The Effect of Sample Date on the Propagation Success Rate of the grape, *Vitis aestivalis* 'Norton/Cynthiana.'

by

Amanda Lynn Uhls

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Amanda Lynn Uhls

APPROVED:

____
Dr. John DuBois
Department of Biology

____
Dr. Lynn Boyd
Department of Biology

____
Dr. Tony Johnston
School of Agribusiness & Agriscience
Honors Council Representative

____
Dr. Philip E. Phillips, Associate Dean
University Honors College

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Abstract

Vitis aestivalis 'Norton/Cynthiana' is a grape native to the southeast region of the United States. The purpose of this research was to determine the appropriate time of year and/or time after last freeze that will yield the most successful propagation rate for *Vitis aestivalis*. It was hypothesized that the highest rate of propagation would be in early summer. Forty cuttings were taken semi-monthly over one year at the local vineyard, treated with 0.1% IBA and placed in the plant growth room located in the MTSU Biology greenhouse. Daily temperatures were recorded every day as well. After six weeks, cuttings were evaluated for root production. Rooting success rates were highest in the month of June at 15.0-27.5%, and 0-7.5% for other sampling dates. It was determined that the best time to propagate *Vitis aestivalis* is in June, ten weeks after the last temperature below 0°C or eight to nine weeks after the first budding.

TABLE OF CONTENTS

Acknowledgements……………………………………………………..iii
Abstract……………………………………………………………….iv
List of Tables…………………………………………………………vi
List of Figures…..……………………………………………………vii

Introduction……………………………………………...…………...1

Materials and Methods

I. On-Site Sampling……………………………………………….3

II. Preparation of Cuttings……………………………………........3

III. Evaluation of Cuttings………………………………….........4

IV. Temperature Log………………………………………………..4

Results

I. Sampling Dates…………………………………………........6

II. Successful Rooting Percentage………………………………........7

III. Daily High and Low Temperatures…………………………….8

IV. Seasonal Look at the Cynthiana Vines………………………........8

Discussion……………………………………………………….....10

Conclusion…………………………………………………...….........12

Literature Cited………………………………………......................13

List of Tables

Table 1: The sampling and analysis dates over the duration of the study, from September 1, 2015 – October 3, 2016…………………………......………........6

List of Figures

Figure 1: The percent successful rooting versus date sampled from September 1, 2015 to August 31, 2016.………..…………………………....………......7

Figure 2: The daily high and low temperatures in Murfreesboro, TN from September 1, 2015 to August 31, 2016...…………………………....................8

Figure 3: Seasonal look at the *Vitis aestivalis* 'Cynthiana/Norton' vines in the Rutherford County Agricultural Extension Service/MTSU vineyard.….........9

Introduction

Vitis aestivalis 'Norton/Cynthiana' is a species of grape native to the Southeastern United States. Industry vineyards have developed an interest in the production of *Vitis aestivalis* for its quality of grapes, disease resistance, and hardy nature (8). The Norton/Cynthiana grape grows in medium-sized clusters that are dark blue-purple in color. When processed into wine, *Vitis aestivalis* demonstrates remarkable antioxidant properties, adding to its already interesting characteristics (9). A notable characteristic is its fungal endophyte that lives inside the plant, which may contribute to its overall hardiness and disease resistance. *Vitis aestivalis* protects itself against pests, diseases, and drought, as well as demonstrating heat and sun tolerance (13). The characteristics of this grape are very appealing to industry vineyards; however, vineyards are facing difficulty in propagating vines with dormant cuttings typically taken just after the last frost, the industry standard. This practice is mainly due to tradition, but is also supported by scientific research that states, "Studies have shown that root development must precede bud break to successfully propagate a new vine" (1). This conventional practice of harvesting dormant cuttings of the 'Norton/Cynthiana' grape has resulted in very low rates of successful propagation, between 9.4-22.0% (7;14), causing difficulties at vineyards that are attempting propagation.

There has been speculation regarding the best procedure that will yield the greatest propagation rate. Attempts have been unsuccessful, to date, at substantially increasing propagation rates. For example, Enderton *et al.* (4) studied the propagation rates of *Vitis aestivalis* when under the influence of bottom heat and rooting hormone treatment. They determined that bottom heat aided in the total percentage of rooting, but were only able to achieve a 40.9% success rate. This may seem like a breakthrough, but vineyards cannot realistically implement such conditions because they would not have the ability to administer bottom heat to as many cuttings necessary to yield sufficient results. Keeley *et al.* (10) studied the propagation rates of *Vitis aestivalis* when the bases of the cuttings were treated with indole-3-butyric acid (IBA). This method has shown improvements in root initiation, up to four times greater rooting than those not treated with IBA (2). These studies are two of many that show the stubborn propagation nature of this species of grape, and information obtained from the latter has pointed this study in the direction of using the supplement, indole-3-butyric acid (IBA).

