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The Effect of Sample Date and Timing of Cuttings for Maximum Propagation Efficiency of the Grape, *Vitis aestivalis* "Norton/Cynthiana"

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Abstract

Vitis aestivalis "Norton/Cynthiana" is a grape native to North America and utilized in the commercial wine industry. 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. During a year-long study (2015-2016), forty cuttings were taken semi-monthly from a vineyard, treated with 0.1% IBA and placed in a plant growth room set to spring conditions. Daily temperatures were recorded every day as well. In a follow-up study, cuttings were taken weekly from early spring before vine budding until late fall (2017) and treated as previous. After six weeks, cuttings were evaluated for root production. In both studies, rooting success rates were the highest in the month of June at 10.0% - 27.5%, with rates less than 7.5% for all other sampling dates. It was determined that the best time to propagate *Vitis aestivalis* is in June, 10 - 12 weeks after the last temperature below 0˚C or eight to ten weeks after the first budding.

Keywords

Vitis aestivalis, Norton, Cynthiana, Grape, Propagation, Rooting

1. Introduction

Vitis aestivalis "Norton/Cynthiana" is a species of grape native to North America and is grown as far north as southern Ontario, Canada, as far west as Oklahoma and as far south as Florida [1]. Industry vineyards have developed an interest in the production of *Vitis aestivalis* for its quality of grapes, disease resistance and hardy nature [2]. 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 [3]. 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 [4]. The characteristics of this grape are very appealing to industry vineyards; however, vineyards face difficulty in propagating vines via dormant cuttings, the industry standard. This practice is mainly due to tradition, but, is also supported by scientific research that shows that root development must precede bud break to successfully propagate a new vine [5]. This conventional practice of harvesting dormant cuttings of the "Norton/Cynthiana" grape have resulted in very low rates of successful propagation, between 9.4% - 22.0% [6] [7], causing difficulties at vineyards that attempt 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.* [8] 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 the large number of cuttings necessary to yield sufficient results. Keeley *et al.* [9] 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 [10]. These studies are two of many that elucidate the stubborn propagation nature of this species of grape.

The purpose of this study was to determine if sample date and timing of cutting harvest has a positive effect on *Vitis aestivalis* "Norton/Cynthiana" grape propagation efficiency.

2. Materials and Methods

2.1. Vineyard Sampling

Cuttings of grapevines were taken at the Rutherford County Agricultural Extension Services/MTSU vineyard (Latitude: 35˚51'15.39"N; Longitude: 86˚26'43.32"W). Semi-monthly sampling was accomplished from September 1, 2015 until August 16, 2016. The study was repeated during the active growth season in 2017, beginning April 4, 2017 and ending September 4, 2017. Upon arrival during each site visit, fifty grapevine cuttings were collected randomly in different locations throughout the vineyard. Each tissue selected for cutting came from the growing end of a vine. Each cutting was made below at least the fifth node from the tip of the vine. Cuttings were placed in a bucket of water, with the freshly-cut end submerged in the water to prevent them from drying out. Cuttings were then transported to the MTSU campus.

2.2. Preparation of Cuttings

Cuttings were immediately returned to the MTSU campus. Sterilization procedures for the cuttings were not necessary. For each stem cutting, a fresh cut was made just below the fifth node, ensuring that the node itself would be submerged into the rooting medium. Each freshly cut end was wetted with water and dipped into rooting hormone (0.1% Indole-3-butyric acid), and the cutting was then placed into the rooting mixture. The rooting mixture was a 50:50 (V:V) mixture of Perlite: Vermiculite. This mixture helped to aerate the soil, as well as provide a higher level of water retention [11] [12]. Six 15cm diameter plastic pots were filled with the Perlite: Vermiculite mixture up to about two centimeters from the top of the pot. Pots were placed on trays, which allowed for sufficient watering. Six to seven cuttings were placed into each of the pots for a total of forty samples at each sample date. The trays of samples were placed into a 2.2 M × 3.8 M controlled-environment plant growth room, watered and allowed to root for six weeks. The plant growth room was set to springtime conditions for Middle Tennessee (12 hours light/12 hours dark; 70% relative humidity; with temperatures of 22˚C during the day and 15˚C during the night).

2.3. Evaluation of Cuttings

After six weeks in the growth room, the samples were removed from the Perlite: Vermiculite mixture and carefully inspected for rooting. If there was no rooting, the sample was discarded. If there was rooting, the cutting was recorded as such.

2.4. Temperature Log

Local daily high and low temperatures according to the weather service, AccuWeather [13], were recorded for the entire year of sampling (September 2015 to August 2016) and for the second study (March 1, 2017 to October 1, 2017).

