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Towards recovery of an endangered Pacific island endemic: Experimental evidence for shortening juvenility of *Serianthes* plants using a shade and trellis system to generate vigorous growth and precocious flowering

Thomas E. Marler[1]

Abstract

Background and Research Aims: Conservationists aiming to establish seed orchards or restoration plantings benefit from methods that reduce tree juvenility. Previous studies suggest that seedling and sapling mortality is the major limitation to natural population recovery of the critically endangered *Serianthes nelsonii*. To enable effective restoration actions new methods are required to reduce sapling mortality.

Methods: *Ex situ* experiments were used to determine if trellised, shaded growth conditions would reduce juvenility of *Serianthes* saplings. *Serianthes kanehirae* was used as a surrogate for *S. nelsonii*. Stem growth was trained horizontally to a wire trellis in shaded tunnels of differing lengths (0, 3, 6, 9 or 12 m). Time to reach the end of each tunnel, and subsequent vertical stem growth in full sun were recorded until flower production commenced.

Results: The shortest *S. kanehirae* juvenile phase occurred under 12 m tunnels, with first flower production within a year (351 ± 10 days, mean ± SE). The juvenile phase increased in duration as tunnel length shortened. The control plants did not produce flowers during the study.

Conclusion: Horizontal trellised support of shaded stems was effective in reducing juvenile phase duration of *Serianthes* saplings.

Implications for Conservation: *Serianthes* seed orchards may use shaded trellises to produce seeds on young plants to rapidly boost species recovery efforts. The production of seeds in managed conservation gardens may improve recovery efforts by reducing the need to remove seeds from *in situ* communities. Therefore, this simple protocol may prove beneficial for both *in situ* and *ex situ* conservation of all federally listed woody plant species in the region.

Keywords
Island endemic, Fabaceae, Guam, Håyun lågu, Mariana islands, precocious, precocity, restoration, recovery, Rota, *Serianthes kanehirae*, *Serianthes nelsonii,* Trongkon guåfi, Trongkon fi'a

Introduction

Woody perennial spermatophyte species may be characterized by prolonged juvenile periods during which initiation of reproductive growth is not possible. Seedlings of many woody species may require decades of growth before commencing flower production for angiosperms or cone

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production for gymnosperms (Hackett, 1985). The duration of this juvenile phase is governed by complex interactions of genetic and environmental factors in a multifaceted process that remains not fully understood, and is more complex in perennial plants than in annuals (Basheer-Salimia, 2007, Chalupka & Cecich, 1997, Sun et al., 2022).

Management protocols designed to shorten juvenility are of importance in plant breeding programs (Basheer-Salimia, 2007, Brewer et al. 2008, De la Rosa et al., 2006). Conservation actions that support formal recovery plans of threatened plant species are more efficient and cost-effective if practitioners understand how to shorten juvenility. One plant factor that correlates with precocity is plant size or distance of stem tissue from roots (Basheer-Salimia, 2007, Fisher, 1994, Hackett, 1985, Robinson & Wareing, 1969). For this reason, fostering rapid growth of seedlings is often employed in efforts to produce precocious plants in managed plantings (Aldwinckle, 1975, Basheer-Salimia, 2007, Chalupka & Cecich, 1997, De la Rosa et al., 2006, Longman, 1976, Santos-Antunes et al., 2005, Van Nocker & Gardiner, 2014, Zimmerman, 1972). Additionally, bending of stems or forcing horizontal growth has shortened juvenility of some woody species (Brewer et al., 2008, Robinson & Wareing, 1969).

Serianthes nelsonii Merr. is a critically endangered Fabaceae tree species that is endemic to the remote Pacific Mariana islands of Guam and Rota (Wiles & Williams, 2017). The global *in situ* population of mature trees declined from 122 in 1994 to less than 50 today, with only a single mature tree known from Guam (Marler et al., 2021). A lack of understanding of the causes of plant mortality severely limits *S. nelsonii* species recovery. This includes mortality of *in situ* seedlings before they reach the sapling stage (Marler & Cascasan, 2015, Marler & Cruz, 2019). The goals outlined in the 1994 national recovery plan for this attractive canopy tree (adults can reach 36 m) included the addition of thousands of *in situ* seed-bearing trees by 2010 (United States Fish and Wildlife Service, 1994). To date, hundreds of Guam saplings have been transplanted from conservation nurseries to *in situ* forest locations, but most transplanted plants died shortly after they were placed in competitive forest communities and none have reached maturity (Marler et al., 2021).