During the spring and summer of 2015, professors from MTSU (3) performed a preliminary study into the propagation of the 'Norton/Cynthiana' grape at the Rutherford County Agricultural Extension Service/MTSU vineyard. For that study, clippings of the vines were harvested in the months of March and June. These cuttings were treated with a rooting hormone composed of 0.1% indole-3-butyric acid (IBA), and incubated in a mixture of 50% Perlite and 50% Vermiculite in a growth room set to mimic spring lighting and temperature conditions. Results showed that the June cuttings had much greater success rate at rooting (40%) than the cuttings taken in March (4%), the time that would be typical for traditional grape propagation in middle Tennessee.

Materials and Methods

I. On-Site Sampling

A portion of this research was conducted at the Rutherford County Agricultural Extension Services/MTSU vineyard that contains Murfreesboro's public crop of *Vitis aestivalis*. I travelled to this vineyard semi-monthly for one year to obtain vine clippings that I then used in my research. Upon arrival of each visit, I took fifty vine cuttings, randomly in different locations throughout the vineyard. I chose vines that appeared hardy enough to withstand the 5.5-mile drive back to the MTSU campus, counted down past at least the fifth node – or knot on the stem where a leaf would typically emerge – from the tip and cut the vine using shears. I then placed the cuttings in a bucket of water, with the freshly-cut end submerged in the water to prevent them from drying out, and returned to campus. To avoid the possibility of the Murfreesboro pruning classes clipping away too many of the vines during the winter months, leaving me with insufficient research samples, I had secured twenty random vines throughout the vineyard to be dedicated solely to my research and to remain untouched by other personnel.

II. Preparation of Cuttings

In the Biology greenhouse, I created a 50:50 mixture of Perlite:Vermiculite. This mixture helped to aerate the soil, as well as provide a higher level of water retention (5;6). Six 15cm diameter pots were filled with the Perlite:Vermiculite mixture up to about two centimeters from the top of the pot. Three pots were placed on one tray and three pots were placed on another tray, which allowed for sufficient watering. For each stem cutting, I made a fresh cut just past the fifth node, ensuring that the node itself would be submerged into the medium to encourage rooting. Each sample end containing the fresh cut was wetted with water and dipped into the rooting hormone (0.1% Indole-3-butyric acid) and then placed into one of the pots containing the Perlite:Vermiculite mixture. There were seven cuttings placed in each of four the pots, and six cuttings placed in each of the remaining two pots. This equaled a total of forty samples in six pots that were used at each sample date. The two trays of samples were then moved into the plant growth room, watered and cuttings allowed to root for six weeks. The plant growth room is a controlled-environment room set to springtime conditions for Middle Tennessee, meaning there are equally twelve hours of light and twelve hours of dark in one day, 70% relative humidity, and temperatures at 22^{o}C during the day and 15^{o}C during the night.

III. Evaluation of Cuttings

After six weeks in the growth room, the samples were removed from the Perlite:Vermiculite mixture and carefully inspected for rooting. I overturned each pot and carefully removed each sample to inspect for rooting after rinsing in water. If there was no rooting, the sample was discarded. If there was rooting, the cutting was recorded as such.

IV. Temperature Log

I kept a record of the daily high and low temperatures that occurred in Murfreesboro by gathering data from an online database (11). This record of temperature aided my research by allowing me to not only provide the best time of year at which to take the cuttings, but also an indication of number of degree days after the last freeze at which to collect cuttings. This information was a useful supplement because industry vineyards at different geographical locations will not have the same temperature conditions during the same months of the year as Murfreesboro, Tennessee.

Results

I. Sampling Dates

The original intent in this study was to take semi-monthly cuttings at the beginning and middle of each month (Table 1). Two sampling dates were missed – early due to harsh weather conditions observed in the area and mid-March due to personal error. All other sampling dates were accounted for.

Table 1. The sampling and analysis dates over the duration of the study, from September 1, 2015 – October 3, 2016. Samples were analyzed six weeks after collection.

