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An Investigation of Factors Affecting the Rooting Ability of Hardwood Muscadine Cuttings and Genetic Diversity of Wild and Cultivated Muscadine Grapes (Vitis rotundifolia Michx.)

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An Investigation of Factors Affecting the Rooting Ability of Hardwood Muscadine Cuttings and Genetic Diversity of Wild and Cultivated Muscadine Grapes (*Vitis Rotundifolia* Michx.)

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Abstract

The muscadine (*Vitis rotundifolia* syn. *Muscadinia rotundifolia*) is a grape species native to the southeastern United States. Muscadines are one of three grape species in subgenus *Muscadinia* with a chromosome number of 2n=40 (*V. rotundifolia*, *V. munsoniana*, and *V. popenoei*), making them genetically distinct from the European wine and table grape (*Vitis vinifera*) and other species in subgenus *Euvitis*. Rooting hardwood cuttings from muscadine vines has traditionally been considered an exceptionally difficult task. Many previous studies observed almost no root formation, leading to a general consensus that muscadines should either be propagated by softwood cuttings or vegetative layering. However, the University of Arkansas System Division of Agriculture Fruit Breeding Program has been using a hardwood rooting protocol for muscadines with moderate success for the past ten years. The first chapter of this thesis investigated the effects of cultivar, bottom heat, cold storage, vineyard location, and cutting collection date on the outcome of muscadine hardwood cuttings. The study was conducted during the dormant seasons of 2019-2020 and 2020-2021, and an overall rooting percentage of 16% was observed. There were multiple higher-order interactions affecting rooting efficacy. Cuttings taken in November generally rooted at higher rates, although interactions with vineyard location and cultivar played a significant role in those results. The Ocilla, GA location performed exceptionally well in November with rooting percentages of over 40%. The effects of supplying bottom heat and/or a cold storage treatment on rooting success declined as the dormant season progressed. Other variables such as increased cutting length and diameter were associated with increased rooting success. Ultimately, this research shows that institutions with modest muscadine propagation needs can successfully propagate plants from hardwood cuttings. Muscadine breeding has been continuous since the late 19th century, yet the germplasm that

served as the foundation for early breeding efforts was sourced almost exclusively from the coastal plains of North Carolina. The second chapter of this thesis investigated the diversity of wild and cultivated muscadine populations. We used the rhAmpSeq *Vitis* core panel haplotype markers to genotype 194 *Muscadinia* accessions from five cultivated populations and 15 wild populations collected across their native range. Wild populations from the western half of the native range were generally less genetically differentiated than hypothesized, but were genetically distinct from the material used in both past and present breeding efforts. One population collected from coastal North Carolina grouped closely with *Vitis munsoniana* accessions despite being well outside the reported range for that species. Principal coordinate and *structure* analyses revealed three main groups within the 194 accessions. At K=5, *structure* results showed that more recent muscadine cultivars are further differentiated from wild accessions and varieties. These analyses confirmed our hypothesis that muscadine cultivars are genetically differentiated from their wild counterparts. This study also showed that genetic diversity in *V. rotundifolia* is not equally distributed across its native range and that the limited number of genotypes used in crop improvement efforts have not fully utilized the genetic diversity within the species.

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Literature Review

General background. The muscadine (*Vitis rotundifolia* Michx.) is a member of the grape family native to the southeastern United States. It has been given many names during its cultivated history, from colloquial names such as "bullace," "fox grape," or "scuppernong" to being incorrectly categorized as *V. vulpina* L. decades after it was described by Andre Michaux in 1803 (Basiouny and Himelrick, 2001; Munson, 1909; *Southern Cultivator*, 1872). The muscadine grape is found from Maryland at its northern boundary west to Missouri, and south to Texas and Florida. The main factor limiting its native range is low cold tolerance: cultivated muscadine vines have, under rare circumstances, been documented to survive temperatures as low as -23 °C, but localities that attain winters lows of -18 °C are considered unsuitable for commercial muscadine production (Clark, 2001; Dearing, 1947; Munson, 1909).

Significant morphological and genetic differences have warranted researchers to divide *Vitis* into two subgenera: *Euvitis* and *Muscadinia*. Over fifty species from both Eurasia and America are placed in *Euvitis* (commonly referred to as "bunch grapes") based on a chromosome number of 2n=38 and three American species (*V. rotundifolia, V. munsoniana,* and *V. popenoei*) are placed in *Muscadinia* based on a chromosome number of 2n=40. *V. munsoniana* has a range from southern to central Florida and along the Gulf Coast into Texas. *V. popenoei* was first described in 1940 in southern Mexico and is only known to grow in the tropical regions of Central America (Fennell, 1940). At this time, muscadines remain the only economically important species in *Muscadinia*, although individuals from *V. popenoei* and *V. munsoniana* have been used in breeding efforts.

Muscadine flowers emerge 2 to 3 weeks after *Euvitis* species with similar geographic distribution and need approximately 100 d for fruit to reach maturity (Basiouny and Himelrick, 2001; Goldy, 1992). The fruit itself can vary greatly in size, but many cultivars are noted for large berries, often exceeding 2.5 cm in diameter (Basiouny and Himelrick, 2001; Anderson, 2006; Conner, 2009). Other significant morphological characteristics that distinguish muscadines from *Euvitis* species include unbranched tendrils, continuous pith through nodes, grape abscission at maturity, and smooth bark (Comeaux et al., 1987). The clusters of fruit are much smaller than those of *V. labrusca* L. or *V. vinifera* L., usually only four to ten grapes each, with notable exceptions having up to 40 on a single cluster (Olien, 1990; Young, 1920). Muscadine fruit in the wild is generally dark purple or black and rarely a bronze or greenish color. Breeding efforts have resulted in both black and bronze cultivars as well as pinks and lavenders (Basiouny and Himelrick, 2001; Comeaux et al., 1987; Conner and Maclean, 2013).

Wild germplasm is almost entirely dioecious, with staminate vines being more common. The notable exception to this rule is 'Hope,' a wild perfect-flowered vine discovered approximately one mile from an experiment station outside of Raleigh, NC in 1910. The significant distinction between this vine and other fruiting muscadine vines is a morphological change in flower structure that is believed to have restored self-fertility to this plant. Stamens were upright with fertile pollen, which contrasts this vine with the "reflexed" stamens of pistillate vines (Reimer and Detjen, 1910).

The first recorded description of muscadines comes from the writings of a French explorer in 1524 who described "many vines growing naturally that without doubt would yield excellent wines" along the Cape Fear River in North Carolina (Morton, 1988). Further descriptions of muscadines' prevalence on the landscape came in 1584 along the coast of North

Carolina. Captains Amadas and Barlowe reported to Walter Raleigh that the "very beating and surge of the sea overflowed with them" and further that "in all the world the like abundance is not to be found" (Winsor, 2016). Actual use of these "abundant and excellent grapes" was not reported until 1565. Captain John Hawkins, while traveling in northern Florida, reported that colonists had produced twenty hogsheads (approximately 4500 liters) of wine out of native grapes that "taste much like our English grapes" (Winsor, 2016). These native grapes were likely muscadines, which would make *V. rotundifolia* the first American grape species to be cultivated by Europeans, although this certainly was not the first time they had been used. Native Americans have been drying grapes over open fires for centuries (Bartram, 1791). Since then, muscadines have maintained some level of cultural significance across the southeastern United States. In addition to its longstanding use as a wine grape, muscadines are commonly processed into other products such as juice and preserves, as well as sold fresh as table grapes. However, despite the long tradition of use from the earliest European Americans and cultural impact of the muscadine, commercial acreage of bunch grapes is three times as much as that of muscadines in southeastern states. As of 1990, muscadine production across the entire southeast region was estimated to be 1600 ha. In Arkansas, muscadines are only 3% of total grape acreage (Olien, 1990; Basiouny and Himelrick, 2001). However, commercial plantings were not present in the state until the mid-1970s (Moore, 1972; Clark, 2001).

Breeding efforts. For centuries after muscadines were first used by colonists on the east coast of North America, they were either harvested from wild vines or from cultivated vines that themselves were propagated from exceptional wild vines. "The Big White Grape," as 'Scuppernong' was known to early settlers in the American Southeast, was propagated and spread outwards from its original location of Roanoke Island. 'Scuppernong' would remain the

most widely cultivated bronze muscadine into the 20th century (Reimer and Detjen, 1914). It was not until 1835 that a commercial vineyard was first founded in North Carolina by planting twelve acres of muscadines (Morton, 1988). Concerted and recorded breeding efforts did not begin until the 1860s. J. Van Buren (1871) of Clarksville, Georgia, reported early experiments with 'Scuppernong' seedlings in *Southern Cultivator*. With the goal of developing new cultivars, Van Buren planted thousands of these open-pollinated seedlings through the late 1860s. Only a handful produced "white" or bronze grapes and he noted that approximately three-fourths of the progeny were staminate vines. Van Buren selected three specimens he felt were worthy release from his efforts over three years and approximately six thousand seedlings (Van Buren, 1871). Much of the breeding effort during this era was focused on hybridization attempts between muscadines and *V. vinifera*, while cultivar development was still in its earliest stages (Reimer and Detjen, 1914). Even as the first formal muscadine breeding programs began, wild varieties were still being recommended to the public as potential improvements upon the limited number of muscadine cultivars available (Dearing, 1917).

The USDA and North Carolina State University cooperatively founded the first public muscadine breeding program in 1908. In 1917, the program reported the first perfect-flowered muscadine vines, referred to as "H1" and "H2" (Dearing, 1917). H1 (source of hermaphroditism 1) was developed from a cross between the pistillate muscadine cultivar 'Eden' and the *V. munsoniana* pollinizer 'Mission Male.' H2 (source of hermaphroditism 2) was the result of a cross between two muscadine cultivars, 'Scuppernong' and 'New Smyrna.' Both crosses were made in 1907 at a commercial vineyard in New Smyrna, Florida, before being transferred to the experiment station in North Carolina (Dearing, 1917). A third hermaphroditic seedling of novel parentage was also reported from a cross made in 1913, but it has since vanished from the

academic record and is not in the pedigree of any released varieties (Dearing, 1917; Goldy, 1992; Loomis and Williams, 1957). It was not until 1946 that the first group of self-fertile muscadine cultivars derived from crosses with H1 and H2 were released. This also signaled the end of pistillate cultivar releases from the North Carolina State University breeding program (Goldy, 1992). Although other programs continued to release pistillate varieties, the release of self-fertile muscadine cultivars represented a significant shift for modern breeding efforts.

Morphological and genetic differences between the two original sources of hermaphroditism were quickly apparent to researchers. Self-pollinated populations of H1 segregate at a 3:1 hermaphrodite:female ratio, while H2 segregates at a 9:3:4 hermaphrodite:female:male ratio when self-pollinated (Loomis, 1954; Conner, 2017). Dearing's initial report showed that H1 had smaller berries but bigger clusters, likely an effect of its *V. munsoniana* parentage, and H2 was similar to 'Scuppernong' in color and fruiting habit, but lower in quality (Dearing, 1917). Biasi and Conner (2016) found that H1-derived cultivars had more flowers per cluster, in line with Dearing's initial conclusions, and that the germination rates of pollen from H1-derived cultivars was higher than H2-derived cultivars, though not enough to warrant favoring one over the other (Conner, 2016).

Another muscadine breeding program began in Experiment, Georgia (now located in Tifton, GA) a year after the NC State program began (Conner, 2010). Other early breeding efforts include the work of T.V. Munson. His work to hybridize muscadines with *V. vinifera* at first appeared to have been successful, and multiple putative hybrid cultivars such as 'La Salle' and 'San Jacinto' were released. However, later investigations determined that these cultivars were likely derived from muscadine and *V. munsoniana* crosses rather than true *Muscadinia* x *Euvitis* hybrids (Dearing, 1917; Detjen, 1917).

Many of these early breeding programs are no longer operating. The private programs of Van Buren, Munson, and others did not continue past their deaths. The original Georgia program operated until 1938, but was restarted in 1951 and is one of the country's leading breeding programs (Conner, 2009). The USDA-ARS began another program in Mississippi in 1941 that ran for only 24 years, but continues today in a research capacity (Olien, 1990; Schwartz, 1975).

Current programs breeding for muscadines include the aforementioned programs at the University of Georgia and University of Arkansas System Division of Agriculture (UA) as well as Florida A&M University and private breeder Jeff Bloodworth's program in collaboration with 'Gardens Alive!' Other research programs are maintained at North Carolina State University, the University of Florida, and the USDA-ARS at Poplarville, Mississippi. The goals of breeding programs include improving yield, color, and fruit quality, while maintaining the inherent disease resistance and adaptation in muscadines (Basiouny and Himelrick, 2001). One major improvement in recent releases is an increased percentage of dry scars after harvest. Many early cultivars and wild types had a thin pedicel that tore when the berry was harvested, resulting in leaking and mold growth. Releases from the University of Georgia have, in some cases, doubled the percentage of berries with dry scars compared to old releases (Conner, 2009).

University of Arkansas breeding program. The UA Fruit Breeding program was initiated by Dr. James Moore in 1964. Table and wine grape breeding was one of the original activities of the UA fruit breeding program, but the muscadine breeding program was not initiated until 2007. As of 2021, over 20,000 seedlings have been planted and 316 selections made. Although there have been no releases at this time, advanced selections are continuously being evaluated. The emphasis for the program has been maintaining the exceptional disease resistance of muscadine grapes while continuing to improve consumer quality and cold hardiness. One great leap forward

for the program was partnering with Jeff Bloodworth, a breeder with 'Gardens Alive!' who has achieved seedlessness in muscadine grapes. Pollen from this seedless germplasm was first used for crossing in the UA breeding program in 2017, and the first seedless progeny from this work were selected in the 2020 growing season (Margaret Worthington, Personal Communication). Improving cold tolerance is another primary objective of the breeding program. The UA Fruit Research Station in Clarksville, situated in the foothills of the Ozark Plateau, is an ideal location to select for cold hardiness. Other replicated trials also take place at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR and at the Southwest Research and Extension Center in Hope.

Rooting muscadines from hardwood cuttings. Muscadine grapes root poorly from hardwood cuttings compared to other *Vitis* species, notably *V. vinifera* and *V. labrusca*, despite the commonality of aerial roots forming during the growing season (Basiouny and Himelrick, 2001; Turner, 1934). Softwood cuttings have been successful, especially under mist, but for most of the muscadine's cultivated history the preferred method of asexual propagation has been layering (Sharpe, 1954). Increased scientific interest in the muscadine at the start of the 20th century led to research efforts to inform nurseries and farmers on the best methods to propagate their muscadine cultivars. This early research varies in scope and is at times contradictory (e.g. Goode et al., 1982; Whatley, 1974), but a few conclusions can be drawn. Primarily, propagation by hardwood cuttings is not the ideal method when layering is available. Niven (1918) suggests that growers should only use hardwood cuttings when an abundance of wood is available and a low number of rooted plants is required. Husmann and Dearing (1913) gave detailed instructions for the taking of hardwood cuttings but acknowledged that this method is "usually difficult" without providing expected success rates. Woodroof explored various propagation methods to help

inform a government program with the goal of providing two million muscadine plants to farmers during the Great Depression. His study encompassed various treatments and cultivars with the number of cuttings totaling 55,000. He described his efforts to root these cuttings as "virtually fruitless" due to poor callusing and instead concluded that using layering would be significantly more productive towards achieving the government's goal (Woodroof, 1935). However, Newman (1907) contested the idea that layering was the ideal method and stated that layering was ideal only when a "limited number" of plants were desired. Furthermore, Newman (1907) described achieving rooting percentages of 80% when taking cuttings in November and immediately placing them in a greenhouse with uniform bottom heat throughout the winter. Dearing (1947) also suggested that expert propagators could root muscadine cuttings with "relative ease" in a greenhouse that can provide ample bottom heat and maintain high levels of soil moisture. However, a study by Cowart and Savage (1944) found that hardwood muscadine cuttings rooted at approximately 1% when exposed to a variety of media and rooting hormones treatments in a greenhouse. Not one cutting out of 1200 planted without bottom heat formed roots in a study by Goode et al. (1982) at the University of Georgia. Cuttings with bottom heat performed better but no treatment exceeded 9% rooting (Goode et al., 1982).

Cultivars also seem to vary in their ability to successfully root from hardwood cuttings. 'Scuppernong' was repeatedly shown to be the least capable of rooting from hardwood cuttings, with rooting percentages varying from 0-10%. 'Thomas' was notably successful among these early studies and had rooting percentages as high as 48%. Other cultivars used in these early studies include 'Hunt,' 'Male,' 'Eden,' and 'James,' which were all considered intermediately good cultivars for rooting (Dearing, 1947; Niven, 1918; Woodroof, 1935).

There is significant variability regarding the recommended time of the year to take hardwood cuttings. In addition, the methods used for "post-harvest" handling of the cuttings reported in the literature were also widely variable. Newman (1907) recommended taking cuttings in November and either planting them immediately or burying bundles of cuttings for six to eight weeks based on research conducted in South Carolina. Niven (1918) suggested taking cuttings in November or December and burying the bundles in mulch until the following April. If cuttings were taking any later, vascular tissue from the propagated vine would leak water, which can lead to disease (Niven, 1918). Dearing (1947) recommended a similar protocol to Niven (1918), but Dearing noted that rooting success was better in northern Florida than eastern North Carolina due to climate and soil differences. Although a specific month was not designated, Dearing (1947) recommended propagating in "early winter". Cowart and Savage (1944) took muscadine cuttings multiple times throughout the dormant season at the Georgia Agricultural Experiment Station over the winters of 1936–37 and 1937–38 for a rooting study. While the authors noted that dormant cuttings taken earlier in the season performed slightly better, no treatment exceeded 1% of cuttings rooted. Consequently, no conclusions were made regarding the significance of cutting date. Cowart and Savage (1944) also found that two months of cold storage did not change the rooting success of muscadine cuttings compared to immediately placing them in media. Goode et al. (1982) also investigated the effects of cutting harvest date and storage length on the success of hardwood cuttings with similar results to Cowart and Savage (1944). Although cuttings taken early in the season during November rooted at 9%, cuttings taken during January and February did not root at all. No cuttings were taken in the month of December.

Asexual propagation by hardwood cuttings is the main method used to propagate muscadine breeding selections at the University of Arkansas Division of Agriculture Fruit Research Station. The muscadine hardwood cutting propagation protocol was modified from the bunch grape protocol used at the station. Cuttings are taken early in December and immediately bundled and placed into cold storage for approximately one month. Afterwards they are treated with rooting hormone and placed into mist beds with six inches of perlite and misted regularly. The rooting hormone used was not standardized and therefore changed year to year. Rooting is expected in 60-90 d, but success varies widely from year to year and cultivar to cultivar. It is not unusual to see rooting percentages as low as 10% some years and as high as 70% other years (David Gilmore, Personal Communication).

The diameter of hardwood muscadine cuttings has been found to have an effect on rooting outcome. Goode et al. (1982) found that large-diameter cuttings rooted at 10% and that most other treatments rooted between 0% and 2%. Numerical values for what constituted a large-diameter cuttings were not provided nor was the rooting percentage of small-diameter cuttings provided. Castro et al. (1994) investigated the rooting efficacy of hardwood muscadine cuttings taken from basal, middle, and apical cane segments and found that the thicker cuttings taken from basal cane segments rooted at higher rates than cuttings taken from apical segments.

Vitaceae phylogeny and genetic work. The grape family (Vitaceae) has been a source of significant phylogenetic discussion. Details surrounding the evolutionary history are not certain, but multiple studies have provided some measure of clarity to the taxonomic nature of the family and genus. Despite the difference in chromosome number between the *Euvitis* (2n=38) and *Muscadinia* (2n=40) subgenera, the monophyletic nature of *Vitis* has been well established (Zecca, 2012; Wan, 2013). Furthermore, these research efforts have revealed that genus *Vitis* is

likely American in origin and that the split between the two subgenera happened in the New World before the *Vitis* common ancestor made the leap to Eurasia (Liu, 2016; Wan, 2013). Recent molecular work in muscadines has found major synteny between the muscadine and *V. vinifera* genomes. Chromosomes 7 and 20 of *V. rotundifolia* were colinear with chromosome 7 of *V. vinifera*, indicating that the two chromosomes likely fused in *Euvitis* sometime after the split of the two subgenera (Blanc et al., 2012; Cochetel et al., 2021; Lewter et al., 2019). Other genera within Vitaceae that are closely related to *Vitis* such as *Ampelopsis*, *Ampelocissus*, and *Parthenocissus* all have diploid chromosome numbers of 40, further suggesting that 2n=40 is the conserved ancestral state of *Muscadinia* (Liu, 2016).

Phylogenetic and molecular work within *Muscadinia* is limited compared to *Euvitis*. However, multiple studies have investigated the genetics of disease resistance in muscadines, notably resistance to powdery mildew (*Erysiphe necator* syn. *Uncinula necator* [Schw. Burr]) and grapevine downy mildew (*Plasmopara viticola* [Berk. & M.A. Curtis] Berl. & De Toni) with the goal of breeding resistance to now globally-distributed pathogens with New World origins into *V. vinifera* Multiple loci and genes have been described, namely *Run1* (Resistance to *Uncinula necator* 1) and *Ren5* (Resistance to *Erysiphe necator* 5) (Merdinoglu et al., 2003; Pauquet et al., 2001; Riaz et al., 2011). Further work has led to the development of markers for flower sex and the mapping of the sex locus in muscadines to a small region on chromosome 2 coinciding with *V. vinifera* sex locus (Conner et al., 2017; Lewter et al., 2019). Park et al. (2021) conducted a genome-wide association study (GWAS) utilizing 1283 haplotype markers from the core *Vitis* rhAmpSeq panel. Significant markers were found for 12 traits such as berry weight and berries per cluster. In addition, a marker was found for berry color in muscadines in the same region as previous research (Lewter et al., 2019; Park et al., 2021).

