Original research article

Chemical and air pruning of roots influence post-transplant root traits of the critically endangered *Serianthes nelsonii*

Thomas Marler and Cameron Musser

Isla Paraiso Environmental Consulting, Alstrom Circle, Mangilao, Guam, 96923 USA.
Corresponding author: T. Marler, E-mail: thomas.marler@gmail.com, Phone: +1-671-686-1949, Fax: +1-671-734-4600

Received on Jun 29, 2015; Accepted on January 14, 2016

Abstract: Container production of *Serianthes nelsonii* Merr. plants for out-planting within the endemic range is a major component of the plan to recover the critically endangered tree species. We exploited the ability to prune roots with copper or with strategically placed air holes in container walls to determine if root quantity or quality would increase in comparison to traditional container design. Following 23 days of growth after transplanting, new root length and root dry weight did not differ among the container types. However, most roots from the control and copper-treated containers developed from the bottom of the root system, and direction of root growth was primarily geotropic. In contrast, the air-pruning containers produced plants with plagiotropic root growth near the soil surface. Root growth was positioned with 58% in the top two-thirds of a rhizotron window for the air-pruning, 29% for the copper-pruning, and 16% for the control containers. Our results indicate that direction but not length or dry weight of post-transplant root growth was changed by use of air-pruning containers. For a critically endangered tree like *Serianthes nelsonii*, the improved root morphology afforded by containers that use air-pruning of roots in the nursery may improve tree stability following transplanting.

Keywords: root egress, RootMaker, *Serianthes nelsonii*, SpinOut

Introduction

Many oceanic islands support limited and declining populations of endemic species. For most of these taxa, very little is known about their biology and ecology (Kueffer et al. 2014). Moreover, biodiversity conservation on oceanic islands is not well supported by a connection between research and applied conservation (Kueffer et al. 2014). *Serianthes nelsonii* is endemic to the island of Rota within the U.S. Commonwealth of the Northern Mariana Islands and the U.S. Territory of Guam. The natural population on Guam has been reduced to one known mature tree (Wiles et al. 1996), and the species is listed as critically endangered in the IUCN Red List (Wiles, 1998). A recovery plan published in 1994 (U.S. Fish and Wildlife Service, 1994) called for research into the factors that are limiting to conservation efforts. Little has been done to address this objective to date.

The western North Pacific is the most active tropical cyclone basin worldwide (Guard et al. 1999, Marler 2014). The Mariana Island Archipelago defines the endemic range of *S. nelsonii* and has a higher probability of being impacted by a tropical cyclone than any other part of the United States (Marler 2001). Tropical cyclones are so controlling of forest physiognomy that Guam's forests have been described as "typhoon forests" (Stone 1971). Experimental research has shown that lateral roots positioned on the windward side of the tree are important for tree anchorage (Coutts 1983, 1986, Ennos et al. 1993). During catastrophic winds, these roots are placed under tension and act as guy wire anchoring. Strong lateral root systems will be essential to the endurance of the Mariana Island trees as climate change predictions indicate severity of tropical cyclones will increase in the coming decades (Elsner et al. 2008; Knutson et al. 2010).

Rigid plastic containers used for nursery production of woody tree species may generate plants with severely deformed roots, especially if the plants remain in the containers for extended

Marler T and Musser C 2016 Chemical and air pruning of roots influence post-transplant root traits of the critically endangered *Serianthes nelsonii*. Plant Root 10:21-25. doi:10.3117/plantroot.10.21

periods of time. Quality of the root morphology and function following transplanting may be impaired for a lengthy period. To address this limitation associated with container production, the inside of horticulture nursery containers have been designed to prune root tips by chemical toxicity or air from strategically placed holes.

The chemical approach has relied on the ability of copper to inhibit root growth upon contact (Appleton 1993). A commercial product based on copper hydroxide has been the most common product used to achieve this goal. Containers with interior walls treated with copper hydroxide (SpinOut®, SePRO Corp., Carmel, Indiana, USA) have been shown to stop root elongation along the container walls. Other commercially available pots use the container wall design to train roots toward strategically placed holes that prune the root tips by contact with air (Appleton 1993). Several products pioneered the dual approach of first training roots toward holes then second pruning of those roots by contact with air (RootMaker®, Lacebark, Inc., Stillwater, Oklahoma, USA).

