Title: Evaluation of a Rooting Protocol for Hardwood Cuttings of Muscadine Grapes

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Abstract:

Muscadine grapes are notoriously difficult to propagate by hardwood cuttings. However, the fruit breeding program at the University of Arkansas System Division of Agriculture has consistently used hardwood cuttings for over a decade despite the literature claiming that this method is ineffective. In this study we sought to determine the efficacy of a clonal propagation protocol for hardwood cuttings used by the Arkansas fruit breeding program and assess the impact of collection date, location, cold storage, and bottom heating on the outcome of hardwood cuttings taken from muscadine grapes. Overall, 57% of the 352 10-cell experimental units in this study had at least one cutting with some root development and the average proportion of cuttings with some root development in each experimental unit was 0.17. The average proportion of rooted cuttings for the sites in Tifton, GA, Fayetteville, AR, Clarksville, AR, and Hillsborough, NC were similar overall (16-19%), but the effects of cultivar and location were different across sites. Cuttings taken in November had much greater rooting success than later dates in GA, while later cutting dates were better in Clarksville, AR, and cutting date had no effect in Fayetteville, AR. There was no significant effect of cultivar on rooting success in Fayetteville or FRS, but Carlos had a significantly lower proportion of rooted cuttings than the other cultivars in GA and NC (Table 4). There was no effect of cold storage or bottom heating in any of the four locations. Although the proportion of cuttings with root development was not commercially viable, this study demonstrates that breeding programs and germplasm repositories may be able to root hardwood cuttings for their modest yearly propagation needs.

Justification and Description:

Muscadines (*Vitis rotundifolia* Michx.) are a species of grape native to the southeastern U.S. This specialty crop is recognized for its disease resistance and thick-skinned, large-seeded berries. Unlike bunch grape species such as *V. labrusca* and *V. vinifera* that are relatively easy to propagate, muscadines are notoriously difficult to propagate by hardwood cuttings (Himelrick,

2001). Thus, most nurseries and breeding programs propagate muscadines with softwood cuttings. In a breeding program, the selection of muscadine seedlings occurs at the end of the growing season in September. A reliance on softwood cuttings requires the postponement of propagation of selected seedlings until the next growing season, generally in June or July. This propagation schedule falls into the busiest time of the year for fruit breeding programs and delays the establishment of plots and evaluation of new selections by a full season. This yearlong delay adds considerable time to the already lengthy process of releasing a variety. The development of a reliable protocol for muscadine propagation by hardwood cuttings would allow propagation work to be conducted after the conclusion of the growing season at a time when work in the field is beginning to slow and would increase the speed of cultivar development.

The literature on propagation of muscadines is sparse and most of the work conducted in this area is very old. Recommendations by Niven (1918) highlight the variability of rooting success for muscadine cultivars and suggest that 8-10% rooting success would be "excellent work." Cuttings were placed in well-prepared soil with only 1-2 inches of wood above the soil surface. Propagation by hardwood cuttings was only recommended if one was willing to get minimal success (Niven, 1918). Later studies at the University of Georgia found rooting percentages from 0 to 3% depending on cultivar; however, there was a noted difference in success when cuttings were taken later in the year. The rooting medium used was fine sand mulched with composted leaves, using wood of various ages. Rooting percentages were improved, but still not commercially viable, when cuttings were taken in November compared to August. These failures were attributed to poor callusing (Woodroof, 1935). Both of the aforementioned studies overwintered propagules in outdoor nursery beds. The application of bottom heat to cuttings in greenhouses greatly increased the success of cuttings taken in November, with rooting percentages as high as 70% (Newman, 1907). More recent investigations into rooting in greenhouses with bottom heat had mixed results. Hardwood cuttings from 'Hunt' were taken in Georgia from November to February on four different dates and treated with and without bottom heat. Rooting of 1-2% of cuttings with bottom heat was observed and no roots were found on cuttings without bottom heat (Goode et al., 1982). Another study found that muscadines rooted readily when hardwood cuttings were held at 4 °C for 60-90 days before potting (Whatley, 1974).