Phylogenetic studies in muscadines to date have been relatively narrow in scope. *Muscadinia* species, when included in larger studies on *Vitis*, make up a small percentage of the accessions and are often included as an outgroup (e.g., Aradhya et al., 2013). A comparison of bunch grapes and muscadines using Random Amplification of Polymorphic DNA (RAPD) markers found that genetic variation among muscadine cultivars was significantly lower than bunch grapes (Qu et al., 1996). Eight muscadine cultivars and eight *Euvitis* hybrids were used in the study. Ancestral backgrounds for the *Euvitis* hybrids ranged from two to eight species, incorporating both European and American taxa (Qu et al., 1996). The higher diversity of bunch grape cultivars in this study was unsurprising given their multispecies background.

Riaz et al. (2008) used SSR markers to create a phylogeny of 57 accessions, including 39 muscadine cultivars, three *V. vinifera* cultivars, three bunch grape hybrids, and 12 *V. vinifera* x muscadine hybrid breeding selections. Because of the lack of SSR markers available specifically for muscadines, 884 markers were tested from multiple *Vitis* sources including 56 markers from newly developed primer sequences for the study. Seventy-eight percent of the primers amplified for muscadines, and approximately two thirds of primers that did not amplify for muscadines did amplify in the *V. vinifera* control accession. Four of the 39 muscadine accessions had genotypes that did not match reported pedigrees (Riaz et al., 2008). Cao et al. (2020) found 12 cultivars with inconsistent pedigrees out of 67 accessions**.** Geographic differences in genetic diversity among wild muscadine populations has been observed. A study that quantified genetic diversity in wild muscadine sub-populations revealed lower genetic diversity among North Carolina sub-populations than Florida sub-populations (Smith, 2010). This molecular work provides a measurable genetic confirmation of the concerns of previous generations of muscadine breeders regarding the constrained geography from which breeding germplasm had been sourced

(Husmann and Dearing, 1913; Onokpise, 1988; Riaz et al., 2008; Riaz et al., 2012; Smith, 2010). An analysis of muscadine pedigrees found that recently-released cultivars had higher inbreeding coefficients than older cultivars and suggested that some of these cultivars may already be suffering from inbreeding depression (Williams et al., 2021).

Although there is no data indicating the necessity of a change in conservation status of wild muscadine grape populations, some have expressed concern about human development throughout their native range (e.g. Basiouny and Himelrick, 2001). The lack of wild muscadine germplasm in most molecular/phylogenetic studies have made it difficult to get a clear consensus of the genetic diversity available in wild populations. However, Cao et al. (2020) used 20 SSRs to fingerprint 67 muscadines cultivars and nine wild muscadine accessions. Across the 20 loci, there were more alleles present in the nine wild accessions than there were in all 67 cultivars combined. Reluctance to include wild accessions into modern breeding programs is founded on the demand for perfect-flowered cultivars and an unwillingness to suffer the short-term setbacks from incorporating untested wild accessions, such as reintroducing dominant male alleles and poor consumer quality, into advanced breeding populations (Basiouny and Himelrick, 2001; Goldy et al., 1989). Wild genotypes have been shown to contain beneficial traits that were unknown to muscadine breeders. Goldy et al. (1989) investigated the pigment quantity and quality of 84 wild accessions collected from across the native range of muscadine grapes using high-performance liquid chromatography (HPLC) and found monoglucoside anthocyanins for the first time. Monoglucoside anthocyanins offer an advantage in the winemaking process in that they are less susceptible to browning while the wine is being aged/stored than the diglucoside anthocyanins that predominate in muscadines (Robinson, 1966). The authors noted that the lack of monoglucoside anthocyanins in cultivated populations because they were missed in previous

HPLC studies. Still, the variation in individual anthocyanin content and chemical composition was significant enough for them to conclude that it was worthwhile for breeders to introgress these traits using wild germplasm (Goldy et al., 1989).

Genetic diversity in wild germplasm. Literature on diversity of wild muscadines is thin compared to *Vitis vinifera* and its wild counterpart, *Vitis vinifera* subsp. *sylvatica*. Wild germplasm both *V. vinifera* and *V. rotundifolia* is almost exclusively dioecious and perfect-flowering is generally only found in cultivated populations (Riaz et al., 2018). Because of the exceptionally long period of cultivation and extensive distribution of *V. vinifera,* researchers must take extra steps to ensure that wild individuals collected are not in fact escaped cultivated vines (Zdunic et al., 2017; De Andres et al., 2011). This issue is less likely to be encountered in muscadines considering the relatively short period of their cultivation and low cultivated acreage across much of their native range, although muscadines have been cultivated in North Carolina for centuries (Basiouny and Himelrick, 2001). Gene flow between vineyards and wild populations has been well documented, although its effects, whether contributing to "genetic swamping" and the extirpation of valuable alleles in wild populations or countering the effects of inbreeding depression due to isolation, are yet unknown (Di Vecchi-Staraz et al., 2008). In the United States, the native species *V. californica* Benth. has populations in which thirty percent of the individuals have hybridized with introduced *V. vinifera* cultivars, some of which had not been grown in the region for around one hundred years (Dangl et al., 2015).

High levels of heterozygosity have been observed in both wild and cultivated grapevine populations. Heterozygosity levels tend to be marginally higher in cultivated *V. vinifera* populations for a few speculated reasons, namely that wild populations are more likely to be isolated and suffer from inbreeding and that cultivated individuals that are the result of breeding

programs are more likely to have ancestries from diverse locations (Zdunic et al., 2017). European and Asian grape populations have been shown to contain significantly varying amounts of genetic diversity. Allelic richness in Georgia was much higher than in France, potentially a symptom of disjunct populations cut off from gene flow (Riaz et al., 2018). Patterns of genetic variation in muscadine populations appear to be consistent with other dioecious plants. The overwhelming majority of genetic diversity in wild muscadines is within populations rather than between populations (Smith, 2010), a trend that is also present in *V. vinifera* and other outcrossing plant species (Riaz et al., 2018; Ward et al., 2008).

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Chapter I

An Investigation of Factors Affecting the Rooting Ability of Hardwood Muscadine Cuttings

Abstract

Rooting hardwood cuttings from muscadine (*Vitis rotundifolia* Michx.) vines has traditionally been considered an exceptionally difficult task. Many previous studies observed almost no root formation, leading to a general consensus that muscadines should either be propagated by softwood cuttings or vegetative layering. However, the University of Arkansas System Division of Agriculture Fruit Breeding Program has been using a hardwood rooting protocol for muscadines with moderate success for the past ten years. The application of this protocol to meet the modest propagation needs of the breeding program has significantly shortened the time required to advance selections. The goal of this research was to more adequately describe the factors affecting the rooting ability of hardwood muscadine cuttings. This research investigated the effects of cultivar, bottom heat, cold storage, vineyard location, and cutting collection date on the outcome of muscadine hardwood cuttings. The study was conducted during the dormant seasons of 2019-2020 and 2020-2021, and an overall rooting percentage of 16% was observed. There were multiple higher-order interactions affecting rooting efficacy. Cuttings taken in November generally rooted at higher rates, although interactions with vineyard location and cultivar played a significant role in those results. The Ocilla, GA location performed exceptionally well in November with rooting percentages of over 40%. The effects of supplying bottom heat and/or a cold storage treatment on rooting success declined as the dormant season progressed. Other variables such as increased cutting length and diameter were associated with increased rooting success. Ultimately, this research shows that institutions with modest muscadine propagation needs can successfully propagate plants from hardwood cuttings.

Introduction

The muscadine (*Vitis rotundifolia* Michx.) is a perennial woody liana native to the southeastern United States that has been cultivated by European settlers for centuries (Basiouny and Himelrick, 2001; Munson, 1909). Although a member of the grape family Vitaceae, there are significant morphological and genetic differences that make this species distinct from the more widely cultivated species such as *Vitis vinifera* L. or *Vitis labrusca* L., such as differences in chromosome number and leaf shape (Comeaux et al., 1987; Liu et al., 2016). This has led taxonomists to divide *Vitis* further into two subgenera: *Euvitis*, or bunch grapes, and *Muscadinia*, which includes muscadines and two tropical grape species with similar morphologies (Basiouny and Himelrick, 2001).

One of the distinctions between muscadines and other grape species is the recommended method of vegetative propagation. Although layering was a standard method of propagation in the 19th century and earlier for nurseries working with perennial plant species, the development of novel protocols for propagation by hardwood and softwood cuttings began to be studied extensively in the 20th century (Hartmann et al., 1997). Concurrent research conducted on propagation by hardwood cuttings in both *V. vinifera* and *V. rotundifolia* revealed highly effective methods for *V. vinifera* and persistent inconsistencies in the rooting ability of *V. rotundifolia* (Cowart and Savage, 1944; Doelle and Mitchell, 1964; Goode et al., 1982). Hardwood cuttings soon became the main propagation method for bunch grapes. Meanwhile, other research revealed that utilizing mist systems in a greenhouse setting were an effective way to propagate *V. rotundifolia* by softwood cuttings (Sharpe, 1954).

Past research into the hardwood propagation of muscadine cuttings evaluated many factors potentially affecting rooting success. Rooting studies in muscadines have been conducted

at vineyards across the southeastern U.S., but the variation in rooting success attributable to the study location has not been investigated. Cutting collection date is mentioned extensively in the literature, although it has not actually been tested as a factor in most studies. November is the most frequent month recommended to collect muscadine hardwood cuttings, although success varied from 0% to 80% among the studies using cuttings taken in November depending on the treatment combination used (Cowart and Savage, 1944; Newman, 1907; Niven, 1918; Woodroof, 1935). It has also been reported that muscadines can root readily when taken in December and that taking cuttings later than December could lead to injury to the vine and poor rooting (Goode et al., 1982; Niven, 1918; Whatley, 1975).

Multiple studies have used cold storage treatments as part of their rooting protocols (e.g. Whatley, 1975). However, the only two studies that specifically compared cuttings that received cold storage and cuttings that did not observed no significant effect (Cowart and Savage, 1944; Newman, 1907). Bottom heat also appears to play a significant role in promoting rooting in dormant cuttings. Goode et al. (1982) had 0% rooting success when muscadine cuttings were not given bottom heat compared to a high of 9% rooting when cuttings were taken in November and given bottom heat. Newman (1907) notes that continuously supplied bottom heat was essential to achieving 80% rooting. The application of various synthetic rooting hormones, although not studied extensively, does not appear to have an effect on rooting success in hardwood muscadine cuttings compared to no hormone application (Cowart and Savage, 1944; Goode et al., 1982). Differences in rooting success attributable to genotype are another factor of interest in previous studies. Dearing (1947) noted that some staminate cultivars are not difficult to propagate by hardwood cuttings compared to the historically important pistillate cultivar ‘Scuppernong.’ Goode et al. (1982) found that ‘Hunt’ outperformed ‘Cowart’ vines taken from the same

location. Most studies conducted on hardwood propagation in muscadines are at least several decades old, and most of the cultivars used in these early studies have since been replaced by newer improved varieties.

The position on the vine from which a cutting is taken may also have a role in promoting the rooting of dormant cuttings in muscadines. Cane position has been shown to have an effect on rooting efficacy in other *Vitis* species. Cuttings taken from basal cane segments rooted at a higher rate than cuttings taken from apical segments in both hardwood and softwood cuttings of *Vitis vinifera* and *Vitis aestivalis* Michx. (Daskalakis et al., 2018; Keeley et al., 2003). To date, a single study has directly investigated the effect of cane position on rooting in muscadines and observed moderately increased rooting success in cuttings taken from basal positions on the cane (Castro et al., 1994). Although not directly comparable to cane position, Goode et al. (1982) saw increased rooting in cuttings with larger diameters.

By the end of the 20th century, significant advances had been made in propagation technology, and the nursery industry was adopting newer, more advanced techniques for propagation. However, despite the labor involved and space required, layering was still a recommended propagation method for muscadines (Hartmann et al., 1997). Nurseries with the ability to maintain the high humidity necessary for softwood cuttings to succeed expect to have rooting success of 90% or more (Basiouny and Himelrick, 2001). The lack of a scientific consensus on the effectiveness of rooting from hardwood cuttings and the relative ease with which muscadine can be propagated by softwood cuttings led to the abandonment of hardwood propagation of muscadines.

Neither of the two widely accepted methods of vegetatively propagating muscadines are ideal for breeding programs. Softwood cuttings are generally taken in June and must be

maintained throughout the summer, which adds to the workload of the fruit breeding personnel during an already busy season. Propagation by layering requires a significant amount of field space and labor to be effective (Hartmann et al., 1997), which directly limits the number of seedlings that can be evaluated by the breeding program. The most significant reason that these methods are not ideal, however, is the amount of time required from when propagation is initiated until plants are ready to be transplanted into the field.

The breeding cycle for muscadines is similar to that of other perennial crops: from the time of making the cross to releasing a cultivar generally requires at least 10-15 years. A muscadine vine will usually not produce fruit until its third year. If a seedling is determined to be worthy of selection, it will be vegetatively propagated and tested at multiple locations and observed for multiple years (Basiouny and Himelrick, 2001; Fehr, 1991). The time required to release a cultivar is a significant impediment in the field of crop improvement, and significant amounts of time and money have been spent determining new plant breeding methods that could accelerate the plant breeding cycle. Propagation by hardwood cuttings instead of softwood cuttings or layering is one way a muscadine breeding program can shorten its cycle relatively easily.

Muscadines flower from mid-May to mid-June and require approximately 100 days to fully ripen (Goldy, 1992). For areas in the northern extent of its native distribution, this can be as late as September or October. Plant breeders therefore taste fruit and make selections well after the recommended time to take softwood cuttings (Basiouny and Himelrick, 2001; Sharpe, 1954). Breeding programs must, therefore, wait until the following June in order to propagate the selected seedling vine for replicated trials, and these softwood cuttings will not be ready for transplanting into the field until too late in the growing season. The plants must be overwintered

in a greenhouse and planted the following spring after the frost free date. In summary, an entire growing season is taken up by the propagation process when using softwood cuttings in the context of a muscadine breeding program. Hardwood cuttings, on the other hand, are taken during the dormant season in the months after the breeder has made selections in August or September. By the following spring planting season, the hardwood cuttings have had months to establish and can be planted without delay. Therefore, an effective and reliable hardwood propagation protocol is able to reduce the muscadine breeding cycle by an entire year.

The staff of the University of Arkansas System Division of Agriculture (UA) Fruit Breeding Program applied a hardwood rooting protocol developed for bunch grapes to muscadine grapes and discovered that hardwood muscadine cuttings rooted at low rates, but still produced enough viable plants to meet the program's modest needs. These cuttings were taken in early December and placed into cold storage until early January, when they were dipped in rooting hormone and placed into a 100% perlite rooting medium under a mist system. This protocol has been the main propagation method for the UA muscadine breeding program for over 10 years, despite the scientific consensus that hardwood propagation of muscadines could not be done or was too difficult to be effective. Conservative estimates of rooting success were around 10% year-to-year, with significant differences between the success of different genotypes (David Gilmore, Personal Communication).

This study aims to accomplish two goals. The first is to evaluate the efficacy of a bunch grape hardwood rooting protocol that has been applied to muscadines over the last 10 years in the UA Fruit Breeding Program. The second is to evaluate five factors identified within the literature that likely affect the success of rooting muscadines from hardwood cuttings.

Materials and Methods

Collection of hardwood cuttings. The study was conducted over two years in a greenhouse at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR from Nov. 2019 to June 2020, and Nov. 2020 to June 2021. Hardwood cuttings were taken from three different vineyards to represent diverse muscadine growing regions: the aforementioned Milo J. Shult Research and Extension Center (36.0991 N, -94.1722 W), the UA Fruit Research Station in Clarksville, AR (35.5332 N, -93.4037 W), and Paulk Vineyards near Ocilla, GA (31.5746 N, -83.0806 W). Three cultivars were selected for this study: 'Fry,' 'Carlos,' and 'Supreme.' Cuttings were collected from mature vines at each vineyard location. Cuttings were approximately 15-20 cm long and 5-10 mm wide, with a minimum of three nodes. If leaves were present, they were removed at the time cuttings were collected. The cuttings were cut perpendicular at the base and at a 45° angle at the top to ensure polarity was maintained and facilitate water runoff from the mist system. Hardwood cuttings were collected at the beginning of each month during November, December, January, and February. Collection dates for the 2019-2020 season were 4 Nov., 4 Dec., 6 Jan., and 4 Feb. and collection dates for the 2020-2021 season were 2 Nov, 1 Dec., 4 Jan., and 2 Feb. At the time of collection, half of all cuttings received a cold storage treatment of 4° C for 30 d before planting.

Mist bed and greenhouse conditions. The rooting containers used for this study were SureRoots® Deep Cell 50-cell plug trays (T.O. Plastics, Clearwater, MN) with 12.7 cm deep cells. Trays were cut into 10-cell experimental units to facilitate replication and randomization within the study. The rooting media was 100% perlite. Cuttings were dipped in 0.1% Indole-3-butyric acid powder (Bonide Products Inc., Oriskany, NY) before being inserted into the rooting

media such that one node was fully submerged in the rooting media and a second node was level with the surface of the rooting media. One cutting was planted into each cell.

Ambient temperatures in the greenhouse were maintained between 18-24 °C through the course of the study. Two greenhouse benches measuring 1.5 m x 3.0 m were used as mist beds. A 1.9 cm in-line sprinkler valve (Rain Bird Corp., Azusa, CA) was connected to a standard hose valve at native city water pressure. A Galcon 8056S AC-6S (Galcon USA LTD., Simi Valley, CA) programmable irrigation controller was wired to the valve. The valve was programmed to run the mist system for 15 s every 10 min with an irrigation window of 6:00 am to 6:00 pm. The irrigation line was 0.64 cm in diameter and suspended approximately 0.61 m above the mist benches. Three Coolnet Pro foggers (Netafim Irrigation Inc., Fresno, CA) were spaced evenly lengthwise across each bench. These foggers were a four-nozzle system and each nozzle flowed at 7.6 $L.h^{-1}$. In addition, an internal check valve inside each fogger ensured that shut off happened quickly after valve closure to maintain a consistent 15 s mist interval. The media was also hand watered to field capacity approximately twice a week during the study as well as immediately after cuttings were placed in the media to ensure adequate moisture availability.

Half of all cuttings received continuously supplied bottom heat at 26 °C. The bottom heat treatment was applied using 1.5 m x 53 cm Redi-Heat™ heavy-duty propagation mats (Phytotronics Inc., Earth City, MO) programmed to maintain an average temperature of 26 °C with a Redi-Heat™ digital thermostat. The attached soil probe was inserted approximately 5 cm into the perlite rooting media.

Data collection. Hardwood cuttings were removed from the media and phenotyped 90 d after they were placed in the mist beds. For each year of the study, the first date of data collection fell in the first week of February and the last date of data collection occurred during the first week of

June. Rooting occurrence was recorded as both a binary outcome and a quantitative measure of the number of roots and the length of the longest root in cm for each cutting in the 10-cell experimental units. Achieving perfect uniformity of the cuttings was not possible. Therefore, three additional measurements were taken: cutting diameter, cutting length, and number of nodes. Cutting diameter was recorded in mm at the top and bottom of the cutting. The overall length of the cutting in cm and the number of nodes per cutting were also recorded. The average of these two measurements was calculated for each experimental unit for the final analysis.

Experimental design and analysis. Cuttings were placed in 10-cell experimental units with one unit for each block. The proportion of cuttings that rooted in each experimental unit was analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC) as a mixed model with five fixed effect treatments, two random effects, and a negative binomial distribution. Bottom heat was the main plot effect and the other four factors (collection date, cold storage, cultivar, and location) were completely randomized within the split plot. Block and year were analyzed as random effects. Four and five-way interactions were dropped from the statistical model to only include main effects and interaction effects that were assumed to have any biological significance as proposed by Harrell (2015). In order to interpret higher-order interactions, the results of mean separations tests were sliced by location when applicable. Mean separations were performed with Tukey's Honest Significant Difference. Graphical representations of data were constructed using the ggplot2 package in RStudio (Wickham, 2016). After analyzing the initial model, a second model was created so that the results of this study could be distilled into a concise protocol for rooting hardwood muscadine cuttings. Instead of five fixed effects, cultivar and location were treated as random effects and only data from the

November harvest date was used. Only the heat and storage factors were analyzed as fixed effects.

Results

First frost dates and chill accumulation. The three vineyard locations selected for this study experienced different climatic conditions across the two-year study period. For both years, the first frost occurred at the Fayetteville location in mid-October and at the Ocilla location in the first week December. At Clarksville, the first frost was 31 Oct. in 2019 and 30 Nov. in 2020 (Table 1). Chill hours were calculated using the Below 45 °F model starting 1 Oct. and the cumulative chilling amount by the first of each month during the trial period was reported for each location At the first collection date in November, the Clarksville, AR, Fayetteville, AR, and Ocilla, GA locations received an average of 41, 193, and 0 chill hours, respectively (Table 1). By the end of the study in February, the Clarksville, AR, Fayetteville, AR, and Ocilla, GA locations had received an average of 1160, 1541, and 550 chill hours, respectively (Table 1).

