ }^{\circ}\text{C}$ ($-7\text{ }^{\circ}\text{F}$) in 1989-1990. More recently, we have had the opportunity to evaluate muscadine cold injury and survival after low temperatures reached $-26\text{ }^{\circ}\text{C}$ ($-15\text{ }^{\circ}\text{F}$) February 16, 2021 and $-18\text{ }^{\circ}\text{C}$ ($-1\text{ }^{\circ}\text{F}$) on December 23, 2022. Distinct genotypic differences in cold hardiness were observed after the arctic vortex of 2021 with the processing cultivars ‘Carlos’ and ‘Noble’ and the fresh-market breeding selection AM-70 surviving $-26\text{ }^{\circ}\text{C}$ with little to no damage and producing full crops in the 2021 season. In contrast, mature vines of all fresh-market cultivars planted at the Clarksville, AR site sustained severe injury to cordons or were killed to the ground, including ‘Black Beauty’, ‘Doreen’, ‘Fry’, ‘Granny Val’, ‘Ison’, ‘Nesbitt’, ‘Sugargate’, ‘Summit’, ‘Supreme’, and ‘Tara’ (Worthington and McWhirt, 2022). Interestingly, many ‘Noble’ vines planted at FRS and in nearby commercial vineyards in Altus, AR have subsequently collapsed due to residual cold injury to trunks in 2021 and new damage inflicted in late December 2022, while ‘Carlos’ and AM-70 have continued to produce healthy crops.

Controlled studies on cold hardiness of muscadine grapes are limited. Clark et al. (1996) investigated the physiology of muscadine grape cold hardiness by conducting differential thermal analysis (DTA) to determine whether muscadine buds supercool. Mean low-temperature exotherms (MLTE) were calculated for buds excised from cuttings collected in November, December, and January from mature vines of ‘Carlos’, ‘Summit’, and ‘Mars’ planted in Clarksville, AR. ‘Mars’, was chosen as comparison cultivar because it is a cold hardy *Vitis* grape hybrid with *V. labrusca* in its pedigree. The bud hardiness of all three cultivars was similar throughout the study and increased from Nov. 5, 1993 to Jan. 7, 1994 as the vines acclimated to winter temperatures. The MLTEs for ‘Carlos’, ‘Summit’, and ‘Mars’ in January, 1994 were $-21.5\text{ }^{\circ}\text{C}$, $-23.4\text{ }^{\circ}\text{C}$, and $-22.4\text{ }^{\circ}\text{C}$, respectively (Clark et al. 1996). A follow-up study conducted with 11 muscadine cultivars and ‘Mars’ also found that all 11 muscadine cultivars had supercooled buds and performed similarly to ‘Mars’ (Clark and Watson 1998).

Despite these results indicating that muscadine grape buds supercool, muscadine cultivars show symptoms of winter injury after exposure to much milder temperatures than predicted based on these DTA results (Clark and Moore 1990; Worthington and Clark 2019) and are decidedly less cold hardy than ‘Mars’. Winter injury symptoms include reduced bud break, spur and cordon damage, trunk splitting, aerial roots, and whole vines killed to the ground (Worthington and Clark 2019). Based on these observations, it has been suggested that vine components other than buds limit the hardiness of muscadine grapes (Hegwood and Himelrick 2001). Electrolyte leakage (EL) tests of vascular tissue collected on different dates throughout the winter would be beneficial to assess the hardiness of this tissue and determine whether cultivars vary in their vascular hardiness. No EL tests of vascular hardiness have ever been published for muscadine grapes.

Materials and Methods

Plant materials

A total of nine cultivars were used in this experiment: four muscadine genotypes (Mighty Fine™, ‘Carlos’, ‘Supreme’, ‘Paulk’), three bunch grape genotypes (‘Jupiter’, ‘Reliance’, ‘Chardonnay’), and two muscadine-bunch grape hybrids (Oh My!®, E4-12). Vines were sourced from two locations for this experiment. The muscadines, muscadine hybrids, and bunch grape cultivars except for ‘Chardonnay’ were planted at the University of Arkansas Fruit Research Station in Clarksville, AR (35.529782, -93.405044). A second testing location for ‘Reliance’ was at the Cornell AgriTech research station in Geneva, NY (42.893183, -77.008658) and the ‘Chardonnay’ vines were located at a nearby commercial vineyard (42.843981, -77.003209). The Oh My!® vines used for this experiment died during the growing season of 2024 and were replaced with E4-12 for the second year of data collection since both are seedless muscadine-bunch grape hybrids from the Gardens Alive breeding program. Material from the Arkansas location was sent to Cornell AgriTech via overnight shipping for analysis.

Experimental Design and Data Collection

The study was conducted over the course of two winters (2023-2024 and 2024-2025). For bud hardiness measurements, collections were made every two weeks starting in early November until budbreak in the spring. Similarly, monthly stem hardiness measurements were also collected from November to budbreak. Low temperature exotherms (LTEs) were measured by placing 10 excised buds from each cultivar onto a moistened thermoelectric module. Three thermoelectric modules were used at each timepoint for each cultivar. The LTE peaks were visually called using the BudProcessor software (v.1.0, Brock University, St. Catherines, ON, CA).

For stem cold hardiness measurements, electrolyte leakage was performed using 9 temperatures below freezing (from -10 °C to -50 °C in 5 degree intervals) and one unfrozen control (4 °C). Tissue for this analysis was collected from dormant one-year old stems sections approximately 2.5 cm wide no smaller than 7mm wide. Stem tissue samples were collected from internodes to

avoid leakage from vegetative buds. Three replicate stem tissue samples were prepared for each cultivar at each timepoint. Each tissue sample was placed in a 50 mL centrifuge tube with 30 mL of distilled water and exposed to one of the 10 temperature treatments. The centrifuge tubes were left at room temperature to thaw and shaken for 24 hours to ensure thorough mixing of the sample. Electrical conductivity (reported in microsiemens (mS)) of each sample was taken using a Mantech Automax 402 autotitrator equipped with a Mantech 4510 conductivity meter . Samples were then placed in a -80 °C freezer overnight to fully kill the tissue and maximize membrane perforation and subsequent electrolyte leakage. Samples were again thawed and shaken, followed by a second conductivity reading. Hourly weather data for both years of data collection was collected at each site.

Data Filtering and Analysis

The index of injury (IoI) for each sample was calculated using a modified version of the methods found in Lim *et al.* (1998). In this method, the mean conductivity reading of the 3 unfrozen control samples for each genotype within a given sampling period was used as the theoretical minimum leakage for the remaining samples of that genotype. The conductivity reading for each sample after the -80 °C killing freeze was used as the maximum amount of conductivity from cold damage that sample could yield. The index of injury then calculates the proportion of damaged tissue using the mean unfrozen control conductivity as the conductivity for zero damage and the conductivity after the -80 °C as total tissue damage. Log-logistic regression curves were generated for each genotype at each timepoint using the R package *drc* by extracting the LT25 parameters for each curve.

Cold hardiness differences between cultivars and tissue types using the entire dataset (i.e., all timepoints throughout winter) were determined with a mixed-effects model with ‘timepoint’ as a nested effect within ‘experiment year’ modeling the LT25 or bud hardiness using the R package *lme4*. Mean separation was performed using the *emmeans* package using Tukey’s HSD to control the experiment-wise error rate for buds and the FDR method to control experiment-wise error rate for canes. A second model examining midwinter cold hardiness measurements (the timepoints for EL and DTA in January/February) was created with the same model architecture.

