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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Name, Mailing and Email Address of Principal Investigator(s):

Principal Investigator:

Dr. Margaret Worthington, Associate Professor, Department of Horticulture, 316 Plant Science Building, University of Arkansas, Fayetteville, AR 72701, mlworthi@uark.edu

Co-Principal Investigators:

Carmen Johns, Assistant Fruit Breeder, Department of Horticulture, 316 Plant Science Building, University of Arkansas, Fayetteville, AR 72701, kes056@uark.edu

Public Abstract

Muscadine grapes often suffer winter injury in areas where temperatures dip below 10 °F, limiting the areas where they can be produced. Previous research has indicated that muscadine buds have comparable hardiness to *V. vinifera* and grape hybrids, but they experience much more severe winter injury when subjected to the same conditions. The goal of this study was to assess the cold hardiness of muscadine grapes and *Vitis* table grapes grown in Clarksville, AR using differential thermal analysis (DTA) of bud hardiness and electrolyte leakage tests (EL) of vascular hardiness at regular intervals throughout the winter and early spring. The conditions during the winter of 2023-2024 reinforced our hypothesis that extreme midwinter temperatures, rather than frosts in early fall or late spring, is the limiting climatic factor determining muscadine survival during dormancy. These results also suggest that vascular hardiness is the limiting factor for survival of muscadine grapes and *Vitis* x *Muscadinia* hybrids.

Introduction

The area 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 °C (10 °F), and definitely not in areas where temperatures reach below -18 °C (0 °F). These recommendations have been cited frequently since then, with many researchers claiming that vine damage begins at -12 °C and mortality occurs at -18 °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 °C (-9 °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°31'5" N, long. 93°24'12" W, USDA plant hardiness zone 7b) where mid-winter low temperatures are almost always below -12 °C (10 °F), and often below -18 °C (0 °F). Clark and Moore (1990) found that many vines planted in Clarksville, AR survived a winter low of -21.6 °C (-7 °F) in 1989-1990. More recently, we have had the opportunity to evaluate muscadine cold injury and survival after low temperatures reached -26 °C (-15 °F) February 16, 2021 and -18 °C (-1 °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 °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 °C, -23.4 °C, and -22.4 °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.

The goal of this study was to assess the cold hardiness of muscadine grapes grown in Clarksville, AR using differential thermal analysis (DTA) of bud hardiness and electrolyte leakage tests (EL) of vascular hardiness at regular intervals throughout the winter and early spring. These tests can provide evidence of whether vascular hardiness is the limiting factor for survival of muscadine grapes and whether cultivars vary in the hardiness of their vascular tissue.

Methods

Plant Materials

Seven grapevine and muscadine cultivars were selected for this study based on their varying cold hardiness and availability of plant material growing at FRS. ‘Carlos’, ‘Supreme’, ‘Paulk’, and ‘AM-70’ were the four pure *Muscadinia* cultivars. The seedless *Vitis* x *Muscadinia* hybrid cultivar ‘Oh My!’ was also included in the study. Two table grape cultivars (mix of *V. vinifera* and *V. labrusca* in pedigree) developed in the UA Fruit Breeding program with varying hardiness were also included: ‘Reliance’ (very cold hardy) and ‘Jupiter’ (moderately cold hardy).

Harvesting Cuttings

Hardwood cuttings were collected from each genotype grown at FRS every 14 days starting on November 6 and continuing through March 11 (10 total collection dates) and mailed overnight to Cornell University for DTA and EL assays conducted by Dr. Jason Londo and PhD student Kenneth Buck. The terminal third of each cane was discarded before preparing cuttings for shipment. Because only one small vine of Paulk was available at FRS, cuttings from Paulk were only collected from December 6 2023 to February 14 2024 and only used for differential thermal analysis.