Rooting percentages and correlations among response variables. The percentage of cuttings that formed roots for the two years of this study were 17.1% and 15.0%, respectively, for an overall rooting percentage of 16.0%. There were four higher order interactions that significantly affected the proportion of hardwood muscadine cuttings that rooted: Location x Cultivar x Date, Location x Heat x Date, Heat x Storage x Date, and Location x Cultivar x Storage (Table 2). Cutting length and cutting diameter were both positively correlated with an increased proportion of cuttings that root (Table 3). The number of nodes per cutting was not correlated with rooting outcome (Table 3). There were multiple significant positive correlations between rooting success and length and diameter of the cutting (Table 3). Overall length of the cutting was positively correlated with rooting success ($r = 0.22$, $P < 0.001$). The number of roots per experimental unit

was also positively correlated with cutting length ($r = 0.11$, $P < 0.05$). Cutting diameter was significantly correlated with all three rooting variables: rooting success ($r = 0.25$, $P < 0.001$), root number ($r = 0.15$, $P < 0.01$), and length of the longest root ($r = 0.11$, $P < 0.05$). The number of nodes per cutting was not significantly correlated with any of the rooting variables.

Location x cultivar x storage interaction. Because the interaction effect of Location x Cultivar x Storage was significant ($P = 0.004$, Table 2), means separation was performed after slicing results by location. Only the Fayetteville, AR location had significant differences between the Cultivar x Storage treatment combinations (Fig. 1). 'Carlos' was the only cultivar that rooted significantly better after receiving a cold storage treatment in the Fayetteville location; 18.9% of stored cuttings rooted compared to 6.2% of cuttings that did not receive the cold storage treatment. There was no difference in the rooting ability of the three cultivars when cuttings were not stored. However, stored cuttings of 'Carlos' and 'Fry' from Fayetteville rooted significantly better (18.9% and 18.7%, respectively) than stored cuttings of 'Supreme' (6.5%).

Heat x storage x date interaction. The interaction of Heat x Storage x Date was significant ($P = 0.004$, Table 2). Therefore, means separation was performed after slicing results by date. For cuttings taken in November, 37.8% of cuttings that were not stored and received bottom heat rooted. This treatment combination performed significantly better than the November treatments that did not receive bottom heat, but it was not significantly different than the treatment that received both bottom heat and cold storage (Fig. 2). Furthermore, cuttings that received bottom heat and cold storage rooted significantly better (27.5%) than cuttings that were cold stored but did not receive bottom heat (11.7%). There was no difference between the rooting percentage of cuttings that received storage and those that did not when bottom heat was not supplied (Fig. 2).

In December, the experimental units that received no cold storage and no bottom heat had significantly lower rooting percentages (1.0%) than all other treatment combinations (Fig. 2). Cuttings that were stored and received bottom heat performed significantly better (21.7%) than cuttings that were stored but did not receive bottom heat (9.9%). There was no difference in rooting ability between the treatment that received bottom heat but not storage and the treatment that was stored but did not receive bottom heat. In January, for cuttings that were not stored, supplying bottom heat significantly increased rooting percentages (15.3% vs 5.3%). There were no other differences between treatment combinations. In February, there were no significant differences between treatment combinations. The four treatment combinations ranged from 6% to 11% during this month, the worst overall rooting percentages observed for any of the months in this interaction (Fig. 2).

Location x cultivar x date interaction. For the Clarksville, AR location, there were significant differences in rooting percentages between cultivars within each collection date for all months except for November (Fig. 3). In December, 'Fry' had lower rooting success than 'Carlos' and 'Supreme' (5.6% vs 10.6% and 15.3%, respectively). However, in January 'Fry' rooted at more than double the rate of 'Supreme' and 'Carlos.' 'Supreme' had significantly fewer cuttings with roots in February (3.2%) than it did in any other month in Clarksville (13.4% average). 'Carlos' rooted equally well in Clarksville across all four dates tested (Fig. 3).

For the Fayetteville, AR vineyard location, there were no significant differences between cultivar rooting efficacy within each month. The only significant differences were across dates and cultivars (Fig. 3). 'Carlos' rooted significantly better in November (26.7%) than in December (7.1%), January (10.2%), or February (7.1%). 'Supreme' rooted significantly better in November (18.3%) than in December (4.7%) and February (7.4%) but not in January (8.8%).

There were no differences in rooting ability for ‘Fry’ across the four collection dates in Fayetteville.

The Ocilla, GA location in November had the highest rooting percentages observed across the entire study (Fig. 3). ‘Fry’ cuttings collected in November rooted at 48.8%, more than four times the average rooting percentage of the other three collection dates (11.7%). Similarly, 42.9% of ‘Supreme’ cuttings rooted, also more than four times the average of the other collection dates (9.6%). ‘Carlos’ rooted at 29.7%, which is more than four times the average of the other collections dates (7.1%) but only slightly more than the rooting percentage of the ‘Carlos’ from Fayetteville in November (26.7%). Unlike ‘Carlos,’ ‘Fry’ and ‘Supreme’ rooted at much higher rates in Ocilla, GA when cuttings were taken in November compared to the other two locations (Fig. 3). Rooting success for cuttings taken from Ocilla, GA in December, January, and February was comparable to the rates observed in cuttings collected in the same month at Fayetteville, AR and Clarksville, AR.

Location x date x heat interaction. For the Clarksville, AR, location there were no significant differences in rooting percentages for cuttings with and without bottom heat within each month of collection. Nor were there any differences in rooting success across months for cuttings that were not supplied bottom heat (Fig. 4). However, supplying bottom heat significantly increased the rooting ability of cuttings in November (24.8%) compared to February (6.2%). Overall, there were fewer significant comparisons between cuttings taken in Clarksville than the other two study sites.

For the Fayetteville location, cuttings taken in November that were supplied bottom heat (30.4%) outperformed cuttings that received bottom heat in December (9.8%) and February (7.0%). That treatment combination also outperformed cuttings in every month but November

that were not supplied bottom heat. Within each month, there were no differences between cuttings supplied bottom heat and those without (Fig. 4).

As seen in the results of the Location x Cultivar x Date interaction, the cuttings collected in the Ocilla, GA site in November significantly outperformed most other treatment combinations (Fig. 4). Cuttings taken in November and supplied with bottom heat rooted at 47.4%, although that is not significantly different from cuttings taken in November that did not receive the heat treatment (33.1%). Among cuttings taken in Ocilla, GA, the November cuttings supplied with bottom heat outperformed cuttings not supplied with bottom heat in every other month. For cuttings that were supplied bottom heat, November was a significantly better date to attempt to collect cuttings than January and February. Ocilla, GA, also had the only significant within-month difference for the heat treatment. In December, cuttings supplied with bottom heat rooted at 22.6%, while those without bottom heat rooted at only 2.9%.

For the second model run to specifically test the effect of heat and storage on cuttings taken in November, there was no significant effect of supplying bottom heat on the rooting ability of hardwood muscadine cuttings. There was also no interaction effect between the cold storage and bottom heat factors. However, cuttings taken in November that were given a cold storage treatment rooted significantly worse (19.4%) than cuttings not given the storage treatment (27.2%).

Discussion

This study represents the first investigation into the rooting ability of hardwood muscadine cuttings in over 25 years. Past research used single vineyard locations to evaluate rooting success across a narrow range of collection dates. The combination of multiple important factors within this study (collection date, bottom heat, cold storage, cultivar, and vineyard

location) allowed for a comprehensive investigation and analysis of the interactions between these variables and the treatment combinations most likely to yield a higher percentage of rooted cuttings. The two-year average rooting percentage of 16% reported here is higher than many of the less-successful rooting studies of the 20th century (Goode et al., 1982; Woodroof, 1935) but far lower than the few papers that concluded hardwood propagation in muscadines is relatively easy (Newman, 1907; Whatley, 1975). While the multiple significant higher-order interactions add considerable complexity to the interpretation of the results, they also provide possible explanations as to why past studies came to radically different conclusions from one another.

The Location x Cultivar x Storage effect was significant, but sliced results by location showed that only the Fayetteville vineyard had significant differences between treatment means. Furthermore, the only cultivar that performed differently based on storage in that interaction was 'Carlos' (Fig. 1). Fayetteville is the northernmost location and receives more chill hours than the other two vineyard sites (Table 2). It would be expected that a crop with low chilling requirements like muscadines, generally only requiring 200-300 chill hours (Basiouny and Himelrick, 2001), would not see significant differences in rooting due to an added cold storage treatment. However, a high number of chill hours (>2000) have been shown to increase the number of roots per cutting but not overall rooting percentage in *Euvitis* hardwood cuttings (Keeley et al., 2000). Regardless of the physiological effects of chill hours on rooting ability, cold storage treatments have been used previously in muscadine rooting protocols. Whatley (1975), based in Alabama, reported that muscadines root readily from hardwood cuttings taken in December when cold stored for 60-90 d, significantly longer than the 30 d storage treatment applied in this study.

The effects of heat and storage on rooting success varied depending on the collection date of the cuttings (Fig. 2). The attenuated effects of these treatments in January and February are possibly a result of climatic conditions cuttings experienced during the study. If the number of chilling hours accrued by *Vitis* vines do in fact have an effect on rooting success (Keeley et al., 2000; Smith and Wareing, 1972), it is possible that those requirements have been fulfilled by January or February at the vineyard locations used in this study. In that case, a month-long cold storage treatment (or lack thereof) would not affect the physiology of the cuttings in a way that promotes rooting.

In addition, conditions in the greenhouse were warmer during the 90 d rooting period for cuttings taken relatively late in the winter. Cuttings taken in February and given the cold storage treatment were not removed from rooting media for data collection until the beginning of June, when supplemental bottom heat may not be as necessary for stimulating rooting due to high ambient temperatures in the greenhouse. Cuttings taken in November that did not receive the storage treatment but were supplied bottom heat rooted at 37.8%, significantly higher than 12 of the 15 other treatment combinations in the comparison of Heat*Storage*Date treatment effects (Fig. 2). Of the three treatment combinations within the Heat*Storage*Date interaction that were not significantly different from one another, two were from November and one from December. The effect of date and bottom heat on rooting ability is mentioned extensively throughout the available literature. Newman (1907) and Dearing (1947), located in South and North Carolina, respectively, reported rooting success as high as 80% when cuttings were taken in November and supplied with bottom heat while Goode et al. (1982), located in Georgia, observed 0% rooting in cuttings taken from late November to February and not supplied bottom heat. It appears that

supplying bottom heat early in the dormant season may prevent these cuttings from achieving full dormancy and therefore help stimulate rooting.

It is expected that rooting success is dependent to some extent on the cultivar being propagated, although in this study no cultivar consistently outperformed the others. Cultivars such as 'Thomas' and 'Hunt' have been shown to root better than 'Scuppernong,' although these cultivars are no longer widely planted (Dearing, 1947; Niven, 1918; Woodroof, 1935). None of the cultivars used in this study have been used in previous research on propagation. It was observed in our research that the rooting success of muscadine cuttings depended not only on the cultivar, but also on the location and date in which the cutting was taken (Fig. 3).

Previous studies rarely mention the effect of location on rooting success, although Dearing (1947) felt that some observed differences between sites could be attributed to soil types and overall climatic differences. To date, no other study has specifically tested the effect of vineyard location on the rooting ability of hardwood muscadine cuttings. Fluctuations in rooting ability through the winter season have been well-documented in other perennial fruit crops and are generally linked to physiological factors related to dormancy (Bassuk and Howard, 1981; Guerriero and Loreti, 1975; Smith and Wareing, 1972). The native range of muscadines covers various climatic regions, resulting in variations between when vines enter dormancy. Thus, the prescriptive recommendations of previous studies regarding when hardwood cuttings should be taken may not be applicable to all locations where muscadines are grown. In this study, cuttings taken from Ocilla, GA (USDA Hardiness Zone 8b) performed exceptionally well in early November, well before the first frost date at that location for both years of the study and when no chilling hours had been accumulated (Table 2, Fig. 3). We hypothesize that the high rooting percentages from cuttings taken in November in Ocilla, GA could be due in part to the

incomplete dormancy of the vines at this location. The precipitous drop in rooting percentages in Georgia after November would be explained by the vines fully entering dormancy (Fig. 3 and 4). At the Clarksville and Fayetteville locations, the more consistent rooting percentages from month-to-month would be explained by the fact that by early November these vines were already fully dormant (Table 2). The UA Fruit Breeding Program intends to mimic the results observed in this study from cuttings taken in November from Ocilla, GA by taking hardwood cuttings from Clarksville, AR in early October this year (Margaret Worthington, Personal Communication). The goal of changing this date to October from December is to potentially take advantage of the vines in Clarksville not being completely dormant while still keeping the propagation schedule outside the busy growing season.

Vegetative buds play an important role in rooting *Vitis* species from cuttings by serving as endogenous sources of auxin hormones that promote adventitious rooting (Kawai ,1996; Thomas and Schiefelbein, 2004). In this study cuttings were collected with at least three nodes. There was no observed correlation between the number of nodes per cutting and rooting success (Table 1), although the exogenous rooting hormone application may have affected these findings by supplying a large amount of synthetic auxin.

Significant positive correlations with increased rooting percentage were found for both the overall length of the cutting and cutting diameter. The average recommended length of hardwood cuttings is often mentioned throughout the literature as part of rooting protocols but has not been studied as a factor in rooting success of muscadines (Dearing, 1947; Newman, 1907; Woodroof, 1935). Increased cutting length has been observed to affect rooting in other fruit crops (Aljane and Nahdi, 2014; Wainwright and Hawkes, 1988), although the correlation between cutting length and rooting success is not always positive as it was in this study

(Exadaktylou et al., 2009). Our results also support previous findings on the association of increased cutting diameter and higher rooting success (Castro et al., 1994; Goode et al., 1982). The differentiation of rooting ability based on cane position is well-documented in both other *Vitis* species and other woody plant species (Hartmann et al., 1997; Keeley et al., 2000). In this study, cane position was not recorded at the time of cutting collection and cuttings were taken from a variety of positions along the cane. Cutting diameter may function as an estimate for cane position and might explain the observed correlation. The differences in rooting ability were possibly due to a gradient in moisture, nitrogen levels, and chemical composition that has been observed from proximal portions of the cane to the growing point of the plant (Geny et al., 2002; Hartmann et al., 1997; Tukey and Green, 1934).

A second statistical model was run after initial data analysis to provide better recommendations for those attempting to root muscadines from hardwood cuttings. The dataset was consolidated to include only cuttings taken in November as those treatment combinations regularly outperformed cuttings taken during other months. The model was simplified to only test the effects of the heat and storage treatments. The location and cultivar factors were made random factors to control for those effects since hardwood propagation efforts are unlikely to be done on the three cultivars or three vineyard locations used in this study. The negative effect on rooting ability observed when cuttings taken in November were given a cold storage treatment supports our hypothesis that taking cuttings before vines are completely dormant is the ideal hardwood propagation method. However, even cuttings taken in November that were given the cold storage treatment had a numerically higher rooting percentage (19.4%) than the average rooting percentage for the entire study (16.0%).

Conclusion

This research provides significant evidence that rooting muscadines from hardwood cuttings is a more effective method of propagation than many previous studies have concluded. The complex experimental design of the study allowed for the higher-order interactions affecting the rooting success of muscadine hardwood cuttings to be elucidated. Cuttings taken from Georgia in early November formed roots more than 40% of the time, the highest percentages for any treatment combination in this study. When cuttings from November were analyzed alone with the location and cultivar factors considered random effects, applying a cold storage treatment reduced rooting percentages from 27% to 19%. Increased cutting length and cutting diameter were significantly correlated with increased rooting success while an increased number of nodes was not. While the rooting percentages reported in this study may not allow for commercially successful propagation of muscadines by hardwood cuttings, breeding programs or germplasm repositories with modest needs may find that transitioning to an off-season propagation protocol may save time and money.

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Table 1. First frost dates and chill hours accumulated by the first of each month cuttings were taken for each vineyard location during 2019-2020 (year 1) and 2020-2021 (year 2).

Date	First frost		1 Nov.		1 Dec.		1 Jan.		1 Feb.	
Year	1	2	1	2	1	2	1	2	1	2
Clarksville	31 Oct.	30 Nov.	50[z]	32	314	277	684	750	1001	1318
Fayetteville	12 Oct.	16 Oct.	185	201	586	453	1053	969	1540	1541
Ocilla	3 Dec.	1 Dec.	0	0	132	42	268	399	457	642

[z]Chill hours calculated using the Below 45 °F Model.

Table 2. Analysis of variance results of the main and interaction effects for the five factors used in this study, including collection date, vineyard location, cultivar, cold storage treatment, and bottom heat treatment.

Effect	Num. DF[y]	Den. DF[x]	*P* value
Date[z]	3	495	<0.001
Location	2	495	0.659
Cultivar	2	495	0.018
Storage	1	495	0.011
Heat	1	1	0.137
Location*Date	6	495	<0.001
Cultivar*Date	6	495	0.016
Date*Storage	3	495	<0.001
Heat*Date	3	495	<0.001
Location*Cultivar	4	495	0.179
Location*Storage	2	495	0.489
Location*Heat	2	495	0.130
Cultivar*Storage	2	495	0.229
Cultivar*Heat	2	495	0.271
Heat*Storage	1	495	0.002
Location*Cultivar*Date	12	495	0.020
Location*Date*Storage	6	495	0.287
Location*Heat*Date	6	495	0.028
Location*Cultivar*Storage	4	495	0.004
Location*Cultivar*Heat	4	495	0.828
Cultivar*Heat*Storage	2	495	0.581
Heat*Date*Storage	3	495	0.017

[z] Date = collection date, location = vineyard location, storage = cold storage treatment, heat = bottom heat treatment.
[y]Numerator degrees of freedom for the F test.
[x]Denominator degrees of freedom used for the F test.

Table 3. Pearson's correlation coefficients for variables measuring rooting success and cutting attributes. Rooting success was measured as the proportion of cutting per experimental unit that formed roots by the end of the 90 d rooting period. The root number and longest root variables were the sum of the number of roots in each experimental unit and the sum of the longest root per cutting in each experimental unit, respectively.

	Rooting success	Root number	Longest tip	Nodes	Cutting length	Cutting diameter
Rooting success	.	0.823***	0.717***	0.008^{NS}	0.220***	0.249***
Root number	0.823***	.	0.758***	-0.015^{NS}	0.112*	0.154**
Longest root	0.717***	0.758***	.	0.075^{NS}	0.063^{NS}	0.113*

^{NS}, Nonsignificant; *, **, *** Significant at $P < 0.05$, 0.01, 0.001, respectively.

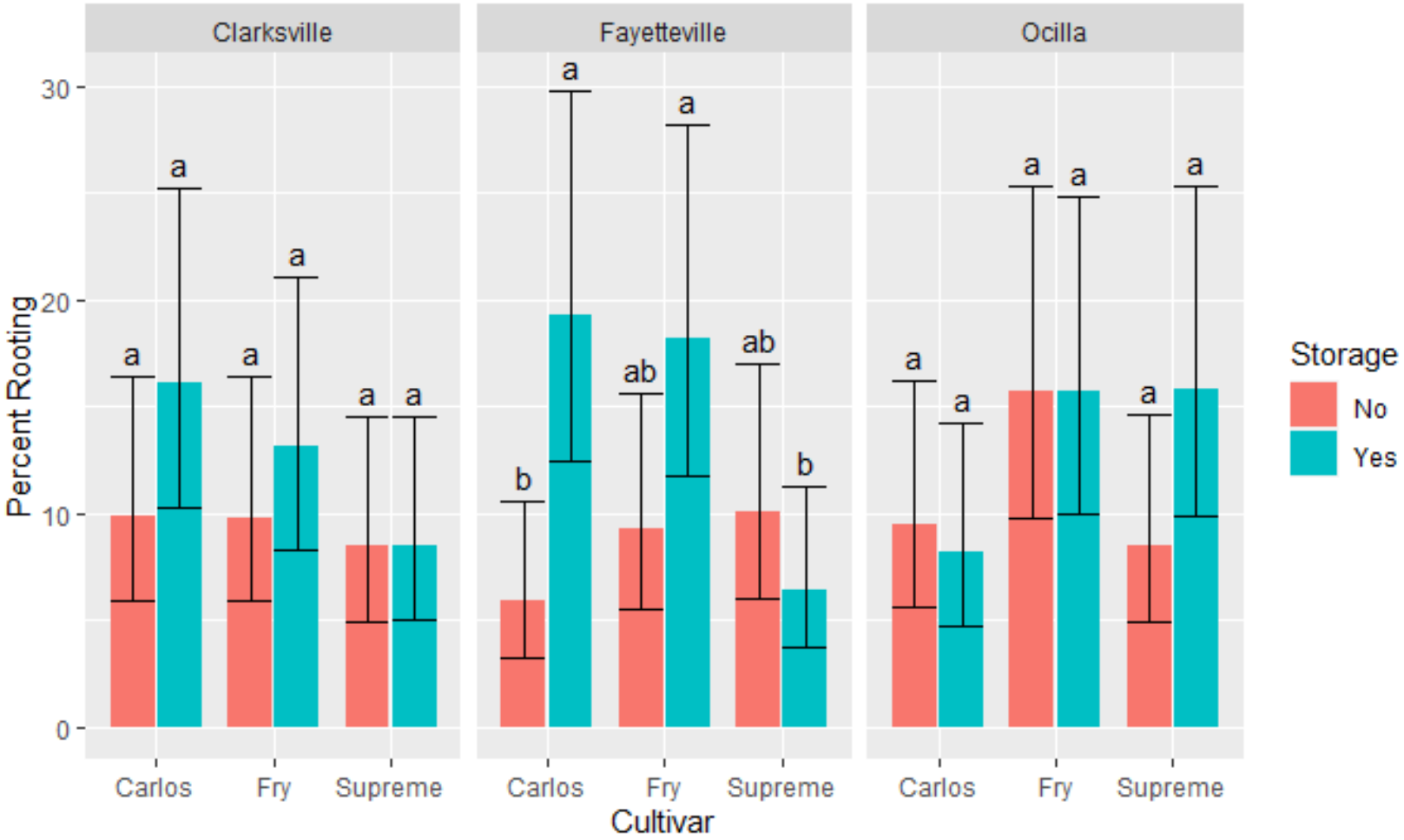

Fig. 1 The effect of Location*Cultivar*Storage treatment combinations on rooting success. Results were sliced by location. Means followed by different letters within each location are significantly different using Tukey's honestly significant difference at α=0.05.