The fruit breeding program at the University of Arkansas System Division of Agriculture has consistently used hardwood cuttings for its modest yearly propagation needs for over a decade despite the literature claiming that this method is ineffective (David Gilmore, Personal Communication). Success rates are highly variable depending on both cultivar and year, ranging from 10-70%. The methods used at the Fruit Research Station are adapted from the protocol used for hardwood propagation of bunch grapes. Minor adjustments to the protocol have been made from year to year, and the protocol described below is the result of combining the most successful observed practices.

Currently, cuttings of the current season's hardened-off growth are collected in early December. Six to eight inch long cuttings with widths ranging from a pencil to the size of one's little finger (~8 - 20 mm) are used. Each cutting has at least three nodes; however, there is no upper limit to the number of nodes on a cutting. All cuttings are trimmed so that they have a 45º angle running across the top to prevent water from the mist system accumulating on exposed vascular tissue and to aid in the orientation of cuttings during planting. The bottom of the cuttings are cut at a 90º angle between nodes. Cuttings are immediately bundled and placed into refrigeration until early January (~4 weeks). Rooting hormone is applied by first dipping each cutting into water followed by an immediate coating of powdered hormone (different hormones have been used with roughly equivalent success). The rooting media is approximately six inches of perlite placed into mist beds in a greenhouse. Cuttings are spaced one to two inches apart and submerged in the media so that the bottom node of each cutting is entirely submerged and the second node is level with the top of the perlite. Misting occurs every five minutes. Noticeable rooting is expected as soon as late February but can take to the end of March, approximately 60 to 90 days.

It is unclear what factors make the propagation of hardwood cuttings successful in the Arkansas Fruit Breeding Program, while others have found this procedure so ineffective. We hypothesis that the high chill environment of Clarksville, AR may play a role in our success with hardwood cuttings. The refrigeration of cuttings over approximately four weeks during the winter holiday may further assist in the accumulation of chill hours.

In this study we evaluated the effect of location, collection date, cultivar, cold storage, and bottom heating on the success of rooting hardwood muscadine cuttings. This study is the first step in the development of a reliable protocol for propagation of hardwood cuttings of muscadines that would be extremely helpful for breeding programs, germplasm repositories, and commercial propagators.

Methods:

Muscadine cuttings were taken on four different dates through the dormant season: 4 Nov. 2019, 4 Dec. 2019, 6 Jan. 2020, and 4 Feb. 2020. The three cultivars selected for this study were 'Fry', 'Carlos', and 'Supreme'. Cuttings were approximately 15-20 cm long and 5-20 mm wide with a minimum of three nodes taken from mature vines. Cuttings were perpendicular at the base and at a 45° angle at the top to ensure that proper polarity was maintained when placing the cuttings in media and facilitate water runoff so that the mist system did not result in the accumulation of water at the top of each cutting. Four locations were selected for this study to represent major muscadine production environments across the Southeast with varying climatic conditions. Three study locations were research vineyards at the Division of Agriculture Experiment Stations in Clarksville (35.5332 N, -93.40378 W) and Fayetteville, AR (36.09910 N, -94.17223 W) and the Coastal Plain Experiment Station in Tifton, GA (31.47985 N, -83.52137 W). The fourth location

was a private vineyard near the city of Hillsborough, NC (36.12383 N, -79.07387W). The 'Fry' vine at Hillsborough, NC was removed before the study began, so only Supreme and Carlos were collected from the North Carolina site.

Cuttings were rooted in a greenhouse located in Fayetteville, AR. 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 were maintained between 18-24 °C through the course of the study. Three greenhouse benches measuring 1.5m x 3.0m were used as mist benches. A Rain Bird 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 was suspended approximately 0.61 m above the mist benches. Three Netafim 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 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 media.

Half of the cuttings from each location for each date were randomly selected for a cold storage treatment and subsequently placed in a 4 °C cooler for one month before planting. The other half of the cuttings were placed into media the day after collection to allow for shipping from Georgia and North Carolina. A bottom heating treatment was also applied to one-half of the cuttings. Heat was applied using 1.5m x 53cm 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 five centimeters into the rooting media.