Differential Thermal Analysis (DTA)

Thirty buds from each cultivar were excised from the cuttings after each collection date with a razorblade and used for DTA following Londo and Kovaleski (2017). Buds were placed cut surface down on a lightly moistened Kimwipe tissue within a thermoelectric module (TEM) chamber placed in a Tenney Model TC2 programmable freezer (Thermal Products Solutions). The temperature was lowered from room temperature to 4 °C, held for 1 hr, and then ramped to -40 °C over 13 hr, before being slow ramped back up to room temperature. This program results in a cooling rate of ~0.06°C/min or 3.4°C/hr. The low temperature exotherm (LTE) peaks resulting from bud killing was recorded using a Kiethley 2700 (Tektronix, Inc.). Data results were manually curated in Microsoft Excel with up to 30 primary bud peak LTEs identified per cultivar for each freezing run.

Electrolyte leakage (EL)

Electrolyte leakage tests to assess the cold-hardiness of vascular tissue were conducted every 28 days for each cultivar. On the day after cuttings were received at Cornell, the internodal tissue was chopped in 60 1-inch segments. The cane segments were placed into 3 replicate tubes for each of 10 different temperature treatments [Control (4 °C), -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -35 °C, -40 °C, -45 °C, -50 °C]. Each tube was filled with 30 mL dH20 and placed in a programmable freezer with cooling rate of 5 °C/hour. The freezer was set to -5 °C for 2 hours to freeze solid, then ramped down to each setpoint temperature. The temperature was held at each setpoint temp for 1 hour, then tubes assigned to that setpoint temperature treatment were removed from the freezer. Tubes were then thawed for a day and shaken overnight at room temperature to release ions. On the third day each sample was run on an AM402 autosampler robot to measure electroconductivity (EC). Each tube was then recapped and moved to kill freeze at -80 °C for at least 4 hours or overnight. Tubes were then thawed for a day and shaken overnight at room temperature to release ions. Final EC after the killing freeze was then measured on an AM402 autosampler robot on day five of the assay. We then divided EC data from T0/TF to get a relative damage metric based on EC. This metric was converted to percent injury by normalizing the control treatment to 0, and highest damage EC (-50 °C) to 100%. We compared effects of treatment and plotted a dose response curve, comparing curves between cultivars at the LT50, which is the inflection point in the dose response curve.

Comparison with winter injury symptoms in the field

Naturally occurring winter injury to the cultivars evaluated in this study grown at FRS was assessed following budbreak on April 25, 2024. Each vine was assessed on a 0-5 scale of winter injury where 5 indicated no symptoms of winter injury, 4 indicated mild spur damage, 3 indicated moderate spur damage and/or death of cordon tips, 2 indicated severe damage or death of one or both cordons, 1 indicated severe damage or death of the trunk with surviving suckers that can be used to retrain vines, and 0 indicated death with no suckering. Photographs documenting winter injury and notes on specific symptoms (e.g. aerial roots, trunk diseases, leaking/cracked cordons and trunks) were collected and compared to the data from laboratory assays.

Statistical Analysis

The LTE values from DTA of primary buds and percent injury of vascular tissue will be analyzed by analysis of variance in R as a split-plot in time with the whole plot factor as cultivar and the subplot factor as collection date. Mean separation was performed using Tukey’s HSD.

Results and Discussion

Winter Injury Symptoms in the Field

Both ‘Jupiter’ and ‘Reliance’ grapes had no symptoms of winter injury following budbreak on April 25, 2024 and were given ratings of 5 (Figure 1). Among the muscadines, ‘Carlos’ was the only cultivar that exhibited no symptoms of winter injury aside from aerial roots, which had formed in previous seasons. ‘AM-70’, ‘Supreme’, and ‘Paulk’ all had some mild spur damage and were rated 4. Only the seedless *Vitis* x *Muscadinia* hybrid ‘Oh My!’ sustained severe winter injury. ‘Oh My!’ was killed to the ground, but was able to be retrained from suckers during the 2024 season.