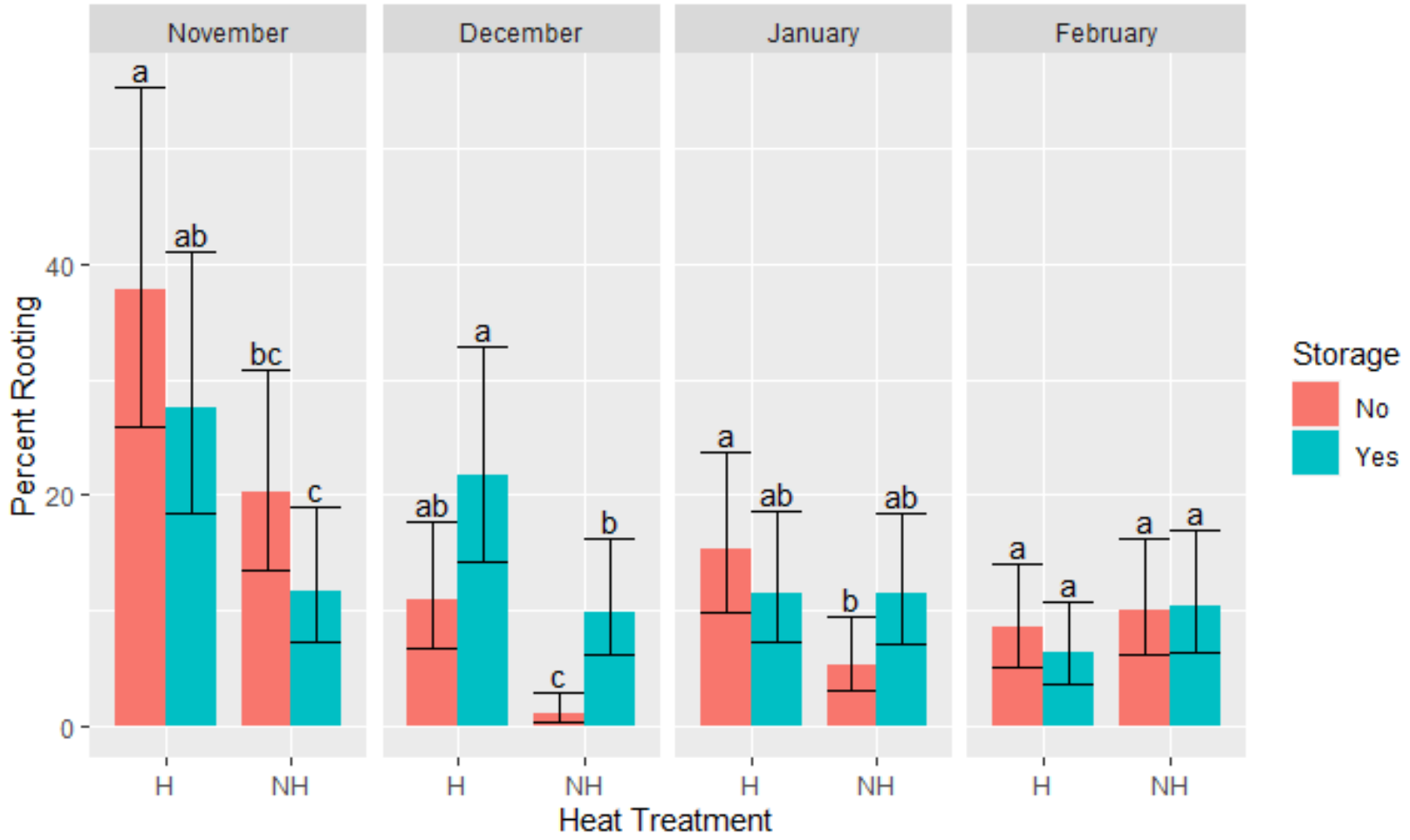

Fig. 2 The effects of the Heat*Storage*Date treatment combinations on rooting success. Results were sliced by collection date. Means followed by different letters within each collection date are significantly different using Tukey's honestly significant difference at α=0.05.

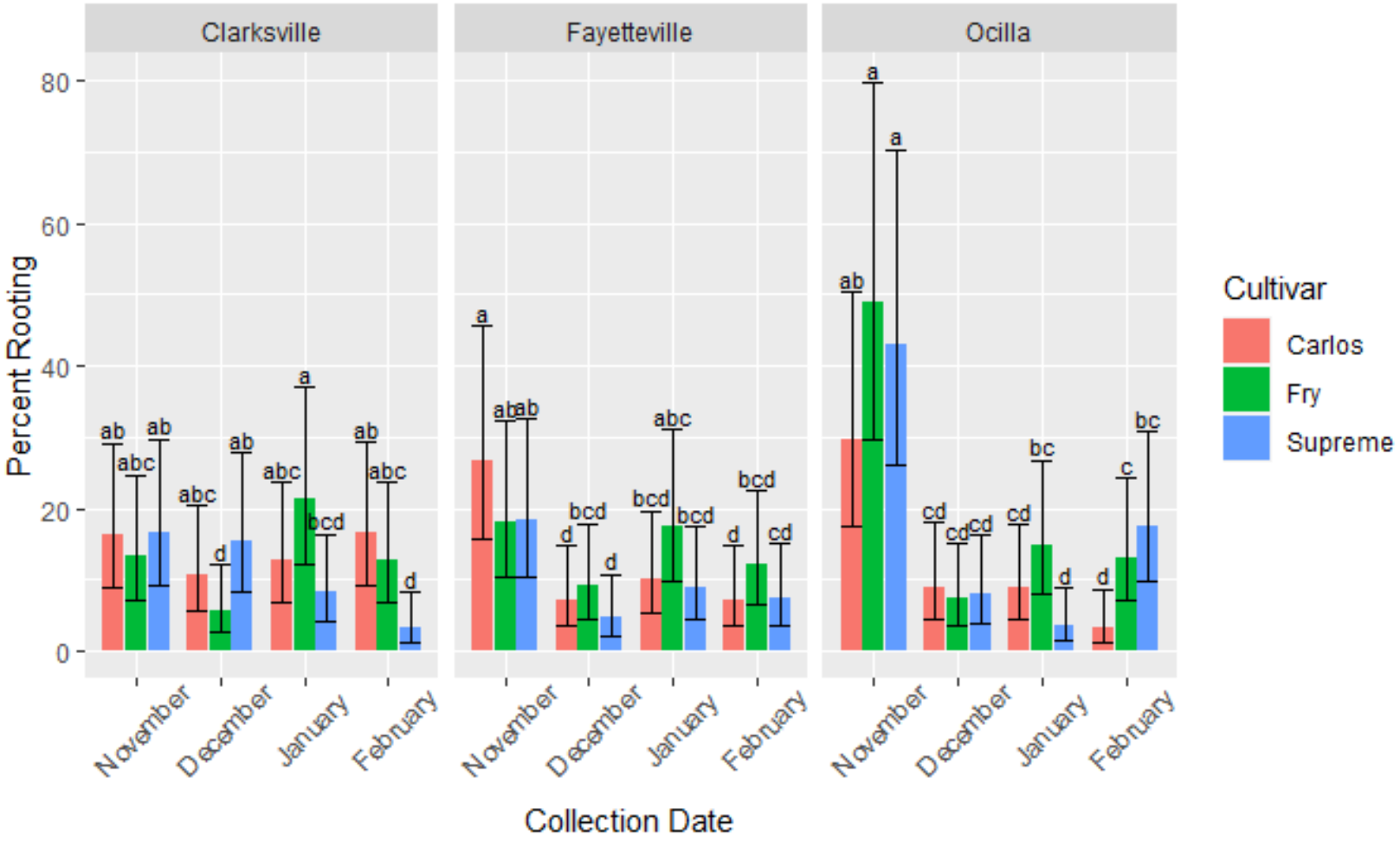

Fig. 3 The effect of the Location*Cultivar*Date treatment combinations on rooting success. Results were sliced by location. Means followed by different letters within each location are significantly different using Tukey's honestly significant difference at α=0.05.

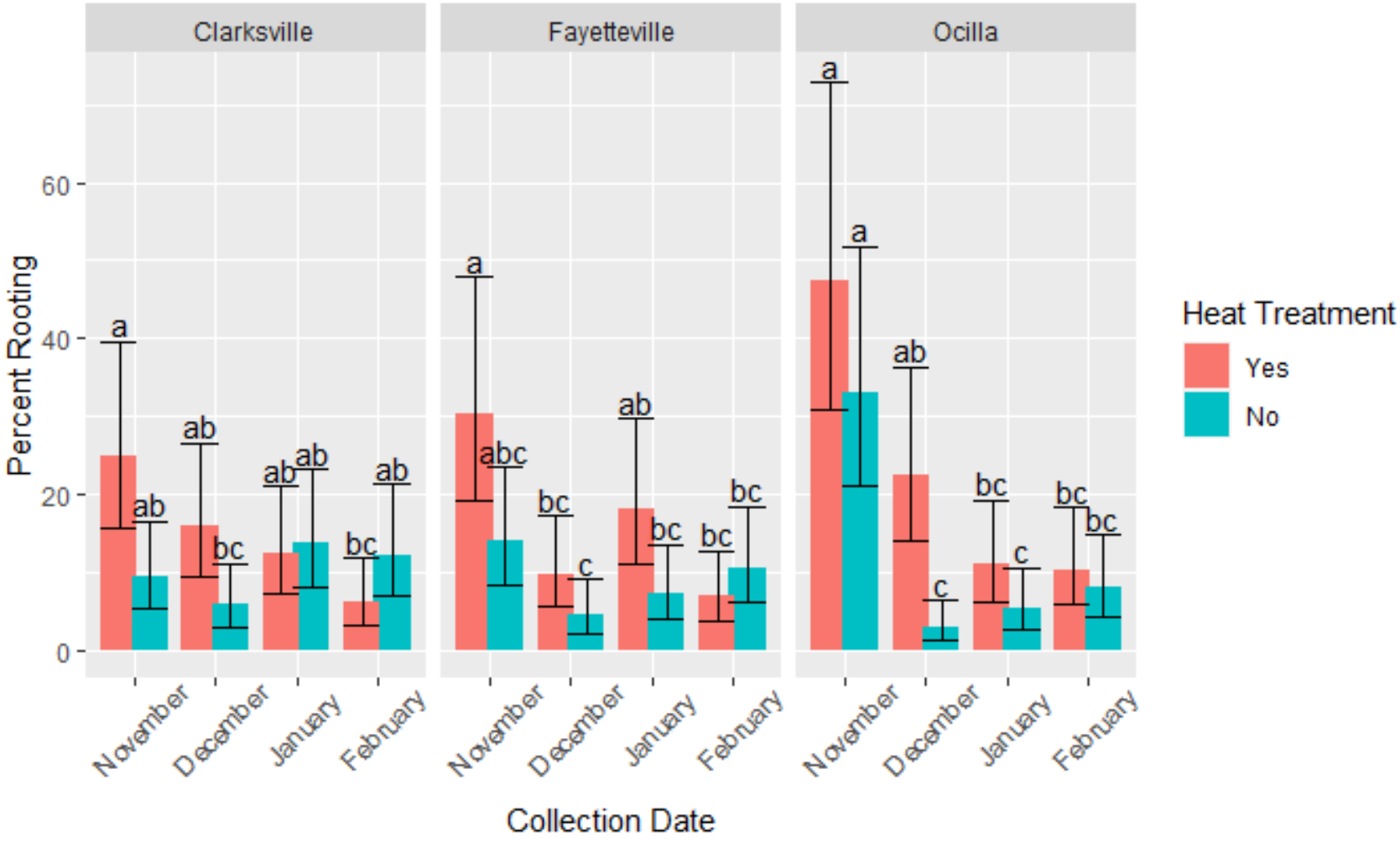

Fig. 4 The effect of the Location*Date*Heat treatment combinations on rooting success. Results were sliced by location. Means followed by different letters within each location are significantly different using Tukey's honestly significant difference at α=0.05

Chapter II

Genetic Diversity of Wild and Cultivated Muscadine Grapes (*Vitis Rotundifolia* Michx.)

Abstract

The muscadine (*Vitis rotundifolia* syn. *Muscadinia rotundifolia*) is an American grape species native to the southeastern United States that has been cultivated for centuries. Muscadines are one of three grape species in subgenus *Muscadinia* with a chromosome number of 2n=40 (*V. rotundifolia*, *V. munsoniana*, and *V. popenoei*), making them genetically distinct from the European wine and table grape (*Vitis vinifera*) and other species in subgenus *Euvitis*. Crop improvement efforts have been continuous since the late 19th century, yet the germplasm that served as the foundation for early muscadine breeding efforts was sourced from a relatively small portion of their native range, mostly in the coastal plains of North Carolina. This study used the rhAmpSeq *Vitis* core panel haplotype markers to genotype 194 *Muscadinia* accessions from five cultivated populations and 15 wild populations collected across their native range. Wild populations from the western half of the native range were generally less genetically differentiated than hypothesized, but were genetically distinct from the material used in both past and present breeding efforts. One population collected from coastal North Carolina grouped closely with *Vitis munsoniana* accessions despite being well outside the reported range for that species. Principal coordinate and *structure* analyses revealed three main groups within the 194 accessions: one for cultivated material, one for wild *V. rotundifolia*, and one for *V. munsoniana* and *V. popenoei*. At K=5, *structure* results showed that more recent muscadine cultivars are further differentiated from wild accessions and varieties. These analyses confirmed our hypothesis that muscadine cultivars are genetically differentiated from their wild counterparts. This study also showed that genetic diversity in *V. rotundifolia* is not equally distributed across

its native range and that the limited number of genotypes used in crop improvement efforts have not fully utilized the genetic diversity within the species.

Introduction

The muscadine (*Vitis rotundifolia* Michx. syn. *Muscadinia rotundifolia* Simpson ex Munson) is a member of the grape family (Vitaceae) native to the southeastern United States. For centuries this species has been used for both wine and fresh market production. The native range of the muscadine is from Maryland west to Texas and south to the Gulf Coast. Perhaps the most important factor limiting the range of the species is cold hardiness. Although there are documented instances of muscadine vines surviving temperatures of -23 °C, regions that regularly attain winter lows of -18 °C are considered unsuitable for commercial muscadine production (Clark, 2001; Dearing, 1947; Munson, 1909).

Taxonomically, the genus *Vitis* can be split into two subgenera based on differences in chromosome number: *Euvitis* or bunch grapes (2n = 38) and *Muscadinia* (2n = 40). Major synteny between the *Euvitis* and *Muscadinia* genomes have been observed despite the difference in chromosome number and barriers to hybridization. Collinearity between chromosomes 7 and 20 in muscadines and chromosome 7 in bunch grapes suggests a chromosome fusion in *Euvitis* grapes sometime after the split in the two subgenera (Blanc et al., 2012; Cochetel et al., 2021; Lewter et al., 2019). In addition to significant genetic differences, there are multiple phenotypic distinctions between muscadine grapes and the more familiar species in *Euvitis* such as the European wine and table grape (*Vitis vinifera* L.). Muscadines are resistant to many pathogens such as Pierce's Disease (*Xylella fastidiosa* Wells et al.), grapevine downy mildew (*Plasmopara viticola* [Berk. & M.A. Curtis] Berl. & De Toni), and powdery mildew (*Erysiphe necator* syn. *Uncinula necator* [Schw. Burr]) that make *V. vinifera* cultivation in the

southeastern U.S. difficult (Hopkins et al., 1974; Merdinoglu et al., 2003; Riaz et al., 2011; Ruel and Walker, 2006). Muscadine flowers emerge 2 to 3 weeks later than *Euvitis* species in the same location and require approximately 100 d for the fruit to reach maturity (Olien, 2001; Goldy, 1992). Fruit size in muscadines is relatively large, often exceeding 2.5 cm in diameter, and fruit appears in clusters of five to ten berries (Anderson, 2006; Conner, 2009; Olien, 1990). By contrast, *V. vinifera* fruit are smaller and appear in much larger clusters. Other morphological differences that distinguish *Muscadinia* from *Euvitis* species are unbranched tendrils, continuous pith through the nodes, grape abscission at maturity, and smooth bark (Comeaux et al., 1987; Olien, 1990).

Muscadines are the only economically important member of *Muscadinia*, although the other two species (*V. munsoniana* Simpson ex Munson and *V. popenoei* J. L. Fennell) have been used in some breeding efforts (Goldy and Onokpise, 2001). *Vitis munsoniana*, sometimes classified as a subspecies of *V. rotundifolia*, is endemic from southern Florida along the Gulf Coast to Texas and has not been reported in the interior regions of the South (Dearing, 1947; Munson, 1909). *Vitis popenoei* is a tropical grape species native to Central America and first described in southern Mexico (Fennell, 1940). It has been used sparingly in breeding efforts, most importantly in the pedigree of the cultivar 'Southern Home' (Mortensen et al., 1994). The USDA National Clonal Germplasm Repository (NCGR) maintains a single accession of *V. popenoei* (DVIT 2970) collected from Veracruz, Mexico, affording the species a minor presence in previous molecular studies (e.g. Cao et al., 2020; Wan et al., 2013).

Muscadines have likely been cultivated by European colonists in the Americas since at least the 16th century, and by indigenous peoples for far longer (Bartram, 1791). Colonists in northern Florida were producing wine in the 1560s from large native grapes that were likely

muscadines (Winsor, 2016). The wild variety ‘Scuppernong’, selected for its unique bronze fruit, has been cultivated for centuries and served as the foundation of the muscadine wine industry even into the early 20th century (Morton, 1988; Reimer and Detjen, 1914). Despite the long and well-recorded history of use in the southeastern U.S., the commercial muscadine industry did not begin until 1835 in North Carolina (Morton, 1988). By the early 20th century, one of the most popular wines in the U.S. was made from muscadines until Prohibition severely hampered the muscadine industry (Gohdes, 1982). By 1990, production had dropped to approximately 1600 ha across the entire southeastern U.S. (Olien, 1990). In Arkansas, commercial muscadine vineyards were not present in the state until the mid-1970s (Clark, 2001; Moore, 1972) and the most recent literature estimates that muscadine production only accounts for approximately 3% of the total grape acreage in the state (Olien, 1990).

The earliest breeding efforts for muscadines began in the second half of the 19th century, with efforts to improve on the dominant variety ‘Scuppernong’ and attempts at hybridizing muscadines and *Vitis vinifera* (Munson, 1909; Van Buren, 1871). Improvement from these private, individual-led programs were limited as hybridization efforts between the two subgenera were largely unsuccessful. Varieties originating from wild selections still constituted the majority of cultivated vines into the early 20th century (Husmann and Dearing, 1913). The USDA and North Carolina State University cooperatively founded the first public muscadine breeding program in 1908. Significant advancees were made in this program, notably the development of perfect flowered muscadine cultivars (Dearing, 1917). Another breeding program at the University of Georgia (UGA) began in 1909 and has made significant strides in increasing vine yield, berry size, and fresh market berry quality over the last century (Conner, 2009). Currently, there are muscadine breeding efforts at the University of Arkansas System Division of

Agriculture (UA), Florida A&M University, UGA, 'Gardens Alive!' LLC, and the USDA-ARS Southern Horticultural Research Station in Poplarville, MS.

As the epicenter of muscadine culture and the location of the first public muscadine breeding program, eastern North Carolina was the main source of germplasm for early breeders (Husmann and Dearing, 1913). Wild vines from central Florida were also used in the very early stages of crop improvement. Before the establishment of the USDA cooperative vineyard in North Carolina, crosses were made between cultivated pistillate vines from North Carolina and wild pollinizers at a private vineyard in New Smyrna, FL (Dearing, 1917; Dearing, 1947). The UGA breeding program only used three female varieties, 'Flowers', 'Scuppernong', and 'Thomas', to begin the program, and these were selected from varieties already in use by the North Carolina program (Stuckey, 1919). T.V. Munson, whose grape breeding efforts were based in east Texas, reported spending significant time canvassing the surrounding countryside in Texas and Oklahoma for exceptional wild grapes to use in his breeding efforts. Yet he never mentions incorporating any wild muscadine vines from the region in his breeding efforts (Munson, 1909). In fact, there does not appear to be any record of wild accessions from west of the Appalachian Mountains used in any breeding program.

