**Short Communication**

# Potential stressors leading to seedling mortality in the endemic Håyun lågu tree (*Serianthes nelsonii* Merr.) in the island of Guam

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**Abstract**
Potential agents of seedling mortality for Guam's only known *Serianthes nelsonii* (Håyun lågu) tree were determined from the *in situ* crop of emerging seedlings. We used insecticides to mitigate exotic arthropod pressure, fertilizer to mitigate nutrient deficiency, fungicide to mitigate root pathogen pressure, and supplemental lighting to mitigate low light stress. The fungicide treatment was the only treatment that elicited a substantial increase in seedling longevity. Past reports based on general observations point to insect and ungulate herbivory as likely agents of seedling mortality. Our results identify root pathogens as an additional cause of mortality at the study site. Results may be useful for designing more complex experiments to improve species recovery efforts.

**Keywords**: Janzen-Connell model, mortality agents, pathogens, seed-to-seedling transition, *Serianthes nelsonii*

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## Introduction

The Janzen-Connell model [1,2] has served as a benchmark for decades of attempts to explain tropical forest diversity, and it is still frequently invoked to underpin current research. For many species that conform to Janzen-Connell spatial effects, seeds deposited under the maternal parent lie in a death zone where they are doomed to fail. This model predicts that natural pressures of seed predators, seedling herbivores, and host-specific pathogens define the escape curve. But many studies verifying Janzen-Connell spatial effects do not attempt to directly address the underlying mechanisms [3,4].

Typically, protocols invoked for this line of research require numerous replications to test the effect of emerging seedlings throughout the natural seed shadow [4]. For this approach to be feasible, the post-dispersal seed input must include a sufficient volume of seeds away from the parent tree. However, for species that have no contemporary seed vector, no natural seedlings occur away from the maternal parent tree. Other protocols use seed collection and experimental planting of plots at the maternal parent tree versus away from the maternal parent tree [5,6]. For critically endangered tree species, however, the permit restrictions may not allow planting seeds in sites away from the parent tree. To comply with these constraints, we studied various components of the Janzen-Connell hypothesis by observing *in situ* germination, seedling emergence, and seedling mortality on a sustained basis. Studying these naturally emerging seedlings can reveal causes of mortality for seedlings close to the maternal parent tree.

*Serianthes nelsonii* Merr. (Håyun lågu) is a rare tree limited to the southern Mariana Islands (Fig. 1). From bi-monthly site visits to Guam's only known remaining mature tree, we learned that numerous seedlings emerge beneath the tree throughout the year. Also, we determined that seedling longevity is compromised, and most seedlings are short-lived. For example, the mean longevity of 488 seedlings that we monitored from 2012-2014 was 5.9 weeks.

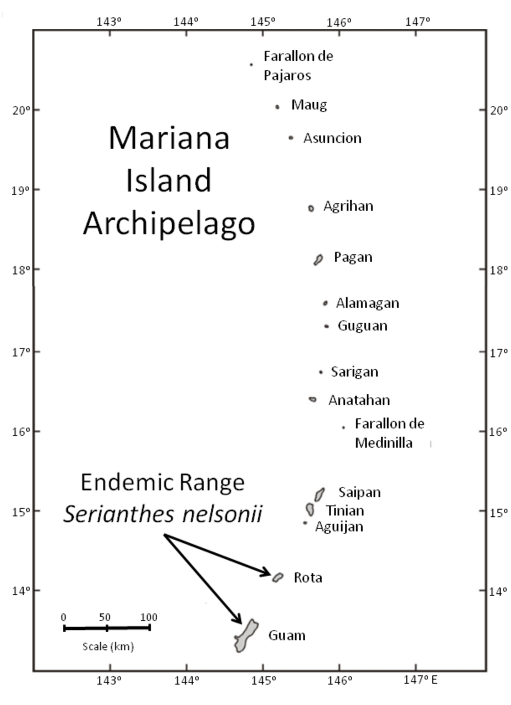


**Fig. 1. The United States Commonwealth of the Northern Mariana Islands and the United States Territory of Guam make up the Mariana Island Archipelago. The endemic range of *Serianthes nelsonii* includes Guam and Rota.**

For our study, we determined potential agents of seedling mortality at the site and then attempted to mitigate the effects of some agents as new seedlings emerged each week. While this method does not offer the statistical breadth that other ecological studies may offer, it is a non-destructive means by which potential causal mechanisms of recruitment failures can be identified for a critically endangered species where seed collections are strictly regulated and limited.

## Methods

This research was conducted under the United States Endangered Species Act Recovery Permit TE-84876A-0 at the site of the only known remaining mature *S. nelsonii* tree on Guam. The site is protected from wild ungulate pressure with a functional exclusion fence. All exotic plant species were removed from the site in 2012. We used pairs of seedlings as they emerged as a replication, and assigned a mitigation treatment to one seedling and utilized the other as the control. For each of the treatments, we used 20 seedlings to provide 10 replications. This procedure was initiated in October 2014, and the final date of seedling mortality was in May 2015.