Cold Hardiness of Muscadine Cultivars

Differential thermal analysis (DTA) and electrolyte leakage (EL) methods for determining the hardiness of different tissue types showed significant differences between genotypes and across timepoints. The cold hardiness curves of muscadines reflect those of other deciduous species, with a long and gradual acclimation period followed by a comparatively short and steep deacclimation time. Even the least cold hardy cultivars such as ‘Paulk’ and ‘Supreme’ had vegetative buds that were much hardier than necessary during acclimation and deacclimation (Figure 2). For most of November and December, temperatures at the Fruit Research Station (FRS) rarely dipped below freezing, but average bud hardiness was consistently below 20 °F for all cultivars. Bud hardiness is similarly sufficient during the spring. Temperatures did not fall below freezing for the last month of the experiment, but even the sharp deacclimation observed would not have resulted in bud damage.

This first year of results demonstrated that deep midwinter temperatures are the bottleneck for muscadine survivability- both in bud and stem tissues. ‘Carlos’ and ‘AM-70’, the most cold-tolerant muscadines in this experiment, had bud and stem cold hardiness of approximately -5 °F during January, when the most extreme temperatures of the experiment occurred (Figure 3). At every point during December and January, ‘Supreme’ and ‘Paulk’ were the least hardy cultivars, although these significant differences had disappeared by February. The close tracking of bud/stem hardiness in the most cold tolerant cultivars provides support to our hypothesis that improvement in muscadine cold hardiness can be traced to improved hardiness in stem tissues. Furthermore, the extreme weather event in central Arkansas- when temperatures did not rise above freezing for four days and fell to a low of 0.5 °F on January 16th- revealed the varying plasticity in muscadine genotypes’ ability to respond to external weather conditions. For example, ‘AM-70’ avoided damage during the January deep freeze without any notable shift in stem hardiness, while ‘Carlos’ responded with a full 5 °F shift within the span of one week (Figure 4).

Bunch Grape Hardiness

The two bunch grape cultivars, ‘Jupiter’ and ‘Reliance’, were included to compare the differences in hardiness between the two subgenera of *Vitis* at FRS. Bunch grapes are generally much hardier than muscadines (e.g., no muscadines are present in the Geneva, NY USDA germplasm repository while ‘Reliance’ is) but results from the first year of this study show that bud hardiness is remarkably similar during the acclimation period (Figure 5). From the end of December to the end of January, there were no significant differences in bud hardiness between the two bunch grape cultivars, ‘Jupiter’, and ‘Reliance.’ From February until the end of the experiment in mid-March, the two cold-hardiest muscadine cultivars (‘AM-70’ and ‘Carlos’) had hardier buds than the bunch grapes, even if the numerical differences were slight (Figure 5).

In contrast, there were stark differences between the stem hardiness of muscadines and bunch grapes. By mid-January, there was a nearly 20 °F difference in stem hardiness between ‘Reliance’ and the hardiest muscadines (Figure 6). This extreme difference in phenotype appears to be unique to midwinter, as the EL values during acclimation and deacclimation are similar. This adds further evidence to our hypothesis that vascular tissues are the ‘weak link’ in a muscadine’s ability to survive midwinter cold snaps, and that the similar DTA values between muscadines and bunch grapes are indicative of bud hardiness playing a lesser role in whole-vine survivability.

Hardiness of ‘Reliance’ At Two Locations

At every time point except December 1st, ‘Reliance’ collected in Geneva was more cold hardy than ‘Reliance’ collected in Arkansas. The long tail of deacclimation is particularly clear with this comparison, as the longer winter of New York ensured that bud hardiness of ‘Reliance’ was still 0 °F in March while the trajectory of ‘Reliance’ was quickly deacclimating. By contrast, the deep freeze experienced by Arkansas in January briefly pushed the stem hardiness of that planting lower than the hardiness of the New York planting.

Conclusions

The conditions during the winter of 2023-2024 reinforced our hypothesis that extreme midwinter temperatures, rather than frosts in early fall or late spring, is the limiting climatic factor determining muscadine survival during dormancy. Similarly, early evidence is indicative that vascular tissues are weaker than vegetative buds in muscadines, in part supported by the fact that bunch grape buds are no hardier than muscadine buds despite bunch grapes surviving in more extreme environments than muscadines are capable of. Interestingly, the *Vitis* x *Muscadinia* hybrid was the only cultivar that sustained severe winter injury, while all pure muscadine cultivars survived with mild winter injury. Given the differences between DTA and EL for generating cold hardiness estimates, statistical methods are still being developed to compare the two statistics. A second year of data collection, already underway, will be used to further enhance our understanding of muscadine physiology.