Multiple studies have utilized subgenus *Muscadinia* germplasm in phylogenetic research, although they often either represent a small percentage of the accessions analyzed or are specifically used as an outgroup for *Euvitis*-specific research (Aradhya et al., 2013; Wan et al., 2013; Zecca et al., 2012). Muscadine-specific conclusions that can be drawn from these studies are limited. There are relatively few studies specifically investigating the genetic diversity of muscadines. One comparative study found that bunch grapes had significantly more genetic variation than muscadines, although those results are difficult to interpret due to the low number

of accessions compared and the interspecific background of the bunch grape accessions included in the study (Qu et al., 1996). Past studies using simple sequence repeat (SSR) markers to quantify genetic diversity in cultivated muscadines have found that observed heterozygosity was higher than expected (Cao et al., 2020; Riaz et al., 2008). However, pedigree analysis has found that recently released varieties have higher inbreeding levels than historical varieties (Williams et al., 2021). Marker data also shows that allelic richness appears to decline between historical cultivars and cultivars released after 1970 (Cao et al., 2020). Despite the limited genetic base available to muscadine breeders and a loss of genetic diversity over time, inbreeding depression does not yet appear to be a significant issue in cultivated muscadines (Goldy and Onokpise, 2001), although low vine vigor has been cited as a potential effect of inbreeding already present in some breeding material (Williams et al., 2021).

Past research showed wild muscadine populations are sources of significant genetic variation. In one study, just nine wild muscadines accessions contained as many SSR alleles as 67 cultivated accessions (Cao et al., 2020). However, there is evidence to suggest that not every region harbors equal levels of genetic diversity. Smith (2010) conducted the only genetic research on *de novo* collected wild muscadine populations and found a diversity gradient running north-south, from more diverse populations in Florida to less diverse populations in North Carolina. Wild muscadine populations generally also appear to have lower observed heterozygosity than expected, although this did not result in a lack of genetic diversity as measured by the total number of alleles present compared to cultivated populations (Cao et al., 2020; Smith, 2010). Wild muscadines are potentially important sources of phenotypic variation for horticulturally important traits. In muscadines, bronze-colored grapes are preferred for winemaking because they lack the diglucoside anthocyanins that are highly susceptible to

browning which predominate in black-fruited cultivars (Robinson, 1966). Monoglucoside anthocyanins were thought to not exist in black muscadine fruit until they were detected by high-performance liquid chromatography (HPLC) in wild muscadine fruit samples collected from North Carolina and Arkansas (Goldy, 1989). Variation in resistance to Pierce's Disease was observed in wild and cultivated muscadine accessions (Ruel and Walker, 2006). Although all muscadine vines used in that study were determined to be tolerant, differences in bacterial concentrations collected from various accessions indicated quantitative differences in resistance that may be important as pathogen pressures shift over time.

To date, no muscadine genetic diversity study has included a significant number of accessions from outside Florida or North Carolina. Considering that these states are also where wild vines were collected for use early breeding efforts, it is possible that a significant amount of genetic diversity is present in wild muscadine populations from unsampled regions, particularly the western half of its native range. This research investigates both the population structure and genetic diversity of cultivated populations and wild populations collected *de novo* from previously unsampled locations in the southeastern U.S.

Materials and Methods

Wild population sampling. Wild populations of *V. rotundifolia* and *V. munsoniana* were sampled from fifteen sites in Alabama, Arkansas, Florida, Mississippi, North Carolina, Oklahoma, Tennessee, and Texas (Table 1). When multiple populations were collected within a state, populations were labelled from east to west with increasing numbers. These sampling locations included areas that had appeared in a previous phylogenetic study (Smith, 2010), regions that served as major germplasm sources for early breeding efforts, and areas in the western range of muscadines that have received little scientific attention. In addition, the collection sites

represented a wide variety of environments to which muscadines are adapted (e.g. Ozark uplands, palm hammocks). At least 10 individual vines within a 1 km^2 area were sampled from each collection site with a minimum of 5 m between individuals. After data filtration, the FLA2 population had only four individuals and was combined with the FLA population for analysis due to the proximity of the collection sites (25.1 km, Table 1). In addition to sampling 15 wild populations, a further four wild vines from the Ozark highlands region of Arkansas were collected and genotyped. Three of these accessions (ARK5-10, ARK5-11, ARK5-13) were collected at relatively high elevations in the Ozarks (>425 m) after being identified as vigorous and cold hardy. The remaining sample (ARK5-12) was collected on UA Fruit Research Station (FRS) property approximately 400 m from the muscadine vineyard used for the UA breeding program. These four vines were included in the ARK5 population for all analyses requiring an individual be assigned to a population. Despite ranging from 20 to 65 km away from the ARK5 collection site, all individuals were collected from the Ozark Plateau Another accession collected in Arkansas (ARK2-11) was included with the closest wild population (ARK2) despite ARK2-11 being 50 km from the ARK2 collection site because both sampling locations were located in the Ouachita Mountains.

Cultivated materials. In order to quantify the genetic diversity of cultivated muscadines as well as compare between cultivated and wild populations, 72 additional muscadine accessions were included in this study (Table 1; Supplemental Table 1). These vines represented wild selections that played a role in early crop improvement efforts, accessions maintained by germplasm repositories for diversity purposes, historical cultivars, recently released cultivars, and advanced selections from the UA breeding program. Sources for this germplasm included the NCGR at Davis, CA, UGA, UA, and a private collection maintained by Gardens Alive! Inc. muscadine

breeder Jeff Bloodworth. Accessions were assigned to five groups: *Muscadinia* varieties and accessions (MUS, n=7), wild *V. rotundifolia* varieties (VAR, n=9), historical cultivars released prior to 1970 (HIS, n=24), recent cultivars released since 1970 (REC, n=18), and unreleased selections from the UA muscadine breeding program (ARK, n=14). Varieties were defined as cultivated vines selected from the wild, while cultivars were defined as cultivated vines resulting from controlled crosses in formal breeding programs. The *Muscadinia* (MUS) population was composed of wild *V. munsoniana* accessions (DVIT 2242, DVIT 2248, 'Thornhill', 'Barrett Mtn', and 'Marsh'), one accession with *V. popenoei*, *V. munsoniana*, *V. rotundifolia* parentage ('Fennel's 3-way hybrid'), and the single *V. popenoei* (DVIT 2970) accession maintained by the NCGR. The 14 individuals within the Arkansas selections (ARK) population are advanced, unreleased genotypes from the UA breeding program. Each of these selections is preceded by the code "AM" (Arkansas Muscadine).

DNA extraction and genotyping. Young leaf tissue was collected from wild muscadines for DNA extraction and genotyping and immediately placed in labelled 2 mL centrifuge tubes. Locational data for wild populations was recorded using OnX mapping software (OnX Maps, Missoula, Mont.). Observational data such as plant health, flower sex, and growing environment was noted at the time of collection when possible. Fresh tissue from the 72 additional accessions was sent to UA for extraction. DNA extraction followed a modified CTAB protocol (Porebski et al., 1997). The quantity of double-stranded DNA was verified using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, Mass.). Final solution volume was diluted to 60 µL and stored at -80 °C.

Genotyping was performed using RNase H2 enzyme-dependent amplicon sequencing (rhAmpSeq), a method that specifically targets amplicons within the genome (Dobosy et al.,

2011). The accessions were genotyped with 2000 rhAmpSeq markers distributed across all chromosomes with an average distance of 200 kb between each marker. Marker development was accomplished using two publicly available *V. vinifera* genomes and de novo assemblies of seven *Euvitis* genomes, including wild American species such as *V. cinerea* and *V. rupestris*, although *V. rotundifolia* was not included in the development process (Zou et al., 2020). Allele calls were generated using a pipeline designed to analyze the multiplexed amplicon sequencing data, resulting in a matrix composed of genotypes and markers with both alleles and read depth.

Data filtration was accomplished using RStudio and the package *adegenet*. A minimum read depth threshold of five reads per allele call was used. Monomorphic loci and loci with >20% missing data were removed. Population structure was analyzed using *structure* 2.3 software (Pritchard et al., 2000). The number of hypothesized populations (K) was run from 2 to 10 without *a priori* assumptions of population delineations and an admixture model. A burn-in period of 300,000 iterations was followed by 800,000 Markov-Chain Monte Carlo (MCMC) repetitions. The simulation was run three times for each K value. The most likely number of populations present in the data was determined using the delta K statistic (Evanno et al., 2005). Visualizations of the *structure* output were constructed using the *pophelper* package. An Analysis of molecular variance (AMOVA) was performed following Excoffier et al. (1992) in the R package *poppr* to investigate genetic variation based on the *a priori* assumption that each collection site sampled represented a distinct population. A dendrogram was created using an unweighted pair group method with arithmetic mean (UPGMA) tree based on Nei's genetic distance using the packages *poppr* and *ape* (Nei 1978). For each population, observed heterozygosity, expected heterozygosity, average number of alleles per locus, and the number of private alleles (alleles found in only one population) were calculated using the package *hierfstat*.

Principal component analysis (PCA) was conducted using the package *ade4*. The package *ade4* was also used to conduct a Mantel test of the *de novo* wild populations using a genetic distance matrix calculated using *hierfstat* and a physical distance matrix calculated using *geosphere* (Mantel, 1967).

Results

In this study 152 wild *V. rotundifolia* accessions collected *de novo* and 72 additional wild and cultivated *Muscadinia* accessions were genotyped. Of the 2000 markers, 1276 had little to no amplification (defined as >80% missing data) and 37 loci were not polymorphic in this dataset. After filtering loci for missing data, 30 of the 152 wild accessions failed to amplify at >90% of loci and were removed. The failed reactions disproportionately affected the final sizes of the ARK3 (n=4), TEX (n=3), and NC3 (n=4) populations (Table 1). Approximately 34.4% of the loci from the rhAmpSeq genotyping panel were polymorphic and had <20% missing data. The number of alleles per locus ranged from 2 to 24 with an average of 5.54. The final dataset was composed of 194 unique genotypes and 687 haplotype loci.

Heterozygosity, private alleles, and alleles per locus. Observed heterozygosity ranged from 0.26 to 0.39 across the 15 wild and five cultivated populations (Table 1). The ARK5 and ARK3 populations, located in western Arkansas, had the lowest observed heterozygosity and the wild varieties (VAR) had the highest. Only the Arkansas selections (ARK) population had an observed heterozygosity higher than expected. The average number of alleles per locus for each population ranged from 1.73 to 3.16 (Table 1). TEX had the lowest number of alleles per locus as well as the lowest number of overall accessions. Only two wild populations (FLA and MIS) and one cultivated population (HIS) had an average of more than 3 alleles per locus. The Arkansas selections (ARK) had the lowest alleles/locus of the cultivated populations (2.41). The

number of private alleles per population ranged from two to 214, with an average of 45. The wild varieties (VAR), Arkansas selections (ARK), historical cultivars (HIS), and recent cultivars (REC) all had lower than average number of private alleles despite having a higher than average number of individuals in each population (Table 1). The seven accessions in MUS had 163 private alleles. The three wild populations with the highest number of private alleles were FLA (214), MIS (193), and NC1 (120). TEX, ARK3, and NC3 each had five or fewer private alleles but also fewer than 5 individuals each (Table 1). Four individuals (ARK5-3, 'Spalding', 'Stuckey', and 'Oh My!') were excluded from calculations of private alleles and alleles per locus in their respective populations. The accession ARK5-3 appeared to be admixed with *V. munsoniana*, which was likely the result of an error during laboratory preparation of samples for genotyping. 'Spalding' and 'Stuckey' were excluded because initial results showed that they grouped with *V. munsoniana* despite there being no indication of *V. munsoniana* in their reported pedigrees. The cultivar 'Oh My!' was developed from a seedless *V. vinifera* cultivar crossed with *V. rotundifolia* that was then backcrossed to *V. rotundifolia* multiple times, resulting in a cultivar with 23.1% of its genetic makeup from *V. vinifera* (Bloodworth, 2019). Due to its interspecific background, 'Oh My!' inflated the number of private alleles in the recent cultivars (REC) population from 2 to 81 when it was included.

Population structure. Four levels of K (2-5) are presented in this research. The Evanno et al. (2005) statistic showed the highest probability for K=3 (Fig. 1). The hybrid nature of some wild populations warranted further investigation into population structure beyond K=3 to better understand the genetic background of these populations. Cultivated populations also showed noteworthy admixture patterns at K values higher than three. The results when the number of

theoretical populations assumed in the *structure* analysis were greater than five were uninformative.

At K=2 the cultivated materials (ARK, REC, HIS, VAR) and *Muscadinia* (MUS) populations formed the first cluster, while the wild populations from west of the Appalachian Mountains (ALA, ARK1, ARK2, ARK3, ARK4, ARK5, OKL, TEN, TEX) formed the second. Five populations (MIS, NC1, NC2, NC3, FLA) collected within 250 km of the Atlantic or Gulf coasts showed moderate levels of hybridization between the two clusters. Each of the vines from the NC1 and FLA populations consistently had approximately 20% assignment to the second cluster, while the admixture levels in the MIS and NC2 populations were more variable from individual to individual.

At K=3, the cultivated populations generally grouped as one cluster with some notable exceptions. The historical cultivars ‘Spalding’ and ‘Stuckey’ had over 90% and 50% assignment, respectively, to the same cluster as MUS. The ARK population showed no levels of admixture with any other cluster while the other cultivated populations showed varying levels of admixture within each accession. The FLA, MUS, and NC1 populations were assigned to the second cluster with low to moderate levels of admixture with the other two clusters. Populations from Alabama, Arkansas, Oklahoma, Texas, and Tennessee (ALA, ARK1, ARK2, ARK3, ARK4, ARK5, OKL, TEN, TEX), generally from the temperate regions of the southeastern U.S., formed a third cluster with low levels of admixture compared to other populations. The KIL, NC2, and NC3 populations appeared to be mixtures of all three hypothetical populations assumed for *structure* simulation. Despite being geographically equidistant from the populations collected in North Carolina and the populations collected in Arkansas, the ALA and TEN populations showed no

admixture with material from North Carolina and grouped exclusively with material from Arkansas, Oklahoma, and Texas (Fig. 2).

At K=4 the MIS population became a distinct cluster that was only observed at minor frequencies in other populations. However, MIS continued to show admixture from the remaining clusters. Each individual within MIS had low to moderate levels of admixture with the cluster of Arkansas, Alabama, Oklahoma, Tennessee, and Texas populations. The K=5 *structure* simulation split the cultivated populations into two groups. In the VAR population, only the cultivar 'Thomas' had a majority assignment the old second cluster and all other accessions in VAR had majority assignment to the new fifth cluster. For the HIS population, 6 cultivars were majority assigned to second cluster and 11 to the new fifth cluster. In recently released cultivars (REC), 14 of the 18 cultivars were assigned to cluster 2. Only one accession in the ARK population, AM-77, had majority assignment to the new fifth cluster (Fig. 2).

Principal component analysis (PCA) produced three clusters corresponding to the *structure* results at K=3 (Fig. 2). The first axis accounted for 17.9% of variation, with cultivated material to the left of the axis and wild material on the right side of the axis. A second axis accounting for 8.7% of observed variation separated the subgenus *Muscadinia* (MUS), Florida (FLA), and coastal North Carolina (NC1) populations from the cultivated populations and the wild populations collected in Arkansas, Alabama, Oklahoma, Tennessee, and Texas. Three populations (NC2, NC3, MIS) that showed significant admixture in the *structure* analysis grouped closest to the wild populations but were distinct from the tightly-grouped cluster of populations from Arkansas, Alabama, Oklahoma, Tennessee, and Texas.

Pairwise F_{st} values ranged from -0.01 to 0.31, indicating that there was significant structure among the populations in this study (Fig. 3). The highest observed F_{st} values were between the

ARK population and the Arkansas Ozarks populations ARK5 and ARK4 (0.30 and 0.31, respectively). The NC1 population had pairwise F_{st} values greater than 0.20 for most wild populations collected west of the Appalachian Mountains. Both FLA and MUS had pairwise F_{st} values ranging between 0.11 and 0.24 when compared to the 18 other populations used in this study. The populations collected in Alabama, Arkansas, Oklahoma, Tennessee, and Texas were generally undifferentiated from one another, the majority of comparisons having F_{st} values of less than 0.15. The cultivated populations in this study also showed relatively little differentiation. Historical cultivars (HIS) and wild varieties (VAR) had the lowest observed F_{st} of all the comparisons of -0.01. Pairwise F_{st} values for the Arkansas selections (ARK) compared to other cultivated populations decreased from the wild varieties (VAR; 0.12) to historical cultivars (HIS; 0.11) to recent cultivars (REC; 0.05). AMOVA results supported the high F_{st} values between populations, showing that just over 14.5% of genetic variation was among populations, 4.7% of variation was within populations, and 80.8% of variation was within individuals. The Mantel test showed a significant positive association between genetic distance and physical distance ($r = 0.47$, $P = 0.002$)

An UPGMA tree was constructed using genetic distances calculated following Nei (1978) that further detailed the population structure of this dataset (Fig. 4). One clade was formed by two accessions: the *V. popenoei* sample (DVIT 2970) and 'Fennell's 3-way Hybrid', which is one half *V. popenoei*, one quarter *V. munsoniana*, and one quarter *V. rotundifolia* by pedigree. The FLA population grouped most closely with the *V. munsoniana* material included in this study. The NC1 population, consistent with both the *structure* and PCA results, was a separate clade despite being closely related to the MUS and FLA material. Cultivated and wild materials generally formed separate clades. Only one cultivated accession ('San Rubra') grouped

with wild material. Two genotypes from the NC2 population (NC2-1 and NC2-12) grouped within the cultivated material.

Discussion

This research represents the most extensive *V. rotundifolia* genetic diversity study conducted to date. One hundred and ninety-four individuals and 684 loci were used to evaluate population structure and quantify genetic diversity of wild and cultivated muscadine populations. Prior genetic diversity work in muscadines focused mainly on cultivated accessions or the relationship between muscadines and *Euvitis* grapes (Cao et al., 2020; Riaz et al., 2008; Qu et al., 1996). One previous study used nine wild accessions available through the NCGR at Davis, CA, but did not incorporate wild genotypes collected *de novo* (Cao et al., 2020). Other research has sampled from multiple wild populations, but the geographic focus of that study was limited to North Carolina, South Carolina, and Florida (Smith, 2010). The results described in this paper represent the first instance wild populations from the western range of muscadines have been sampled for genetic characterization. Additionally, 65 cultivated accessions and 7 germplasm accessions previously characterized as *V. munsoniana*, *V. popenoei*, and other subgenus *Muscadinia* interspecific hybrids were included in the study. Wild varieties, early improved genotypes, cultivars released since 1970, and advanced breeding selections were included in this analysis.

Effectiveness of rhAmpSeq in muscadine grapes. The rhAmpSeq *Vitis* core panel of haplotype markers used in this study was developed to have marker transferability >90% between species within subgenus *Euvitis* that diverged up to 20 mya (Zou et al., 2020). However, the three species in subgenus *Muscadinia* likely diverged from subgenus *Euvitis* species approximately 37 mya (Liu et al., 2016) and none of these species were included in the design and optimization of

the *Vitis* core markers. Still, major synteny between muscadine and bunch grape genomes has been found despite the significant time since divergence and difference in chromosome number (Cochetel et al., 2021; Lewter et al, 2019; Park et al., 2021). In this study, we found 34.4% of rhAmpSeq markers developed for *Euvitis* grapes were transferable to diverse *Muscadinia* germplasm. One previous study using the *Vitis* rhAmpSeq markers for a GWAS analysis in a muscadine breeding population was able to utilize 1283 markers (64.2% transferability) after a data imputation process (Park et al., 2021). The lower number of transferable *Vitis* rhAmpSeq markers useful markers discovered in this study compared to Park et al. (2021) can likely be attributed to fact that we required a minimum read depth of five for allele calling and did not attempt imputation for missing genotype data.

Comparisons to previous genetic studies. Multiple differences were found between the results of this study and past muscadine genetic diversity studies. The exceptionally high genetic diversity observed in a small set of wild muscadine germplasm accessions by Cao et al. (2020) was not observed in all populations collected for this study. The majority of the western wild populations collected for this study had relatively few private alleles. The five populations collected in Arkansas, despite being collected from diverse climatic and ecological regions of the state, contained three to 35 private alleles each. These values represent less than 20% of the number of private alleles found in MIS (193) or FLA (214). The MIS and FLA populations each had more private alleles and a higher average number of alleles per locus than the MUS population, which consisted of seven individuals from interspecific backgrounds: one *V. popenoei* accession, two wild *V. munsoniana* accessions, three *V. munsoniana* varieties, and the interspecific accession 'Fennel's 3-way hybrid'. Directly comparing the number of private alleles found in this study to Cao et al. (2020) is difficult. The latter used 20 SSR markers specifically selected for high

differentiation in muscadines, while this study used 687 haplotype markers designed for amplification and polymorphism in diverse *Euvitis* material. Another reason for the high observed diversity the nine wild accessions from Cao et al. (2020) is that this group represented all three species in subgenus *Muscadinia* and was sampled from across the native range of *V. rotundifolia* using accessions available through the USDA germplasm repository and a private collection, including one accession each from Arkansas and Louisiana.

