# Effect of Container Size and Design on Morphological Attributes of *Cercocarpus ledifolius* Nutt. (Curlleaf Mountain Mahogany) Seedlings

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## Abstract

We conducted an experiment to evaluate the effects of four common seedling containers on the morphology and plant biomass production efficiency of *Cercocarpus ledifolius* Nutt. (curlleaf mountain mahogany) seedlings. All four types produced well-balanced shoot-root ratios. The largest container (Styro-20) produced the largest seedlings and greatest total plant biomass production per unit bedspace area, but also produced the most inconsistently sized seedlings. Among the smaller containers, cell spacing density proved more important than cell volume. The smallest container (Stubby-10) produced seedlings comparable to or greater than the Styro-10 and RL-10, with a high degree of crop consistency and efficient use of growing medium. The RL-10 produced the smallest seedlings by all measures, and plant biomass per unit volume of growing medium was lower than any of the three Copperblock™ containers. Despite its shortcomings, the versatility of the RL system may make it a worthy selection for those nurseries where seedling germination or survival has been problematic.

## Introduction

*Cercocarpus ledifolius* Nutt. (curlleaf mountain mahogany) has high potential as a restoration species for degraded, arid upland habitats throughout the Interior West of the United States. Interest in this native dryland shrub has been spurred by the species' ability to establish and survive in harsh edaphic conditions. Additionally, it is a nitrogen-fixing, pioneer species that enhances long-term nutrient availability for itself and other species (Lepper and Fleschner 1997). Because *C. ledifolius* is a palatable shrub with high protein and digestibility ratings, it often serves as an important winter food source for ungulates (Davis and Brotherson 1991).

In the northern Rocky Mountains, *C. ledifolius* is typically prescribed for sites best characterized by dry, rocky, south-facing slopes with little to no vegetation present, and consisting primarily of exposed mineral soil, the topsoil often having eroded away. Dealy's (1975) study of the morphological development of *C. ledifolius* described a growth habit of vigorous early root development and very little shoot development; that habit likely contributes to its successful outplanting at harsh sites. Studies comparing stocktype success in hot, dry environments have generally shown that seedlings with deep, well-established root systems contribute to their survival (e.g., Lloret et al. 1999, Chirino et al. 2008). At such sites, a lower shoot-root ratio is desirable to minimize transpirational surface area while seedlings are establishing (Cregg 1994).

Yet, for growers interested in supplying *C. ledifolius* seedlings for restoration projects, knowledge about propagation practices for this species is lacking. It is generally understood that container type (cell volume, cell shape, etc.) can directly affect the morphological characteristics of nursery-grown seedlings (NeSmith and Duval 1998). This study was prompted by a need to identify the most effective container for nursery production of *C. ledifolius* seedlings for restoration of a droughty site in the Bitterroot Valley of Montana. We designed an experiment to compare morphological attributes of seedlings grown in four different containers during one growing season. The objective was to isolate the impacts of container type and volume on aspects of *C. ledifolius* seedling morphology, holding other determinants of plant growth as constant as possible. Analyses tested the following hypotheses:

1. Seedling shoot weight, root weight, shoot height, and root collar diameter will positively correlate with container cell volume.
2. No differences in the above seedling attributes will be observed for containers of equal cell volume but different shape/material.
3. Shoot-root ratio will be unaffected by container cell size or type.

Additionally, we evaluated the relative nursery production efficiency per container by comparing total seedling biomass produced per unit volume of soilless medium, and per unit area of nursery bedspace.

## Methods

### Treatments

In March 2014, uniformly sterilized, stratified seeds were direct-sown into four different types of sterilized containers (figure 1). Containers consisted of Copperblock™ Styrofoam containers (Beaver Plastics, Alberta, Canada) and Ray Leach Cone-tainer™ single cells in plastic trays (Stuewe & Sons, Corvallis, OR). Containers ranged in cell volumes from 125 $cm^3$ (Stubby-10) to 336 $cm^3$ (Styro-20), and in cell densities from 213/$m^2$ (Styro-20) to 528/$m^2$ (RL-10)(table 1). We used eight full units of each container type (block or tray), with container type as the experimental unit (replications) and seedlings within container type as the sampling unit.