Results

Weather Data and Sampling Changes

During the 2023-2024 season, a mid-January polar vortex event affected central Arkansas. Temperatures were projected to fall to -20 °C, threatening the experiment if this winter storm proved lethal to some of the muscadine genotypes. To capture the effects of this unusual cold event, electrolyte leakage sampling was expanded from the planned single January timepoint to three: the scheduled collection, an additional pre-storm sample on January 13, and a post-storm

sample on January 24. This added sampling used more tissue than expected, leaving insufficient material for the planned February electrolyte leakage assessment. Actual temperatures during the polar vortex were slightly milder than predicted. Temperatures dropped below freezing on the afternoon of January 13 and did not rise above 0 °C until January 17. The minimum temperature reached –17.5 °C on January 16. Aside from this event, the 2023–2024 winter remained relatively warm: December highs occasionally reached 16 °C, and late-February highs exceeded 24 °C on three days. Sampling ended on March 13 due to budbreak in the bunch grape cultivars.

The 2024–2025 winter followed a similar warmer-than-average pattern. Temperatures stayed above 0 °C until December 2, and early- to mid-February highs again reached 24 °C before dropping to the seasonal minimum of –14.9 °C on February 20. As in the previous year, sampling ended in mid-March (March 19) following budbreak in the bunch grapes. Limited budwood availability reduced the frequency of ‘Reliance’ sampling in both Arkansas and New York: Arkansas vines were sampled biweekly until tissue was depleted in February, while New York vines were sampled monthly throughout the season.

Bud Hardiness - Arkansas

Across two dormant seasons and 20 sampling dates, more than 3,300 LTE peaks were recorded for ten cultivars. Because sampling schedules differed between years, winters were analyzed separately. In 2023–2024, muscadine cultivars Mighty Fine™ ‘Carlos’, ‘Paulk’, and ‘Supreme’ all reached mean bud hardiness levels below –18 °C during at least two of the three January–February sampling dates (Fig 1). ‘Carlos’ and Mighty Fine™ showed the greatest hardiness, remaining below –20 °C at all three January dates and reaching lows of –21.2 °C and –20.9 °C, respectively. ‘Supreme’ was the only muscadine cultivar that had not begun to swell or break bud by mid-March. In comparison, the bunch grapes ‘Jupiter’ and ‘Reliance’ reached mean bud hardiness below –20 °C at only one sampling date (January 3) (Fig. 2).

Patterns were similar in 2024–2025: ‘Carlos’ and Mighty Fine™ again exceeded –20 °C hardiness on both January dates, with lows of –21.6 °C (‘Carlos’) and –21.4 °C (Mighty Fine™) (Fig. 3). ‘Paulk’ never exceeded –18 °C during this season, while ‘Supreme’ reached –21.6 °C on one January date. ‘Reliance’ and ‘Jupiter’ showed their maximum midwinter hardiness on January 22 (–22.0 °C and –20.6 °C, respectively). Earlier in January, ‘Reliance’ was comparable to the hardiest muscadines (–20.2 °C), whereas ‘Jupiter’ was less hardy (–18.7 °C) (Fig. 4).

The seedless muscadine cultivars Oh My!® (tested in 2023–2024) and E4-12 (tested in 2024–2025) displayed hardiness levels similar to the pure muscadine cultivars (Fig. 2, 4). Oh My!® remained below –20 °C at all three January 2024 timepoints. E4-12 closely tracked the hardiness of ‘Carlos’ and Mighty Fine™ except at the January 8, 2025 sampling (–17.1 °C), and reached –23.2 °C on January 22—the lowest mean value recorded in Arkansas that season.

Bud Hardiness - New York

‘Chardonnay’ and ‘Reliance’ had similar cold hardiness levels throughout the midwinter 2023-2024 season, but ‘Chardonnay’ deacclimated at a faster rate during the final two timepoints (Figure 5). During the 2024-2025 season, low vine vigor in ‘Reliance’ allowed for only four bud collections during the season. These four timepoints still showed that ‘Reliance’ buds are capable of extreme winter hardiness. During the Feb. 18th, 2025 sampling timepoint, mean hardiness was -33 °C while ‘Chardonnay’ hardiness was -26.8 °C (Figure 6).

Whole-Winter and Mid-Winter Bud Hardiness Comparisons

The mixed-effects model comparing bud hardiness levels of the cultivars collected in Arkansas found multiple significant differences. Mighty Fine™ was the hardiest of the Arkansas material when compared across the entire winter (average bud hardiness of -16.4 °C) but was not significantly harder than ‘Carlos’ (-16.2 °C), E4-12 (-16.2 °C), Oh My!® (-15.9 °C), or Reliance (-15.8 °C). ‘Supreme’ and ‘Paulk’ had the weakest cold hardiness (-14.9 °C and -14.4 °C, respectively), and were significantly less hardy than all other cultivars tested except ‘Jupiter’, which was intermediately hardy at -15.2 °C. ‘Jupiter’ was, however, significantly less cold hardy than Mighty Fine™, ‘Carlos’, and E4-12 (Table 1). Significant differences were also present in the mixed-effects model comparing ‘Reliance’ and ‘Chardonnay’ collected in New York to the ‘Reliance’ collected from Arkansas. The New York ‘Reliance’ was over 5 °C harder than the Arkansas ‘Reliance’, with ‘Chardonnay’ approximately 4 °C harder on average than the Arkansas ‘Reliance’ (Fig. 5, 6).

To test differences between bud hardiness during midwinter, when each genotype would be at its most hardy, a second mixed-effects model was tested using only the material from Arkansas during January and February sampling dates over both winters. Similar to the whole-winter model, ‘Paulk’ was the weakest of the Arkansas material (-17.0 °C) and was significantly less hardy than every genotype but ‘Jupiter’ (-17.7 °C), which itself was significantly less hardy than every genotype but ‘Paulk’ and ‘Supreme’ (-18.2 °C). Two of the muscadine genotypes (Mighty Fine™ and ‘Carlos’) were significantly harder than ‘Reliance’ by 1.1 and 1.2 °C, respectively. The seedless muscadines E4-12 and Oh My!® were not significantly different from ‘Reliance’ (Table 1).

Cane Hardiness

Over 2,500 electrical conductivity readings were made while measuring grape cane cold hardiness over the two years of this experiment. In the same manner as the bud hardiness analysis, mixed-effects models were tested comparing LT25 values across cultivars for whole-winter hardiness, midwinter hardiness, and the New York material compared to ‘Reliance’ from Arkansas.

Arkansas Material - 2023-2024

Of the four tested muscadine cultivars in 2023-2024, Oh My![®] and ‘Supreme’ acclimated much slower than ‘Carlos’ and Mighty Fine[™], with neither cultivar’s LT25 value falling below -20 °C until mid-January of 2024. Mighty Fine[™] and ‘Carlos’ both acclimated steadily from the November to December timepoint followed by slow acclimation through the January timepoints, amounting to only a few degrees Celsius. The hardiness differences between the final January electrolyte leakage experiment and the final experiment of the season on March 1st demonstrated that muscadines also deacclimated at different rates: ‘Supreme’ and Mighty Fine[™] deacclimated very little (<1 °C) while Oh My![®] and ‘Carlos’ had undergone over 3 °C and 5 °C of deacclimation, respectively (Figure 7). ‘Reliance’ and ‘Jupiter’ had much steeper acclimation and deacclimation periods than any of the muscadines in the 2023-2024 winter. All the Arkansas material had cane LT25 values of approximately -15 °C at the November EL timepoint and no muscadine acclimated further than -23.4 °C (Mighty Fine[™], Jan. 18th) while both bunch grapes had calculated LT25 values below -30 °C. By the March 1st sampling point, ‘Jupiter’ had deacclimated approximately 15 °C, and ‘Reliance’ had deacclimated 10 °C (Figure 7).