Heterozygosity of the wild material collected in this research was generally lower than previously reported in other studies. Every wild population sampled for this study had observed heterozygosity lower than expected heterozygosity. Smith (2010) sampled 24 wild populations and calculated observed heterozygosity levels ranging from 0.27 to 0.65. Cao et al. (2020) found an observed heterozygosity level of 0.71 for the set of wild accessions used in that study. However, the observed heterozygosity was lower than the expected heterozygosity, as we found in this study. In this study observed heterozygosity levels ranged from 0.27 to 0.35 in the 15 wild populations. As with the calculations alleles per locus, the type of markers used may have impacted our results. Smith (2010) and Cao et al. (2020) each used 15-20 SSR markers selected for their high levels of polymorphism in muscadines. When the dataset used in this study was adjusted to include only the 30 loci with the highest number of alleles, the range of observed heterozygosity levels increased from 0.27-0.39 to 0.50-0.75 across the 20 populations. The population with the lowest observed heterozygosity in this study (ARK5) is located at the northernmost extent of the native range of muscadines in the Arkansas Ozark Plateau. Other populations collected near the westernmost extent of the native range (ARK3, ARK4, OKL, TEX) also had lower observed heterozygosity compared to the wild populations collected in eastern and central Arkansas, Alabama, Florida, Mississippi, North Carolina, and Tennessee.

The cultivated material used in this study also had lower observed heterozygosity than other examples in the literature. Riaz et al. (2008) evaluated 57 cultivated genotypes with 15 SSR loci and found an average observed heterozygosity of 0.76, which was higher than the expected heterozygosity of 0.69. Cao et al. (2020) found a similar trend of cultivated material having higher heterozygosity than expected for both historical and current cultivars. In this study, only the Arkansas selections (ARK) had higher observed than expected heterozygosity, although ARK had also had the lowest observed heterozygosity of the four cultivated populations. Observed heterozygosity levels decreased gradually from the wild varieties (VAR), to the historical cultivars (HIS), recent cultivars (REC), and Arkansas selections (ARK), potentially indicating a loss in genetic diversity over time across breeding germplasm. A similar trend is observed with the number of alleles per locus, although HIS is a notable exception (Table 1). One reason for this could be that HIS is by far the largest population examined in this study with 24 accessions. The number of private alleles is low for each cultivated population relative to many of the wild populations, particularly when accounting for the larger sample sizes of the cultivated populations. This is not unexpected, particularly in the case of the Arkansas Selections (ARK) as many of the most important cultivars used as parents in the founding of the program that could have been the source of unique alleles have also been included in the study.

Population structure of subgenus Muscadinia and wild populations. The taxonomic distribution of muscadines and the other 2n=40 *Muscadinia* species is a matter of some discussion, made more difficult by the overlapping ranges that these species inhabit (Aradhya et al., 2013; Olien, 1990). *Vitis munsoniana* is at times considered a subspecies of *Vitis rotundifolia* that is simply better adapted to semitropical environments (Olien, 2001), although T.V. Munson felt the differences between *V. rotundifolia* and *V. munsoniana* were clearer than many of the differences

between American *Euvitis* species (Munson, 1909). *V. munsoniana* is native to Florida and the Gulf Coast and has not been reported in the interior of the Southeast (Husmann and Dearing, 1913). Two populations (MIS and FLA) were collected within the documented geographic range of both *V. rotundifolia* and *V. munsoniana* (Dearing, 1947; Munson, 1909). At K=2 and K=3, the MIS population appeared to be the result of hybridization between western *V. rotundifolia* populations and coastal *V. munsoniana* populations. At K=4 and K=5, it appeared that some of this admixture was from a unique population, perhaps a coastal *V. rotundifolia* population, that only appeared sparingly in other accessions in this study. The MIS samples also clustered between the three major groups of wild and cultivated material in the PCA. At all levels of K, the MIS population showed some level of admixture with cultivated populations. Approximately 13 km south of the MIS collection site are the remains of Brown's Vineyard, a historical muscadine vineyard that grew 'Scuppernong' on at least 15 acres for decades (Stafne, 2014). It is possible that historical admixture between local wild vines and the vineyard have resulted in the unique genetic profile observed in the MIS population.

Results from the PCA, *structure* analysis, and pairwise F_{st} comparisons showed that FLA was highly related to MUS, indicating that the wild population was likely almost entirely *V. munsoniana* in origin (Table 1, Figs. 1-2). One unexpected result of this study was the close relationship between the NC1, MUS, and FLA populations (Figs. 1-5). The NC1 population was sampled from a maritime forest in Ft. Macon State Park on the southern Outer Banks of the Atlantic coast of North Carolina, well outside the reported native range of *V. munsoniana*. However, this population appeared much more closely related to *V. munsoniania* than the other muscadine germplasm included in this study, including other populations sampled from the North Carolina Piedmont (NC2 and NC3) and historically important cultivated varieties (VAR)

that were largely sourced from the North Carolina Coastal Plain. In contrast, Smith (2010) found that the wild populations from Florida were genetically distinct from those of North Carolina, although three of the 14 North Carolina populations from that study were sampled from sites on the Outer Banks and the FLA population in this study was sampled from the same location as one of Smith's eight Florida populations. The NC1 population is located in USDA Plant Hardiness Zone 8a and experiences colder temperatures than the semi-tropical regions of the Gulf and Atlantic coasts that traditionally have been considered the historical range of *V. munsoniana*. Munson (1909) noted that *V. munsoniana* was almost as cold hardy as *V. rotundifolia* and had fruited for multiple years his vineyard in Denison, Texas (also Zone 8a). Thus, it seems possible that the far southern Outer Banks could be a sort of isolated refugium for *V. munsoniana* or an underexplored intermediate bridge between the two species or subspecies. This hypothesis should be explored further by sampling coastal populations in South Carolina and Georgia.

Diversity of wild populations from Alabama, Arkansas, Oklahoma, Tennessee, and Texas. The wild populations from Alabama, Arkansas, Oklahoma, Tennessee, and Texas in this study were more genetically similar than expected. *V. rotundifolia* has a large native range, and it was hypothesized that genetic diversity within the species would be evenly distributed across the southeastern U.S. The *structure* and PCA results showed that even populations such as ALA and ARK3 that were collected more than 800 km apart were genetically similar (Fig. 2 and 5), although a Mantel test did find that increased physical distance was positively associated with genetic distance. It is not immediately clear why these western interior populations are not more genetically differentiated. Results from this study show that coastal areas are major regions of diversity for subgenus *Muscadinia*. It is possible that the regions sampled for this study have had

shifts in climate to make them more suitable to muscadines since the last glacial maximum during the ice age approximately 10,000 years ago.

One notable exception to the lack of differentiation in western populations was the accession ARK5-3, which appeared to be admixed between the western population cluster and the subgenus *Muscadinia* cluster. However, these results are likely due to a laboratory plating error, as there is no evidence to support that there are *V. munsoniana* x *V. rotundifolia* hybrids occurring naturally in the Arkansas Ozarks. Two accessions in the ARK2 population showed low levels of admixture with cultivated populations. A possible explanation for this finding is that a small muscadine vineyard had recently been planted on the property where the ARK2 population was sampled, and some of the wild vines used in this study were young enough to be the result of hybridization between wild and cultivated material. In contrast, one vine sampled on the UA Fruit Research Station property (ARK5-12) showed no admixture with cultivated populations despite its proximity to the muscadine vineyard used by the UA breeding program. Admixture between cultivated and wild populations has been shown to decrease the unique genetic diversity of wild populations in a process known as genetic swamping. This phenomenon has been observed in *Vitis* previously, such as regular hybridization between *V. californica* Benth. and *V. vinifera* leading to genetic diversity being lost in the former (Dangl et al., 2015). The small sample of wild vines growing near vineyards in Arkansas used in this study shows that while some level of admixture occurs between these populations, it does not appear that cultivated populations are swamping out local Arkansas wild populations of *V. rotundifolia*.

Diversity of central North Carolina populations. The two populations collected in the Piedmont of North Carolina (NC2 and NC3) showed more admixture in the *structure* and PCA analyses than many of the other wild populations sampled in this study. Most of the wild vines that were

cultivated by growers in the 19th and early 20th centuries that were later used as founding parents in breeding programs were sourced from forests in the Coastal Plains of North Carolina, between the location of these two populations and the NC1 population. Both populations showed low levels of admixture with the MUS, FLA, and NC1 populations at all tested levels of K (Fig. 1), likely a result of their proximity to the coastal NC1 population. The varying admixture levels with cultivated material observed in the NC2 population could be the result of feral vines that escaped cultivation. The two accessions from NC2 that showed significant admixture with cultivated material, NC2-1 and NC2-12, grouped most closely in the UPGMA dendrogram with the cultivar 'Chowan', a bronze-fruited cultivar released in the 1960s. Bronze fruit were observed on NC2-1, a trait that is exceedingly rare in wild muscadines and common in cultivated material. These findings suggest that NC2-1 and NC2-12 probably resulted from accidental cross pollination between cultivars grown at old homesteads and wild populations in the surrounding forest. Smith (2010) also observed the bronze phenotype in wild material from collections made around Raleigh, NC. Therefore, the long cultivation history of muscadines in North Carolina may have led to more genetic swamping in North Carolina than in Arkansas.

Use of wild populations in cultivar development. The results of this study indicate that the wild muscadine populations from the western part of the native range are highly differentiated from the germplasm used in muscadine breeding during the last century. The PCA axis that accounts for the highest variation (17.9%) showed the wide gulf between the material collected in Alabama, Arkansas, Oklahoma, Tennessee, and Texas and both recent cultivar releases and the advanced breeding selections within the Arkansas muscadine breeding program (Fig. 2). This finding is not surprising considering that the founding germplasm used in muscadine breeding programs was sourced from a very limited portion of their native range. The origins of nearly

every muscadine variety in the early 20th century can be traced to wild vines collected in eastern North Carolina (Husmann and Dearing, 1913). Wild *Muscadinia* germplasm from Florida has also been important in muscadine breeding. The 'H1' source of hermaphroditism in muscadines was selected from a cross between North Carolina varieties and wild Florida pollinizers and used extensively in subsequent breeding efforts (Dearing, 1917; Smith, 2010). The importance of wild Florida germplasm in historical breeding material can be observed in our results. 'Tarheel', an important historical cultivar and parent that is the closest existing relative of the 'H1' source of hermaphroditism is predicted to be 12.5% *V. munsoniana* by pedigree. We found that 'Tarheel' had 27% assignment to the *structure* cluster with the MUS, FLA, and NC1 populations at K=5 and grouped closer to the *V. munsoniana* material than other cultivated material in the PCA.

Although the wild populations from the western portion of the native range of muscadines had lower genetic diversity than the coastal populations (FLA, MIS, NC1) sampled in this study, the presence of unique alleles within each population indicates the potential for beneficial alleles in these populations. Cold tolerance is of particular importance to the UA muscadine breeding program because of its location near the northern limits of the native range of muscadines. Damage from winter temperatures was rarely observed on wild muscadine vines during tissue collection in this study. The ARK5 population was collected from a site further north and at higher elevation than the UA breeding program vineyard in Clarksville, AR. Vines at the ARK5 collection site were regularly observed to survive past juvenility, indicating that this was an established population adapted to local winter conditions. Crossing with wild muscadine vines from the southern Ozarks could help achieve a major breeding goal of the UA muscadine breeding program by serving as a source of cold hardiness. It would not be the first time that wild material was found to contain beneficial traits. Bronze-fruited muscadines are considered

superior for winemaking due to a lack of diglucoside anthocyanins that cause unsightly browning during the aging process. Using HPLC, Goldy (1989) characterized the anthocyanin content of black fruit from wild muscadines in Arkansas and North Carolina and found superior monoglucoside anthocyanin profiles for winemaking compared to current cultivated material.

Differences among cultivated material. Results from the PCA show a steady differentiation over time of cultivated material from the two clusters of wild populations (Fig. 5). Historical cultivars (released prior to 1970) and wild varieties were relatively dispersed across the PCA plot compared to both wild and other cultivated populations. The dispersion of the historical cultivars is expected as the categorization of "historical cultivar" as defined in this study is quite broad compared to the other cultivated population categorizations. In this study, a historical cultivar could be the offspring of two wild varieties such as 'San Jacinto' (and therefore relatively undifferentiated from wild types) or the result of multiple generations of controlled crossing such as 'Chowan'.

The UA muscadine breeding program began in 2007 and has utilized recently released cultivars such as 'Tara,' 'Supreme,' and 'Southern Home' as a base for genetic improvement. 'Southern Home' is an interspecific hybrid with *V. rotundifolia*, *V. popenoei*, and *Euvitis* material in its pedigree (Mortensen et al., 1994). It is therefore unsurprising that the advanced selections within the program represent the most distal cluster along both axes in the PCA plot. A small decrease in heterozygosity levels was observed along the progression from the VAR, HIS, REC, and ARK populations, representing an increase in inbreeding as crop improvement efforts transitioned from selecting vines in the wild to breeding efforts over 100 years later. The increasing levels of inbreeding in more recently released muscadine cultivars has also been documented by pedigree analysis (Williams et al., 2021). The low number of private alleles and

the decrease in the average alleles per locus from historical cultivars to recent cultivars and the unreleased material in the UA breeding program also represent a challenge for breeders. Without new beneficial alleles, genetic gains will be more difficult to achieve. Interestingly, the number of private alleles in the REC population increased from two to 81 when 'Oh My!' a *Euvitis* x *Muscadinia* hybrid that is predicted to be 23.1% *V. vinifera* by pedigree (Bloodworth, 2019). Therefore, crosses with wild germplasm from diverse parts of the native range and with diverse *Vitis* species are both options to increase genetic diversity in cultivated muscadine breeding material.

Potential misidentifications in muscadine germplasm. T.V. Munson produced a number of putative hybrids between 'Scuppernong' and *Euvitis* grapes (Munson, 1909), yet the Munson cultivars included in this study ('San Jacinto', 'La Salle', 'San Alba', and 'San Rubra') do not appear to be of *Euvitis* descent. Uncertainty about the provenance of these cultivars had been expressed by Dearing as early as 1917, and genetic research conducted with these varieties has provided further evidence (Dearing, 1917; Cao et al., 2020). In this study 'San Jacinto', 'La Salle', and 'San Alba' grouped closely together with the eastern North Carolina wild variety 'James' in the UPGMA dendrogram and clustered with the other historical cultivars in the PCA plot. Previous research found similar marker profiles between these cultivars and ruled out 'Scuppernong' as a parent for 'San Jacinto' and 'La Salle' (Cao et al., 2020). 'San Rubra', another Munson cultivar, clustered with the historical material as expected in the *structure* analysis but groups with wild populations from Arkansas and Texas in the dendrogram. This conflicting finding likely is likely an artifact of the way UPGMA analysis handled the higher percentage of missing data for 'San Rubra' rather than a real indication that 'San Rubra' is closely related to wild material from that region. Other misidentifications observed in previous

genetic studies were also confirmed by our research. Genetic and phenotypic inconsistencies reported for 'Creswell', 'Irene', 'Stuckey', and 'Spalding' also appear in our dendrogram (Cao et al., 2020; Riaz, 2008). 'Stuckey' and 'Spalding' in this study appear to be *V. munsoniana* in origin despite their reported pedigrees being exclusively *V. rotundifolia* (Fig. 4-5). It is likely that these accessions are mislabeled in the NCGR germplasm collection.

Conclusion

Although a Mantel test found that genetic distance between wild populations increased with physical distance, *structure* and principal component analyses showed that many of the muscadine populations collected west of the Appalachian Mountains were more genetically homogenous than expected. However, the same analyses confirmed that these populations have not been incorporated in either historical or present breeding efforts and could serve as a source of beneficial alleles for current breeding programs. Coastal populations of *V. rotundifolia* and *V. munsoniana* were the most diverse wild populations examined in this study. One of these populations, collected in the Outer Banks of North Carolina, appeared to be *V. munsoniana* in origin despite its location far north of that species' previously documented range. The *structure* and principal component analyses also showed that the last century of muscadine breeding efforts have resulted in significant differentiation from the wild material that formed the foundation of early muscadine breeding programs. Calculations of heterozygosity, private alleles, and the average alleles per locus for these cultivated populations reinforced the findings of recent studies showing increased inbreeding coefficients and low numbers of unique alleles in recent muscadine cultivars. The 2000 rhAmpSeq *Vitis* core panel markers, despite being developed for subgenus *Euvitis* material, had 34.4% transferability to the subgenus *Muscadinia* populations used in this study. The final dataset comprised of 194 muscadine accessions and 687

haplotype markers, representing the largest investigation of genetic within subgenus *Muscadinia* to date. Low observed heterozygosity levels, likely an artifact of marker design, did not prevent rhAmpSeq from being an effective genotyping platform for cultivar fingerprinting and establishing geospatial patterns of population structure in both cultivated and wild muscadine grapes.

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Table 1 Population names, number of individuals per population, locational data for each population, and summary statistics for basic population genetics parameters.

Pop. ID	Pop. description	N[z]	Latitude	Longitude	Elevation (m)	No. of private alleles	H_O[y]	H_E[x]	Alleles/locus
ARK	Arkansas selections	14	n/a	n/a	n/a	15	0.34	0.33	2.41
REC	Recent cultivars	18	n/a	n/a	n/a	2[w]	0.36	0.38	2.65
HIS[w]	Historical cultivars	24	n/a	n/a	n/a	21	0.38	0.40	3.16
VAR	Wild varieties	10	n/a	n/a	n/a	7	0.39	0.43	2.82
MUS[v]	*Muscadinia*	6	n/a	n/a	n/a	163	0.3	0.48	2.90
FLA	Anastasia, FL	11	29.8701	-81.2765	2	214	0.35	0.46	3.13
FLA2[u]	Nocatee, FL	n/a	30.0753	-81.3892	5	n/a	n/a	n/a	n/a
NC1	Ft. Macon, NC	12	34.6956	-76.6985	2	120	0.34	0.40	2.79
NC2	Umstead, NC	8	35.8702	-78.7468	130	24	0.34	0.43	2.83
NC3	Birkhead Mountain, NC	4	35.6370	-79.9040	192	6	0.32	0.41	2.01
MIS	Kiln, MS	11	30.4083	-89.4363	8	193	0.34	0.47	3.05
ALA	Blevin's Gap, AL	9	34.6741	-86.5293	239	31	0.32	0.37	2.47
TEN	Harry Carter Area, TN	11	35.1224	-85.9200	386	16	0.32	0.38	2.63
OKL	Broken Bow, OK	7	34.1287	-94.6866	206	16	0.3	0.35	2.18
TEX	Indian Mounds, TX	3	31.3118	-93.6968	59	4	0.3	0.37	1.73
ARK1	Village Creek, AR	10	35.1557	-90.7206	100	9	0.31	0.39	2.68
ARK2	Pellegrino, AR	9	34.5180	-93.0002	311	13	0.34	0.37	2.57
ARK3	'Y' City, AR	4	34.7304	-94.0688	255	3	0.27	0.35	2.57
ARK4	Wildcat Mountain	10	35.2768	-93.8050	208	30	0.28	0.32	2.21
ARK5	Jack Creek, AR	13	35.7085	-94.0960	286	16[t]	0.26	0.33	2.17

[z]Number of accessions.

[y]Observed heterozygosity.

[x]Expected heterozygosity.

[w]Three accessions (‘Oh My!’, ‘Stuckey’, and ‘Spalding’) were not used to calculate private alleles. ‘Oh My!’ is a hybrid with *V. vinifera* and was excluded from the recent cultivar summary statistics calculations. Both ‘Stuckey’ and ‘Spalding’ do not match their reported pedigrees and therefore were excluded from the historical cultivars.

[v]*Muscadinia* is composed of multiple *V. munsoniana* accessions, one *V. popenoei* accession, and one interspecific hybrid between all three subgenus *Muscadinia* species.
[u]The population FLA2 had only four accessions after data filtration (FLA-11, FLA-12, FLA-13, FLA-14) and was combined with the FLA population that is only 25.1 km away.
[t]The accession ARK5-3 was excluded from summary statistic calculations due to a probable plating error.

