



Communication

Serianthes nelsonii Seed Germination and Seedling Behavior are Minimally Influenced by Chemical and Light Treatment

Thomas E. Marler

College of Natural and Applied Sciences, University of Guam, Mangilao, Gu 96923, USA;
thomas.marler@gmail.com; Tel.: +1-671-735-2100

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Abstract: The role of seed imbibition and light during germination are not known for the critically endangered *Serianthes nelsonii* Merr. Scarified seeds were pre-soaked in gibberellic acid (GA₃) up to 300 mg/L and nitrate solutions of 3000 mg/L to determine if germination was influenced by these treatments. Scarified and imbibed seeds were incubated in high red:far red and low red:far red light to determine the influence of light quality on germination traits. The GA₃ and nitrate treatments did not influence germination percentage or timing, but did increase the height of newly emerged seedlings. Moreover, GA₃ extended the longevity of cotyledons and shortened the window of time that seedlings required to resume height growth. These growth responses were not sustained, and all seedlings reached heights of 30 cm at a similar number of weeks. The light treatments did not influence any of the germination response traits. The results indicate that imbibing seeds with chemical solutions and providing light in a range of light quality treatments exerted a minimal influence on *S. nelsonii* seed germination behaviors. Imbibing seeds with water and germinating in darkness is sufficient for achieving the germination of this endangered tree species.

Keywords: endangered species; Guam; imbibition; priming

1. Introduction

The seed-to-seedling transition is an important phenological transition and every plant species exhibits a unique suite of requirements for germination and seedling establishment [1–3]. The determination of these requirements is a prerequisite for successfully growing tree species that are important in large-scale horticulture and silviculture. Similarly, the germination requirements for tree species that are threatened with extinction must be known for successful conservation nursery management with limited access to seeds.

Serianthes nelsonii is a critically endangered legume tree species restricted to the islands of Guam and Rota [4]. Thousands of container-grown transplants will be required to fulfill the goals of the published species recovery plan [5,6]. Yet germination treatments that maximize the propagation success of this species have been only minimally studied. A single mature tree remains as the sole seed source for Guam's population of *S. nelsonii*. Even a modest increase in the germination success of this severely limited seed source may benefit species recovery efforts.

The first objective of this study was to determine the influences of nitrogen and gibberellic acid (GA₃) pre-soak treatments on *S. nelsonii* germination traits. The second objective was to determine the influence of light quality on *S. nelsonii* germination traits. These treatments have been widely used as seed treatments to improve the germination of many plant species. The continued investigation of *S. nelsonii* seed germination traits may provide important information for improving species recovery.

2. Materials and Methods

Serianthes nelsonii seeds were collected in Guam from May 2013 through January 2014 (Recovery Permit TE-84876A-0) and stored in darkness in ambient conditions. The influence of light and pre-soaking seeds with chemical solutions on seed germination was studied in October 2014 through February 2015. For all treatments, the methods to mitigate physical dormancy followed protocols as previously described [7]. Scarification of the seed coat was accomplished prior to imbibition by opening a small hole at a position that was 90° from the hilum by scratching on 50-grit sandpaper until cotyledon tissue could be seen beneath the testa. Seeds were subsequently soaked in a 10% bleach solution for 3 min, then soaked in tap water or chemical solutions for a total of 1 h. The water or solution was decanted and replaced every 15 min to ensure adequate oxygenation. A fully imbibed seed exhibits greater seed size and the testa becomes less dark and wrinkled (Figure 1a).

Each experimental unit consisted of five imbibed seeds which were sandwiched within wet paper towels and placed in 18 × 19 cm plastic ziploc bags. Germination was checked every 12 h and the paper towels were re-moistened if needed. A seed was recorded as germinated when the radicle reached 1 mm in length, and was removed from the incubation bags immediately upon germination. Germination for each replication continued until every seed germinated or when un-germinated seeds were unmistakably dead. Seeds of this species that do not germinate have an unambiguous appearance and odor.

Germinated seeds were planted individually in 0.625 L containers using a 60% peat and 40% perlite medium. The plants were placed under 45% sunlight transmission and grown as previously described [8]. The initial growth phase of *S. nelsonii* seedlings includes the full extension of the hypocotyl, retention of the cotyledons, full extension of one internode above the cotyledons, and the expansion of an opposite pair of bi-pinnately compound leaves [5]. Following this, a quiescent stage occurs for several days during which no stem extension is observed (Figure 1b). When resumption of seedling height growth commences, the stem growth is continuous, and the pinnately compound leaves are borne with an alternate arrangement and become larger and more complex with height. At the end of the germination growth phase, the length of the hypocotyl and epicotyl were measured for each seedling. Each seedling was subsequently observed daily to record when both cotyledons had abscised, then when growth resumption of the stem occurred following the quiescent period. The seedlings were grown until they reached a height of 30 cm (Figure 1c). The number of weeks required to reach this height was recorded for each seedling.