Arkansas Material - 2024-2025

In the 2024-2025 dormant season, E4-12 and Mighty Fine[™] were the only of the four muscadine cultivars to have a measured LT25 value of less than -20 °C (Figure 8). At the Jan. 24th, 2025 sampling timepoint Mighty Fine[™] had a LT25 measurement of -23.8 °C while E4-12 was -23.1 °C. The acclimation and deacclimation periods were much shallower in the second experimental year for ‘Carlos’ and ‘Supreme’. From the November to the January timepoint, ‘Carlos’ acclimated 0.6 °C (from -18.3 °C to -18.9 °C) while ‘Supreme’ acclimated 1.9 °C (from -16.7 °C to -18.6 °C). While all cultivars had begun deacclimating By the March 21st, 2025 timepoint, ‘Carlos’ had a much higher deacclimation rate and had a much higher LT25 measurement than the other three muscadines. Similar to the muscadine stem hardiness curves for the 2024-2025 winter, acclimation and deacclimation was not as obvious as in 2023-2024, nor did stem hardiness levels reach the same levels as in 2023-2024. ‘Reliance’ was at its most cold hardy on the Dec. 20th, 2024 timepoint with an LT25 value of -24.2 °C, but ‘Jupiter’ was not at its most cold hardy until the Feb. 20th, 2025 timepoint at -21.2 °C. With only four timepoints for ‘Reliance’ we are unable to comment on its deacclimation rate during 2024-2025, but the deacclimation rate of ‘Jupiter’ closely matched that of ‘Carlos’ (Figure 7).

Cane Hardiness - New York Material

Upstate New York experienced mild winter conditions during the 2023-2024 dormant season, leading to relatively high LT25 values for ‘Chardonnay’ (> -25 °C) over the entire sampling period. ‘Reliance’ (NY) acclimated to -28 °C by the January timepoint. The winter storm that brought temperatures of -18 °C to the Clarksville experiment vineyard drove ‘Reliance’ (AR) stem hardiness to an LT25 below -30 °C, the only time during 2023-2024 that the hardiness curve for ‘Reliance’ (AR) crossed below the hardiness curve for ‘Reliance’ (NY). Hardiness

curves during the 2024-2025 season identified a period of moderate deacclimation for the NY vines from the December to the January sampling timepoint, followed by a strong acclimation pressure for both genotypes at the February timepoint. This strong acclimation led to a 15 °C difference in LT25 values for ‘Reliance’ (AR) and ‘Reliance’ (NY).

LT25 Statistical Comparisons

The mixed-effects model using LT25 estimates from the entire winter showed few statistically significant differences between the cultivars in this experiment. ‘Reliance’ was significantly hardier than all other genotypes besides E4-12, which was not significantly different from any of the genotypes (Table 2). The midwinter-only model, which used only the Arkansas material that had two years of data, found that both ‘Reliance’ and ‘Jupiter’ were significantly hardier than ‘Carlos’ and ‘Supreme’, but that only ‘Reliance’ was significantly more hardy than Mighty Fine™. For the mixed-effects model comparing the NY-collected material to ‘Reliance’ (AR), no statistically significant differences were found between the three genotypes, despite a 3.5 °C difference in LT25 values between ‘Reliance’ (NY) and ‘Reliance’ (AR).

Discussion

This experiment demonstrated important physiological differences between the cold hardiness of stem/bud tissues in cultivars from subgenus *Euvitis* and subgenus *Muscadinia*. Analysis conducted on the over 3,000 LTE peaks identified during this study found that, including data from the entire winter, there were no significant differences between the bud hardiness of muscadines and bunch grapes at the Arkansas location. When using only the midwinter bud sampling dates for statistical analysis, the two hardiest muscadine cultivars (Mighty Fine™ and ‘Carlos’) were actually more cold hardy than the bunch grape cultivars. By contrast, midwinter measurements of cane hardiness indicated that bunch grape material was more hardy than the muscadine cultivars.

Comparing MLTE to LT25 Values

Although the electrolyte leakage and differential thermal analysis methods both measure hardiness in degrees Celsius, direct comparisons of these methods are not always practical. Many studies measuring electrolyte leakage to determine cold hardiness use the LT50 (lethal temperature for 50% of tissue) as parameter of interest- but the biological relevance of LT50 measurements is unclear. In some experiments, LT50 measurements correlated with observed tissue browning damage from freeze treatments (Kovaleski and Grossman, 2021), but the association between the percentage of vascular tissue damage and vine survival itself is unclear. Reporting the LT25 or the LT50 as the primary parameter of interest varies between studies. The rationale for using one parameter over the other is rarely discussed and instances of both in the literature have been published for decades (Doullis et al., 1993, Lim et al., 1998). Methodological investigations into electrolyte leakage have found instances where an LT50 measurement can be extremely low, well below the reasonable hardiness of the plant species

being measured. This was the case in our experiment, where LT50 values were consistently too low to be biologically relevant for the bunch grape material- e.g., -41 °C for ‘Chardonnay’ in Feb. 2025, -49 °C for ‘Jupiter’ in Jan. 2024, or -47 °C for ‘Reliance’ in Feb. 2025. For muscadines, the evidence against using LT50 measurements for cane hardiness is even stronger: a midwinter freeze event that affected the Clarksville, AR sampling site in 2021 killed mature ‘Supreme’ vines in the vineyard when temperatures dropped to -26 °C (Conner and Worthington, 2022), while midwinter LT50s for ‘Supreme’ in this experiment were as low as -32.5 °C. The same freeze event brought -29 °C temperatures to another muscadine research vineyard in Fayetteville, AR, killing the Mighty Fine™ and ‘Carlos’ vines planted there. Similar to ‘Supreme’, both ‘Carlos’ and Mighty Fine™ had LT50s as low as -34.6 °C and -34.5 °C, respectively. Although previous studies have directly compared bud MLTE values and cane electrolyte leakage LT50 measurements (Horiuchi *et al.*, 2021; Jun *et al.*, 2021), we focused on the LT25 measurement to more closely represent the biological reality of grape cane hardiness. Comparing MLTE and LT25 values directly has its own complications: the MLTEs reported here are a measurement of only the average bud hardiness of the vine that correlate strongly with primary bud hardiness (Wolf and Cook, 1994), not a measurement of lethal temperatures like the LT25 values are estimated to be. In this experiment, we chose to analyze the methods separately.

Grape Bud Hardiness

Despite the limited native range of muscadines to the southeastern U.S., there were few differences in bud hardiness between the *Muscadinia* and *Euvitis* cultivars in this experiment. In fact, both Mighty Fine™ and ‘Carlos’ were nominally more cold hardy than ‘Reliance’, which is capable of surviving in upstate New York. Although ‘Reliance’ is a release from the University of Arkansas breeding program, it is the offspring of two cultivars released from the Cornell grape breeding program, has significant *V. labrusca* genetics in its pedigree, and has been demonstrated to survive winters even when temperatures fall below -30 °C (Clark, 2002; Moore, 1983). The ‘Reliance’ vines at the Arkansas testing location never had a mean bud hardiness below -22 °C, while the New York ‘Reliance’ vines had a minimum mean hardiness of -33 °C. This indicates that bud hardiness levels in Arkansas do not reach their full potential even during winters with instances of extreme weather like the winter storm in January of 2024. This trend is true for muscadines as well, however, given that at this same sampling location many of the genotypes tested in this experiment survived temperatures of -26 °C in 2022 (Conner and Worthington, 2022).