The decades of failures to advance toward the goal of 2000+ mature trees within four managed sites have unfolded without the involvement of published academic scientists in the field work following transplantation from conservation nurseries (Marler et al., 2021). This has led to the current absence of knowledge concerning what has caused the mortality. This tree has also emerged as a symbol of the inability of indigenous CHamoru peoples to have an adequate voice concerning the management of the natural systems that define their heritage and culture (Bevacqua & Bowman, 2018, Frain, 2020). These unique facets of conserving this charismatic tree indicate an urgent need for federal decision-makers to conduct truly integrated conservation efforts with transparency and the best available knowledge.

Empirical studies designed to inform *S. nelsonii* conservation decisions have been lacking, and one question that remains unanswered is how to shorten the juvenility phase in an effort to propagate more plants to reach the species recovery goals (Marler et al., 2021). Determining the efficacy of forcing vigorous stem extension to shorten juvenility would benefit conservation efforts. Stem extension rates of *S. nelsonii* plants were increased by supplying shade (Marler et al., 2015). However, stem strength was compromised, the shaded plants exhibited an inability to maintain an orthotropic orientation, and experimental application of mechanical stress reversed the shaded stem response (Marler, 2019). This *S. nelsonii* knowledge concerning the use of shade to increase stem extension rates and decrease stem strength was exploited in a shaded trellis system to generate excessive primary stem growth of *Serianthes* seedlings. The limitations on *S. nelsonii* research imposed by federal handling permits has led to the use of *Serianthes kanehirae* Fosberg as a surrogate for conservation research to aid in recovery of *S. nelsonii* (Marler et al., 2021). Canopy and inflorescence appearance is similar for these two species (Figure 1). Therefore, this study was conducted with *S. kanehirae*. The objectives were to exploit a shade and trellis system to generate vigorous stem extension rates and shorten the duration of the juvenile phase of *Serianthes* juveniles.

Methods

The study was conducted in Angeles City, Philippines. The site was in full sun and the soil was an entisol (Coarse loamy, isohyperthermic, Typic Untipsamment) which was unstructured, well-drained, and low in fertility (pH = 6.4, total nitrogen = 1.9 ± 0.2 $\text{mg}\cdot3\text{g}^{-1}$, available phosphorus = 88.7 ± 10.5 $\mu\text{g}\cdot\text{kg}^{-1}$, exchangeable potassium = 43.7 ± 4.6 $\mu\text{g}\cdot\text{kg}^{-1}$). The experimental plants were initially grown in tubes (5-cm diameter, 12-cm depth) under shade screen providing 25% sunlight transmission. The growing medium was river sand, and the seedlings were three to four months old when the field studies were initiated.

Quonset-shaped tunnels were constructed with a maximum height of 80 cm and basal diameter of 80 cm, and covered with shade screen to exclude 75% of incident sunlight. The shade screen extended from the top of each tunnel to a height of 35 cm on each side. Tunnel length was 3, 6, 9, or 12 m. A control was included with transplanted seedlings receiving no shade for a total of five tunnel length treatments with six replications. The total footprint of the study was 16 m × 14 m with 30 individual plants.

A one-wire trellis was installed at a height of 30 cm in the center of each tunnel with gauge 6 wire (4 mm diameter). Each planting hole was located at one end of each tunnel and excavated to 0.5 m depth and 1.0 m diameter. The excavated soil was mixed with 8 g phosphorus supplied as $Ca(H_2PO_4)_2$

then returned to the holes to approximate 100 kg phosphorus per ha.

The *S. kanehirae* seedlings were sourced from Yap island in the Federated States of Micronesia from a single tree and were half-siblings. They were 31.2 ± 1.2 cm tall with a basal diameter of 4.8 ± 0.2 mm at the initiation of the study on September 14, 2015. The seedlings were planted in the center of each prepared planting hole. Rapid stem growth was enabled by supplying a weekly drench of soluble fertilizer solution at 200 ml per plant for the first two months, 400 ml per plant for months three and four, 600 ml per plant for months five and six, then 1 L per plant for the duration of the shade tunnel growing period. The stock solution was comprised of water-soluble fertilizer (24% nitrogen, 3.5% phosphorus, 13.2% potassium, 0.02% boron, 0.07% copper, 0.15% iron, 0.05% manganese, 0.0005% molybdenum, 0.06% zinc) at 1 $g \cdot L^{-1}$ and calcium nitrate at 0.5 $g \cdot L^{-1}$. Additionally, irrigation was supplied by drip irrigation with two emitters per plant, positioned 30 cm away on opposite sides of each plant. On days without rainfall, the system was turned on for one hour and each emitter supplied 3.79 L.

