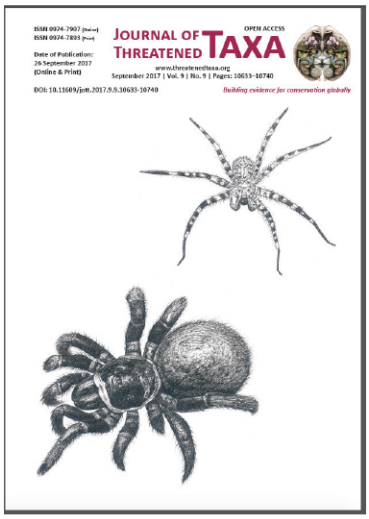

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Response & Reply

Response to article on Adventitious rooting of mature *Cycas micronesica*

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Response to article on Adventitious rooting of mature *Cycas micronesica*

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A recent article by Marler & Cruz (2017) in JoTT states that the stem cutting can be used as planting material to save the endangered cycads. I list a few questions and comments that may be addressed by the authors to clarify some points.

1. Generally, in vegetative propagation methodology, the new vegetative cutting are maintained in mist chambers to enhance the rooting percentage. Was there any methodology adopted in this study?

2. From the article it can be presumed that cuttings were maintained in open air nurseries. A mention of the climatic condition of the study site will help in understanding the success of vegetative propagation.

3. What was the criteria in choosing the concentration of indole-3-butyric acid (IBA), is there any previous study in this regard? Instead of comparing the efficiency of cycad specialist and a general forestry nursery specialist, the authors would have attempted to compare the different concentration of rooting hormone on root formation.

4. Further, a photo showing the root formation in stem cutting would have made the article more vibrant.

5. Regarding the success rate between the two nursery conditions, one having 34 and the other having 15 stem cuttings, what was the actual number of plants that rooted out?

6. Has this sort of adventitious rooting been reported for other cycads?

These are important issues that an article propounding vegetative propagation of an endangered species requires clarification.

Reference

Marler, T.E. & G.N. Cruz (2017). Adventitious rooting of mature *Cycas micronesica* K.D. Hill (Cycadales: Cycadaceae) tree stems reveals moderate success for salvage of an endangered cycad. *Journal of Threatened Taxa* 9(4): 10565–10570; http://doi.org/10.11609/jott.3523.9.8.10565-10570

Best protocols for cycad propagation require more research

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Knowledge of cycad biology and conservation is limited by the paucity of empirical studies. When adaptive management research protocols are envisioned in conservation programs, the best available science is first amassed, but then is distilled down to prioritize study methods. The life experiences of the research team are of paramount importance in this process. Due to the historical abundance of *Cycas micronesica* throughout Guam, both authors interacted with this tree throughout childhood. Additionally, after becoming

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professionally involved in the plant sciences, we accrued a combined 32 years of studying the behavior of this and other *Cycas* species in natural and cultivated settings. A brief explanation for why we employed some methods instead of others in our recent publication is that we believed them to be the most important in fulfilling the objective. More specific reasons are detailed below.

Mist Chambers. Small adventitious stems called "pups" in the cycad nursery industry are routinely removed and nurtured to produce adventitious roots. The protocols employed in our unprecedented large-scale attempt to propagate from large apical cycad stem cuttings were primarily based on those methods, which can be accessed by any reader from multiple online resources. Based on all available knowledge, the use of intermittent mist systems would kill every experimental unit, and adding mist as a treatment in asexual propagation of cycads would not be considered by any competent research team.

Climate conditions: Cycad research is best conducted in settings that match in situ conditions. With the exception of the subpopulation on the peak of Guam's highest elevation, all all of the island's cycad subpopulations occur in similar conditions. Our nursery was located ca. 600m west of an in situ subpopulation of *C. micronesica*. General climatic conditions of Guam can be accessed by any reader using online resources. For the duration of the propagation period, mean high temperature was 29°C, mean low temperature was 25°C, mean daily precipitation was 5.3mm, and mean daylength was 12:45hr.

Auxin and multiple nursery choices: The purpose of this adaptive management study was to determine if the protocols for asexual propagation using large stem cuttings could be scaled up to rescue the the gene pool from thousands of *C. micronesica* trees that reside within forests that will be cleared during construction activities in the near future. Using two nursery locations in the study was mandated in the project's statement of work from the funding agency, therefore we had no choice but to include this as part of the study. Comparing dose levels of laboratory grade auxin was not considered because future Guam contractors would have difficulty obtaining the product. A retail auxin product that can be purchased locally was used to adhere to protocols that are commonly used for rooting cycad pups in the international horticulture industry. The number of experimental units needed to provide adequate replication of a dose-response treatment was not available.