Similar bud hardiness levels between some muscadines and bunch grape cultivars has been observed in previous studies- once when Clark and Watson (1998) compared 11 muscadine cultivars to the bunch grape cultivar ‘Mars’. During their two midwinter sampling timepoints, the majority of muscadine cultivars were of equal or greater hardiness than ‘Mars’, which has been demonstrated to be quite cold hardy (Moore, 1985). At their February bud sampling, the

cultivar ‘Sugargate’ was actually significantly more cold hardy than ‘Mars’ (Clark and Watson, 1998).

Not all muscadine cultivars have equal bud hardiness to bunch grapes; both ‘Supreme’ and ‘Paulk’ were less hardy than ‘Reliance’. Both ‘Supreme’ and ‘Paulk’ are fresh-market varieties released from the University of Georgia muscadine breeding program located in Tifton, GA, where mid-winter temperatures are usually too mild to cause injury to muscadines (Conner, 2009; Conner, 2017, Hoffman *et al.*, 2020). In contrast, ‘Carlos’ and Mighty Fine™, which were bred in colder environments in North Carolina and Arkansas, are both much hardier than the Georgia-released varieties. Of the 5 muscadines significantly less hardy than the bunch grape cultivar ‘Mars’ during the January sampling point in Clark and Watson (1998), three were bred at the University of Georgia (‘Tara’, ‘Triumph’, and ‘Fry’) and a fourth cultivar (‘Loomis’) originated from central Mississippi (Conner, 2006; Lane and Owen, 1989). The work presented here not only replicates previous studies showing that ‘Carlos’ has high levels of bud hardiness, but also that selections from the University of Arkansas breeding program are achieving equal levels of cold hardiness, a primary goal of the breeding program.

Cane Hardiness

Despite anecdotal evidence for muscadine cane hardiness mentioned in previous publications and speculation about differences between cane and bud hardiness in muscadines, this study is the first experiment to measure muscadine cane cold hardiness (Clark and Watson, 1988; Conner and Worthington, 2022). Compared to buds, where hardiness levels between bunch grapes and the hardiest muscadines were equivalent, the electrolyte leakage data showed that bunch grapes were significantly hardier than muscadines in most circumstances. ‘Reliance’ was the hardiest of the tested material, averaging over 2 °C hardier than the next highest cultivar, E4-12. This is unsurprising given that ‘Reliance’ is grown in upstate New York, has survived winters temperatures below -34 °C, and is the offspring of NY-bred cultivars: ‘Suffolk Red’ and ‘Ontario’ (Einset, 1972, Hedrick, 1908).

Although the cane hardiness of ‘Reliance’ and E4-12 were not significantly different, E4-12 was only measured for a single year (2024-2025) and was not measured during the polar vortex of 2023-2024 when the differences in cane hardiness between muscadines and bunch grapes were most apparent. The statistical equivalence of cane hardiness between ‘Reliance’ and E4-12, despite an average 2.0 °C of difference over the 2024-2025 winter, is likely due to ‘Reliance’ not reaching its full acclimation potential during that winter and rather than evidence of E4-12 being capable of surviving extremely harsh winters. When midwinter timepoints alone were used for hardiness comparisons, the differences in cane hardiness between muscadines and bunch grapes is more stark. In this instance Oh My!® and E4-12 were excluded because of how few timepoints a single year of midwinter data yielded. ‘Reliance’, as expected, was much hardier than any of the muscadine material. Mighty Fine™ had the hardiest canes of the

muscadine material sampled was 4.8 °C less hardy than Reliance in January and February. ‘Jupiter’, while significantly hardier than both ‘Carlos’ and ‘Supreme’ (, was not significantly hardier than Mighty Fine™. Similar to ‘Reliance’, ‘Jupiter’ is also a release from the University of Arkansas grape breeding program with significant parentage from Cornell-released varieties (Clark and Moore, 1999). However, ‘Jupiter’ is noted as less hardy than ‘Reliance’ during extremely cold winters, and the New York varieties in its pedigree (such as ‘Seneca’ and ‘Glenora’) are considered to be only moderately hardy (Clark and Moore, 1999; Howe, 1933; Pool, 1977). It should be noted as well that no significant differences in cane hardiness were observed between the muscadines during midwinter, despite a 2.2 °C difference between Mighty Fine™ and ‘Supreme.’

Cane Hardiness as the Weak Link

The sampling before and after the polar vortex event in the 2023-2024 winter season further demonstrate that cane hardiness in muscadines is the limiting factor for winter survival. The extreme changes in winter temperature, punctuated by a winter low of -17.5 °C and nearly four straight days where temperatures did not rise above freezing, had essentially no effect on muscadine cane hardiness. By contrast, both ‘Reliance’ and ‘Jupiter’ acclimated strongly (approximately 6 °C and 10 °C, respectively) over this two week period. The results from this study represent an important change in our understanding of how cold hardiness varies across tissues in muscadines, with implications for future breeding and management efforts. In subgenus *Euvitis* grapes, canes are generally 3-5 °C more cold hardy than vegetative buds (Jones *et al.*, 1998; Miller *et al.*, 1988), therefore the primary focus for breeding efforts and climate modeling have been on vegetative bud hardiness. Although muscadines are less hardy than hardy than *Euvitis* species, previous research has indicated that, over a single winter and only a few timepoints, some muscadine cultivars are equal in bud hardiness to bunch grapes (Clark *et al.*, 1996; Clark and Watson, 1998). Our results indicated that the hardiest muscadines are actually have slightly hardier vegetative buds than the bunch grape cultivars during the midwinter months, while the electrolyte leakage results showed exactly the opposite. This confirms the suspicions of Conner and Worthington (2022), that muscadine hardiness is in fact limited by weak cane tissues. Besides previous investigations into bud hardiness, anecdotal evidence for this has been observed in Arkansas vineyards after harsh winters: some muscadine vines grow extensive masses of aerial roots from the cordons, indicating a hormonal balance and vascular discontinuity (Margaret Worthington, Personal Communication). This research demonstrates that muscadine cane tissues are the ‘weak link’ in their dormant season physiology, greatly limiting their native range. Future investigations into muscadine cold hardiness, as well as efforts to improve muscadine cold hardiness, should focus on cane hardiness in place of vegetative bud hardiness as in bunch grapes.

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Table 1. Statistical analysis of bud hardiness for the Arkansas-collected material combined over two seasons. The first table shows mean bud hardiness from all collection dates, while the second table shows mean bud hardiness only from sampling dates during January and February. Means followed by different letters are significantly different at the $p = 0.05$ level using Tukey's HSD.

Cultivar	Whole-Winter Mean	Group		Cultivar	Midwinter Mean	Group
Mighty Fine™	-16	a		Mighty Fine™	-19.8	a
Carlos	-15.8	a		Carlos	-19.7	a
E4-12	-15.7	a		Oh My!®	-19.2	ab
Oh My!®	-15.7	a		E4-12	-19.2	ab
Reliance	-15.5	a		Reliance	-18.6	b
Jupiter	-14.7	b		Supreme	-18.2	bc
Supreme	-14.5	bc		Jupiter	-17.7	cd
Paulk	-14	c		Paulk	-17	d

Table 2. Statistical analysis of cane hardiness for the Arkansas-collected material combined over two seasons. The first table shows mean cane hardiness from all collection dates, including the cultivars Oh My!® and E4-12, which were only collected in 2023-2024 and 2024-2025, respectively. The second table shows the mean hardiness from the January and February collection dates, including only those cultivars that were collected in Arkansas for two years. Means followed by different letters are significantly different at the $p = 0.05$ level using the FDR method.