The vigorous stem extension growth was inspected weekly and attached to the single wire trellis with polyvinyl chloride stretch tie tape to ensure the trellis supplied mechanical support of the stems. This procedure ensured there was no mechanical stress imposed on the *Serianthes* stems. All lateral bud growth was pruned weekly such that the only active primary growth was that of the apex. The result was an unbranched stem exhibiting minimal secondary growth for the length of each tunnel. When the stem growth reached 50-cm from the end of each tunnel, weekly fertilization and lateral stem pruning were ceased but irrigation was continued. Subsequent apical stem extension was tied to the trellis for the final 50 cm of shaded growth, then was allowed to reorient to orthotropic (vertical) growth under full sun exposure (Figure 2). The plants were inspected weekly to determine when the first flowers were produced on each replication. The study was terminated on January 18, 2018 when the final

Figure 1. Canopy and inflorescence of *Serianthes* species exhibit similar gestalt appearance. (a) Canopy of *Serianthes nelsonii* street tree in Rota showing stem dieback. (b) Inflorescence of *Serianthes nelsonii*. (c) Inflorescence of *Serianthes kanehirae.*

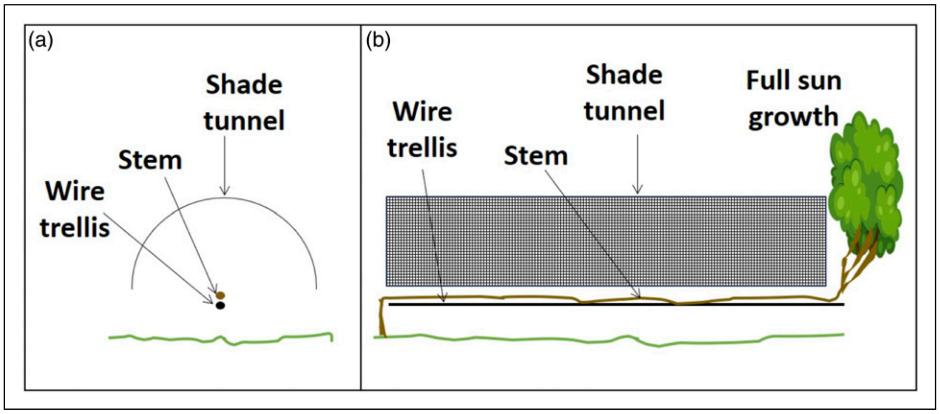

Figure 2. Depiction of shade tunnels used for *Serianthes* juvenility studies. (a) Profile view. (b) Side view.

replication from the shade tunnel treatments produced flowers. The height of all plants was measured on this date.

The response variables that were measured and calculated were time to reach the end of the tunnel (day), duration of growth in full sun until flower production (day), length of stem growth in full sun (cm), length of stem from first flower to root collar (cm), total time required to produce the first flower (day), growth rate of stems in the tunnels ($\text{cm}\cdot\text{day}^{-1}$), and growth rate of stems in full sun ($\text{cm}\cdot\text{day}^{-1}$). The control plants did not exhibit these response variables prior to the termination of the study. Therefore, the data from plants receiving 3, 6, 9, or 12 m tunnel treatments were evaluated for satisfying prerequisites for parametric tests, and time to reach the end of the tunnel, growth rate of stems in the tunnels, and growth rate of stems in full sun were subjected to one-way ANOVA. The remaining response variables did not meet parametric requirements due to unequal variances, and were therefore subjected to the Kruskal-Wallis H test. For response variables that were significant according to the F or H tests, regression models were fitted to the data to understand the relationships of plant behavior among the tunnel length treatments. All statistical analyses and regressions were performed using SAS (SAS Institute, Cary, NC, USA).

Results

No mortality occurred among the experimental plants. The growth rate of *S. kanehirae* seedlings under the tunnels did not differ among the tunnel lengths ($F_{3,20} = 0.619$; $P = 0.611$). The mean growth rate in the shaded tunnels was 4.1 ± 0.1 $\text{cm}\cdot\text{day}^{-1}$ (n = 24). The time required for the seedlings to reach the end of the tunnels differed among the tunnel lengths ($F_{3,20} = 219.2$; $P < 0.001$). A linear increase in time occurred from 3 m to 12 m tunnel lengths (Figure 3(a)).