The efficacy of container treatments to improve root quality of container-grown plants is variable among plant species, therefore the usefulness of the approaches should be determined for each species. Moreover, use of these approaches to improve containerized root system structure and behavior has not been adequately applied to conservation programs for endangered species. We have considered these approaches for improving nursery plant quality while studying various aspects of conservation biology in attempts to improve efforts toward recovery of the critically endangered *Serianthes nelsonii*. The actions enumerated in the United States (U.S.) Fish and Wildlife Recovery Plan for *Serianthes nelsonii* (U.S. Fish and Wildlife Service 1994) call for growing and out-planting hundreds of individuals in numerous locations throughout its endemic range. To date, research to improve nursery protocols for this endeavor has been limited. Our objective was to determine *S. nelsonii* root growth potential and root morphology following transplanting from containers that employed chemical- or air-pruning approaches.

Materials and Methods

Three distinct container treatments included air-pruning pots (RootMaker®), chemical-pruning pots (SpinOut®), and untreated rigid plastic pots as the control. Container shape and size were standardized among the treatments, and container volume was 290 ml. All containers were filled with a medium consisting of 60% peat and 40% perlite. We germinated *S. nelsonii* seeds in moist filter paper, and planted seeds as they germinated on October 28-31, 2014. Seedlings did not establish adequately and were supplanted with seedlings of the same age on November 17, 2014. The plants were arranged in a Randomized Complete Block layout with five replications, grown underneath 50% shade, watered daily, and fertilized weekly. The fertilizer solution supplied all essential macronutrients and most micronutrients (Miracle-Gro, Scotts, Marysville, Ohio, USA), approximated 7.5 mM N, and each plant received 50 ml per week.

The plants were grown until March 18, 2015, when roots were pot-bound in the control containers. Stem height and basal diameter were measured, and leaf number was counted. The plants were removed from their containers and planted in #16 silica sand within rhizotrons on March 18, 2015. Each rhizotron was 2.1 L in volume and contained one plexiglass window 12 cm in width and 15 cm in height. The edge of the root system from the container was placed 4-cm from the window. The windows were covered with black plastic sheets to exclude light. The weekly fertilizer applications were continued and applied to the sand medium.

The black sheets were briefly removed one time per day from each rhizotron in order to observe roots, and the location of each root tip was marked on the surface of the window. The date on which the first root reached the window was recorded for each replication. Root extension to the nearest mm was recorded daily until the experiment was terminated.

Final measurements were made on April 10, 2015, and included stem height, basal stem diameter, and leaf number. In order to determine the direction of root growth, the number of root tips that first touched the window in the top, middle, and bottom portions of the window were counted. The sand medium was carefully washed from each rhizotron to expose roots that had grown into the sand between March 18 and April 10. These roots were removed from the original root system, placed in plastic bags, and stored at 21ºC until root length was estimated. The line-intersect method (Newman 1966, Tennant 1975) using a 2-cm grid was employed to calculate total root length. Roots were dried at 75ºC for 24 hours before being weighed to the nearest 0.1 mg. Specific root length was calculated from root length and root dry weight.

All data were analyzed using SAS ver. 9.3 (SAS Institute, Cary, North Carolina, USA). Stem and leaf data from the end of the nursery phase were subjected to ANOVA as a randomized complete block with five replications. Stem, leaf, and root data from the end of the rhizotron phase were subjected to ANOVA in a completely randomized