Cultivar	Whole-Winter Mean	Group		Cultivar	Midwinter Mean	Group
Reliance	-22.9	a		Reliance	-25.9	a
E4-12	-20.8	ab		Jupiter	-24	ab
Mighty Fine™	-19.4	b		Mighty Fine™	-21.1	bc
Jupiter	-19.3	b		Carlos	-19.5	c
Carlos	-17.4	b		Supreme	-18.9	c
Supreme	-17.2	b				
Oh My!®	-16.2	b				

Muscadine Bud Hardiness: 2023-2024

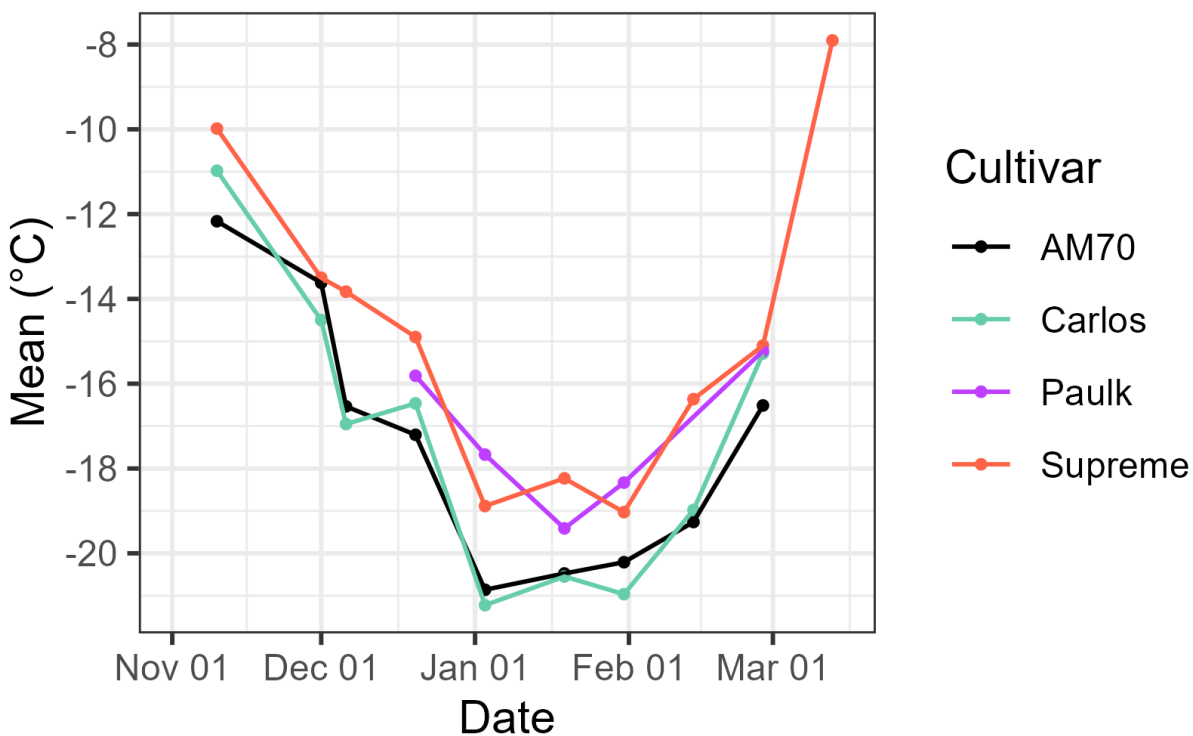


Figure 1. Cold hardiness of vegetative muscadine buds measured using differential thermal analysis from November 2023 to March 2024. Both 'Carlos' and Mighty Fine™ (AM-70) had mean bud hardiness levels below -20 °C during at 3 of the midwinter timepoints, while 'Paulk' and 'Supreme' were less cold hardy at those sampling points.

Grape Bud Hardiness: 2023-2024

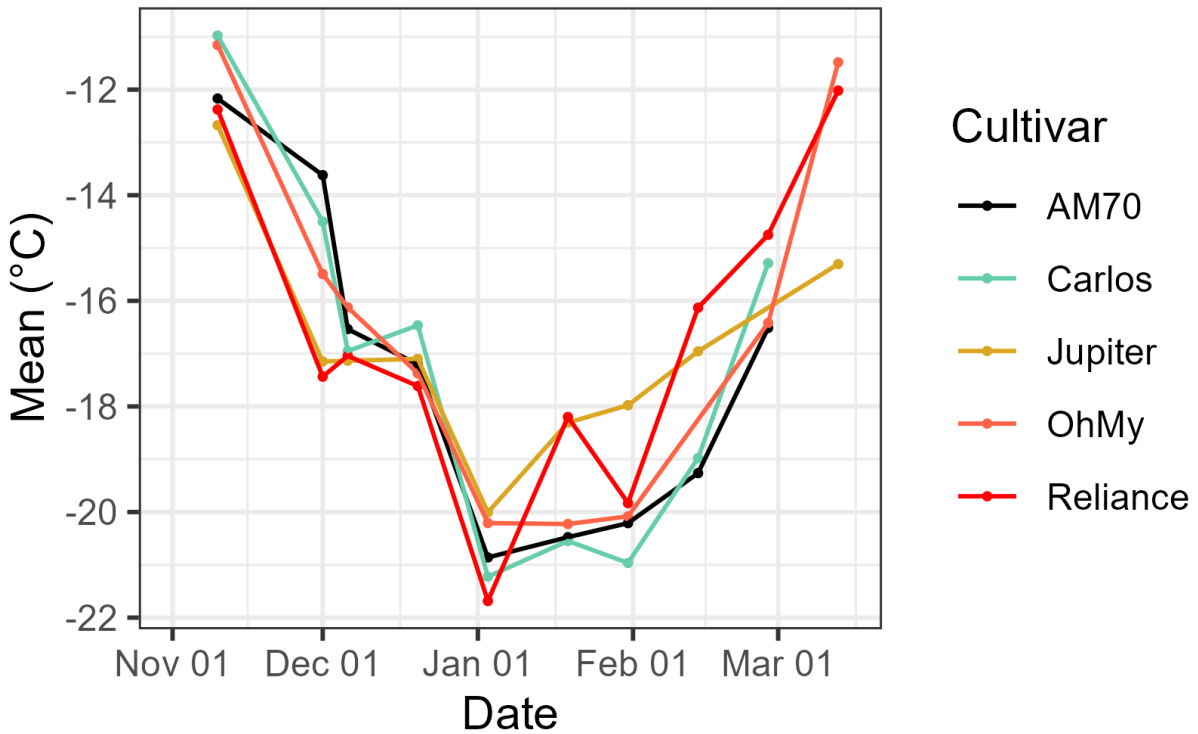


Figure 2. Cold hardiness of vegetative muscadine and bunch grape buds during the winter of 2023-2024 measured using differential thermal analysis. Despite the unseasonably cold temperatures experienced in mid-January of 2024, the bunch grapes 'Reliance' and 'Jupiter' began deacclimating during midwinter while muscadines maintained their hardiness levels into February. AM-70 = Mighty Fine™.