Protection of seedlings from arthropod herbivory was accomplished with granular application of 61 mg Imidacloprid (Bayer Corp., Kansas City, Missouri, USA) per seedling as a soil topdress on the initial date of emergence, and a weekly foliar spray of 1.26 $mg \cdot L^{-1}$ carbaryl solution (TechPac LLC, Atlanta, Georgia, USA). Our experiences indicated the Imidacloprid product is highly successful for protection against Hemiptera pests, and carbaryl is successful for protection from Lepidoptera pests. All ten replications were initiated in October 2014.

Mitigating potential nutrient deficiencies was done with a weekly drench of 500 ml fertilizer solution. We alternated between two commercial soluble fertilizer products each week, and used the commercially recommended solution concentration. The two products were 2.1 $g \cdot L^{-1}$ Miracle-Gro (8N-3.5P-13.3K; Scotts, Marysville, Ohio, USA) and 2.6 $ml \cdot L^{-1}$ Vigoro (10N-4.3P-8.3K, Spectrum Group, St. Louis, Missouri, USA). The first replication was initiated in October 2014, and the final replication was initiated in November 2014.

Soil-borne pathogens were suppressed with the fungicide Ridomil Gold® (Syngenta, Basel, Switzerland) delivered in a 0.04 $ml \cdot L^{-1}$ solution. A drench of 500 ml per plant was applied as a one-time treatment on week 1. This product has been used for other Janzen-Connell studies [6,7]. All ten replications were initiated in November 2014.

Supplemental light was supplied with a solar 12-volt system that powered LED lamps that were turned on at 10:00 hr and turned off at 15:30 hr every day. Photosynthetically active radiation (PAR) was measured directly with a quantum sensor (Model SKP200; Skye Instruments, Ltd., Powys, United Kingdom). During periods without sunflecks, control seedlings received a mean of 62 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and seedlings supplied with supplemental light received 205 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The first replication was initiated in January 2015, and the final replication was initiated in February 2015.

Control seedlings received a volume of water to match the volume of fungicide and fertilizer solutions applied to the treatment seedlings. Results from each of the four experiments were subjected to paired *t*-test.

## Results

Insecticide applications did not influence seedling longevity (Fig. 2). Seedlings in this experiment lived for 4.8 weeks. Fertilizer applications did not influence seedling longevity, either (Fig. 2). Seedlings from this experiment lived for 5.2 weeks. Longevity of seedlings receiving fungicide applications was significantly increased to 2.4 times that of the untreated control seedlings (Fig. 2). The treated seedlings lived for 15.8 weeks. Supplemental light significantly increased seedling longevity. Longevity of seedlings receiving supplemental light was 1.7 times that of untreated control seedlings (Fig. 2).

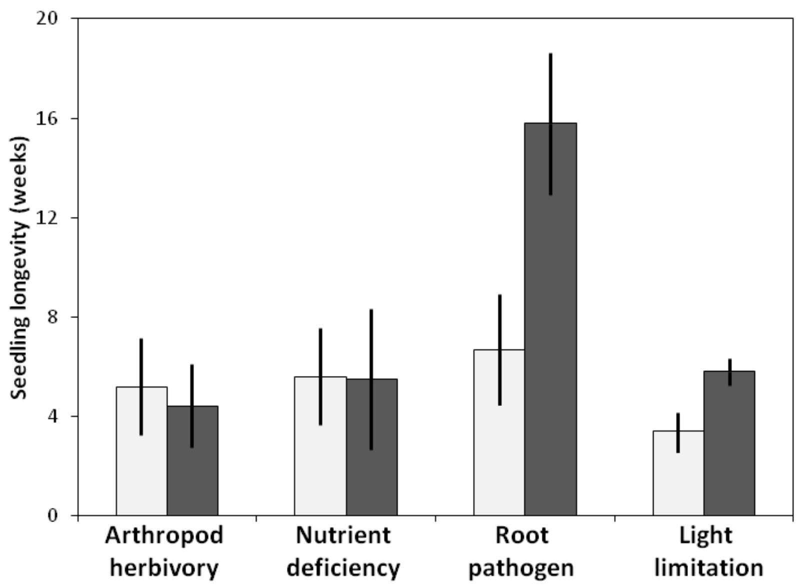


**Fig. 2.** ***In situ Serianthes nelsonii*** **seedling longevity as influenced by mitigating arthropod herbivory with insecticides (*P* = 0.3668), mitigating nutrient deficiencies with fertilizers (*P* = 0.4844), mitigating root pathogens with fungicide (*P* = 0.0021), or mitigating limited light conditions with supplemental light (*P*≤ 0.0001). Mean ± SE, n = 10.**

## Discussion

Experimental testing of the Janzen-Connell model's spatial effects has not been accomplished adequately for tropical forests [5], and even less so for endangered species on oceanic islands [8]. Here, we employed *in situ* experimental techniques commonly used in Janzen-Connell research protocols to determine the influence of potential agents of mortality on the limited seedling longevity beneath Guam's only known naturally occurring *S. nelsonii* tree.