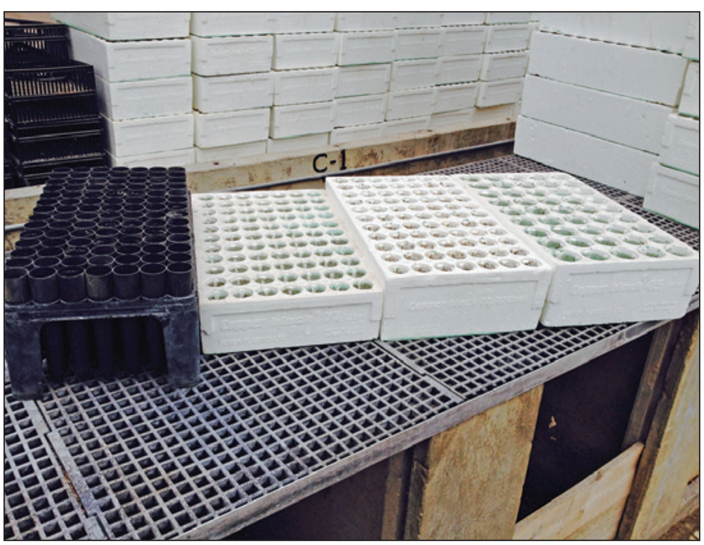


**Figure 1.** Four container types were compared in this study (L to R): Ray Leach Cone-tainer™ (RL-10), and Copperblock™ Styrofoam containers (Stubby-10, Styro-10, Styro-20). (Photo by Christopher Keyes, 2016)

**Table 1.** Attributes of the four containers evaluated in this study.

| Container | Cell Depth (cm) | Cell Volume ($cm^3$) | Cell Density (cells/$m^2$) |
|---|---|---|---|
| Ray Leach Cone-tainer™ SC10; "RL-10" | 21.0 | 164 | 528 |
| Copperblock™ 415D; "Styro-10" | 15.2 | 164 | 364 |
| Copperblock™ 412A; "Stubby-10" | 11.7 | 125 | 364 |
| Copperblock™ 615A; "Styro-20" | 15.2 | 336 | 213 |

The soilless growing medium consisted of a manually blended 1:1 mixture (by volume) of *Sphagnum* peat moss and perlite. Sowing occurred within a 5-day timeframe to ensure consistency. Following sowing, a thin layer of 5-mm granite poultry grit covered each cell. Copperblocks were sown with two seeds per cavity and were later thinned (as needed) to one seedling per cavity. Germination was excellent, and about half of the cells required thinning down to one germinant. A very small number of cells per block (less than approximately 5 percent) remained empty. Cone-tainer™ cells were sown with one seed per cavity, but with additional units sown as potential substitutes. After germination was complete, empty cells (approximately 10 to 20 percent of each tray) were removed and replaced with substitute cells to produce complete cell trays.

Seedlings were grown under conditions designed to be representative of a low-intensity native plant nursery, with cultural methods aimed at producing seedlings of uniform quality. The percent saturation block weight method (Dumroese et al. 2015) determined the watering schedule (80 percent threshold during establishment and rapid growth, 70 percent thereafter), with saturation block weights updated during the growth period. Fertilizer consisted of commercial water-soluble Miracle-Gro® 24-8-16 (Scotts Miracle-Gro Company, Marysville, OH) at 250 ppm nitrogen during the rapid growth phase, applied in conjunction with irrigation via siphon injection (Hozon™ Brass Siphon Mixer, Phytotronics, Inc., Earth City, MO). Seedlings were germinated and grown indoors at a greenhouse (University of Montana) until early June, at which point they were moved outdoors to a shade-house (Vander Meer's Wildland Conservation Nursery, LLC, Missoula, MT), where they were grown for the remainder of the experiment (October 2014). To reduce the potential for bias associated with microsite, blocks and trays were shuffled monthly during residency at each facility. The four container types were kept in groups, and those four groups were shuffled monthly; on the same occasions, the eight replications within each container-type group were shuffled as well.

### Measurements

After one full growing season (March–October), eight seedlings per container were randomly selected for destructive measurement. To ensure that the sample only included seedlings that germinated promptly and received the full growing season, discrimination rules were applied to constrain potential seedling selection to those taller than 15 mm. Seedlings were selected on an X-Y axis grid based on random number generation. If the random number generation produced a cell that was empty or had a seedling less than a minimum height of 15 mm, then a new, randomly generated cell replaced it.