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Figure 1. Vines used in the study as observed after budbreak on April 25, 2024. a. Carlos, b. AM-70, c. Supreme, d. Paulk, e. Oh My!, f. Reliance, g. Jupiter

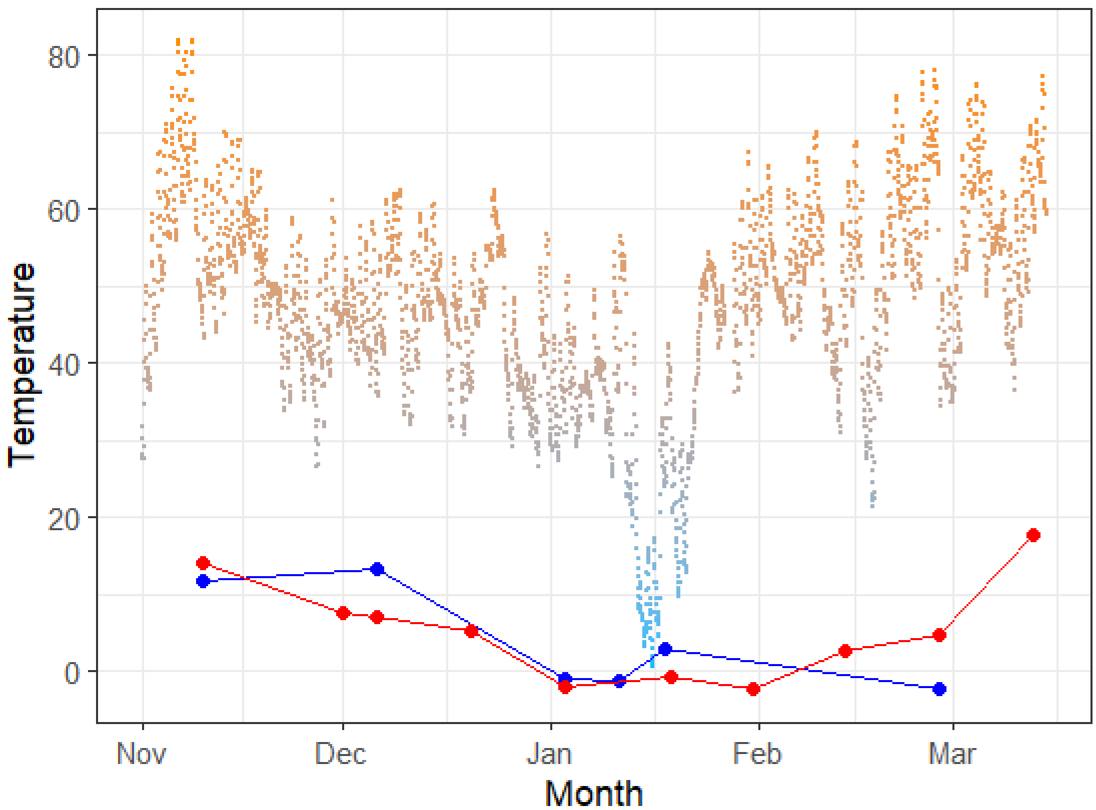

Figure 2. Hourly temperature data from the FRS weather station plotted as points from Nov. 1 2023 to March 15 2024. The red line shows average bud hardiness values and the blue line represents computed stem hardiness values for the muscadine cultivar ‘Supreme.’