Root formation on cuttings: Large cycad stem cuttings do not produce zygotic roots. A zygotic root system may

Image 1. (Left) Seedling root system developing on a *Cycas micronesica* seedlings showing a dominant taproot and subordinate lateral roots. (Right) Adventitious root system on *Cycas revoluta* stem cutting showing dominant lateral roots and no taproot.

Image 2. Adventitious root formation followed by resumption of new stem growth (blue arrow) occurred 14 months after preparing this *Cycas micronesica* pup for asexual propagation.

Image 3. Snapped *Cycas micronesica* stem sections during Guam's recurring tropical cyclones naturally develop adventitious roots then resume stem growth (yellow arrow). The decapitated standing stem also resumes growth by developing adventitious lateral buds (blue arrows). The photograph was taken 15 months after the tropical cyclone that broke the stem.

be retained by excavating a portion of a tree's existing root system, and subsequent root growth would egress from existing root tissue that can be traced back to the original radicle that emerged from the embryo. The phenotype of a developing zygotic root system is shown in Image 1A. When large rootless stems are tended in a nursery to induce new roots, these are adventitious roots because they develop through *de novo* organogenesis from stem tissue and cannot be ontogenetically traced back to any historical root tissue. We did not bare-root any of the large rooted transplants to observe the adventitious roots, as this would have imposed unnecessary stress on the valuable transplants. However, the phenotype of an adventitious root system that developed on a *Cycas revoluta* stem cutting is shown in Image 1B. Any reader can calculate that 41% of 34 experimental units was 14 rooted transplants.

Other cycad species: No cycad horticulturist would purposefully cut off large apical stem cuttings from 49 healthy trees just to try to clone each tree. Therefore, no citations exist for other cycad species describing attempts to develop adventitious roots on large stem cuttings. This was an unprecedented opportunity to determine the success of rooting large stem cuttings from cycad trees that were located within a forested site that was destined to be destroyed for construction activities.

Additional remarks: Cycad plants are highly skilled at what they do, but they are never in a rush. When experienced horticulturists begin to grow cycad plants using methods they have developed from successfully managing common angiosperms, they often kill several specimens before learning that cycad plants often just need to be left alone. Overwatering or use of container medium that lacks adequate aeration are often the causes of these initial failures. Indeed, patience and resisting the urge to do something may be the two most important horticultural skills for managing sexual and asexual cycad propagation. Most cycad horticulturists share our experiences of patiently waiting more than one year for a cycad pup to develop adventitious roots then resume stem growth (Image 2).

No cycad horticulturist will be surprised that we were successful even though the use of large stem cuttings has never been employed for large-scale propagation. We used methods that adhered to the most important facets of asexual propagation, which included covering the exposed wound tissue with a sealant of some type, ensuring extremely high aeration within the rooting medium, respecting the need for hygiene when handling the cutting and container medium, and under-watering throughout the propagation phase. The fact that more than half of the cuttings were unsuccessful was due to the extremely poor health status of the source trees. Storage organs on healthy cycad plants contain copious levels of non-structural carbohydrates, and we believe that chronic damage by the non-native *Aulacaspis yasumatsui* armored scale infestations cause mortality by way of depleting these storage resources over time. Healthy trees with no history of herbivory damage would likely yield large stem cuttings that exhibit 100% success in developing adventitious roots. We do not envision an opportunity to verify this directly because sacrificing large healthy arborescent cycad trees just to determine if adventitious roots could be induced on large stem cuttings would be difficult to justify. But we propose that this can be studied indirectly by quantifying the response of *Cycas* stem and root non-structural carbohydrate pools to terminal infestations of *Aulacaspis yasumatsui*.

The recurrent tropical cyclones that characterize life on Guam may dislodge large structural stem sections from in situ *C. micronesica* trees. In the absence of subsequent non-native insect and mammal herbivore damage, these stems develop adventitious roots where they are in contact with the soil surface, then resume stem growth (Image 3). This natural form of cloning is evidence that cycad stems do not need anthropogenic treatments like synthetic auxins or mist chambers in order to successfully produce adventitious roots. Expanded cycad propagation research is not necessarily needed to increase cloning success rates, but is needed to speed up the process such that more established transplants can be produced in a shorter time-frame.

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