Response variables measured were: shoot height, shoot weight, root collar diameter (RCD), root weight, total seedling weight, and shoot-root ratio. Seedling samples were removed from their cells and growing medium was gently washed off. Shoots were cut from roots. Shoot heights were measured as length from the root collar to the top of the terminal bud. Seedling RCDs were measured with a digital caliper. Plant materials were placed in paper envelopes and oven-dried at 60°C for 48 hours, then weighed with a digital balance to determine root and shoot dry weights. Shoot-root ratios were calculated from those dry weights.

To estimate the potential production efficiency tradeoffs among containers, the sum of total seedling biomass produced was relativized to per unit volume of growing medium used ($m^3$), as well as per unit area of nursery bedspace ($m^2$) used, and those relativized values per container were compared. For those relative contrasts, we assumed filled cells for all four containers.

### Experimental Design and Analyses

We used a randomized complete block design for this study with eight replications (blocks or trays) of the four container types. We used analysis of variance (ANOVA) F-tests to identify differences among container types for all response variables ($\alpha=0.05$), using transformed and untransformed data as appropriate. The normality assumption was evaluated via the D'Agostino-Pearson omnibus test of skewness and kurtosis (D'Agostino et al. 1990) at $\alpha=0.05$. The equal variances assumption was evaluated via the Brown-Forsythe test (a nonparametric data-means version of the Levene's test; Brown and Forsythe, 1974) at $\alpha=0.05$. Untransformed RCD, and log-transformed shoot height and shoot-root ratio, met both assumptions. For the remainder (all responses related to weight), log transformations resolved normality issues, but no transformation successfully resolved variance heteroscedasticity. We used ANOVA for all responses because the samples sizes among treatments were equal, and ANOVA F-tests are robust against variance heteroscedasticity when sample sizes are equal. Where the tests indicated a significant treatment effect, two-tailed Tukey-Kramer multiple comparison tests were subsequently applied to distinguish differences among all-possible treatment pairs ($\alpha=0.05$). All tests were performed using NCSS version 11 statistical software (NCSS 11 Statistical Software 2016).

## Results and Discussion

As expected, the largest cells (Styro-20) produced the largest seedlings by every measure (figures 2 and 3). This result was unsurprising, since the Styro-20 cell volumes were more than double those of the other container types. Seedlings grown in the Styro-20 containers had mean shoot height 146 to 193 percent taller and mean RCD 32 to 45 percent larger than the three smaller stocktypes (figure 2). Accordingly, the Styro-20 produced seedlings with the largest mean shoot and root weights, with an average of 116 to 227 percent more total seedling weight than seedlings grown in the other three containers (figure 3).

Among the smaller containers, cell spacing density seemed to determine seedling morphologies more so than cell volume (figure 2 and 3). The Stubby-10 and the Styro-10 had the same cell density and produced seedlings with similar attributes, despite the fact that the latter's cell size was 31 percent greater in volume. In contrast, the RL-10, the most densely spaced of the four stocktypes, produced the smallest seedlings, even though its cell volume was identical to the Styro-10 and larger than the Stubby-10. Despite differences in shoot and root weights, the balance between those attributes was very similar among container types (figure 3).