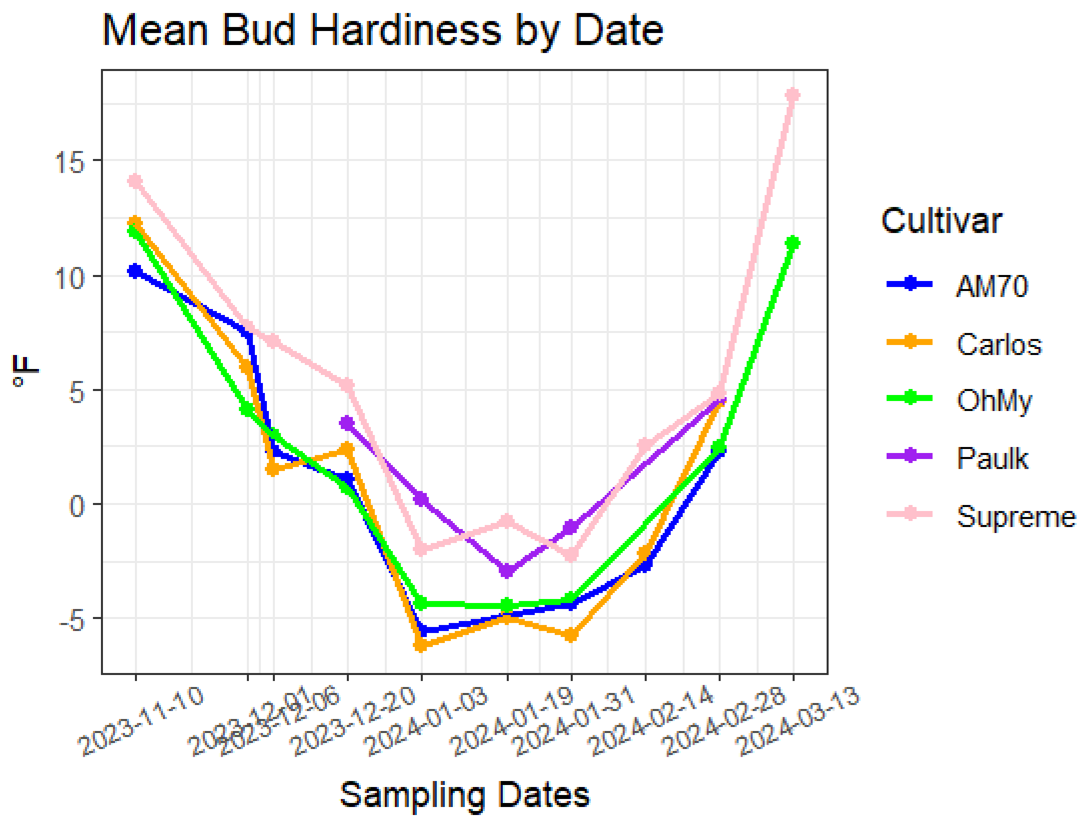

Figure 3. Average bud hardiness for five muscadine cultivars showing a steady rate of acclimation until early January followed by rapid deacclimation from mid-February to mid-March.