2.5. Vine Status Log

The physical appearance and general vine status of the grapevines was assessed and photographed during every site visit to the vineyard. This assessment was done to record initial budding in the spring, along with approximate dates for flowering and fruiting.

2.6. Statistical Analysis of Data

The percent rooting success rates for each sampling date were analyzed using a t-test (SigmaStat Version 3.1). The 2016 study and the 2017 study were analyzed separately.

3. Results

3.1. Sampling Dates

Sample dates for the year-long study (2015-2016) were completed on a semi-monthly schedule. Sample dates for the second study (2017) were completed weekly in an attempt to better define the optimal sampling time. In both studies, some sample dates had to be adjusted for harsh weather conditions that prevented vineyard site visits.

3.2. Rooting Success Rates

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. For the year-long study, the highest percent success rates were observed in the cuttings taken during 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.

In the second study (2017), the highest success rates were also observed in the month of June (Figure 2). The June 5, 2017 sampling showed a 5% success rate,

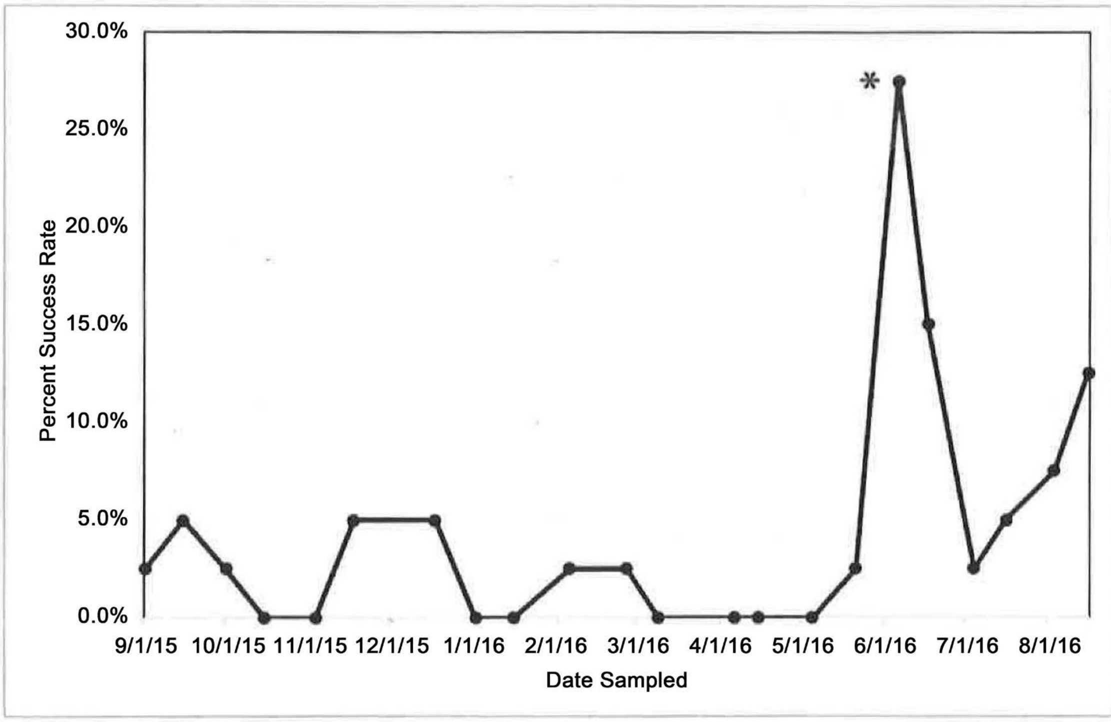

Figure 1. The percent successful rooting versus date sampled from September 1, 2015 to August 31, 2016. * Statistically significant (t-test; $p < 0.001$).