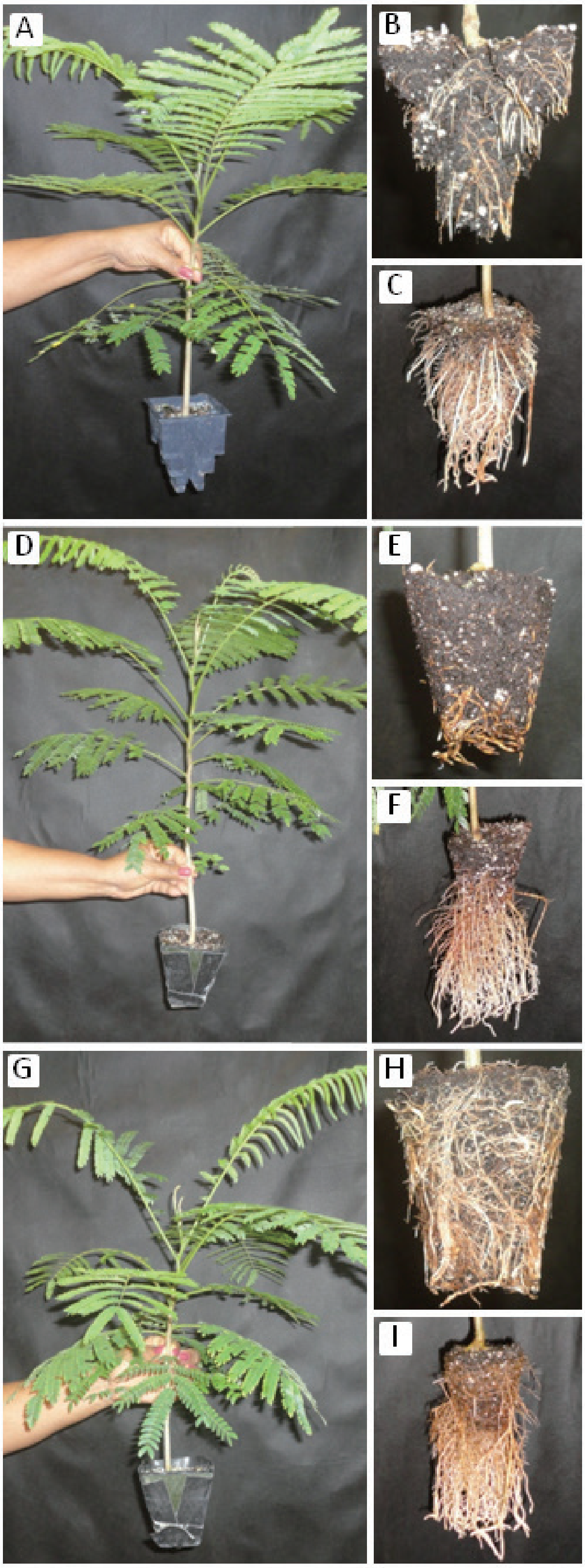

Fig. 1. *Serianthes nelsonii* plants grown in air-pruning (A), chemical-pruning (D), or control (G) following ca. 20 weeks of growth. Phenotype of surface roots following removal from air-pruning (B), chemical-pruning (E), or control (H) containers. Phenotype of root growth after 23 days following transplanting from air-pruning (C), chemical-pruning (F), or control (I) containers.

design. Means separation for variables that exhibited significant differences was conducted by Least Significant Difference.

Results

At the end of the nursery phase, container type had no influence on stem height (P = 0.1992), and the overall mean was 31.0 cm. Stem diameter was not influenced by container type (P = 0.1677), and the mean was 6.0 mm. Similarly, leaf number did not differ among the container types (P = 0.5509), and the plants exhibited a mean of 13 leaves.

Stem height was likewise not influenced by container type after the rhizotron phase of the study (P = 0.7684), and averaged 37.8 cm. The plants had a mean stem diameter of 6.8 mm, which did not differ among the treatments (P = 0.5027). The final mean number of leaves per plant was 15, and leaf number did not differ among the treatments (P = 0.4666).

The plants that were grown in the air-pruning containers (Fig. 1A), chemical-pruning containers (Fig. 1D), and control containers (Fig. 1G) were robust and healthy throughout the study. Few roots could be seen on the surface of the medium following removal from the air-pruning containers, and these roots were trained toward one of the air holes (Fig. 1B). No roots were visible on the side surfaces of the medium following removal from the chemical-pruning containers (Fig. 1E). However, the bottom surface of the container had some surface roots. In contrast, every surface of the control container medium was covered by copious root growth following plant removal (Fig. 1H).

Container type strongly influenced the number of days required for the first root to reach the rhizotron window (P = 0.0004). Plants in control containers required the greatest number of days to develop roots on the windows, and plants in the air-pruning containers required the least number of days (Table 1). Container type also strongly influenced root position on the rhizotron windows, as the proportion of roots in the top, middle, and bottom thirds of the windows differed among the treatments (Table 1). The number of roots in the upper two-thirds of the observation windows was 58% for the air-pruning containers, 29% for the chemical-pruning containers, and 16% for the control containers. Root extension rate was about 12 mm d^{-1} for the plants grown in air- and chemical-pruning containers, and about 8 mm d^{-1} for the control plants (Table 1).