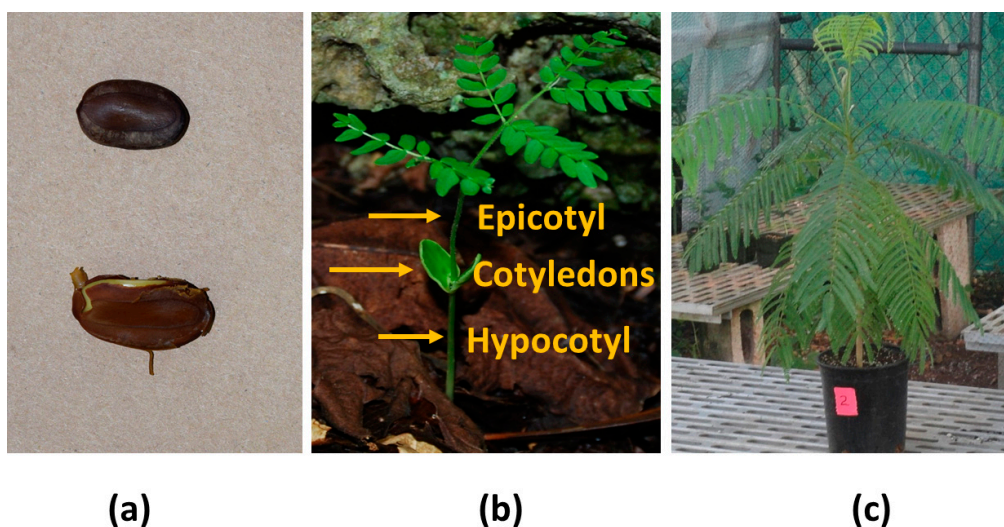


Figure 1. The general appearance of a *Serianthes nelsonii* seedling. (a) Non-scarified seed (top) and scarified + imbibed seed (bottom). (b) At the end of the germination growth phase when a quiescent period occurs. (c) General appearance at about 30 cm height.

2.1. Light Methods

The influence of light quality on seed germination was studied using two protocols. First, incandescent bulbs were employed to provide a low red:far red (R:FR) light of 0.7 at a photon flux density (PFD) of $60 \mu\text{mol}/\text{m}^2/\text{s}$. A Skye SKP200 quantum sensor (Skye Instruments, Llandrindod Wells, Powys, UK) was used to quantify PFD, and a Skye SKR110 R:FR sensor was used to determine incident R:FR for each of the treatments. Fluorescent bulbs were used to provide a high R:FR light of 4.8 at the same PFD. Second, incident sunlight was intercepted by a fresh leaf of *Ochrosia oppositifolia* (Lam.) K. Schum. to provide a low R:FR of transmitted light. The leaf was placed on top of the incubation bag and replaced twice per day to ensure the quality of transmitted light was not altered by leaf desiccation. The R:FR was 0.21 ± 0.3 and the PFD was $64 \mu\text{mol}/\text{m}^2/\text{s}$. Incident sunlight was intercepted by neutral density shade fabric to provide a high R:FR of transmitted light. The R:FR was 1.14 ± 0.01 and the PFD was $61 \mu\text{mol}/\text{m}^2/\text{s}$. For both trials, a dark treatment was also included. For all batches incubated in darkness, the daily check for germination occurred under indirect light from a low wattage incandescent bulb. The amount of light was below the detection limits of the quantum sensor. A high–low recording thermometer was used to quantify temperature. The mean low temperature was 28.7°C and the mean high temperature was 30.7°C . Seeds were scarified and imbibed with water for 1 h prior to placing in the wet paper towels to begin the incubations.