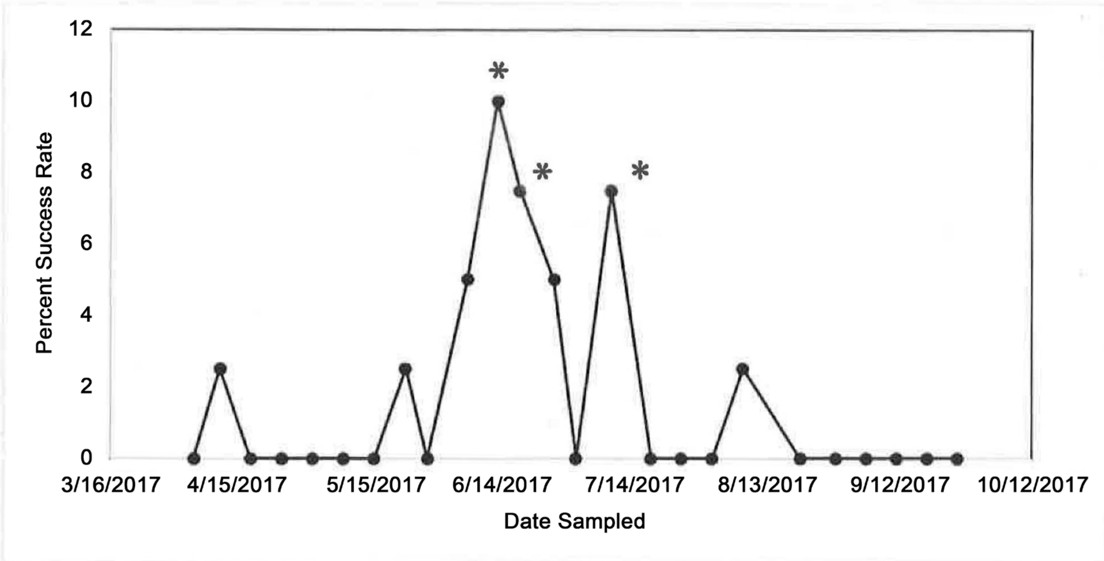

Figure 2. The percent successful rooting versus date sampled from April 4, 2017 to September 4, 2017. * Statistically significant (t-test; $p < 0.001$).

whereas the June 12, 2017 sampling yielded a 10% success rate. Samplings on June 17, 2017 and July 8, 2017 both showed a 7.5% success rate. The success rates for cuttings taken on June 12, 17 and July 8 were found to be statistically significant (t-test; $p < 0.001$). All other sampling dates yielded 0% - 2.5% success rates.

3.3. Temperature Log

The daily high and low temperatures for the year-long study showed the last day with a temperature below 0˚C to be March 26, 2016 (**Figure 3**). For the second study, the last day with a temperature below 0˚C was March 19, 2017 (**Figure 4**).

3.4. Grapevine Status Log

Assessments and photographs of the grapevine status at each sampling show that, for the year-long study, the date of first vine budding occurred on April 6, 2016 (**Figure 5(a)**). For the second study, the date of first vine budding occurred on April 4, 2017 (**Figure 5(b)**). As for flowering and fruiting, by May 17, 2016, vines had completed flowering and immature fruits had begun to form (**Figure 6(a)**). Unfortunately, the late April/early May 2016 sampling was not completed due to harsh weather conditions, so when flowering began that spring cannot be assessed. In the second study, flower inflorescences were first observed on April 17, 2017 and anthesis had occurred by May 15, 2017 (**Figure 6(b)**). Fruiting occurred by May 22, 2017 (**Figure 6(c)**).

4. Discussion

The Norton/Cynthiana grape has proven to be difficult to propagate via the traditional route of propagation, taking cuttings during dormancy. Results of this study demonstrate that traditional propagation cutting collection times (December to March in middle Tennessee) for this grape yield very low success rates (Figure 1 & Figure 2). Excluding the sampling dates during June through early