After excavating the root systems from the sand medium in the rhizotrons, all plants had robust root growth (Fig. 1C, F, I). The geotropic growth of roots from the plants from the chemical-pruning and control containers was readily observable, which was in accordance with the root position data from the rhizotron windows. Container treatment did not influence length or weight of these roots. The overall mean for total root extension into the sand medium was 1,982 cm per plant (*P* = 0.6425), and mean dry weight of these roots was 1,163 mg (*P* = 0.4694). The specific root length of these out-growing roots did not differ among the container treatments (*P* = 0.3171), and the overall mean was 17.2 cm g^{-1}.

Discussion

We have responded to the need for a better understanding of the conservation management protocols for *S. nelsonii* by showing that initial root growth following transplanting from a container nursery can be improved by use of commercial containers that train roots toward strategically placed holes. The *S. nelsonii* plants grown in these containers do not exhibit greater absolute root growth when compared to traditional containers, but they do show improved root system shape with many plagiotropic roots. Although root pruning of *S. nelsonii* plants using commercially available copper hydroxide products was effective in minimizing the surface root growth along the container walls, it did not improve the shape of the root system shortly after transplanting.

These relationships can be seen in our results showing that roots grown in the control containers took twice the length of time of those grown in the air-pruning containers to reach the rhizotron windows. This delay, however, was not a result of slower root growth *per se*, but was caused by geotropic directional root growth. These vertical roots did not reach the rhizotron window, which was positioned 5-cm away from the side of the original root system.

Use of various sources of copper to treat inside walls of containers has been evaluated for decades (Burdett and Martin 1982, Ruehle 1985, Struve 1993, Wenny and Woollen 1989). This approach was highly effective in controlling *S. nelsonii* root growth along the sides of the containers, but not as effective on the bottom surface of the containers. Direct contact with the copper ions generates the cessation of root growth, and free copper ions rapidly complex with the soil or potting media (Crawford 2003). The flat bottom surface of our containers may have contained perched water for extended periods following each irrigation event, which may have created a buffer between the growing *S. nelsonii* root tips and the chemical product. Further research on the use of chemical root pruning of this species may benefit from using a container design that would not enable a perched water layer following irrigation.

The frequency of tropical cyclones in the endemic range of *S. nelsonii* calls for solutions for improving *S. nelsonii* establishment. Air-pruning containers produced superior *S. nelsonii* plants with extensive lateral root growth following transplanting, a feature which will likely increase the tree's resistance to toppling during tropical cyclone winds.

One of the goals of the *S. nelsonii* recovery plan is the eventual establishment of multiple sites of mature *S. nelsonii* trees in managed conservation plantings. A minimum of 2,000 established mature trees is needed to meet the goals of this recovery plan (U.S. Fish & Wildlife Service 1994). Recent

Table 1. Root traits of *Serianthes nelsonii* seedlings grown in air-pruning (RootMaker®), chemical-pruning (SpinOut®), and control containers from October 2014 until March 2015, then transplanted to rhizotrons and grown until April 10, 2015. "Window" refers to 15x18 cm rhizotron window positioned 5-cm away from the original root system

Root trait	Air-pruning	Chemical-pruning	Control	Significance
First root observed on window (days)	9.1±1.1 † a	14.4±1.5 b	19.6±1.2 c	*P* = 0.0004
Proportion of roots in top third of window	0.21±0.06 a	0.09±0.05 b	0 c	*P* = 0.0355
Proportion of roots in middle third of window	0.38±0.04 a	0.20±0.08 b	0.15±0.08 c	*P* = 0.0355
Proportion of roots in bottom third of window	0.41±0.08 a	0.71±0.09 b	0.85±0.10 c	*P* = 0.0224
Root growth rate (cm d^{-1})	11.8±0.9 a	12.3±0.3 a	7.9±1.1 b	*P* = 0.0054

† Mean ± SE, n = 5. Values followed by the same letter within rows are not significantly different according to LSD.

research has shown that shade is required for facilitating the seed-to-seedling transition and facilitate early seedling growth (Marler et al. 2015), *in situ* seedling emergence is substantial but mortality is 100% (Marler and Cascasan 2015), and identified threats to the seedling stage are ungulate damage, arthropod herbivory, and rhizosphere pathogens (Marler and Musser 2015, Wiles et al. 1996). Using our method of root modification during containerized nursery production of *S. nelsonii* may further contribute to species recovery.

Acknowledgements

This research was conducted under United States Endangered Species Act Recovery Permit TE-84876A-0. Support provided by the United States Department of Defense through N40192-14-M-5010, administered by the Environmental Flight, Andersen Air Force Base, Guam. We thank Leanne Obra for development, execution, and administration of the project.