2.2. Chemical Pre-Soak Methods

Two approaches were employed to study 1 h of imbibition of *S. nelsonii* seeds with chemical solutions. First, two forms of nitrate were used at 3000 mg/L to determine if a nitrogen solution influenced germination traits, based on the upper end of the range that is recommended for germination tests [9,10]. The treatments were NH_4NO_3 (37.5 mM), KNO_3 (29.7 mM) and a water control. The mean low temperature was 29.1°C and the mean high temperature was 31.1°C . The incubations occurred in darkness. Second, five dose levels of GA_3 were used to determine if this hormone influenced germination traits. The doses were 0, 50, 100, 200, or 300 mg/L (0, 0.14, 0.29, 0.58, or 0.67 mM, respectively). The mean low temperature was 28.5°C and the mean high temperature was 30.9°C . The incubations occurred in darkness.

2.3. Statistics

The germination and seedling response variables within each trial were subjected to a one-way ANOVA (SAS 9.3; SAS Institute, Cary, NC, USA). For response variables that were significantly influenced by nitrate or light treatments, mean separation was significantly different at $p = 0.05$. For response variables that were significantly influenced by GA_3 dose level, linear and quadratic functions were fitted to the data using the PROC GLM statement in SAS 9.3.

3. Results and Discussion

3.1. Light Treatments

Light quality as supplied by light bulbs with contrasting R:FR traits did not influence any of the response variables in this study (Table 1). The mean germination for this trial was 68.9%, the number of days for both cotyledons to abscise was 8.6 d, the number of days for growth resumption to occur was 8.4 d, and these seedlings reached a height of 30 cm in 17.3 w.

Light quality as supplied by blocking sunlight transmission with a live leaf or with shade cloth did not influence any of the response variables in this study (Table 2). The mean germination for this trial was 72.2%, the number of days for both cotyledons to abscise was 8.5 d, the number of days for growth resumption to occur was 8.1 d, and these seedlings reached a height of 30 cm in 17.3 w.

Table 1. The influence of exposing germinating *Serianthes nelsonii* seeds to darkness or light of 60 $\mu\text{mol}/\text{m}^2/\text{s}$ as low red:far red light (0.7; incandescent bulb) or high red:far red light (4.8; fluorescent bulb) on germination and seedling traits. Mean \pm standard error. Six replications, five seeds each.

Response Variable	Darkness	Incandescent	Fluorescent	Mean	F	p
Germination (%)	70.0 \pm 4.5	66.7 \pm 4.2	70.0 \pm 4.5	68.9	0.1923	0.8271
Initial germination (h)	42.0 \pm 2.7	44.0 \pm 4.0	38.0 \pm 2.0	41.3	1.0294	0.3811
Final germination (h)	58.0 \pm 2.0	58.0 \pm 2.0	54.0 \pm 2.7	56.7	1.0526	0.3734
Germination duration (h)	16.0 \pm 4.0	16.0 \pm 4.0	14.0 \pm 4.8	15.3	0.0725	0.9304
Hypocotyl length (mm)	22.8 \pm 1.7	22.2 \pm 2.1	19.8 \pm 1.6	21.6	1.0038	0.3898
Epicotyl length (mm)	15.7 \pm 1.2	16.3 \pm 1.7	16.2 \pm 1.3	16.1	0.0590	0.9429
Cotyledon death (d)	8.3 \pm 0.7	8.7 \pm 0.6	8.8 \pm 0.6	8.6	0.1977	0.8227
Growth resumption (d)	8.7 \pm 0.6	8.3 \pm 0.8	8.2 \pm 0.6	8.4	0.1643	0.8499
Time to 30-cm height (w)	17.3 \pm 0.6	16.8 \pm 0.9	17.8 \pm 0.9	17.3	0.2848	0.7561

Table 2. The influence of exposing germinating *Serianthes nelsonii* seeds to darkness, low red:far red light of 64 $\mu\text{mol}/\text{m}^2/\text{s}$ (0.21; shade by *Ochrosia oppositifolia* leaf), or high red:far red light of 61 $\mu\text{mol}/\text{m}^2/\text{s}$ (1.14; shade by cloth) on germination and seedling traits. Mean \pm standard error. Six replications, five seeds each.