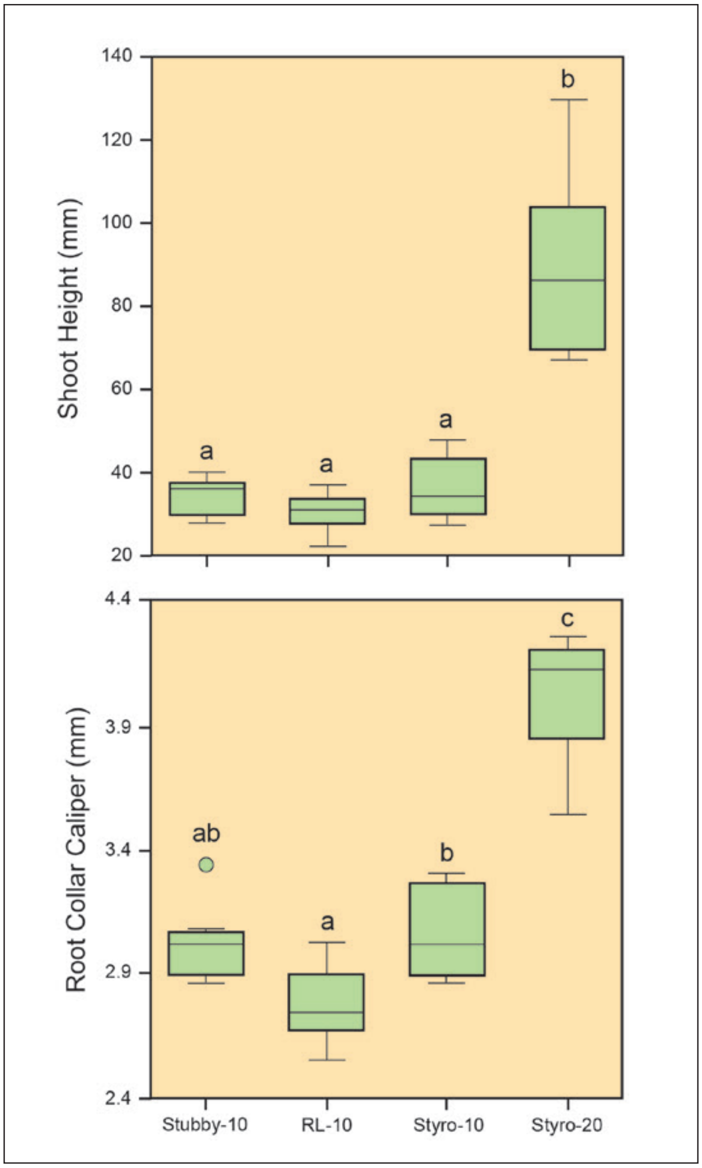


**Figure 2.** *Cercocarpus ledifolius* seedling shoot height and root collar diameter varied by stocktype. Letters denote statistically significant treatment differences. Bars within boxplots denote medians; box boundaries denote interquartile range (IQR); whiskers denote 1.5 times IQR.

The modest differences in root mass among the smallest three stocktypes do not adequately convey the substantial differences in root structure among containers, an observation that was revealed during the extraction of seedlings from cells (figure 4). All the Copperblock™ seedlings possessed fibrous, well-branched root systems with many fine roots that retained the root mass in a plug form with the growing medium attached. In contrast, the RL-10 seedlings possessed vertical roots with much less branching and lateral growth. When those plugs were extracted, the medium often fell away from the roots, leaving an exposed root mass. In practice, those seedlings could be vulnerable to desiccation and J-rooting during outplanting.

Although plant size is certainly important, size consistency and predictability of the seedling crop is also a matter of concern to propagators seeking to achieve a target seedling for consumers. The range of values for the Styro-20 seedlings was great for each measure, indicating a high degree of variability among the seedlings produced by that container (figure 2). In contrast, the Stubby-10, Styro-10, and RL-10 all produced smaller seedlings, but they were consistently similar.

Expressed on a volume-relativized basis, and assuming filled cells for all container types, our results indicate that the three Copperblock™ containers produce comparable amounts of biomass per unit volume of propagation medium (figure 5). The RL-10's performance was by far the worst of the four containers, at just 0.007 g of plant biomass per $cm^3$ of medium, a rate that was on average 37.3 percent less than the combined production rate of all three Copperblock™ containers. Because of the variability in plant morphology seen in the Styro-20 containers, its mean production (0.011 $g/cm^3$) was comparable to that of the Stubby-10 (0.011 $g/cm^3$) and the Styro-10 (0.010 $g/cm^3$), but its range of values was great; the highest recorded seedling biomass production rates per unit medium as well as some of the lowest production rates were measured in that container type.

Expressed on an area-relativized basis, the Styro-20 was a standout, producing a mean 775.3 g of plant biomass per square meter of bedspace, significantly greater (35.2 percent) than the smaller containers combined (figure 5). Among the three smaller containers, differences in mean production efficiency were nonsignificant. Apparently, the compact arrangement of the RL-10 cell trays compensated for their smaller seedlings and resulted in a mean production efficiency comparable to the Styro-10 and Stubby-10.

Seed use represents an additional efficiency metric, but we did not quantify the exact number of blank cells in the Copperblock™ containers nor the exact number of blank RL-10 cells that required replacement with substitutes. Thinning of duplicate germinants was required for about half of the Copperblock™ cells, so seed use efficiency was lower and thinning labor was greater for those containers compared with the trays of Ray Leach Cone-tainer™ cells. That cost, however, was likely offset by the additional growing medium and labor needed for filling, sowing, and growing the substitute seedlings needed to fill the RL-10 trays.