The mist benches were organized as a split-plot with two randomized complete block main plots. The main plot factor was bottom heating and the subplot factors were collection date, cold storage, cultivar, and location. The percentage of cuttings with any degree of root development were measured 90 d after planting for each experimental unit. Other collected data included: length of the longest root, number of roots, diameter at top and bottom of cuttings, whether cuttings leafed out, overall length of cuttings, and number of nodes per cuttings. Data was analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC) as a mixed model with

location, collection date, cultivar, cold storage, and bottom heating and their interactions considered fixed effects and block and block*bottom heating considered random effects. Mean separation was performed with Tukey's Honestly Significant Difference.

Results and Discussion:

Overall, 57% or 251 of the 352 10-cell experimental units in this study had at least one cutting with some root development. The other 151 experimental units had no root development in any of the 10 cuttings. Only 48 experimental units had five or more of the 10 cuttings with some root development. Overall, the average proportion of cuttings with some root development in each experimental unit was 0.17 (Fig. 1). The total number of roots per experimental unit ranged from 0 to 80, with an average of 5.95 and the sum of the longest roots from each cutting in the experimental unit ranged from 0 to 776 mm with an average of 63 mm (data not shown).

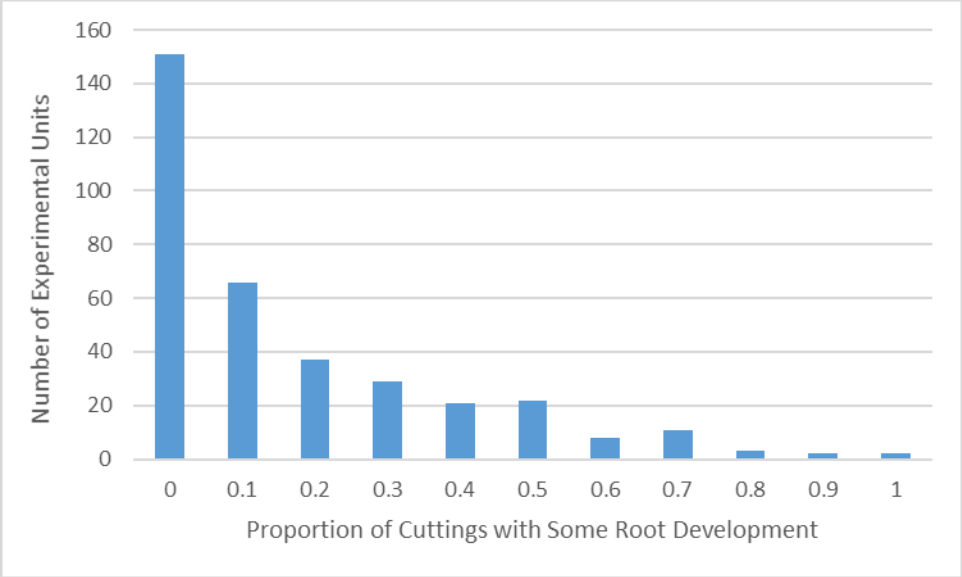

Figure 1. Proportion of cuttings with some root development in each of the 352 experimental units

The proportion of cuttings with some root development, number of roots, and sum of the lengths of the longest root in each cutting were all highly correlated with each other (Table 1). Experimental units which had a high proportion of rooted cuttings, longer roots, and more roots tended to have more cuttings with leaves. Likewise, the proportion of rooted cuttings and number of roots in each experimental unit were positively correlated with average cutting diameter. There was no significant correlation between the sum of the lengths of the longest root in each cutting and the diameter of the cuttings and none of the rooting attributes were correlated with average cutting length or the number of nodes per cutting

Table 1. Correlations between rooting attributes and presence of leaves, diameter, length, and nodes in the cuttings in each 10-cell experimental unit

	Root length (mm)	No. Roots	Proportion of cuttings with leaves	Avg. cutting diameter	Avg. cutting length	No. Nodes
Proportion of cuttings with roots	0.78**	0.83**	0.37**	0.22**	0.06	-0.07
Root length (mm)		0.80**	0.33**	0.07	-0.01	0.02
No. Roots			0.25**	0.15**	0.09	-0.05