Response Variable	Darkness	Leaf Shade	Cloth Shade	Mean	F	p
Germination (%)	73.3 \pm 4.2	70.0 \pm 4.5	73.3 \pm 4.2	72.2	1.5513	0.2441
Initial germination (h)	38.0 \pm 2.0	38.0 \pm 2.0	38.0 \pm 2.0	38.0	0	1.0000
Final germination (h)	52.0 \pm 4.0	52.0 \pm 4.0	56.0 \pm 2.5	53.3	0.4617	0.6666
Germination duration (h)	14.0 \pm 4.8	14.0 \pm 4.8	18.0 \pm 2.7	15.3	0.2985	0.7462
Hypocotyl length (mm)	22.7 \pm 1.8	22.2 \pm 2.0	20.8 \pm 1.9	21.8	0.2503	0.7818
Epicotyl length (mm)	16.3 \pm 1.8	16.0 \pm 1.1	16.2 \pm 1.9	16.2	0.0145	0.9856
Cotyledon death (d)	8.7 \pm 0.8	8.3 \pm 0.6	8.5 \pm 0.5	8.5	0.0664	0.9361
Growth resumption (d)	8.2 \pm 0.7	7.7 \pm 0.7	8.3 \pm 0.8	8.1	0.2490	0.7827
Time to 30-cm height (w)	17.5 \pm 1.5	17.0 \pm 1.3	17.3 \pm 0.9	17.3	0.0426	0.9584

3.2. Nitrogen Treatments

Pre-soaking *S. nelsonii* seeds with nitrate solutions did not influence germination percentage, the time required for the first seed to germinate, the time required for all of the seeds to germinate, or the duration of germination (Table 3). The mean germination for this trial was 76.7%. Hypocotyl and epicotyl lengths were increased by both nitrate sources above that of the water control. The number of days for both cotyledons to abscise was 8.4 d, and was not influenced by the nitrate treatments. The number of days for growth resumption to occur was 10.4 d, and was not influenced by the nitrate treatments. These seedlings reached a height of 30 cm in 16.4 w, and the pre-soak with nitrate solutions did not influence this response variable.

Table 3. The influence of imbibing *Serianthes nelsonii* seeds with water or 3000 mg/L ammonium nitrate or potassium nitrate on germination in darkness and seedling traits. Mean \pm standard error. Six replications, five seeds each.

Response Variable	Water	NH ₄ NO ₃	KNO ₃	F	p
Germination (%)	73.3 \pm 6.7	76.7 \pm 6.1	80.0 \pm 5.2	0.3061	0.7408
Initial germination (h)	46.0 \pm 2.0	44.0 \pm 2.5	46.0 \pm 2.0	0.6919	0.5159
Final germination (h)	62.0 \pm 2.0	62.0 \pm 2.0	62.0 \pm 2.0	0	1.0000
Germination duration (h)	16.0 \pm 4.0	18.0 \pm 4.1	16.0 \pm 2.5	0.1020	0.9036
Hypocotyl length (mm)	21.5 \pm 0.9a ¹	26.5 \pm 1.3b	27.2 \pm 1.2b	6.8609	0.0077
Epicotyl length (mm)	16.2 \pm 0.5a	20.0 \pm 0.3b	19.8 \pm 0.3b	12.5495	0.0006
Cotyledon death (d)	8.3 \pm 0.8	7.8 \pm 0.7	9.1 \pm 0.8	0.5490	0.5887
Growth resumption (d)	9.8 \pm 0.7	10.3 \pm 0.6	11.0 \pm 0.6	0.7312	0.4977
Time to 30-cm height (w)	16.3 \pm 0.4	16.5 \pm 0.6	16.3 \pm 0.4	0.0376	0.9632

¹ Means followed by the same letter within each row are not different according to least significant difference.

3.3. Gibberellic Acid Treatments

Imbibing *S. nelsonii* seeds with GA₃ solutions did not influence the germination percentage, the time required for the first seed to germinate, the time required for all of the seeds to germinate, or the duration of germination (Table 4). Mean germination for this trial was 76.7%. A linear increase in hypocotyl length occurred in response to the GA₃ dose level, but epicotyl length was not influenced. The number of days for both cotyledons to abscise increased in a linear pattern with increased GA₃ concentration, with the plants receiving GA₃ at 300 mg/L exhibiting retention of cotyledons 30% longer than the control plants. The number of days for growth resumption to occur was reduced by GA₃, with the plants receiving GA₃ at 300 mg/L exhibiting a 33% reduction in the number of days for resumption as compared to the control plants. These seedlings reached a height of 30 cm in 16.7 w, and the GA₃ treatments did not influence this response variable.

Table 4. The influence of imbibing *Serianthes nelsonii* seeds with gibberellic acid (mg/L GA₃) on germination in darkness and seedling traits. Mean ± standard error. Six replications, five seeds each.