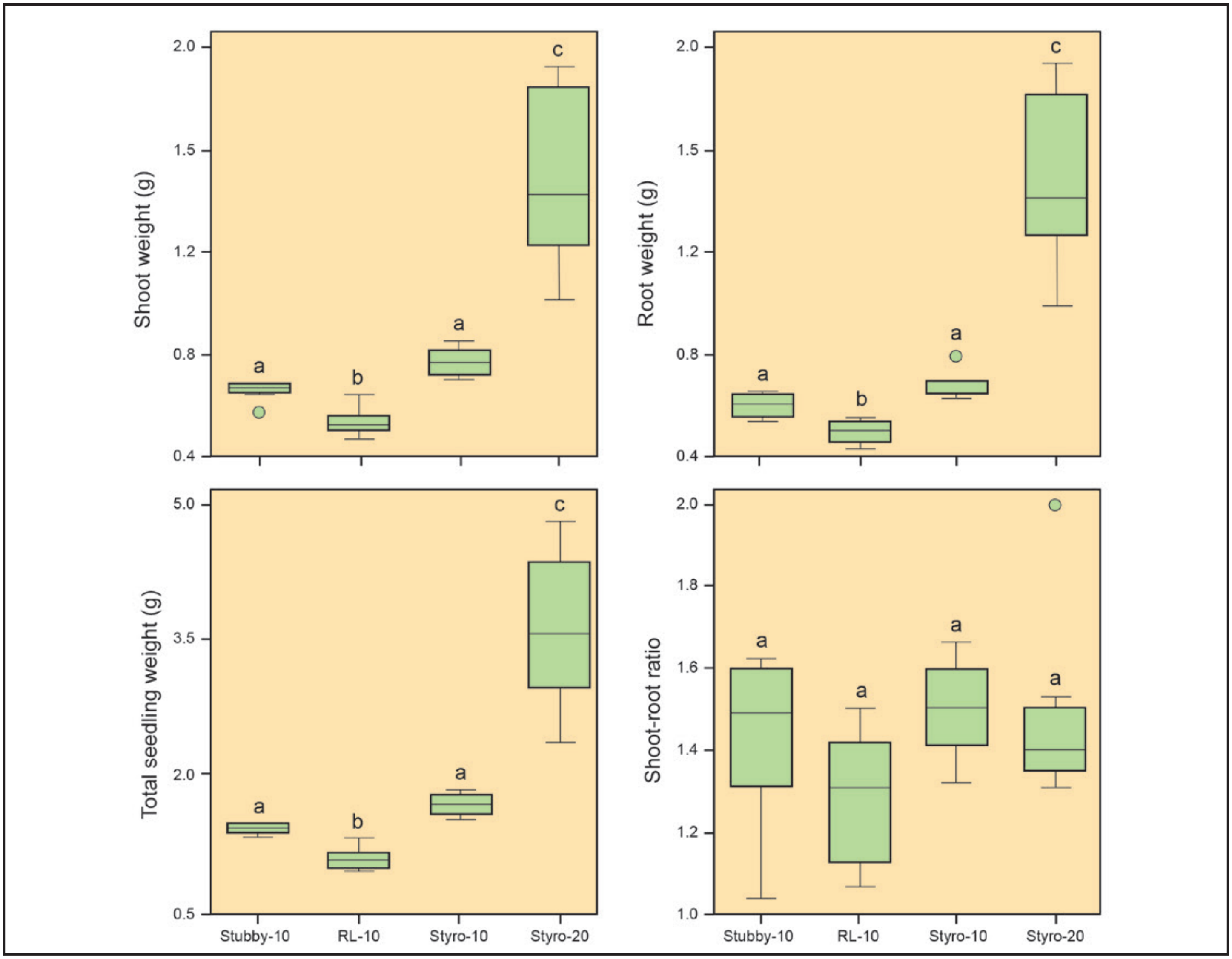


**Figure 3.** *Cercocarpus ledifolius* shoot weight, root weight, total seedling weight, and shoot-root ratio by stocktype. Letters denote statistically significant treatment differences. Bars within boxplots denote medians; box boundaries denote interquartile range (IQR); whiskers denote 1.5 times IQR.