Muscadine Bud Hardiness: 2024-2025

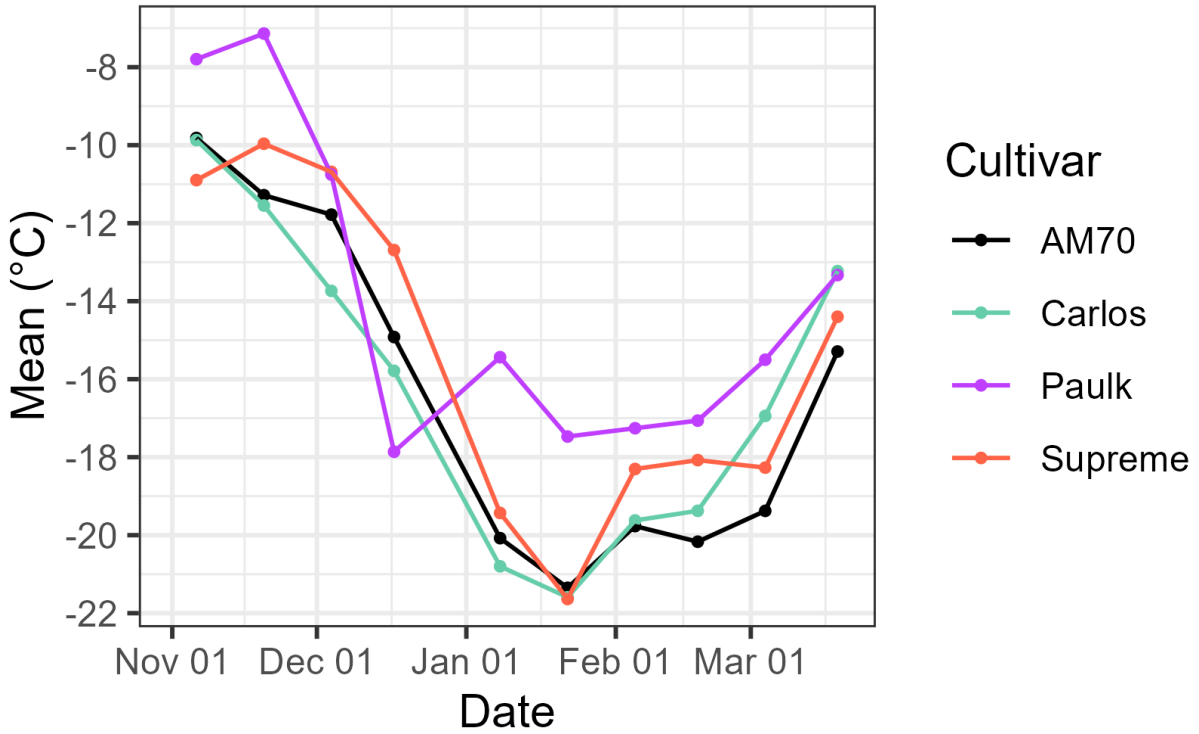


Figure 3. Cold hardiness of vegetative muscadine buds during the 2024-2025 dormant season measured using differential thermal analysis. During this winter, ‘Supreme’ was nearly as cold hardy as ‘Carlos’ and Mighty Fine™ (AM70) during the midwinter timepoints, while ‘Paulk’ was not nearly as hardy, similar to the winter of 2023-2024.

Grape Bud Hardiness: 2024-2025

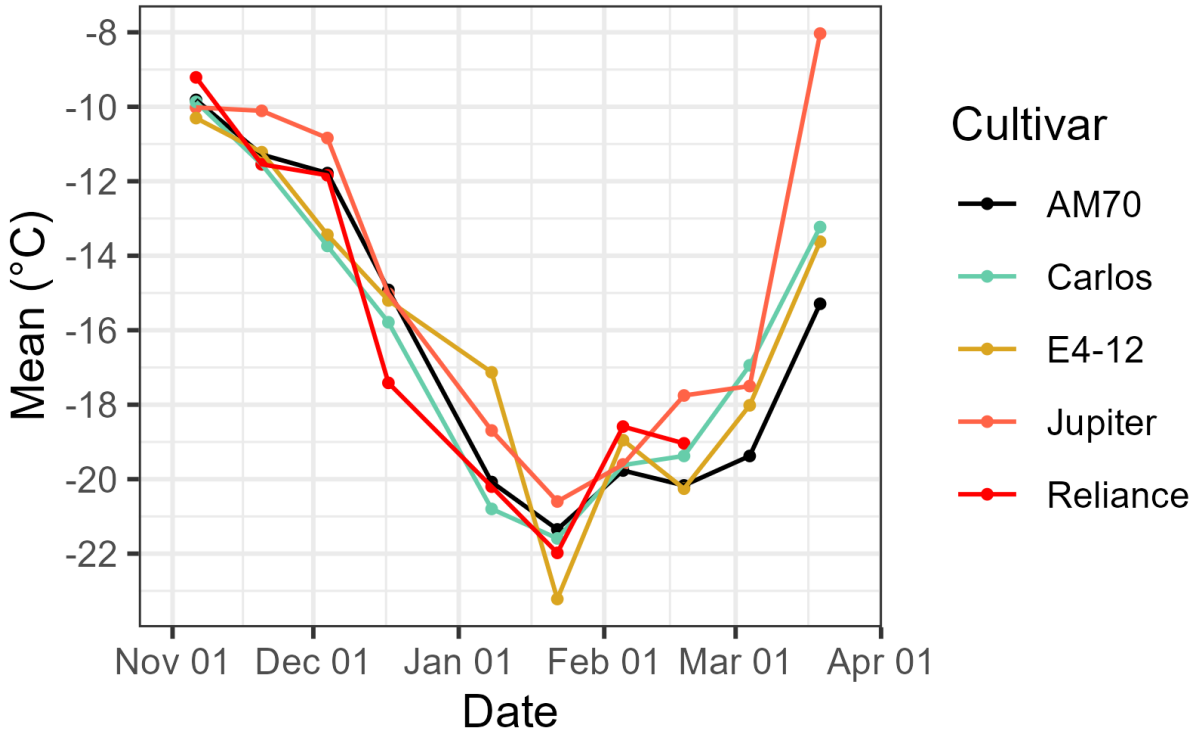


Figure 4. Cold hardiness of vegetative muscadine and bunch grape buds measured during the winter of 2024-2025 using differential thermal analysis. Hardiness levels between the different subgenera of grape were similar during this winter. The early budbreak of bunch grape cultivars compared to muscadines is visible with the rapid deacclimation of 'Jupiter' during the March timepoints.