The time required for the stem growth in full sun to reach the first flower also differed among the shaded tunnel lengths ($H = 21.6$; $P < 0.001$). A non-linear decrease in time occurred with the greatest number of days in 3 m tunnels and the least number of days in 12 m tunnels (Figure 3(b)). The length of stem growth in full sun prior to flower initiation differed among the tunnel lengths ($H = 21.6$; $P < 0.001$). The pattern was similar to that of time in full sun, with a non-linear relationship describing a decrease in time from 3 m tunnels to 12 m tunnels (Figure 3(d)). The growth rate of the stems in full sun differed among the tunnel lengths ($F_{3,20} = 32.29$; $P < 0.001$). A linear increase in growth rate occurred from the 3 m plants to the 12 m plants (Figure 3(f)).

The total number of days these *S. kanehirae* plants required from initiation of the study until the first flower was produced was dependent on tunnel length ($H = 21.6$; $P < 0.001$). The least number of days was required by the 12 m tunnel plants, and a non-linear model described the relationship among the tunnel lengths (Figure 3(c)). The total length of stem from root collar to the node that produced the first flower was least for the 3 m tunnel plants and greatest for the 12 m tunnel plants ($H = 21.6$; $P < 0.001$). The relationship among the tunnel lengths was described by a quadratic model (Figure 3(e)).

The control plants that were grown in full sun for the duration of the study were chlorotic and stunted in appearance despite ample fertilizer and irrigation. These plants were 113 ± 15 cm in height after 856 days of growth during the study, indicating only 0.1 cm of growth occurred per day after subtracting the initial plant height from the ending plant height.

Discussion

Reproductive growth of large woody perennial tree species occurs in the periphery of the canopy where tissues are ontogenetically older but chronologically younger (Hackett, 1985). This knowledge has been used to shorten the juvenile phase of woody plants by forcing vigorous growth to increase stem length (Basheer-Salimia, 2007, Hackett, 1985, Robinson & Wareing, 1969). For some species, forcing prostrate stem growth has also shortened juvenility (Brewer et al. 2008, Robinson & Wareing, 1969). In this study, *S. kanehirae* plants produced flowers after 351±10 days when stems were grown with the support of a prostrate trellis under shade and when plants received ample irrigation and luxurious fertilizer applications. Similar results have been reported for other woody tree species. For example, vigorous stem growth of seedlings was used to produce flowers in three to eight months for *Betula* L. seedlings (Lemmetyinen et al., 1998, Longman, 1976), 16 months for *Malus domestica* Borkh. seedlings (Aldwinckle, 1975), and 29 months for *Olea europaea* L. seedlings (Santos-Antunes et al., 2005).

Recovery of threatened tree species typically requires nursery production of transplants to satisfy various recovery objectives. The production of seeds in managed conservation gardens may greatly benefit recovery efforts by reducing the need to remove seeds from *in situ* communities. Therefore, my methods may prove to be beneficial for all of the federally listed woody plant species in the region (United States Fish and Wildlife Service, 2015).

The influence of exposing *Serianthes* plant growth to full sun conditions is context dependent, and should be viewed through the lens of ontogeny and how much of the plant body is protected by shade. At one end of the ontogenetic spectrum, providing no shade protection at the germination and emerging seedling growth stage is lethal to *S. nelsonii* plants (Marler et al., 2015). At the other end of the ontogenetic spectrum, the *in situ* mature trees in Guam and Rota produce flowers in the emergent portion of the canopy receiving full sun. The *S. nelsonii* leaf is a highly plastic organ that is able to modify morphological and anatomical traits to exploit the incident light conditions (Deloso & Marler, 2020). Interpreting how primary stem growth responds to shade versus sun conditions in natural settings is complicated by the correlation of plant age and shaded plant body size. However,