Sampling Dates	**Analysis Dates**
September 1, 2015	October 15, 2015
September 15, 2015	November 3, 2015
October 1, 2015	November 17, 2015
October 15, 2016	December 1, 2015
November 3, 2015	December 15, 2015
November 17, 2016	January 1, 2016
December 17, 2016	February 1, 2016
January 1, 2016	February 16, 2016
January 15, 2016	March 9, 2016
February 5, 2016	March 24, 2016
February 26, 2016	April 6, 2016
March 9, 2016	April 15, 2016
April 6, 2016	May 6, 2016
April 15, 2016	June 4, 2014
May 5, 2016	July 4, 2016
May 21, 2016	July 4, 2016
June 6, 2016	July 16, 2016
June 17, 2016	August 16, 2016
July 4, 2016	August 21, 2016
July 16, 2016	September 1, 2016
August 3, 2016	September 27, 2016
August 16, 2016	October 3, 2016

II. Successful Rooting Percentage

Success rate was determined by dividing the number of cuttings that showed roots by forty, the total number of cuttings processed for each sample date. The highest percent success rates were observed in the cuttings taken in the month of June 2016 (Figure 1). The June 6, 2016 sampling date yielded the highest percent of successful propagation at 27.5%. This success rate was statistically significant (t-test; $p < 0.001$) compared to all other sampling dates. The June 17, 2016 sampling showed a 15.0% success rate. The next highest propagation success rates occurred in August 2016. The August 3, 2016 sampling date exhibited a 7.5% success in propagation and August 16, 2016 sampling date exhibited successful propagation at 12.5%. All other sampling dates resulted in 0.0-5.0% success rates.