Geneva Bud Hardiness: 2023-2024

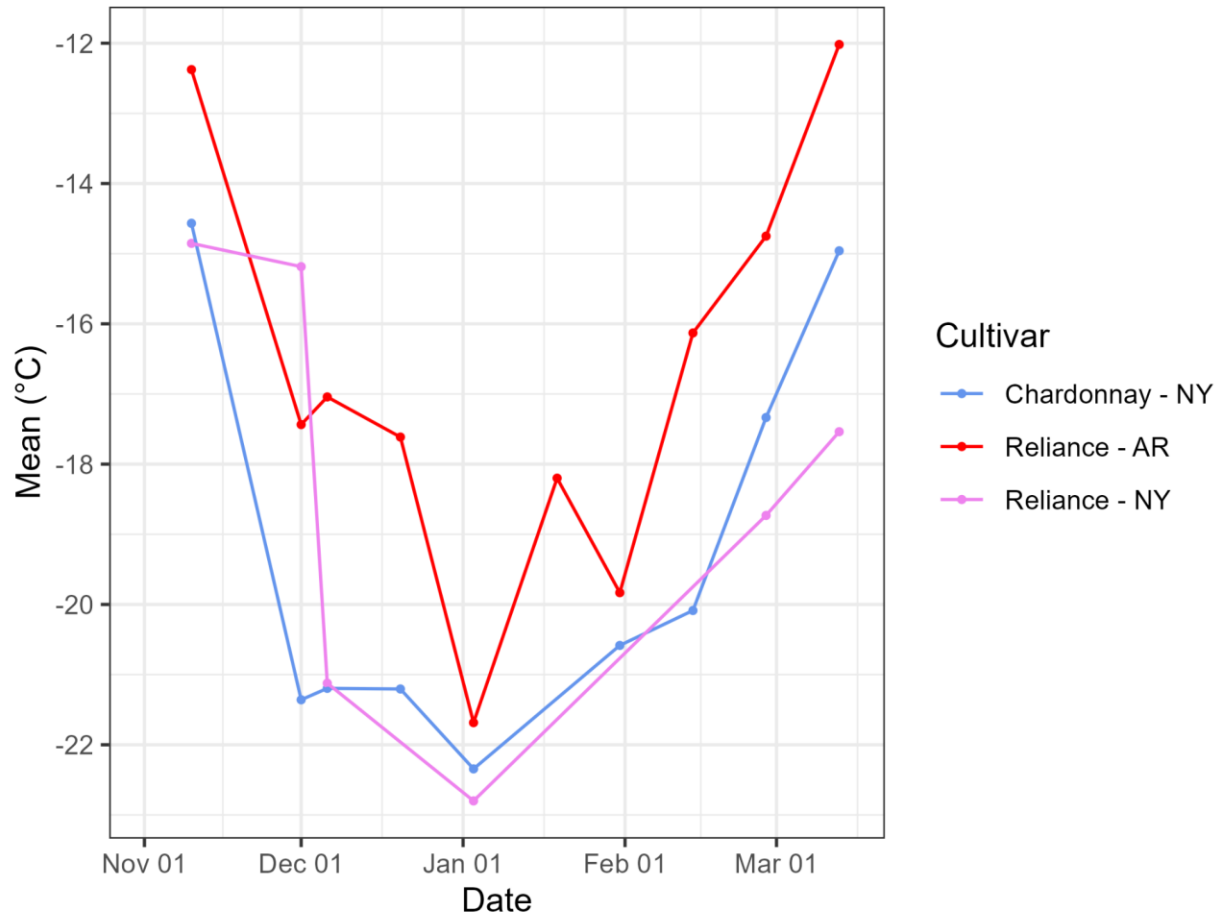


Figure 5. Cold hardiness of grape buds collected in Arkansas and New York, determined using differential thermal analysis. The 'Reliance' vines in New York were significantly more cold hardy than 'Reliance' vines in Arkansas, and were approximately as hardy as 'Chardonnay'.

Geneva Bud Hardiness: 2024-2025

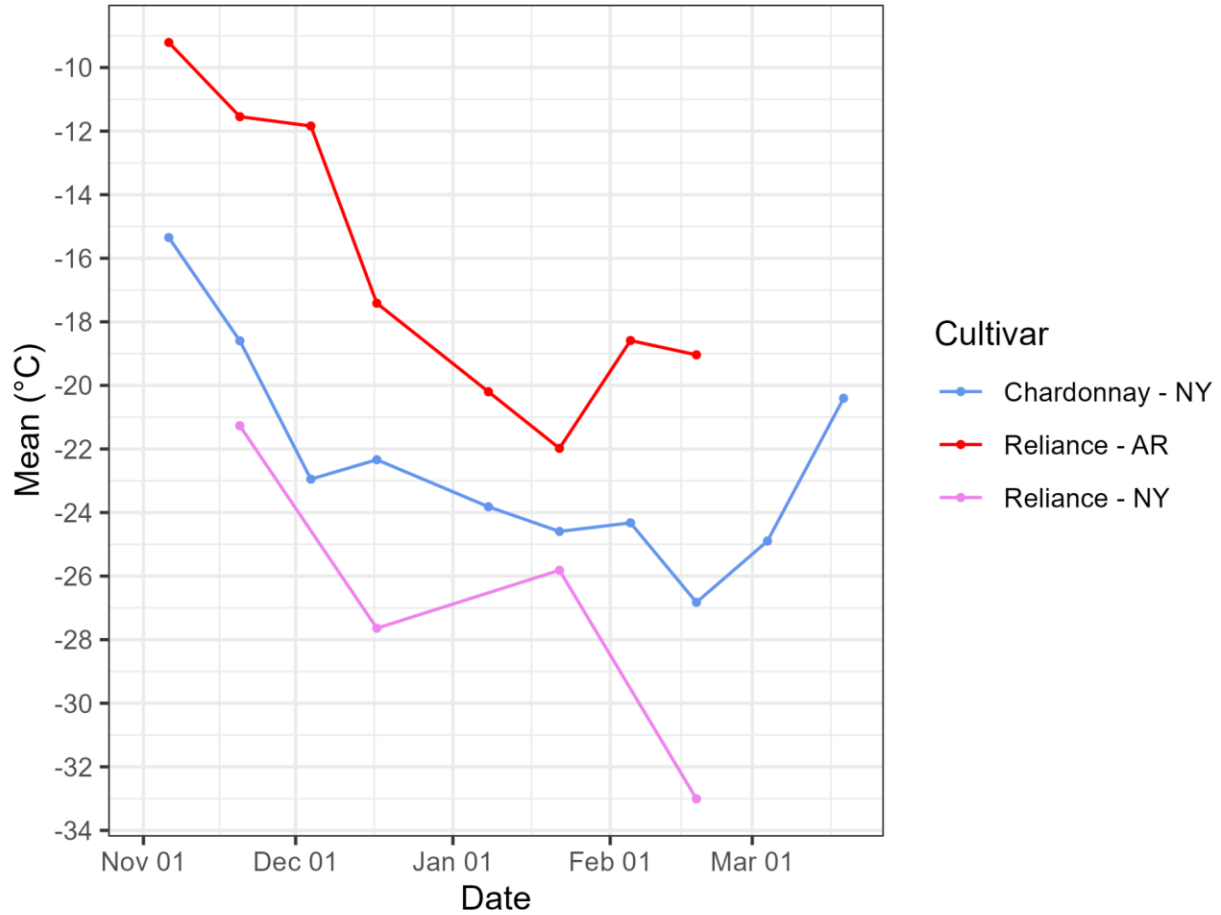


Figure 6. Upstate New York experienced a much colder winter in 2024-2025 than in 2023-2024, leading to much harder vegetative buds for both ‘Chardonnay’ and the New York ‘Reliance’ vines. The New York ‘Reliance’ vines registered the coldest mean hardiness of the entire experiment during the February sampling date: nearly -34 °C.