**Figure 4.** Representative images of *Cercocarpus ledifolius* root mass structure for the RL-10 seedlings (left) and Styro-20 seedlings (right). (Photos by Christine Brissette, 2014)

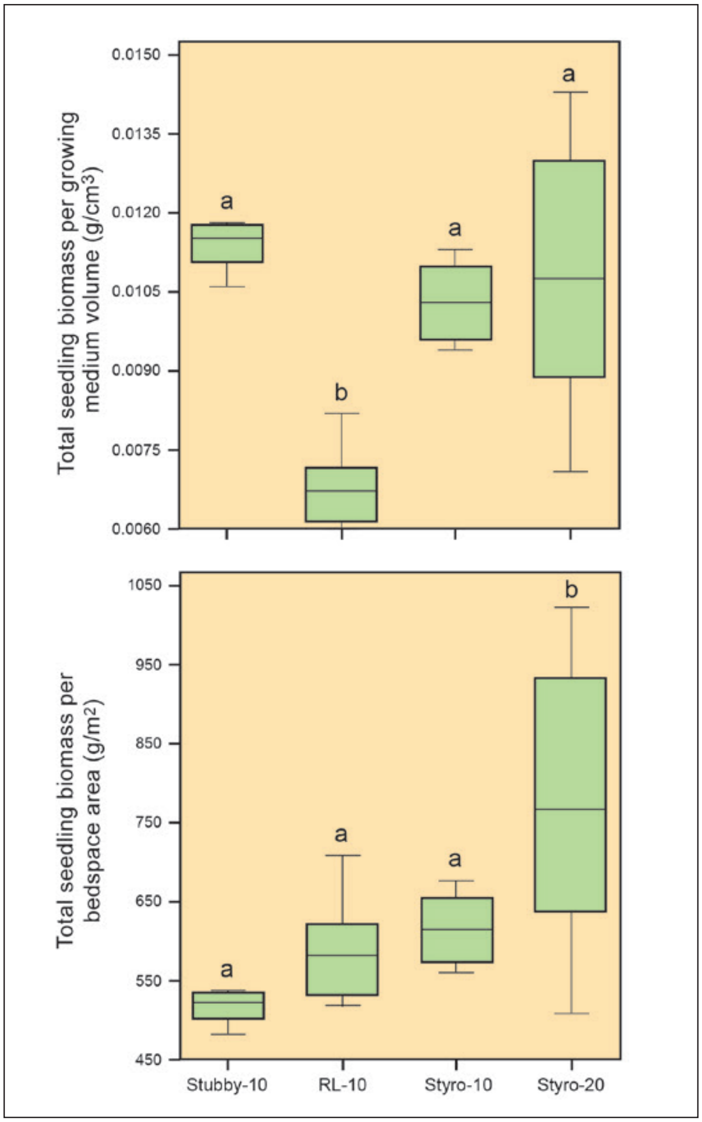


**Figure 5.** Nursery plant production efficiency of each container type was estimated by comparing *Cercocarpus ledifolius* total seedling biomass produced per unit volume of growing medium ($g/cm^3$) and per unit area of nursery bedspace ($g/m^2$). Letters denote statistically significant treatment differences. Bars within boxplots denote medians; box boundaries denote interquartile range (IQR); whiskers denote 1.5 times IQR.

## Conclusions

Revegetation of dry, south-facing slopes is a significant challenge for restoration practitioners. *Cercocarpus ledifolius* offers promise as a pioneer species for these difficult sites, providing stability, cover, and nutrients. This experiment shows that seedling container type can significantly influence *C. ledifolius* seedling morphology and also vary in the biomass production efficiency as expressed per unit volume of growing medium and per unit bedspace area. Highlights of this study's findings are as follows:

- All cell types produced well-balanced seedlings (as judged by shoot-root ratio).
- Styro-20 produced the largest seedlings, but also produced the most variable crop with inconsistent seedling sizes.
- Despite its smaller volume, the Stubby-10 produced seedlings as large as or larger than the Styro-10 or RL-10.
- The RL-10 produced the smallest seedlings with least shoot weight and root weight; RCD and shoot height were also among the lowest.
- All Copperblocks produced similar levels of plant biomass per unit volume of growing medium; the efficiency of the RL-10 in this regard was very low relative to all three Copperblocks.
- The Styro-20 had the highest plant biomass production per unit bedspace area.

Although the RL-10 failed to outperform its competing alternatives in any regard, it did produce balanced seedlings in a consistently sized crop, and the versatility of the RL system (due to moveable cells within trays) may make it a worthy selection for those nurseries where *C. ledifolius* germination or survival has been a problem.

Monitoring the performance of outplanted seedlings from various containers such as those tested here is the next logical step in determining best practices for *C. ledifolius* production for restoration outplantings. Such an analysis could show whether our observed differences in seedling stocktypes translate to differential rates of seedling survival or early growth under field conditions.


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