References

Appleton BL 1993 Nursery production alternatives for reduction or elimination of circling tree roots. J. Arboric. 19: 383-388.

Burdett AN, Martin PAF 1982 Chemical root pruning of coniferous seedlings. HortScience 6: 622-624.

Coutts MP 1983 Root architecture and tree stability. Plant Soil 71: 171-188.

Coutts MP 1986 Components of tree stability in Sitka spruce on peaty, gley soil. Forestry 59: 173-197.

Crawford MA 2003 Copper-coated containers and their impact on the environment. In: Riley LE, Dumroese RK, Landis TD, Eds., National Proceedings RMRS-P-28: Forest and Conservation Nursery Associations-2002. Ogden, Utah, USDA Forest Service, Rocky Mountain Research Station, pp. 76-78.

Elsner JB, Kossin JP, Jagger TH 2008 The increasing intensity of the strongest tropical cyclones. Nature 455: 92-95.

Ennos AR, Crook MJ, Grimshaw C 1993 A comparative study of the anchorage systems of Himalayan balsam *Impatiens glandulifera* and mature sunflower *Helianthus annuus*. J. Exp. Bot. 44: 133-146.

Guard CP, Hamnett MP, Neumann CJ, Lander MA, Siegrist HG Jr. 1999 Typhoon vulnerability study for Guam. WERI Tech. Rpt. 85. Water and Environmental Res. Inst. Western Pacific. Univ. Guam, Mangilao.

KnutsonTR, McBride JL, Chan J, Emanuel K, Holland G, Langsea C, Held I, Kossin JP, Srivastava AK, Sugi M 2010 Tropical cyclones and climate change. Nature Geosci. 3: 157-163.

Kueffer C, Drake DR, Fernández-Palacios JM 2014 Island biology: looking towards the future. Biol. Lett. 10: 20140719.

Marler TE 2001 Tropical cyclones and perennial species in the Mariana Islands. HortScience 36: 264-268.

Marler TE 2014 Pacific island tropical cyclones are more frequent and globally relevant, yet less studied. Front. Environ. Sci. 2: 42.

Marler TE, Cascasan AN 2015 Number of emerged seedlings and seedling longevity of the non-recruiting, critically endangered Håyun lågu tree *Serianthes nelsonii* Merr. (Fabales: Leguminosae) are influenced by month of emergence. J. Threat. Taxa 7: 8221-8225.

Marler T, Musser, C 2015 Potential stressors leading to seedling mortality in the endemic Håyun lågu tree (*Serianthes nelsonii* Merr.) in the island of Guam. Tropic. Conserv. Sci. 8: 738-744.

Marler TE, Cascasan AN, Lawrence JH 2015 Threatened native trees in Guam: short-term seed storage and shade conditions influence emergence and growth of seedlings. HortScience 50: 1049-1054.

Newman EI 1966 A method of estimating the total length of root in a sample. J. Appl. Ecol. 3: 139-145.

Ruehle JL 1985 The effect of cupric carbonate on root morphology of containerized mycorrhizal seedlings. Can. J. For. Res. 15: 586-592.

Stone BC 1971 America's Asiatic flora: the plants of Guam. Am. Scientist 59: 308-319.

Struve DK 1993 Effect of copper-treated containers on transplant survival and regrowth of four tree species. J. Environ. Hortic. 11: 196-199.

Tennant D 1975 A test of a modified line intersect method of estimating root length. J. Ecol. 63: 995-1001.

U.S. Fish and Wildlife Service 1994 Recovery plan for *Serianthes nelsonii*. U.S. Fish and Wildlife Service, Portland, Oregon, 60 pp. http://www.fws.gov/pacificislands/recoveryplans.html (retrieved: December 30, 2015)

Wenny DL, Woollen RL 1989 Chemical root pruning improves the root system morphology of containerized seedlings. West. J. Appl. For. 4: 15-17.

Wiles G 1998 *Serianthes nelsonii*. The IUCN Red List of Threatened Species. Version 2015.1. http://www.iucnredlist.org (retrieved: December 30, 2015)

Wiles GJ, Schreiner IH, Nafus D, Jurgensen LK, Manglona JC 1996 The status, biology, and conservation of *Serianthes nelsonii* (Fabaceae), an endangered Micronesian tree. Biol. Conserv. 76: 229-239.