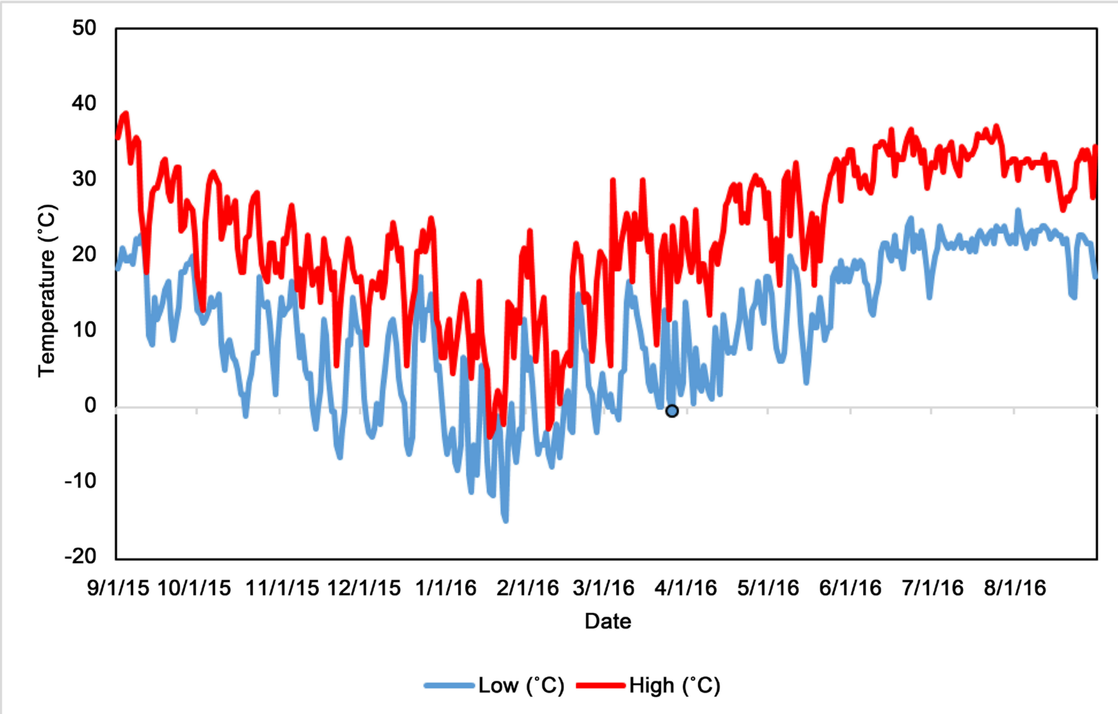

Figure 3. The daily high and low temperatures in Murfreesboro, TN from September 1, 2015 to August 31, 2016. The last date that the temperatures dropped below 0˚C was March 26, 2016, and is noted on the graph with a black circle.

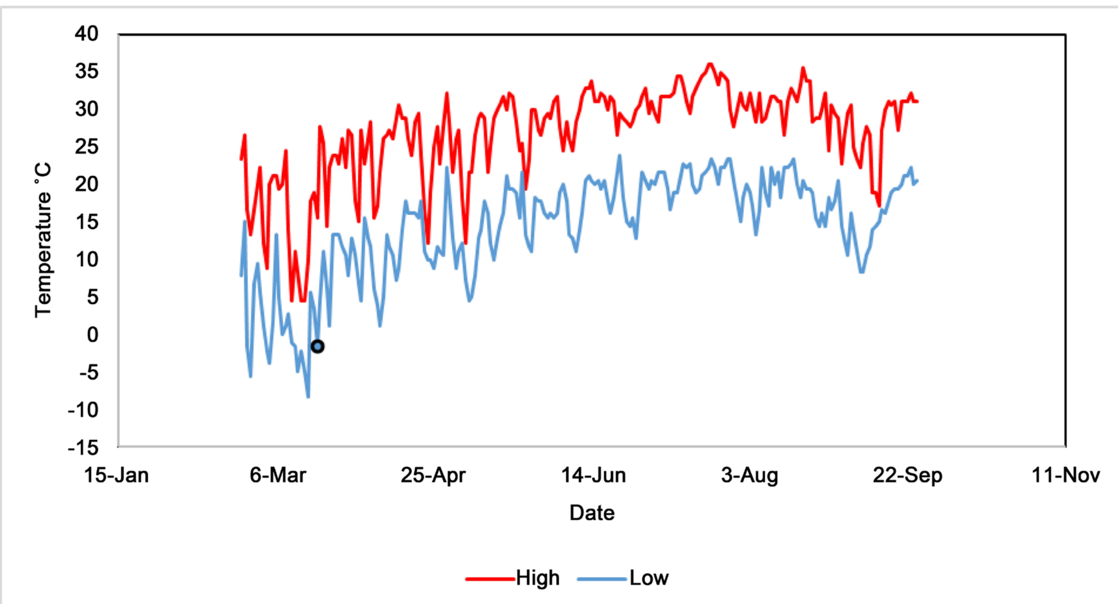

Figure 4. The daily high and low temperatures in Murfreesboro, TN from March 1, 2017 to September 4, 2017. The last date that the temperatures dropped below 0˚C was March 19, 2017, and is noted on the graph with a black circle.

Figure 5. Observed first budding in *Vitis aestivalis* "Norton/Cynthiana" grapevines in middle Tennessee. (a) April 6, 2016; (b) April 4, 2017.