** highly significant correlation at $p < 0.01$

Significant Location*Date interactions were observed for all three rooting traits. Therefore, ANOVA results are presented separately for each location (Table 2). The average proportion of rooted cuttings for each site were similar overall (16-19%), but the effects of cultivar and collection date were very different in each study location. For the Fayetteville, AR site no significant main effects or interactions impacted the success of rooting. The only significant treatment effect was cutting date in Clarksville, AR (FRS), while rooting success was impacted by both cultivar and collection date in Tifton, GA. In Hillsborough, NC significant main effects for collection date and cultivar and date*storage and date*cultivar*storage interaction effects were observed. Bottom heating had no effect on the success of rooting in any of the four study locations and none of its interactions were significant.

Table 2: P values from analysis of variance of percent of cuttings with some root development for each study location.

Effect	GA	Fay.	FRS	NC
Heat	0.34	0.54	0.93	0.77
Date	**<.01**	0.26	**<.01**	**0.02**
Cultivar	**0.02**	0.28	0.15	**<.01**
Storage	0.79	0.12	0.08	0.23
Date*Heat	0.12	0.13	0.06	0.06
Cultivar*Heat	0.76	0.84	0.86	0.14
Storage*Heat	0.87	0.86	0.40	0.28
Date*Cultivar	0.06	0.66	0.09	0.06
Date*Storage	0.72	0.31	0.09	**<.01**
Cultivar*Storage	0.49	0.15	0.08	0.22
Date*Cultivar*Heat	0.69	0.12	0.62	0.36
Date*Storage*Heat	0.32	0.94	0.11	0.15
Cultivar*Storage*Heat	0.72	0.37	0.73	0.84
Date*Cultivar*Storage	0.08	0.41	0.06	**0.03**
Date*Cultivar*Storage*Heat	0.60	0.44	0.52	0.83

Significant effects ($p < 0.05$) are emphasized with bold font

Table 3: Effect of cutting date on proportion of cuttings with some root development for each study location.

	GA	Fay	FRS	NC
November	0.36a[z]	0.20a	0.07c	0.26a
December	0.16b	0.12a	0.12bc	0.09b
January	0.07b	0.21a	0.23a	0.25a
February	0.16b	0.14a	0.22ab	0.12b

[z]Means within columns followed by the same letter are not significant by Tukey's HSD

Cutting date had no effect on rooting in Fayetteville, AR. In Tifton, GA, cuttings taken in November had significantly higher proportion with root development than any other date (Table 3). In contrast, rooting success was greater for cuttings taken in January and February than early in the season in Clarksville, AR. In Hillsborough, NC cuttings taken in November and January had better rooting success than those taken in December and February. It is unclear why the effect of cutting date differed so strongly across sites in this study. It is possible that the vines in Tifton, GA were not completely dormant at the November cutting date and this affected their rooting success. It is also possible that local weather conditions during the days before cuttings were made had an effect on rooting success.

There was no significant effect of cultivar on rooting success in Fayetteville or FRS. However, Carlos had a significantly lower proportion of rooted cuttings than the other cultivars in GA and NC (Table 4).

Table 4: Effect of cultivar on proportion of cuttings with some root development for each study location.

	GA	Fay	FRS	NC
Carlos	0.11b[z]	0.16a	0.18a	0.08b
Fry	0.21a	0.21a	0.19a	.
Supreme	0.24a	0.13a	0.11a	0.28a

[z]Means within columns followed by the same letter are not significant by Tukey's HSD

Conclusions:

Although the 17% rooting success reported in this study is not likely to inspire commercial nurseries to begin hardwood propagation of muscadine grapes, this modest rate of success may be good enough for breeders and germplasm repositories. Hardwood propagation fits better in the yearly cycle for breeding programs and may be beneficial for the USDA germplasm repository in Davis, CA, since this program already executes hardwood propagation of bunch grapes in the collection. Based on this first year of data collection it seems that some cultivars may root more readily than others and that collection date plays an important role in the success

of propagation. There was no evidence that cold storage of cuttings or bottom heating affected rooting. This study will be repeated in 2020/2021.

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