

# Box Huckleberry Roots at Highest Percentages From Softwood Stem Cuttings<sup>1</sup>

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## Abstract

Box huckleberry [*Gaylussacia brachycera* (Michx.) Gray] is a slow-growing, dwarf evergreen woody groundcover that is native to both the mountains and coastal plains of eight states in the mid-Atlantic region of the United States. It grows as a low, dense, spreading mat and is affected by few major disease or insect pests. While uncommon in the wild, it has potentially high ornamental value as an evergreen groundcover, growing well in dry shade and acidic soil. Unfortunately, knowledge on the optimal asexual propagation of box huckleberry is lacking. In order to identify optimal propagation timing, we took stem cuttings from five genotypes of box huckleberry every month for two years. Although some genotypes differed, on average 90% or more of the cuttings rooted for those taken during May to July, 30% or fewer for September to December cuttings, and 40 to 70% for cuttings taken in other months. Cuttings generally formed roots faster in months with a higher rooting percentage. Based on these results, the best time to take stem cuttings for box huckleberry propagation is late spring to early summer which corresponds to softwood or semi-hardwood growth.

**Index words:** native plant, nursery plant, production, propagation.

**Chemicals used in this study:** indole-3-butyric acid (IBA, Hormodin 3).

**Species used in this study:** box huckleberry [*Gaylussacia brachycera* (Michx.) Gray].

## Significance to the Horticulture Industry

Box huckleberry is an evergreen groundcover that is native to eight states in the mid-Atlantic region of the United States. It grows well in dry shade, where few woody plants can thrive. Although it has potential as a niche landscape plant, it is infrequently grown by nursery producers, partly due to uncertainties about clonal propagation. We conducted experiments to determine the optimum time to take stem cuttings of this plant to ensure that nursery growers maximize propagation efficiency. We found that the highest percentage of stem cuttings rooted with cuttings that were taken in May, June, and July. Based on these results, we conclude that the best time to take stem cuttings for box huckleberry propagation is late spring to early summer, which corresponds to softwood or semi-hardwood growth.

## Introduction

Box huckleberry is a slow-growing, dwarf evergreen woody groundcover that is native to both the mountains and coastal plains of Pennsylvania, Virginia, Kentucky, Tennessee, West Virginia, Delaware, Maryland (USDA NRCS 2014) and North Carolina (Wilbur and Bloodworth 2004). It has glossy, dark-green, fine-textured foliage, which emerges red to maroon and changes to red in winter or when stressed. It grows as a low, dense, spreading mat and is affected by few major disease or insect pests. The box huckleberry's global conservation status is listed as G3 (vulnerable, Center for Plant Conservation 2014), and the state listing for Delaware, Maryland and Pennsylvania is S1 (critically imperiled). In the states in which it is native, approximately 100 known populations of this species can be found (Center for Plant

Conservation 2014). Under permit, cuttings or plants of box huckleberry have been collected from 14 native habitats in six states and are currently growing in a protected site at the U.S. National Arboretum (USNA). In addition to the original populations, we also have a number of seed-derived, open-pollinated progeny from these plants. Box huckleberry is considered to be self-incompatible and rarely reproduces by seed due to isolated genotypes. However, diverse genotypes are growing in close proximity in our collection, which allows cross-pollination necessary for seed production. Optimal propagation methods are needed in order to evaluate, select, and distribute these novel genotypes for restoration, conservation, or landscape use.

Although box huckleberry is not commonly grown by nurseries, it can be purchased from a few commercial nurseries in the U.S. It has potentially high ornamental value as a woody, evergreen groundcover that grows well in dry shade in acidic soil. Clonal propagation and evaluation of wild-collected plants or new genotypes from our collection offers the opportunity to extend the range of use and ease of production of this plant. However, studies to optimize propagation of box huckleberry using stem cuttings are lacking. The purpose of our study was to identify optimal stem cutting propagation timing to increase the plant's potential as a nursery crop or to assist in conservation or reintroduction of this native plant.

## Materials and Methods

Box huckleberry stock plants at the U.S. National Arboretum were grown under a wooden lath structure (60 to 70% shade) in beds containing Fafard Nursery Mix (Sungrow Horticulture, Agawam, MA) with a small amount of soil and organic matter taken from the same general area where the plants that were collected in Maryland were growing. We added this organic matter under the hypothesis that box huckleberry may have a mycorrhizal association in the wild. At the time of establishment, compost was incorporated into the nursery mix and plants were mulched with a layer of shredded leaves collected from the Arboretum's wooded areas.

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**Table 1.** Accession information for the five box huckleberry genotypes used in this study.