*Serianthes nelsonii* is listed as endangered in the United States Endangered Species Act [9] and as critically endangered in the IUCN Red List [10], with the global population totaling no more than 120 adult trees [11]. Research into the factors that are limiting the natural recruitment is a major component of the recovery plan for *S. nelsonii* [12]. Yet, no studies have responded to this call for action until recently.

The control seedlings lived an average of 5.3 weeks; this was similar to our previous work showing the mean longevity of 488 seedlings was 5.9 weeks. Independently correcting for insect pressure, nutrient deficiency, or limited light conditions did not substantially increase seedling lifespan. Although supplemental lighting significantly increased longevity, the mean of the treated seedlings was only 5.8 weeks; this treatment effect was of little ecological relevance. In contrast, the use of fungicide to control root pathogens generated a substantial increase in seedling longevity.

This is the first time that experimental manipulation of *in situ* seedlings for *S. nelsonii* has occurred. These results add soil-borne pathogens to the list of potential causes for seedling mortality, with ungulate and arthropod herbivory reported in earlier work [11,12]. Our four experiments were conducted in the absence of ungulate pressure, and our use of insecticides did not affect *in situ* seedling longevity under our experimental conditions.

**Fig. 3. General appearance of healthy growth of *Serianthes nelsonii* foliage, and one-week old seedling at the time the cotyledons usually abscise. (Photo credits: Thomas Marler)**

## Implications for conservation

These results present new insights into the potential reasons that *in situ S. nelsonii* seedlings do not reach the juvenile stage. The role of specialist root pathogens in close proximity to the maternal parent has not been reported as a possible cause of seedling mortality in past reports. Identifying these pathogens may be an important step in future mitigation efforts, because development of protocols may differ for specialist pathogens versus generalist pathogens. Although longevity of 15.8 weeks for the fungicide-treated seedlings was much greater than for the control seedlings, this one-time dose of fungicide did not provide the seedlings the ability to reach juvenile stage. Perhaps more frequent fungicide applications would extend longevity to a greater degree. Moreover, each of the four experimental treatments was applied to seedlings that did not receive the other three treatments. A synergistic effect may occur if a combination of treatments is applied to seedlings, as multiple treatments may extend longevity to a greater degree than any one treatment. Ongoing recovery efforts with nursery-grown plants will provide an opportunity to further evaluate the role of root pathogens in *S. nelsonii* plant health by applying fungicides to a portion of the stock that is outplanted, and comparing plant health to the unprotected portion.

One important caveat to our results is the relevance of space. Ungulate damage to *S. nelsonii* was reported at this site prior to the construction of an exclusion fence [11], and small *S. nelsonii* plants outplanted at other sites without ungulate control would likely suffer from ungulate damage. Moreover, severe arthropod pest infestations have been reported on *S. nelsonii* seedlings in *ex situ* sites [11], so insect herbivory could be a potential cause of seedling mortality at other locations selected for outplanting of trees for recovery efforts. Another caveat to our results is the relevance of time. Repeating these studies during various seasons would reveal whether the results are consistent throughout the year or follow seasonal patterns of abiotic and biotic factors.

The distance effect of the Janzen-Connell model may be used in recovery efforts. If the putative root pathogens are specialist pathogens, then outplanting some of the *S. nelsonii* plants at locations that are distant from established natural or cultivated *S. nelsonii* trees may provide an opportunity for those plants to escape the pressures of any host-specific natural enemy. These choices may include selecting distances away from any other closely related tree species that may provide refuge for *S. nelsonii* pests.

This context-dependent approach to manipulating *in situ* seedlings can be applied to all critically endangered plant species, though it may be time- and labor-intensive if *in situ* specimens are in remote locations. This is a non-destructive means of obeying seed collection restrictions while identifying causes of seedling mortality, which can inform management protocols for species recovery efforts. For example, improved restoration trials may result from documenting small-scale environmental heterogeneity of soils at restoration sites. More specifically, the approach moves beyond anecdote to asking empirical questions about the *in situ* seedling crop.

We have shown that despite permit restrictions, it is possible for resource managers to conduct non-destructive experiments on untested recruitment hypotheses. Since density and distance effects of seedling mortality may not be driven by the same mechanism for all tree species, our method can provide the site- and species-specific contextual information needed to improve recovery efforts. However, we do not recommend this method without initially determining the background level of mortality and general length of seedling longevity for each species.

## Acknowledgements
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