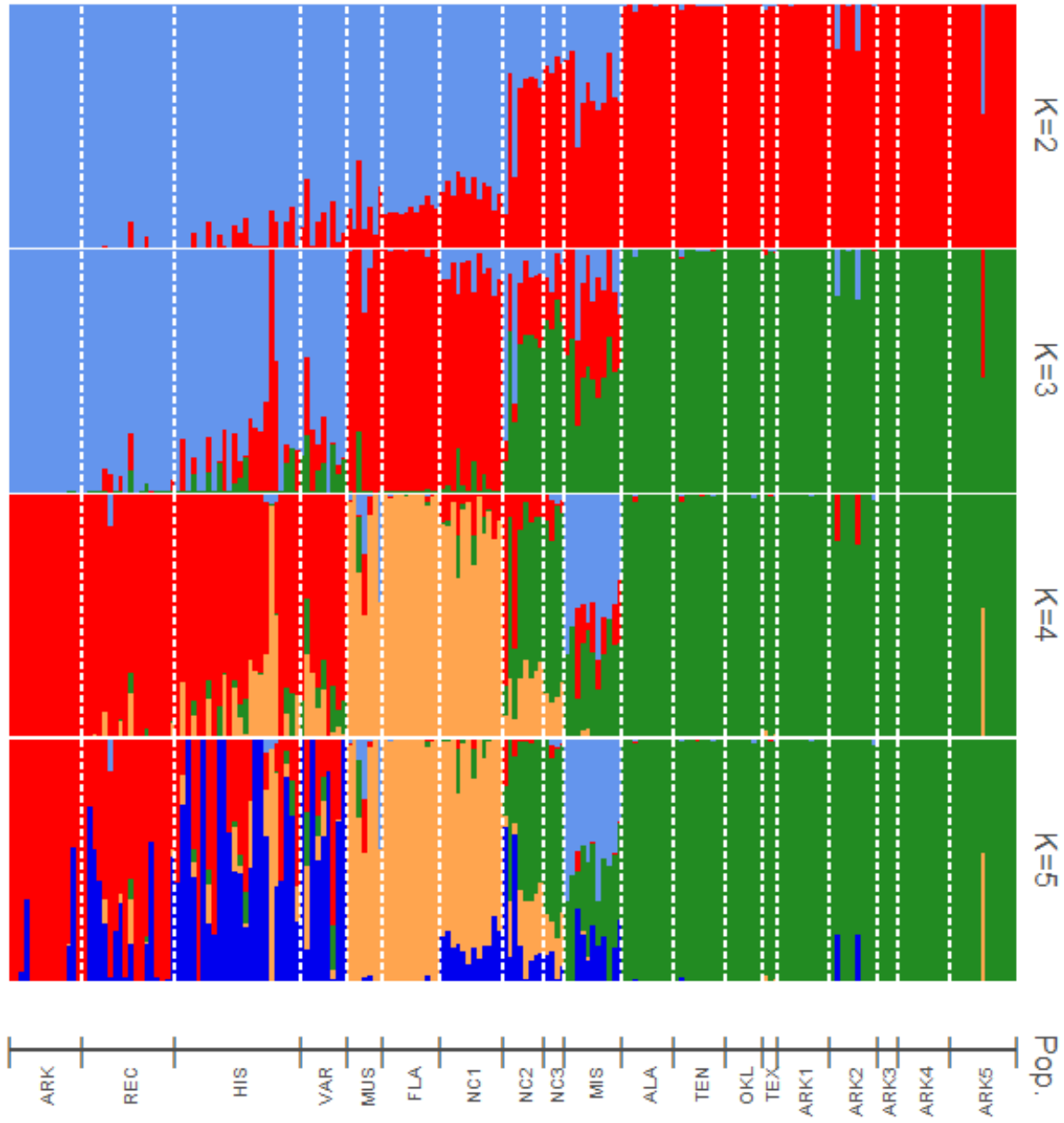

Fig. 1 Bayesian structure analysis results showing assignments to four theoretical levels of K (2-5). Vertical bars indicate the estimated membership coefficients (Q) of each individual for each population cluster.

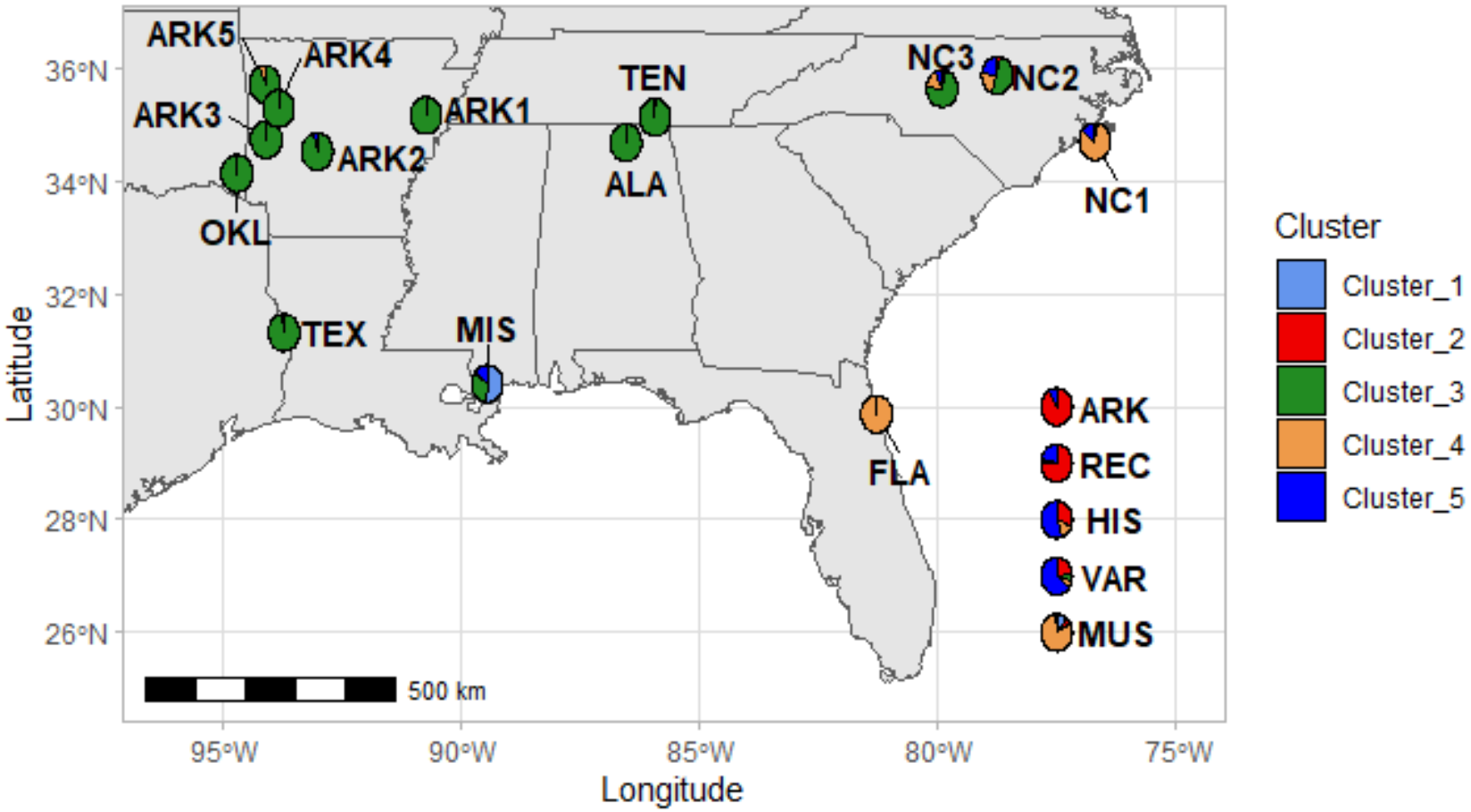

Fig. 2 Map showing the collection sites of the wild populations used in this study as well as corresponding pie charts showing the average assignment to each cluster for each population at K=5.

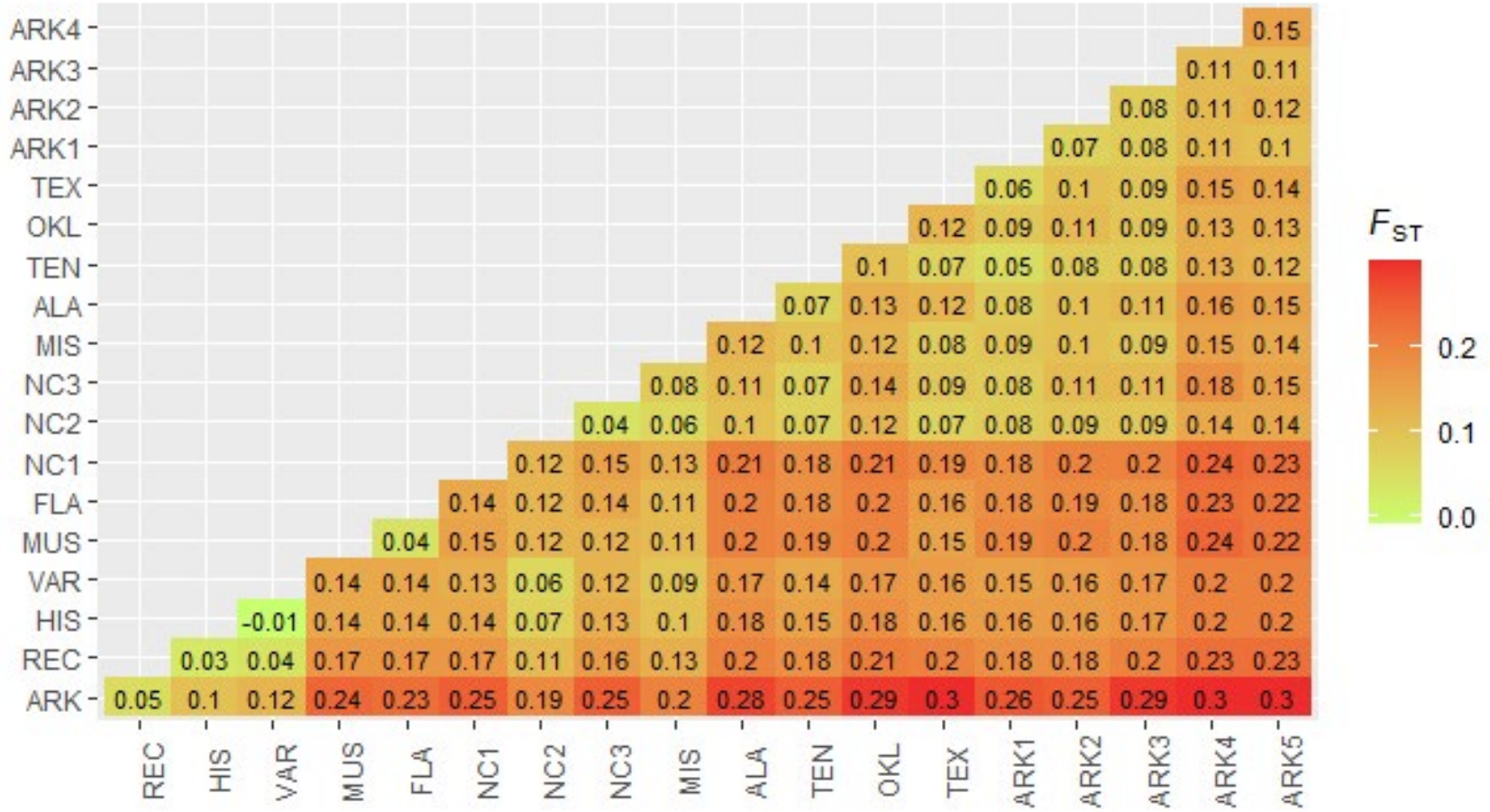

Fig. 3 Pairwise FST values calculated for each population following Weir and Cockerham (1984).

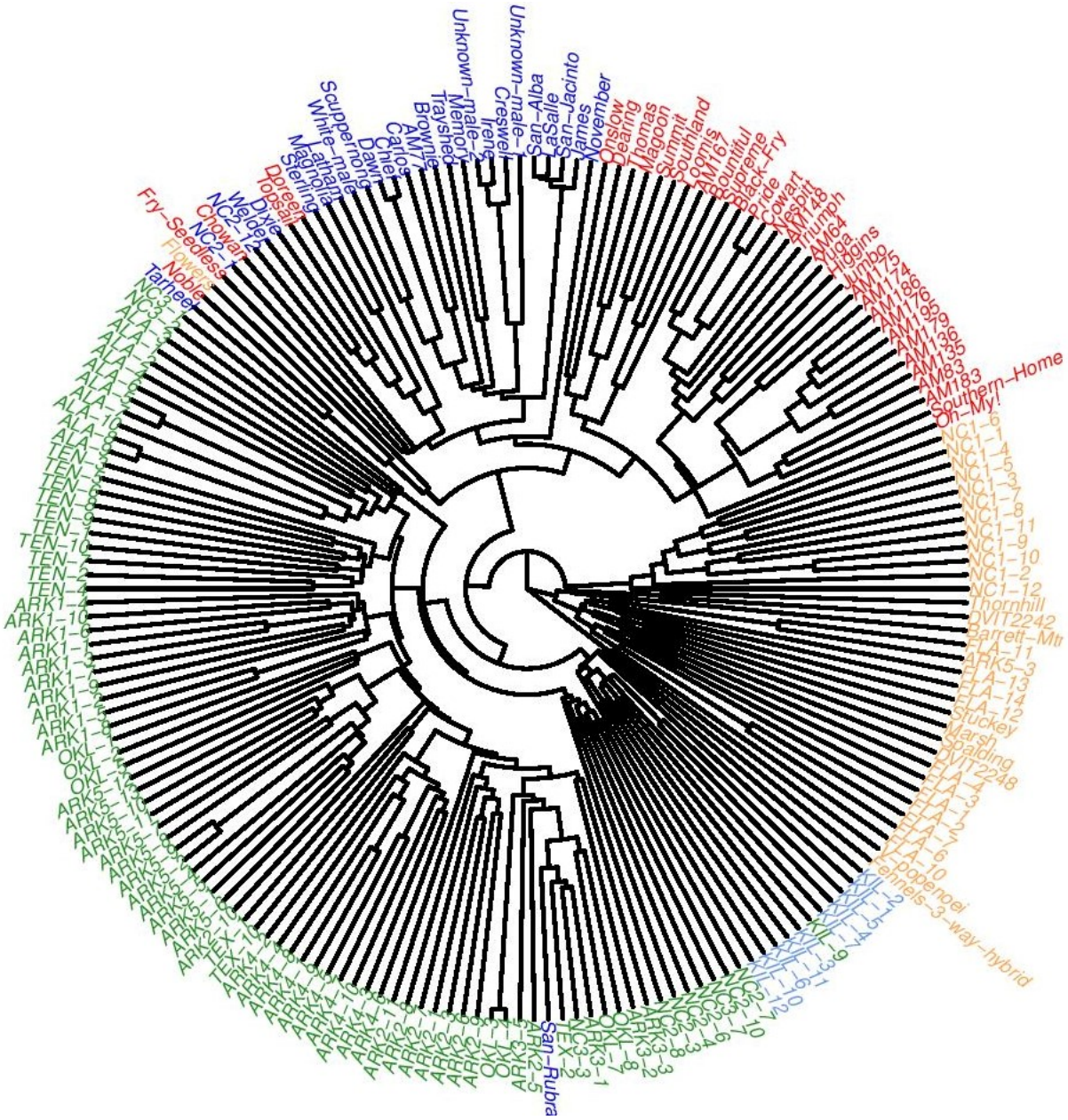

Fig. 4 UPGMA tree of all 194 accessions used in this study colored according to which cluster they were majority assigned to at K=5.

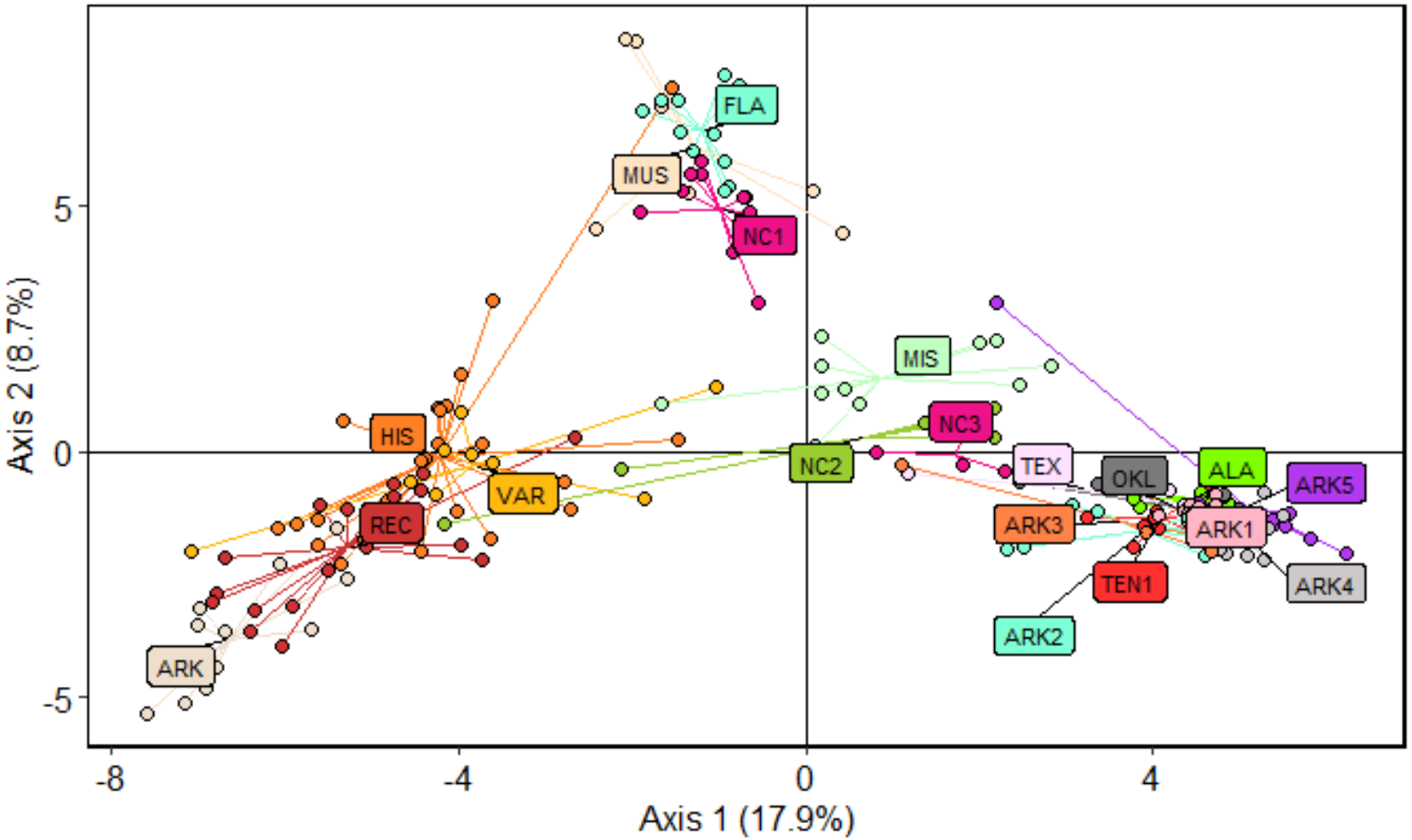

Fig. 5 Principal component analysis (PCA) showing the three distinct clusters of material used in this study. Each point is color coded according to its respective population.

Overall Conclusion

The first chapter of this thesis provides significant evidence that rooting muscadines from hardwood cuttings is a more effective method of propagation than many previous studies have concluded. The complex experimental design of the study allowed for the higher-order interactions affecting the rooting success of muscadine hardwood cuttings to be elucidated. Cuttings taken from Georgia in early November formed roots more than 40% of the time, the highest percentages for any treatment combination in this study. When cuttings from November were analyzed alone with the location and cultivar factors considered random effects, applying a cold storage treatment reduced rooting percentages from 27% to 19%. Increased cutting length and cutting diameter were significantly correlated with increased rooting success while an increased number of nodes was not. While the rooting percentages reported in this study may not allow for commercially successful propagation of muscadines by hardwood cuttings, breeding programs or germplasm repositories with modest needs may find that transitioning to an off-season propagation protocol may save time and money.

The second chapter of this thesis investigated the genetic diversity and population structure of a large collection of wild and cultivated *Muscadinia* populations. Although a Mantel test found that genetic distance between wild populations increased with physical distance, *structure* and principal component analyses showed that many of the muscadine populations collected west of the Appalachian Mountains were more genetically homogenous than expected. However, the same analyses confirmed that these populations have not been incorporated in either historical or present breeding efforts and could serve as a source of beneficial alleles for current breeding programs. Coastal populations of *V. rotundifolia* and *V. munsoniana* were the most diverse wild populations examined in this study. One of these populations, collected in the Outer Banks of North Carolina, appeared to be *V. munsoniana* in origin despite its location far

north of that species' previously documented range. The *structure* and principal component analyses also showed that the last century of muscadine breeding efforts have resulted in significant differentiation from the wild material that formed the foundation of early muscadine breeding programs. Calculations of heterozygosity, private alleles, and the average alleles per locus for these cultivated populations reinforced the findings of recent studies showing increased inbreeding coefficients and low numbers of unique alleles in recent muscadine cultivars. The 2000 rhAmpSeq *Vitis* core panel markers, despite being developed for subgenus *Euvitis* material, had 34.4% transferability to the subgenus *Muscadinia* populations used in this study. The final dataset comprised of 194 muscadine accessions and 687 haplotype markers, representing the largest investigation of genetic within subgenus *Muscadinia* to date. Low observed heterozygosity levels, likely an artifact of marker design, did not prevent rhAmpSeq from being an effective genotyping platform for cultivar fingerprinting and establishing geospatial patterns of population structure in both cultivated and wild muscadine grapes.

Appendices

Appendix A

Supplemental Table S1. Accession name, population, year of release (when applicable), and source of tissue for the 194 accessions used in this study. Included are the *structure* proportional assignment to each cluster at K=5 and the principal componenent analysis coordinates for the two primary axes.