Accession number (NA <sup>z</sup> number)	Collection information/origin	Date collected
1 BX (NA 70976)	Lake Waterford Park, Anne Arundel Co., MD	October 29, 1998
3 BX (NA 70945)	National Clonal Germplasm Repository CGAY7 (TN)	Fall, 1993
7 BX (NA 71192)	Hollywood Township, Monroe Co., WV	August 26, 2001
9 BX (NA 71196)	Daniel Boone National Forest, KY	October 3, 2001
11 BX (NA 71199)	Grassy Gap Ridge, KY	October 4, 2001

<sup>z</sup>NA number = U. S. National Arboretum number.

Stem cuttings were taken from the newest growth from five selected clones (Table 1) each month for two years, from May 2011 to May 2013. Cuttings consisted of terminal stems with 10 to 14 leaves and were approximately 7 cm (3 in) long. The lowest 4 to 5 leaves were removed, and cuttings were dipped in 8000 ppm IBA-talc (Hormodin 3, OHP, Inc., Mainland, PA) and placed in flats containing 50% milled sphagnum peat and 50% coarse perlite. Flats were placed on a bench under mist (30 seconds of mist every 45 minutes) in a greenhouse with 50% solar shade and kept at 24 to 27C (75 to 80F), with bottom heat of 25C (78F) applied to flats. Cuttings were evaluated periodically (generally every two to three weeks) for rooting from 6 to 50 weeks after cuttings were taken by carefully removing cuttings from the growing medium and visually inspecting for roots. In addition to rooting percentage, the number and length of roots were also recorded.

The proportion of cuttings that rooted was fit using a logistic regression, using the GLM function in R (R Development Core Team 2013), with month as the independent variable. Data from different clones were combined, as were data from both years because clonal variance and year-to-year variance were relatively small, and interactions with month were not significant (both  $p > 0.05$ ). However, these additional sources of variation were accommodated in the model by including an over-dispersion parameter. In a logistic regression, the variance of an observation is determined by its mean, as data are assumed to follow a binomial distribution. However, for most biological data, there is additional variability (extra-binomial variation) that needs to be captured by the model so that the standard errors of means are not underestimated. An over-dispersion parameter is one method of accomplishing this; essentially it is a one-number inflation of all theoretical standard errors (based on the binomial distribution) that brings average theoretical variances to average observed ones using a quasi-likelihood approach (McCullagh and Nelder 1989). Multiple comparisons of months were made using the R multcomp package (Hothorn et al. 2008).

## Results and Discussion

The proportion rooting was highest from stem cuttings taken in May, June, and July, with over 90% of cuttings rooting (Fig. 1). The lowest proportion of stem cuttings rooted from cuttings taken in the fall (September through December), with less than 30% of cuttings rooting. This result contrasts with evidence from Dirr (1998), who cited excellent success using fall cuttings, but also noted differences among the two genotypes tested. The over-dispersion parameter, which indicates how much variability the data set had compared to the expected 1.0 from a theoretical binomial distribution,

was 1.855. This value is typical of logistic regression with biological data, here accounting for all sources of variability other than month that might influence the proportions.

Although we recorded data on root number and root length, we did not include these data in the analysis due to irregularities in timing of observations and incomplete data sets. The trend, however, was that the higher the number of roots, the longer the roots (data not shown). In addition, the box huckleberry clones that had the highest rooting percentage also had the highest number of and longest roots.

The variable timing in our recording of rooting success made it impossible to conduct meaningful statistical analysis of how long it took cuttings to root each month. However, we did note that stem cuttings rooted the fastest with cuttings that were taken from May through July (data not shown), which generally rooted in six to nine weeks, especially cuttings from two accessions, 3BX and 7BX. Cuttings that were started in the fall and early winter months took longest to root, if they rooted at all. Interestingly, the fastest rooting tended to occur with cuttings that also demonstrated the highest proportion of rooting. In practical terms, this result means that generally if a plant was going to root, it did so relatively quickly. The higher success of rooting from cuttings taken in May to July likely reflects the physiological state of the plants, as they were initiating new growth. Softwood or semi-hardwood cuttings are often the most successful starting material for propagation of woody plants (Macdonald 1986).