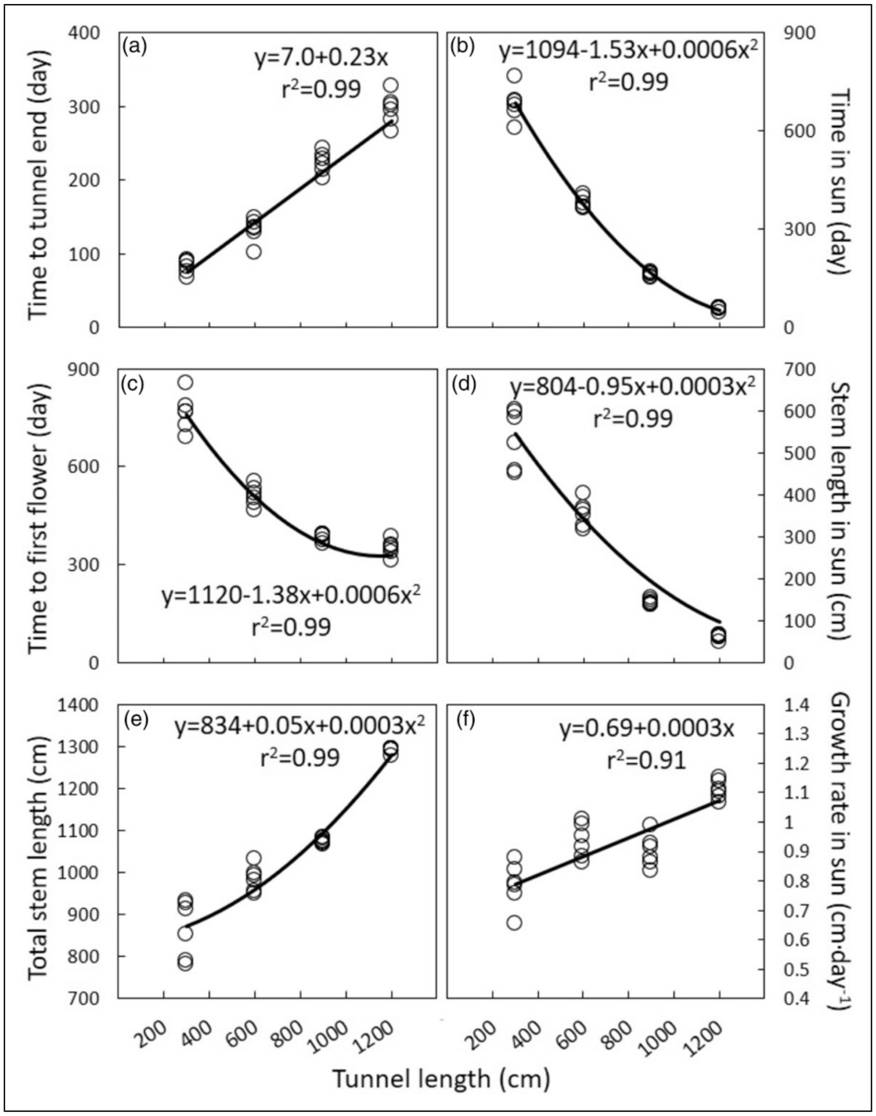

Figure 3. The influence of shaded tunnel length on growth traits of *Serianthes kanehirae* plants. (a) Time required to reach the end of each tunnel. (b) Time of stem growth in full sun conditions required to reach first flower production. (c) Total time from initiation of study until first flower production. (d) Length of stems in full sun between end of tunnel and initial flower. (e) Length of stem from root collar to node with first flower. (f) Stem growth rate in full sun.

in this study every *S. kanehirae* replication was the same age, and yet the relative size of the plant body that was protected by shade exerted a direct influence on primary growth rate of the sun-exposed stem tissues. Stem extension rate did not differ among the tunnel lengths during primary stem growth in the shade. However, stem extension rate in full sun conditions after the stems had reached the end of each tunnel was greater for the plants in the longer tunnels than for the plants in the shorter tunnels. This outcome was likely due to source-sink relations whereby the greater volume of stem non-structural resource deployment and greater number of source leaves in the shade were able to contribute more resources to the sink activity of full sun primary stem growth. The importance of the shaded plant body to support primary growth in the sun-exposed stems appears to be profound. For example, the control plants which received full sun beginning as 30 cm tall transplants grew only 0.1 cm·day^{-1} throughout the course of this study, but the plants which benefitted from 12 m of shaded plant stems and leaves grew sun-exposed stems at 1.1 cm·day^{-1}.