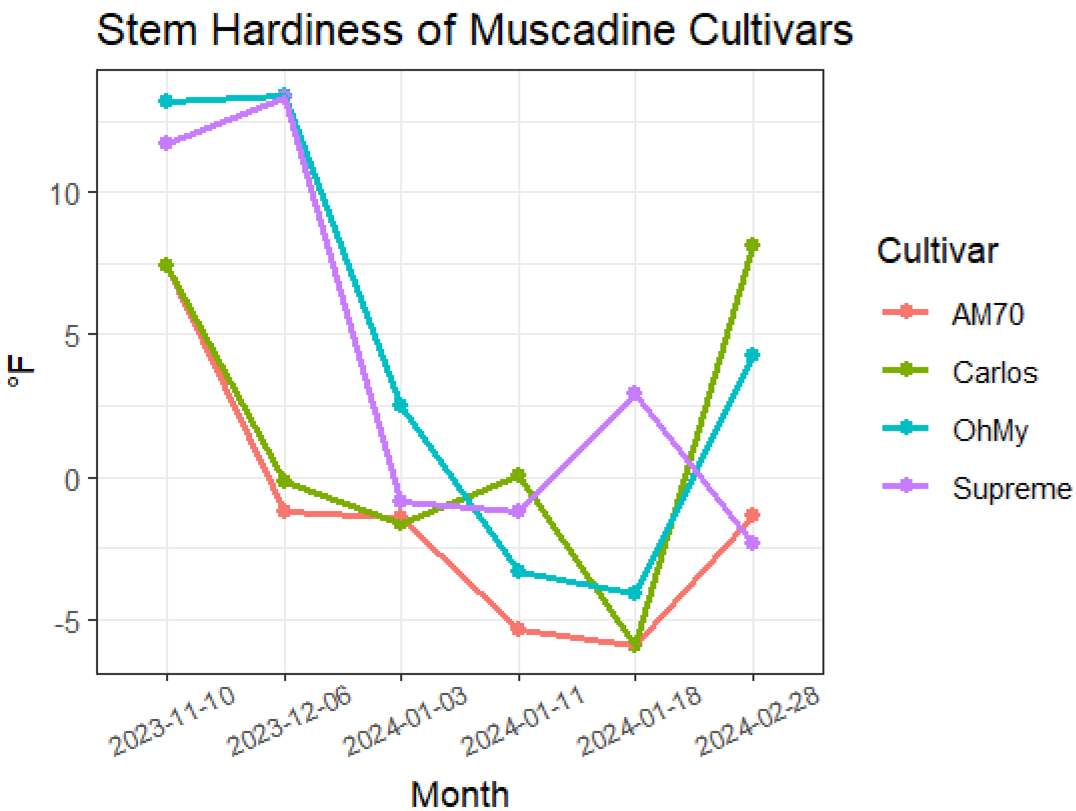

Figure 4. Stem hardiness of muscadine cultivars over the course of the experiment. Curves are generally similar to the bud hardiness curves, with a notable increase in the hardiness of 'Carlos', likely due to the extreme weather between the 1-11-2024 and 1-18-2024 sampling dates.