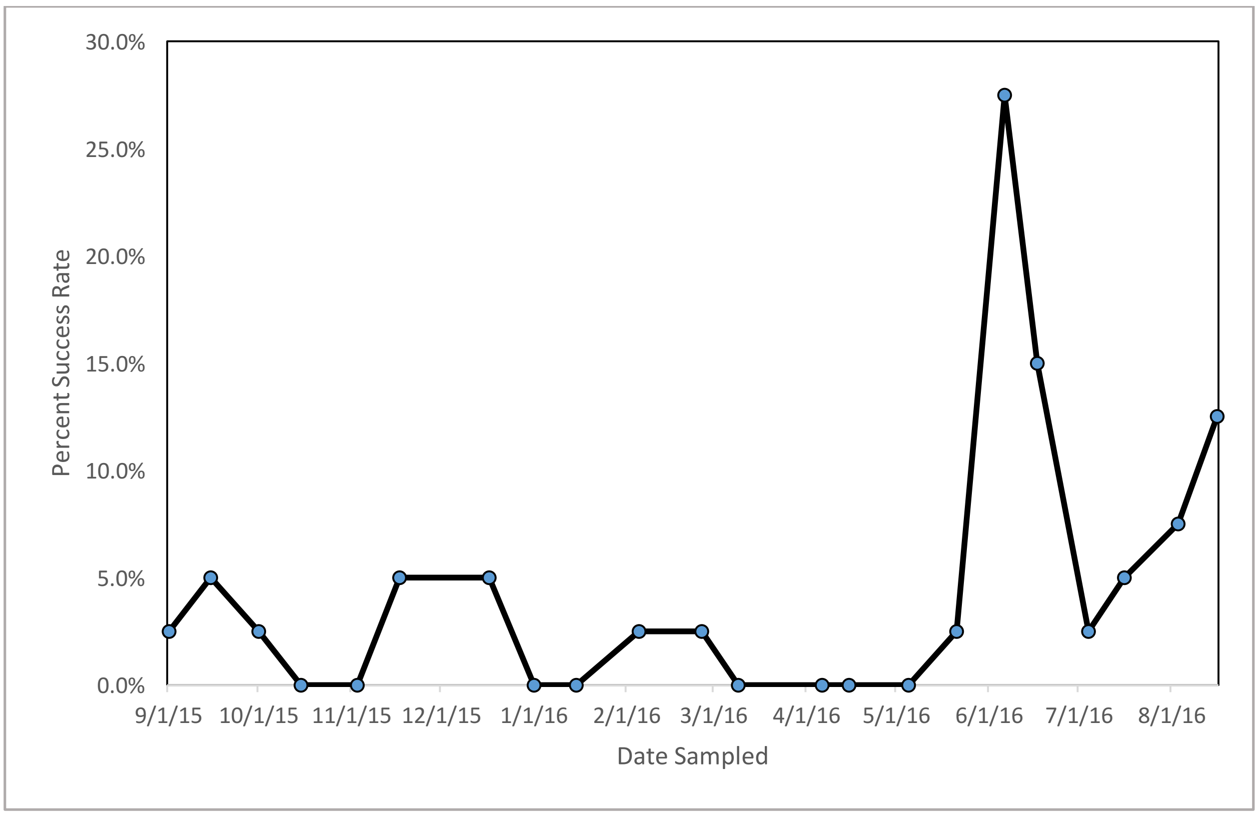

Figure 1. The percent successful rooting versus date sampled from September 1, 2015 to August 31, 2016.

III. Daily High and Low Temperatures

The daily high and low temperatures were recorded for Murfreesboro, TN (Figure 2). The last temperature below 0°C was observed on March 26, 2016.

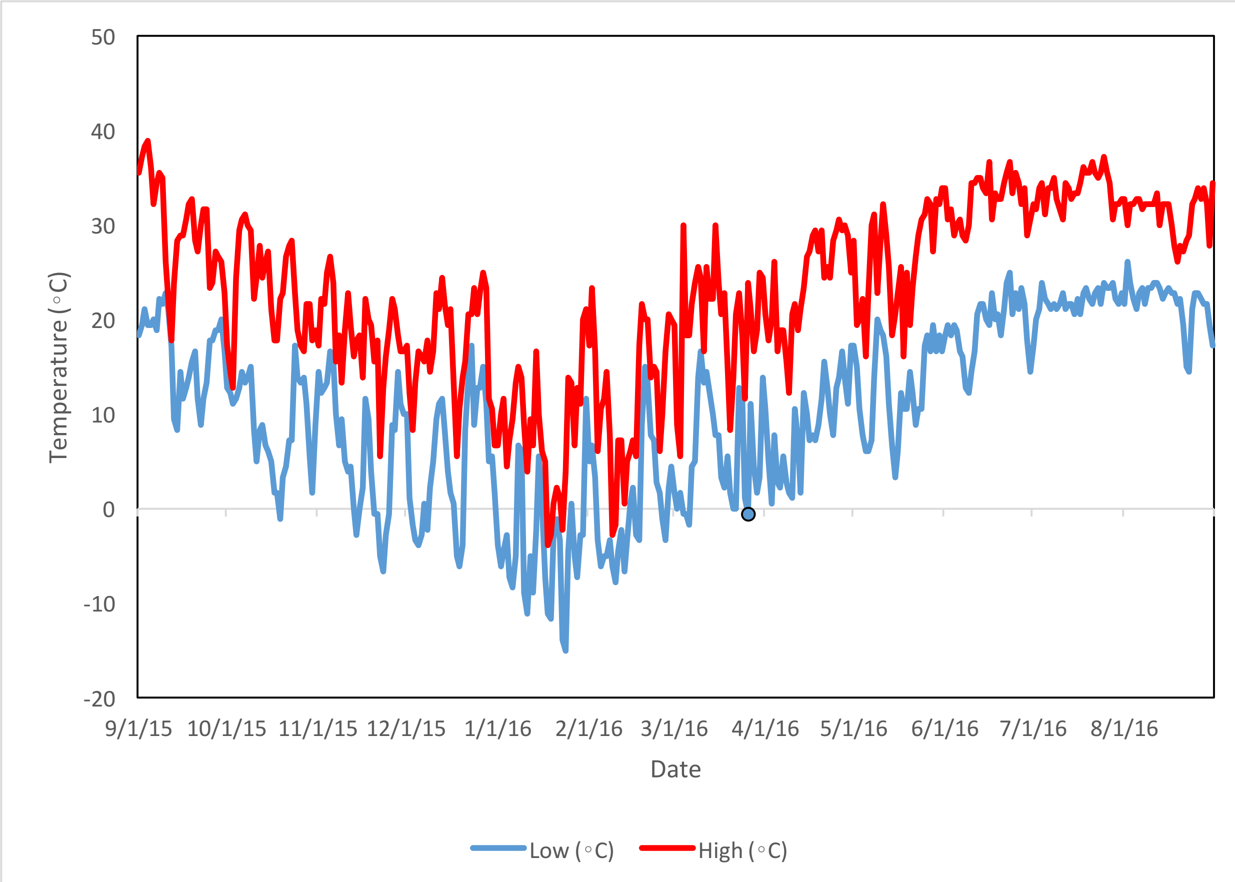

Figure 2. The daily high and low temperatures in Murfreesboro, TN from September 1, 2015 to August 31, 2016 (11). The last date that the temperatures dropped below 0°C was March 26, 2016, and is noted on the graph with a black circle.