Several avenues of further research are urgently needed to provide knowledge for *S. nelsonii* recovery efforts. The greatest contemporary recovery need is to stop the ongoing plant mortality through appropriate adaptive management research by competent specialists (Marler et al., 2021). Approximately 60% of the global mature tree population at the time of the 1994 national recovery plan has died to date, yet no investigations have been funded to understand this ongoing *in situ* mortality. Soliciting research from a tree biologist with expertise in tree ontogeny will be required to stop this attrition. *In situ* regeneration in Guam has been considerable but recruitment to the juvenile stage has been nil (Marler & Cruz, 2019). One preliminary study revealed pathogen pressure and limited light availability may be partly causal of seedling mortality (Marler & Musser, 2015). Funding a knowledgeable ecologist to more comprehensively study all distance and density components of community competition will be required to mitigate the ongoing recruitment failures. Decades of successful production of container-grown nursery plants in Guam have been characterized by post-transplant sapling death (Marler et al., 2021). Post-transplant monitoring research by a community ecologist with skills to tease apart the net-positive and net-negative biotic and abiotic interactions within the restoration sites will be required to stop this form of mortality.

A greater understanding of *S. nelsonii* plant nutrition is also urgently needed. Leaf stoichiometry indicated that phosphorus deficiency is a limitation of *in situ* trees (Marler, 2021). Additions of phosphorus-only fertilizers may mitigate phosphorus deficiencies without substantial changes to stoichiometry among other nutrients (Marler, 2022a). Furthermore, foliar application of aerosol forms of essential nutrients may prove useful for meeting nutritional needs of *S. nelsonii* plants in highly competitive forest settings (Marler, 2022b). Funding a competent silviculturist or horticulturist to continue studying plant nutrition requirements will enable greater evidence-based management decisions for post-transplant saplings.

The physiological and genetic controls over *Serianthes* precocity remain to be determined. Flowering is clearly regulated by complex gene networks, and recent research with other angiosperm species has shown that microRNA molecules are involved in this critical phase transition out of juvenility (Ahsan et al., 2019, Li et al., 2021, Wang et al., 2011, Wu et. al., 2009). These gene networks respond to environmental regulatory signals such as photoperiod and temperature, and endogenous signals such as sugars (Yang et al., 2011, Yu et al., 2013).

Although the use of management decisions to force a greater distance between apical stem tissue and roots is a method shown to advance precocity, the causal mechanisms are not understood. One proposal is that a dilution of root-derived gibberellic acids occurs during translocation to the distal stem tissues (Wareing & Frydman, 1976). Studies designed to use timely applications of antagonists of gibberellin biosynthesis (e.g. paclobutrazol) may improve protocols for forcing earlier precocity of *Serianthes* trees. Girdling of terminal stem sections has shortened juvenility of woody tree species (Basheer-Salimia, 2007, Brewer et al., 2008, Longman, 1976). This simple method of reducing export of phloem-transported resources may also be effective for improving precocious *Serianthes* flower production.

Conclusions

Woody tree conservationists who aim to establish seed orchards or self-sustaining restoration plantings would benefit from methods that reduce the juvenile period. Hortizontal *Serianthes* stem growth was trained to a wire trellis beneath shaded tunnels as a protocol to foster vigorous primary stem growth that shortened the juvenile phase of *S. kanehirae* plants to 351 ± 10 days.

Implications for Conservation

The national recovery plan for *S. nelsonii* was published in 1994 at a time when the known global population was comprised of 122 mature trees, and set a goal of adding thousands of mature trees to the population by 2010 (United States Fish and Wildlife Service, 1994). Implementation of this recovery plan has not been successful, as the global population of mature trees has declined to less than 50 (Marler et al., 2021). One of the proposed reasons for the decades of failure has been the reliance on anecdotal reports and un-vetted communications for informing management plans, which has partly resulted from the choice to exclude academic researchers from the role of knowledge production (Marler et al., 2021). When the production of new knowledge by research scientists is absent from the conservation agenda, the management approach can be based on potentially harmful information (Nogueira et al., 2021). This study adds to the list of recent *Serianthes* publications by reporting on the use of management decisions to force vigorous horizontal growth of juvenile plants to foster an early transition from juvenility to maturity. The plan of adding thousands of mature trees to the *in situ* population has been hindered by seed supply limitations. The installation and management of seed orchards for production of high quality forestry seeds has been utilized in many countries (Liesebach et al., 2021). The protocol described herein could be used by conservation contractors to construct a *S. nelsonii* seed production system to support the seed supply needs.

A qualified community ecologist expert will be required to conduct urgently needed adaptive management studies, and a copious supply of seeds for producing the experimental units for these studies is necessary. The protocols described herein may be used to address these requirements.

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