Grape Stem Hardiness: 2023-2024

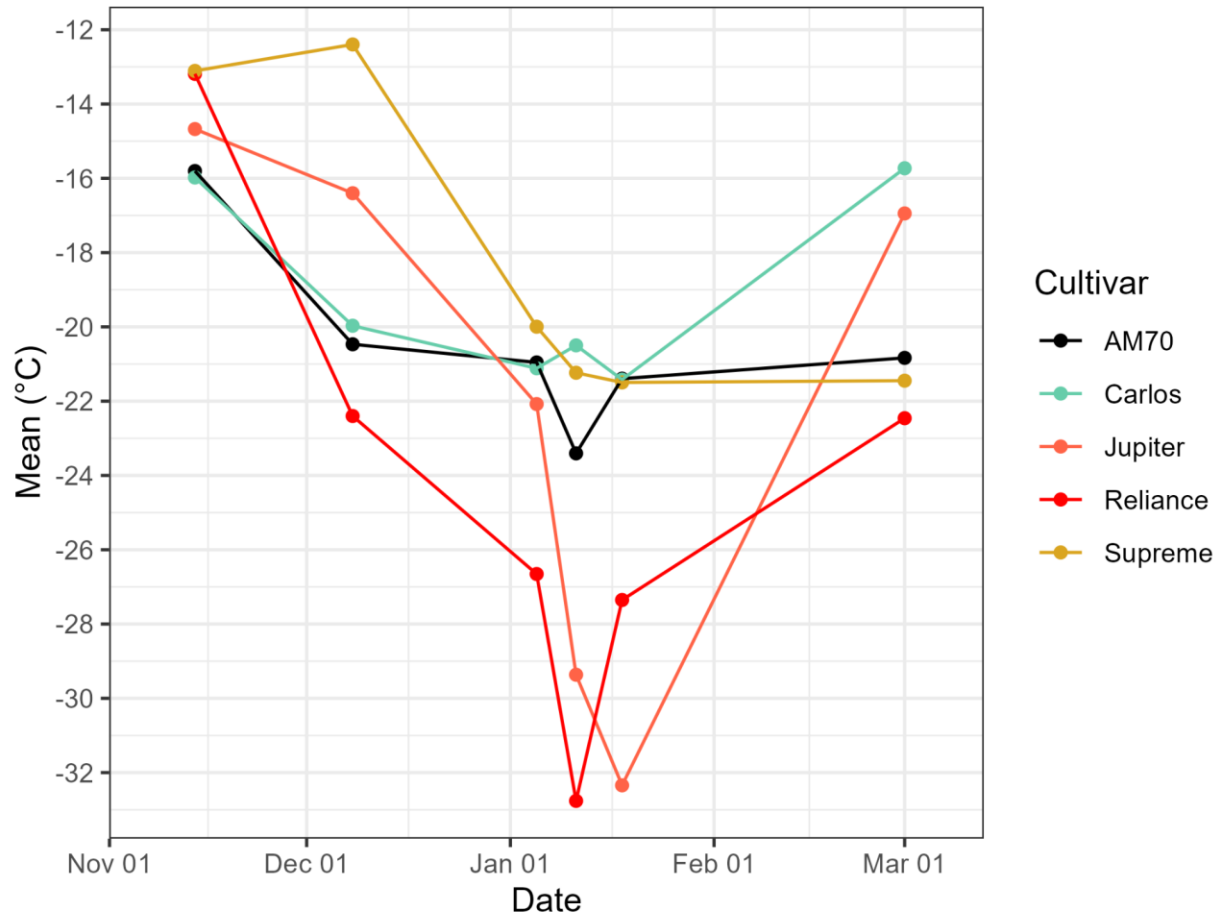


Figure 7. Stem hardiness of muscadine and bunch grape cultivars measured using electrolyte leakage. The polar vortex that brought temperatures as low as $-17.5\text{ }^{\circ}\text{C}$ in January caused significant acclimation in the bunch grape cultivars ‘Reliance’ and ‘Jupiter’ while muscadine cultivars had comparable hardiness levels before and after the cold event. AM70 = Mighty Fine™.

Grape Stem Hardiness: 2024-2025

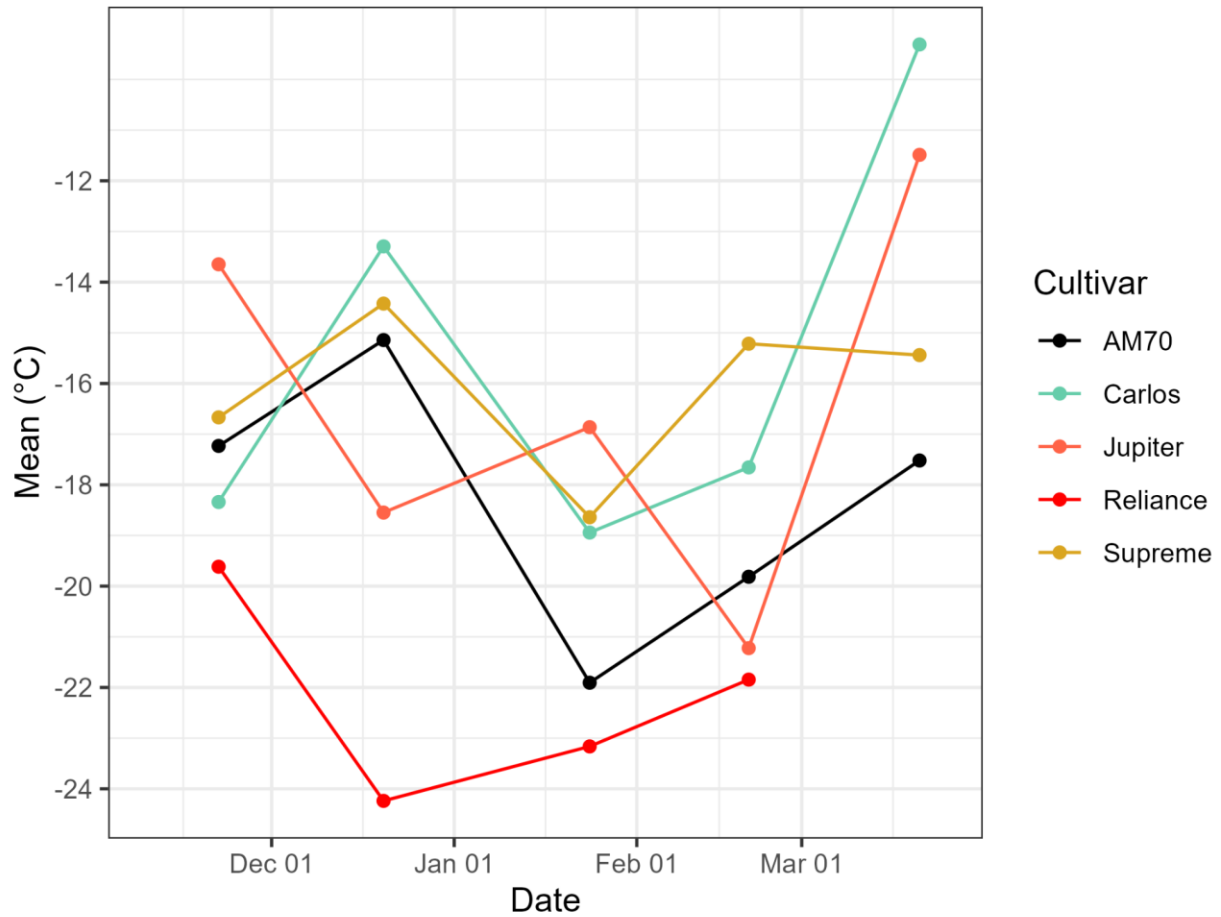


Figure 8. Stem hardiness of muscadine and bunch grape cultivars measured using electrolyte leakage. Arkansas experienced a comparatively milder winter in 2024-2025 than in 2023-2024- this is reflected in the more similar hardiness levels of 'Jupiter' and the muscadine cultivars, while 'Reliance' was still significantly hardier than the muscadines in this experiment. AM70 = Mighty Fine™.