Although the fastest and highest percentage of successful rooting occurred with stem cuttings taken in the late spring and early summer, cuttings that were taken throughout the rest of the year rooted to some extent. However, these cuttings were slower to root (some taking as long as 26 weeks to produce roots), and rooted in lower percentages. We observed differences among the five clones we used in this experiment in terms of rooting time and quality (data not shown). Generally stem cuttings from 3BX and 7BX rooted faster and with more roots than did stem cuttings from clones 9BX and 11BX. Stem cuttings from the clone from Maryland (1BX) rooted less successfully than the other clones. Although we noted these clonal differences, all data were combined in the analysis because these clones are not commercially available, so data on which clone performed best would be of minimal use to a grower. Similarly, data from separate years were combined rather than separately partitioned out because a clear pattern in differences was absent for the same month in the two years.

This study was the first to systematically analyze rooting success in box huckleberry as a function of the month that stem cuttings were taken. We found that across all clones studied, the best time to take stem cuttings of plants grow-

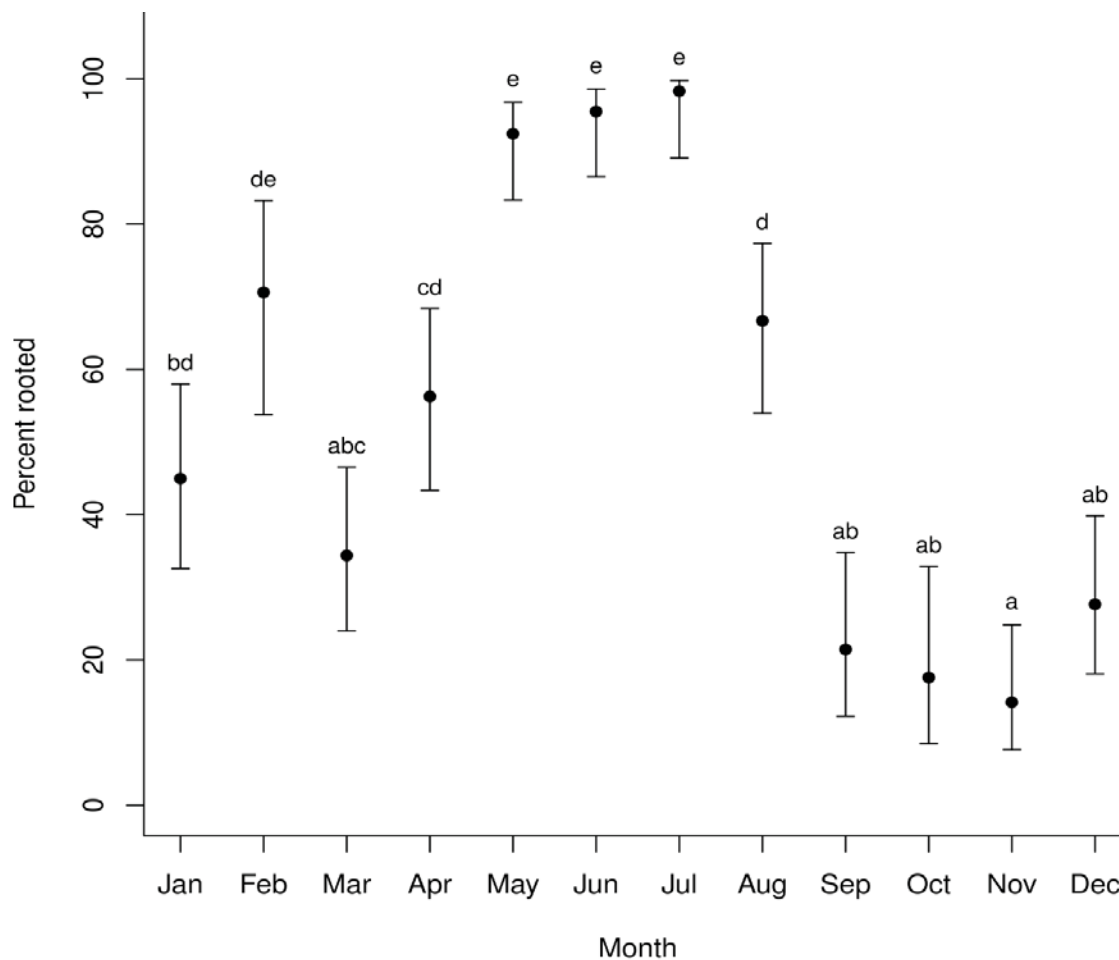


Fig. 1. Percent of box huckleberry cuttings that rooted for each month that cuttings were taken, averaged across all genotypes and over two years. The Y-axis shows back-transformed figures, with a 95% confidence interval on monthly proportions. Means with the same letter are not significantly different based on the R multcomp package (Hothorn et al. 2008).

ing in Washington, DC, was May through July. This timing corresponded to active growth of the plant, and resulted in the fastest and highest proportion of cuttings rooted.

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