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
ALA-1	ALA	n/a	Wild	0.001	0.002	0.996	0.001	0.001	4.43	-1.26
ALA-10	ALA	n/a	Wild	0.001	0	0.998	0	0	4.70	-0.51
ALA-2	ALA	n/a	Wild	0.007	0.011	0.972	0.003	0.008	3.74	-0.80
ALA-3	ALA	n/a	Wild	0.001	0	0.998	0.001	0	5.18	-1.35
ALA-4	ALA	n/a	Wild	0.001	0.001	0.997	0.002	0	3.85	-1.15
ALA-5	ALA	n/a	Wild	0	0	0.999	0	0	4.56	-0.91
ALA-6	ALA	n/a	Wild	0.002	0.001	0.994	0.001	0.002	3.78	-0.98
ALA-7	ALA	n/a	Wild	0.001	0	0.998	0	0	4.56	-0.83
ALA-8	ALA	n/a	Wild	0	0	0.999	0	0	4.86	-1.04
ALA-9	ALA	n/a	Wild	0	0	0.999	0	0	4.82	-0.91
AM135	ARK	n/a	UA	0	0.999	0	0	0	-7.09	-4.40
AM136	ARK	n/a	UA	0	0.999	0	0	0	-7.06	-4.31
AM148	ARK	n/a	UA	0	0.957	0	0	0.042	-5.71	-3.64
AM167	ARK	n/a	UA	0	0.658	0	0	0.341	-6.06	-2.30
AM174	ARK	n/a	UA	0	0.999	0	0	0	-7.02	-3.53
AM175	ARK	n/a	UA	0	0.999	0	0	0	-6.70	-3.67
AM176	ARK	n/a	UA	0	0.999	0	0	0	-7.17	-5.14
AM179	ARK	n/a	UA	0	0.999	0	0	0	-7.40	-4.66
AM183	ARK	n/a	UA	0	0.999	0	0	0	-6.80	-4.38
AM186	ARK	n/a	UA	0	0.999	0	0	0	-7.58	-5.33
AM195	ARK	n/a	UA	0	0.999	0	0	0	-6.91	-4.82

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
AM64	ARK	n/a	UA	0	0.847	0.001	0.001	0.151	-5.28	-2.59
AM77	ARK	n/a	UA	0	0.447	0	0	0.552	-5.41	-1.57
AM83	ARK	n/a	UA	0	0.997	0	0	0.002	-6.99	-3.21
ARK1-1	ARK1	n/a	Wild	0.003	0	0.996	0	0.001	4.36	-1.21
ARK1-10	ARK1	n/a	Wild	0	0	0.998	0	0	4.73	-0.89
ARK1-2	ARK1	n/a	Wild	0.002	0	0.993	0.001	0.002	4.71	-1.07
ARK1-3	ARK1	n/a	Wild	0.002	0	0.997	0	0	4.49	-1.39
ARK1-4	ARK1	n/a	Wild	0	0	0.999	0	0	4.66	-1.60
ARK1-5	ARK1	n/a	Wild	0.001	0.001	0.997	0	0.001	4.42	-1.43
ARK1-6	ARK1	n/a	Wild	0.009	0	0.99	0.001	0.001	4.42	-1.11
ARK1-7	ARK1	n/a	Wild	0.001	0	0.998	0.001	0	4.52	-1.13
ARK1-8	ARK1	n/a	Wild	0.002	0.001	0.996	0.001	0	4.06	-1.31
ARK1-9	ARK1	n/a	Wild	0	0.001	0.997	0	0.001	4.41	-1.26
ARK2-1	ARK2	n/a	Wild	0.001	0.001	0.996	0.001	0.001	4.69	-1.77
ARK2-10	ARK2	n/a	Wild	0.004	0.002	0.797	0	0.197	2.50	-1.96
ARK2-3	ARK2	n/a	Wild	0	0	0.999	0	0	5.09	-1.62
ARK2-5	ARK2	n/a	Wild	0	0.004	0.993	0	0.001	3.07	-1.09
ARK2-6	ARK2	n/a	Wild	0	0.001	0.998	0	0.001	3.36	-1.20
ARK2-7	ARK2	n/a	Wild	0	0.004	0.8	0	0.195	2.33	-1.98
ARK2-8	ARK2	n/a	Wild	0.001	0	0.998	0	0	5.06	-1.43
ARK2-9	ARK2	n/a	Wild	0.001	0.001	0.998	0	0	4.60	-2.14
ARK2-11[y]	ARK2	n/a	Wild	0.023	0.001	0.974	0.002	0.001	4.42	-1.35
ARK3-1	ARK3	n/a	Wild	0.001	0.001	0.995	0.001	0.001	1.11	-0.28
ARK3-2	ARK3	n/a	Wild	0.001	0	0.998	0	0	4.66	-2.02
ARK3-3	ARK3	n/a	Wild	0.003	0	0.997	0	0	4.91	-1.83
ARK3-5	ARK3	n/a	Wild	0.001	0	0.998	0.001	0	3.93	-1.66
ARK4-1	ARK4	n/a	Wild	0	0	0.999	0	0	5.49	-1.32
ARK4-10	ARK4	n/a	Wild	0	0	0.999	0	0	4.85	-2.09

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
ARK4-2	ARK4	n/a	Wild	0	0	0.999	0	0	4.77	-1.78
ARK4-3	ARK4	n/a	Wild	0	0	0.999	0	0	5.34	-1.57
ARK4-4	ARK4	n/a	Wild	0	0.001	0.998	0	0.001	5.20	-1.82
ARK4-5	ARK4	n/a	Wild		0	0.999	0	0	5.08	-2.11
ARK4-6	ARK4	n/a	Wild		0	0.999	0	0	4.77	-2.01
ARK4-7	ARK4	n/a	Wild	0	0	0.999	0	0	5.27	-2.18
ARK4-8	ARK4	n/a	Wild	0	0	0.999	0	0	5.28	-0.84
ARK4-9	ARK4	n/a	Wild	0	0	0.999	0	0	4.78	-1.79
ARK5-12[x]	ARK5	n/a	Wild	0.001	0	0.998	0	0.001	4.68	-1.22
ARK5-7	ARK5	n/a	Wild	0.001	0	0.999	0	0	4.54	-1.04
ARK5-8	ARK5	n/a	Wild	0	0	0.999	0	0	5.49	-1.39
ARK5-9	ARK5	n/a	Wild	0	0	0.999	0	0	5.82	-1.76
ARK5-1	ARK5	n/a	Wild	0	0	0.999	0	0	5.42	-1.48
ARK5-2	ARK5	n/a	Wild	0	0	0.999	0	0	6.23	-2.07
ARK5-3[w]	ARK5	n/a	Wild	0.001	0	0.469	0.529	0.001	2.21	3.01
ARK5-4	ARK5	n/a	Wild	0	0	0.999	0	0.001	5.38	-1.38
ARK5-5	ARK5	n/a	Wild	0	0	0.999	0	0	5.54	-1.52
ARK5-6	ARK5	n/a	Wild	0	0	0.999	0	0	5.09	-2.11
ARK5-10[x]	ARK5	n/a	Wild	0	0	0.999	0	0	5.22	-1.34
ARK5-11[x]	ARK5	n/a	Wild	0	0.001	0.998	0	0	4.60	-1.20
ARK5-13[x]	ARK5	n/a	Wild	0.001	0	0.998	0	0	5.58	-1.28
FLA-1	FLA	n/a	Wild	0	0.001	0.001	0.997	0.001	-1.46	7.13
FLA-10	FLA	n/a	Wild	0.011	0.002	0.001	0.983	0.003	-1.89	6.94
FLA-2	FLA	n/a	Wild	0.001	0.001	0.001	0.996	0.001	-1.45	6.50
FLA-3	FLA	n/a	Wild	0.001	0.001	0.001	0.997	0.001	-0.90	5.38
FLA-4	FLA	n/a	Wild	0.001	0.003	0.002	0.993	0.001	-1.30	6.11
FLA-6	FLA	n/a	Wild	0.001	0	0.001	0.998	0	-0.77	7.46

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
FLA-7	FLA	n/a	Wild	0.002	0	0	0.997	0	-0.93	7.64
FLA-11[v]	FLA	n/a	Wild	0	0.001	0.001	0.997	0.001	-1.06	6.47
FLA-12[v]	FLA	n/a	Wild	0.006	0.005	0.022	0.943	0.024	-0.93	5.89
FLA-13[v]	FLA	n/a	Wild	0.006	0.001	0.001	0.989	0.002	-0.94	5.29
FLA-14[v]	FLA	n/a	Wild	0.002	0.001	0.001	0.994	0.002	-1.67	7.13
Bountiful	HIS	1967	GRIN_D	0	0.589	0.001	0	0.41	-5.08	-1.76
Brownie	HIS	1933	GRIN_D	0.001	0.149	0	0.119	0.731	-5.34	0.64
Chief	HIS	1967	GRIN_D	0	0.002	0	0	0.998	-5.63	-1.39
Chowan	HIS	1962	UGA	0	0.455	0.055	0.058	0.432	-4.83	-1.02
Cowart	HIS	1968	UGA	0	0.997	0	0	0.002	-5.36	-2.27
Dawn	HIS	1938	UGA	0	0.002	0	0	0.997	-5.88	-1.46
Dearing	HIS	1957	JB	0.001	0.54	0.063	0.161	0.236	-2.78	-0.62
Higgins	HIS	1955	UGA	0	0.806	0.001	0	0.193	-5.63	-1.92
Irene	HIS	1919	GRIN_D	0	0.001	0	0	0.999	-4.24	0.16
LaSalle	HIS	1908	UGA	0	0	0	0	0.999	-4.25	0.90
Magnolia	HIS	1962	UGA	0	0.385	0.001	0.001	0.613	-6.09	-1.56
Magoon	HIS	1959	UGA	0.001	0.629	0.134	0.011	0.226	-2.71	-1.16
November	HIS	1920	GRIN_D	0.003	0.339	0.022	0.183	0.453	-3.72	0.17
Onslow	HIS	1946	GRIN_D	0.001	0.474	0.048	0.032	0.445	-4.02	-1.22
San-Alba	HIS	1906	GRIN_D	0	0	0	0	0.999	-4.23	0.83
San-Jacinto	HIS	1908	UGA	0	0	0	0	0.999	-4.14	0.93
San-Rubra	HIS	1906	GRIN_D	0.056	0.15	0.001	0.196	0.597	-1.47	0.26
Spalding[u]	HIS	1920	GRIN_D	0.038	0.001	0.001	0.961	0	-1.53	7.42
Stuckey[u]	HIS	1920	GRIN_D	0.017	0.117	0.003	0.468	0.394	-3.62	3.07
Tarheel	HIS	1946	UGA	0.001	0.255	0.001	0.274	0.469	-3.96	1.56
Topsail	HIS	1946	JB	0	0.582	0.001	0	0.417	-3.63	-1.78
Trayshed	HIS	n/a	UCD	0	0.049	0.057	0.052	0.842	-4.39	-0.14

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
White-male	HIS	n/a	JB	0	0.169	0.147	0	0.684	-4.44	-2.05
Yuga	HIS	1934	UGA	0.002	0.603	0.001	0.146	0.247	-4.44	-0.18
MIS-1	MIS	n/a	Wild	0.666	0	0.333	0.001	0.001	2.19	2.24
MIS-10	MIS	n/a	Wild	0.562	0	0.437	0	0	2.48	1.35
MIS-11	MIS	n/a	Wild	0.463	0.082	0.154	0.001	0.3	-1.67	0.97
MIS-12	MIS	n/a	Wild	0.439	0.002	0.35	0.013	0.197	0.17	1.74
MIS-2	MIS	n/a	Wild	0.505	0.001	0.396	0.01	0.088	1.99	2.23
MIS-3	MIS	n/a	Wild	0.426	0.003	0.339	0.001	0.23	0.18	1.16
MIS-4	MIS	n/a	Wild	0.668	0.001	0.182	0.001	0.147	0.19	2.35
MIS-5	MIS	n/a	Wild	0.493	0.001	0.319	0	0.187	0.45	1.28
MIS-6	MIS	n/a	Wild	0.52	0	0.479	0.001	0	2.83	1.76
MIS-7	MIS	n/a	Wild	0.468	0.011	0.378	0.001	0.142	0.61	0.95
MIS-9	MIS	n/a	Wild	0.338	0.013	0.397	0.001	0.251	0.11	0.11
Barrett-Mtn	MUS	n/a	UGA	0.017	0.008	0.001	0.972	0.001	-1.67	7.03
DVIT2242	MUS	n/a	GRIN_D	0.002	0	0	0.996	0.001	-1.96	8.36
DVIT2248	MUS	n/a	GRIN_D	0.084	0.002	0.238	0.675	0.001	0.41	4.46
Fennells-3-way-hybrid	MUS	n/a	UGA	0.25	0.223	0	0.506	0.02	-2.43	4.54
Marsh	MUS	n/a	UGA	0.008	0.024	0.003	0.939	0.026	-1.36	5.28
Thornhill	MUS	n/a	UGA	0.001	0	0	0.998	0.001	-2.09	8.40
V-popenoei	MUS	n/a	UGA	0.453	0.001	0.001	0.545	0.001	0.09	5.29
NC1-1	NC1	n/a	Wild	0.001	0.001	0.006	0.805	0.187	-0.65	4.29
NC1-10	NC1	n/a	Wild	0	0.001	0.013	0.778	0.208	-0.69	5.18
NC1-11	NC1	n/a	Wild	0	0.001	0.001	0.856	0.143	-1.22	5.63
NC1-12	NC1	n/a	Wild	0.001	0.037	0.184	0.621	0.157	-0.55	3.05
NC1-2	NC1	n/a	Wild	0.001	0.001	0.016	0.857	0.125	-0.70	5.16
NC1-3	NC1	n/a	Wild	0	0.003	0.003	0.922	0.072	-1.32	5.65
NC1-4	NC1	n/a	Wild	0.001	0.039	0.126	0.691	0.143	-0.85	4.05

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
NC1-5	NC1	n/a	Wild	0	0.001	0.001	0.9	0.097	-1.20	5.91
NC1-6	NC1	n/a	Wild	0.001	0.001	0.078	0.769	0.151	-0.66	4.87
NC1-7	NC1	n/a	Wild	0.002	0.001	0.008	0.845	0.144	-0.72	5.19
NC1-8	NC1	n/a	Wild	0	0.001	0.001	0.728	0.27	-1.90	4.86
NC1-9	NC1	n/a	Wild	0	0.001	0.001	0.791	0.207	-1.43	5.32
NC2-1	NC2	n/a	Wild	0.001	0.19	0.122	0.048	0.638	-4.16	-1.47
NC2-10	NC2	n/a	Wild	0.001	0.003	0.662	0.236	0.099	2.17	0.28
NC2-12	NC2	n/a	Wild	0.001	0.073	0.274	0.043	0.609	-2.14	-0.35
NC2-3	NC2	n/a	Wild	0.004	0.004	0.617	0.23	0.144	1.37	0.60
NC2-4	NC2	n/a	Wild	0.002	0.006	0.66	0.319	0.012	2.18	0.88
NC2-6	NC2	n/a	Wild	0.001	0.015	0.651	0.246	0.088	2.04	0.65
NC2-7	NC2	n/a	Wild	0	0.003	0.632	0.258	0.106	2.07	0.80
NC2-8	NC2	n/a	Wild	0.001	0.003	0.592	0.291	0.113	2.05	0.87
NC3-1	NC3	n/a	Wild	0.001	0.003	0.72	0.167	0.109	2.30	-0.42
NC3-2	NC3	n/a	Wild	0.027	0.054	0.672	0.121	0.126	1.82	-0.29
NC3-3	NC3	n/a	Wild	0.03	0.01	0.783	0.17	0.006	0.82	-0.03
NC3-7	NC3	n/a	Wild	0.017	0.007	0.692	0.223	0.062	1.92	0.59
OKL-1	OKL	n/a	Wild	0	0	0.999	0	0	4.82	-0.89
OKL-2	OKL	n/a	Wild	0.001	0.001	0.997	0	0.001	4.89	-1.74
OKL-3	OKL	n/a	Wild	0	0	0.999	0	0	4.98	-1.18
OKL-4	OKL	n/a	Wild	0	0	0.999	0	0	4.87	-1.27
OKL-5	OKL	n/a	Wild	0	0	0.998	0	0.001	4.52	-1.70
OKL-7	OKL	n/a	Wild	0.017	0.001	0.98	0.001	0.001	2.46	-0.60
OKL-8	OKL	n/a	Wild	0.001	0	0.998	0	0	3.35	-0.66
Black-Fry	REC	1986	UGA	0	0.998	0	0	0.001	-6.78	-2.87
Carlos	REC	1970	UGA	0	0.277	0.001	0	0.722	-5.61	-1.10
Dixie	REC	1976	UGA	0	0.452	0.001	0.002	0.545	-4.75	-0.91
Doreen	REC	1981	UA	0	0.583	0.001	0.001	0.416	-5.30	-1.16

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
Fry-Seedless	REC	1990	UGA	0.001	0.656	0.001	0.102	0.239	-4.74	-0.66
Jumbo	REC	1970	UGA	0	0.79	0	0	0.208	-5.07	-1.93
Loomis	REC	1989	UGA	0.001	0.636	0.001	0.042	0.32	-4.43	-0.80
Nesbitt	REC	1985	UA	0	0.982	0	0	0.017	-6.42	-3.68
Noble	REC	1973	GRIN_D	0	0.577	0.08	0.187	0.155	-2.67	0.28
Oh-My![t]	REC	2019	JB	0.136	0.848	0	0.001	0.015	-6.35	-3.24
Pride	REC	1972	UGA	0.001	0.996	0.001	0	0.003	-5.50	-2.44
Southern-Home	REC	1994	UGA	0	0.999	0	0	0	-5.92	-3.14
Southland	REC	1977	UGA	0	0.812	0.029	0.001	0.158	-3.74	-2.20
Sterling	REC	1981	UGA	0	0.424	0	0	0.575	-6.70	-2.16
Summit	REC	1977	UA	0	0.983	0.001	0	0.015	-6.05	-3.98
Supreme	REC	1988	UA	0	0.999	0	0	0.001	-6.85	-3.08
Triumph	REC	1980	UGA	0	0.988	0	0	0.011	-3.98	-1.91
Welder	REC	1972	UGA	0	0.453	0.001	0.031	0.515	-4.41	-0.45
TEN-1	TEN	n/a	Wild	0	0.001	0.997	0	0.001	4.08	-1.56
TEN-10	TEN	n/a	Wild	0.007	0.007	0.969	0.002	0.016	4.06	-1.20
TEN-2	TEN	n/a	Wild	0.001	0	0.998	0	0.001	4.56	-1.75
TEN-3	TEN	n/a	Wild	0.001	0.001	0.997	0.001	0.001	4.80	-0.56
TEN-4	TEN	n/a	Wild	0.001	0.007	0.991	0	0.001	3.24	-1.33
TEN-5	TEN	n/a	Wild	0.001	0.005	0.99	0.001	0.002	3.91	-1.51
TEN-6	TEN	n/a	Wild	0.002	0.002	0.992	0.001	0.003	3.94	-0.79
TEN-7	TEN	n/a	Wild	0.011	0.002	0.985	0.001	0.001	4.01	-1.36
TEN-8	TEN	n/a	Wild	0	0.003	0.995	0.001	0.001	3.77	-1.94
TEN-9	TEN	n/a	Wild	0	0.001	0.996	0.001	0.003	4.81	-1.45
TEX-1	TEX	n/a	Wild	0.003	0.001	0.974	0.02	0.002	4.19	-0.79
TEX-10	TEX	n/a	Wild	0.005	0.005	0.988	0.001	0.001	4.36	-1.08
TEX-2	TEX	n/a	Wild	0.007	0.002	0.98	0.007	0.003	1.18	-0.44
Creswell	VAR	1946	GRIN_D	0	0.001	0	0	0.999	-3.60	-0.22

Genotype	Population	Year released	Germplasm source[z]	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	PCA_Axis1	PCA_Axis2
Flowers	VAR	1816	GRIN_D	0.001	0.298	0.226	0.343	0.132	-1.02	1.31
James	VAR	1866	GRIN_D	0	0	0	0	0.999	-3.98	0.81
Latham	VAR	1855	JB	0	0.319	0.083	0.101	0.497	-4.27	-0.88
Memory	VAR	1868	GRIN_D	0.001	0.154	0.099	0.145	0.601	-3.84	-0.06
Scuppernong	VAR	n/a	UGA	0	0.134	0	0	0.865	-7.09	-2.05
Thomas	VAR	1845	UCD	0.001	0.765	0.186	0.041	0.008	-1.86	-0.96
Unknown-male-1	VAR	n/a	UGA	0.001	0.331	0.002	0.009	0.658	-4.55	-0.62
Unknown-male-2	VAR	n/a	UGA	0	0	0	0	0.999	-4.16	0.01

[z]UA = University of Arkansas, UGA = University of Georgia, GRIN_D = National Clonal Germplasm Repository at Davis, California, UCD = University of California at Davis, JB = Jeff Bloodworth, "Gardens Alive!", Wild = wild accession collected de novo.

[x]A single accession was collected in the Ouachita Mountains of Arkansas outside of the 1 km range for a population in this study. This accession was added to ARK2, the closest wild population.

[y]Accessions that were collected outside the 1 km range set for a wild population in this study. Each accession was collected from the same geographic region as ARK5, the Arkansas Ozarks, and therefore added to the ARK5 population.

[w]The genotype of ARK5-3 is presumed to be the result of a plating error and was not included in population-level statistics.

[v]A second population from Florida was collected for this study, but low-quality marker data resulted in only four accessions being included in the final dataset. Only 25.1 km separated the two Florida populations, therefore the two populations were combined.

[u]The tissue received for 'Spalding' and 'Stuckey' was determined to not match the reported pedigree. Therefore these samples were not included in population-level statistics.

[t]'Oh My!' is an interspecific hybrid between *V. rotundifolia* and *V. vinifera* and was therefore not included in population-level statistics.