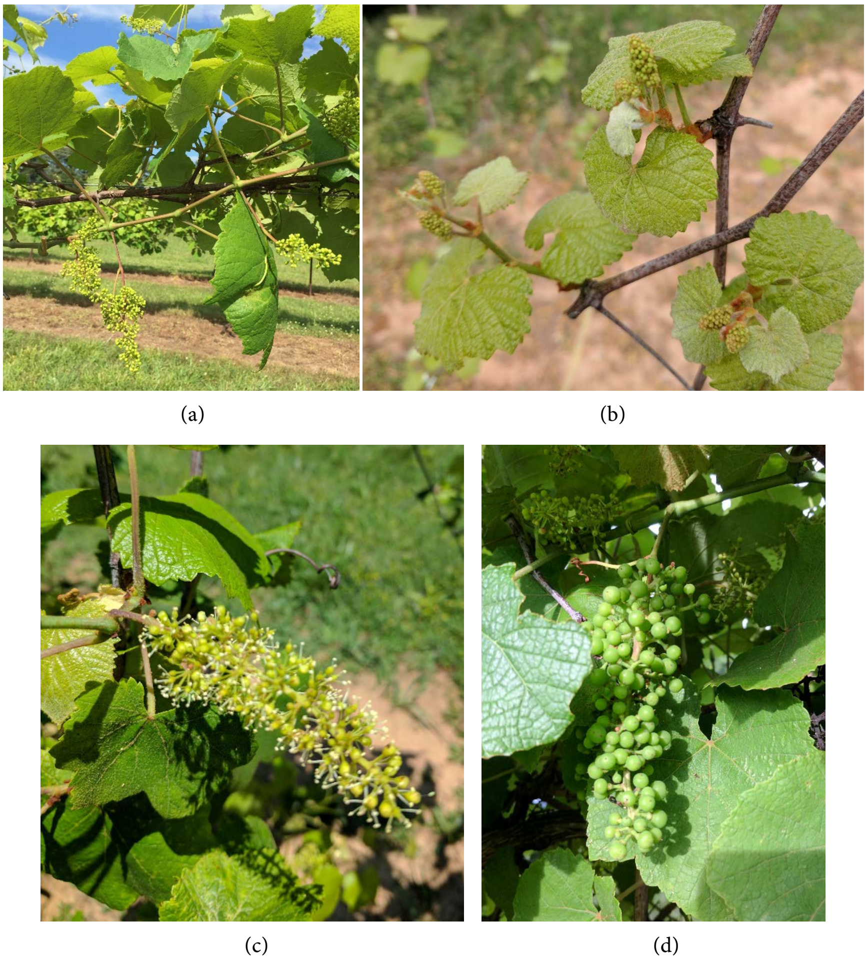

Figure 6. Observed flowering and fruiting in *Vitis aestivalis* "Norton/Cynthiana" grapevines in middle Tennessee. (a) Fruiting May 17, 2016; (b) First floral inflorescences April 17, 2017; (c) Floral anthesis May 15, 2017; (d) Fruiting May 22, 2017.

August, all other sampling dates yielded propagation success rates of 5% and below. In both sampling years (2016 and 2017) the highest success rates for rooting occurred during the month of June. Rooting success rates approached 30% during June 2016.

While Enderton *et al.* [8] 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 invest the extra expense of applying bottom heat to grape cuttings at the scale necessary for large scale production.

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 in this study will not be meaningful. In the year-long study, the first significant success rate in June occurred ten weeks after the last day the temperature fell below freezing (March 26, 2016). In the 2017 study, the highest success rate for propagation occurred 12 weeks after the last day the temperature was below 0˚C (March 19, 2017). Therefore, based upon our studies, the best time to propagate this grape is 10 - 12 weeks after the last temperature below freezing. Commercial vineyards in states outside Tennessee will find this information more beneficial than an actual date when determining the optimal propagation time. The first significant propagation success rate in June also occurred eight to nine weeks after the first day of observed budding (April 6, 2016) in the year-long study and 10 weeks after first budding (April 4, 2017) in the second study. Vineyards in more tropical locations that do not experience a frost or freeze will find this reference to first budding to be beneficial in determining the optimal time to propagate.

High temperatures were evaluated over the period from February 23 to July 22, 2017 by calculating a trendline. Over this period, the slope of the trendline was 0.1142, indicating rising temperatures. As spring gives way to summer, rising daily high temperatures are expected and normal. In addition, daily high temperatures were plotted from June 5 to July 7, 2017, the period of time during which propagation rates were highest. The slope of this trendline was 0.0643. This indicates that the highest propagation efficiency occurred during the time period when daily high temperatures were essentially steady and unchanging.

5. Conclusions

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 fruit set occurs during the months of May and 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. Therefore, our recommendation for commercial vineyards to avoid this problem is to set aside a host of vines which will solely be used in a given year as a propagation stock. It would be highly advantageous to remove all floral inflorescences from these vines before fertilization to promote as much vegetative growth as possible during the growing season, thus, having more vine tissues from which to take cuttings.

For regions outside of middle or west Tennessee that experience different weather patterns, collection of cuttings for propagation of this grape should occur 10 - 12 weeks after the last recorded temperature below freezing. For areas that don't experience a frost or freeze, collection of propagation cuttings should occur 8 - 10 weeks after the first budding is observed.

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