IV. Seasonal Look at the Cynthiana Vines

Figure 3 shows a seasonal look at the grape vines in the vineyard. The first bud opening was observed on the April 6, 2016 sampling date.

Figure 3. Seasonal look at the *Vitis aestivalis* ‘Cynthiana/Norton’ vines in the Rutherford County Agricultural Extension Service/MTSU vineyard. First budding was observed in the spring, on April 6, 2016.

Discussion

Due to the difficult propagation nature of *Vitis aestivalis* 'Norton/Cynthiana,' this study was designed as a starting point on which to base further, more detailed research. The study began on September 1, 2015 with the first cuttings and ended on October 3, 2016 with the final analysis. Cuttings were taken semi-monthly over the course of a year at the beginning and middle of each month with some variation due to inclement weather. Two of the sampling dates, the beginning of December 2015 and the middle of March 2016, were missed due to inclement weather and personal error. After observation of the adjacent sampling dates and their subsequent results, it is hypothesized that had these two sampling dates not been missed, their data would not have altered the overall conclusion of this study.

The Norton/Cynthiana grape has caused difficulty when commercial vineyards have attempted the traditional route of propagation, taking cuttings just after the last frost and before the first budding. Results of my study demonstrate that traditional propagation times (March and April) for this grape prove to yield very low success rates (Figure 1). With the exception of the months of June and August, all other sampling dates yielded propagation success rates of 0.0 – 5.0%. However, during the month of June, successful propagation (i.e. rooting) approached 30%.

During the month of August, successful propagation was observed at 7.5 – 12.5%. Further research will need to be conducted to determine the importance of these results. It is unclear if the August samplings showed another peak in rooting success or if the July samplings showed an abnormal decrease in rooting success. July could have experienced a dry period, causing the success rate to be lower than what could be observed in a future study. A proposed second study would be to repeat the aforementioned methods outlined from the month of May through the month of October in middle Tennessee. This repeat study would further determine the true significance of the propagation success rates seen in August. Regardless, it was determined that the month of June, for middle Tennessee, yields the highest success rate of propagation.

While Enderton *et al.* (4) achieved a higher success rate at 40.9%, their experiment utilized the application of bottom heat on the cuttings. Commercial vineyards may not be willing to go to the extra time and expense of applying bottom heat to their grape cuttings. Therefore, my study provides a simple viable alternative by sampling at a different time of year.

High and low temperatures were recorded daily and the first budding date was observed because this grape is also grown in regions outside of middle Tennessee. These other geographical locations have different growing seasons, so the date of sampling itself will not be meaningful. In this experiment, the first significant success rate in June occurred ten weeks after the last day the temperature reached below freezing (March 26, 2016). Commercial vineyards in states such as Missouri or Kansas may find this information more beneficial when determining the proper propagation time (12). The first significant success rate in June also occurred eight to nine weeks after the first day of observed budding (April 6, 2016). Locations attempting new vineyards – such as the vineyard that Dr. Tony Johnston is helping to create in Honduras (3) – do not experience a frost or freeze. Therefore, relating propagation time to first budding would be beneficial to these tropical locations.

Conclusion

Based on this study, advice to commercial vineyards in middle and/or west Tennessee is that the best time at which to take cuttings of the *Vitis aestivalis* 'Norton/Cynthiana' grape is in the month of June. This can pose a problem due to the fact that the fruits set during the month of June. This is not a good time to take cuttings because it is not wise to cut vegetative tissue while trying to promote fruit growth. My recommendation for commercial vineyards to avoid this problem is to set aside at least 4-6 vines, solely as a propagation stock.

Some regions outside of middle or west Tennessee that experience different weather patterns should propagate this grape ten weeks after the last temperature below freezing. Areas that do not experience a frost or freeze should propagate 8-9 weeks after the first budding.

It would be valuable to repeat this study from May through October to confirm the results seen in June and to further explore the results seen in July and August. This replication would also increase this database and strengthen conclusions.

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