Response Variable	0	50	100	200	300	Significance ¹
Germination (%)	76.7 ± 6.1	73.3 ± 4.2	73.3 ± 6.7	76.7 ± 3.3	83.3 ± 6.1	NS
Initial germination (h)	46.0 ± 2.0	44.0 ± 2.5	46.0 ± 2.0	46.0 ± 2.0	46.0 ± 2.0	NS
Final germination (h)	62.0 ± 2.0	62.0 ± 2.0	62.0 ± 2.0	62.0 ± 2.0	62.0 ± 2.0	NS
Germination duration (h)	16.0 ± 4.0	16.0 ± 3.0	16.0 ± 2.5	16.0 ± 2.5	16.0 ± 2.5	NS
Hypocotyl length (mm)	23.3 ± 1.8	26.2 ± 1.9	28.7 ± 1.2	29.2 ± 1.4	33.8 ± 2.7	L ***
Epicotyl length (mm)	16.2 ± 1.8	16.3 ± 1.4	16.5 ± 1.8	16.3 ± 1.8	16.2 ± 1.9	NS
Cotyledon death (d)	8.2 ± 0.6	8.8 ± 0.5	9.8 ± 0.5	10.2 ± 0.5	10.7 ± 0.4	L ***
Growth resumption (d)	10.7 ± 0.3	8.5 ± 0.4	7.8 ± 0.6	7.3 ± 0.5	7.2 ± 0.4	L *** Q **
Time to 30-cm height (w)	16.7 ± 1.0	17.0 ± 1.0	16.7 ± 0.8	16.0 ± 1.1	17.3 ± 0.8	NS

¹ NS = not significant. L = linear. Q = quadratic. ** $p < 0.01$. *** $p < 0.001$.

3.4. Interpretations

The Recovery Plan for *Serianthes nelsonii* [6] called for more research, and my results contribute to that component of species recovery. Seed imbibition with various ionic, hormonal, or osmotic solutions is a successful technique for the increasing seed germination percentage, increasing the speed of germination, and improving seedling establishment [11,12]. Heydecker [13] was influential in first describing the benefits of the technique. Herein, imbibition of *S. nelsonii* seeds with various solutions exerted minimal influence on the germination traits that were measured. The significant changes in initial seedling height and the speed of seedling growth were ephemeral. The use of hormone solutions for priming seeds may mitigate some of the detrimental effects of abiotic stressors on seed germination [14]. My germination trials were conducted in the absence of abiotic stress. Therefore, the influence of GA₃ on *S. nelsonii* germination traits may be more profound under stressful conditions.

Various forms of GA may induce contrasting plant responses in bioassays and efficacy may differ among the plant species being studied. The influence of GA₃ on germination and seedling behavior has been shown to promote hypocotyl and early stem growth of other species [15–17] which corroborates my findings with *S. nelsonii*. Commercially available GA₄₊₇ is available for seed treatments, and further study with this and other forms of GA may greatly improve the germination protocols for *S. nelsonii*.

Light quantity and quality are environmental factors that influence the seed germination of some species [18–21]. The red:far red ratio is an effective cue that plants use to detect the potential for future shading [22]. Red enriched light is characteristic of forest conditions where gaps or large sunflecks reach the forest floor. Far-red enriched light is characteristic of forest conditions with a closed, dense canopy. In this study, exposing the *S. nelsonii* seeds to various light quality conditions during incubation exerted minimal influence on the germination traits that were measured.

These results lend evidence to the assertion that the seed-to-seedling stage is not a limiting factor for the regeneration of *S. nelsonii*. When the testa was broken and imbibition was initiated, germination was rapid regardless of chemical pre-soak or light treatments during incubation. The longevity of the in

situ seedlings was severely constrained [23], and greater efforts are needed to more fully understand the constraints on the seedling-to-sapling stage. For example, in species such as *S. nelsonii* that germinate in deep shade within a late climax forest community, shade tolerance is required for adequate initial seedling survival. Understanding the lower limits of shade that *S. nelsonii* seedlings can tolerate is an agenda that should be pursued.

3.5. Conclusions

Serianthes nelsonii seeds were minimally responsive to pre-soaking in GA₃ or nitrate solutions, indicating that imbibition treatments are likely not important for improving the germination traits of this species. *Serianthes nelsonii* seeds were not responsive to R:FR light treatments during germination incubation, indicating that the germination of seeds for this tree species is light-independent. The general lack of change in seed germination behavior among an extensive range of seed treatments indicates that the germination stage is not likely a limitation to the regeneration of *S. nelsonii* under natural conditions and the provision of testa scarification and pre-sowing imbibition with water is fully adequate for germination success.

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