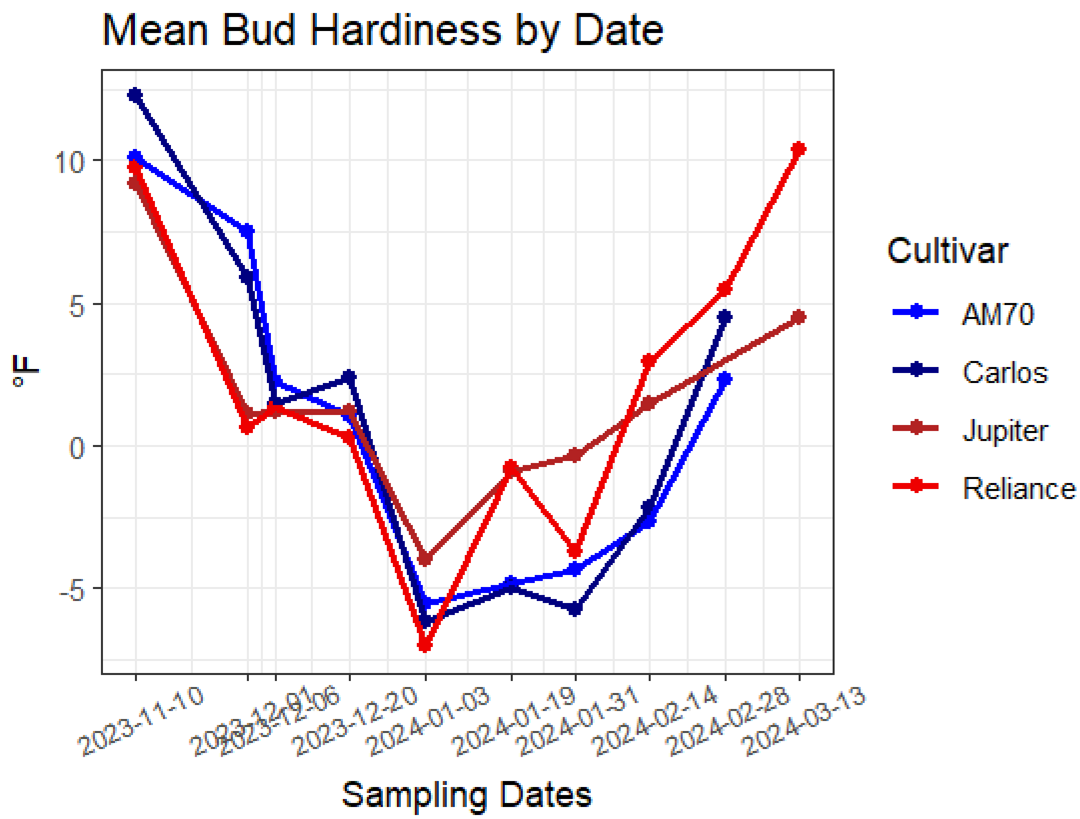

Figure 5. Mean bud hardiness over the course of the experiment for the two hardiest muscadine cultivars (‘AM-70’ and ‘Carlos’) and the two bunch grape cultivars (‘Jupiter’ and ‘Reliance’) in Clarksville, AR. Buds from the bunch grapes had less cold hardiness in late winter than muscadines.

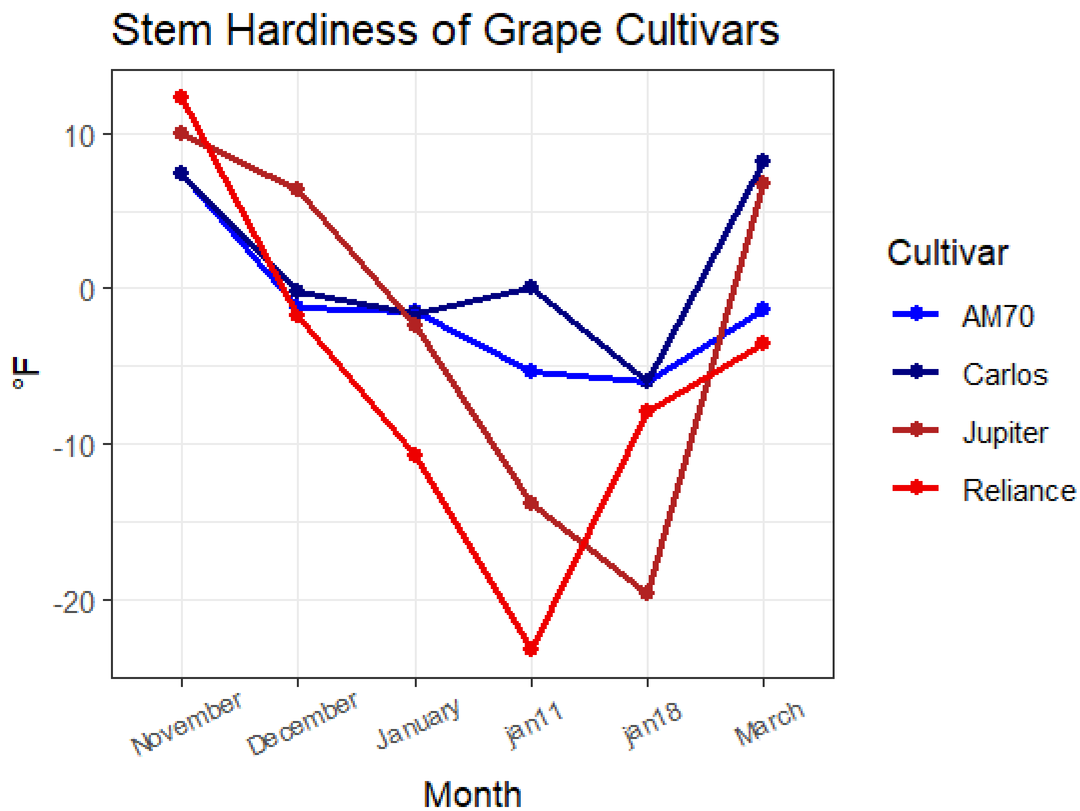

Figure 6. Stem hardiness of the two hardiest muscadines ('AM-70' and 'Carlos') and the two bunch grape cultivars ('Jupiter' and 'Reliance') collected in Clarksville, AR. During midwinter, the bunch grape cultivars are significantly more cold hardy than even the hardiest muscadines.