horticulturae

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Expanding the Cycad Horticulture Toolbox: Air Layer Protocols for *Cycas* Stems

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Abstract

Knowledge of propagation methods is crucial for conserving endangered plant species. Cycads are highly threatened, and propagation protocols using seeds and stem cuttings are well-understood. No air layer technique has been developed for cycad propagation, so the objective of this study was to develop a working protocol for adding this technique for cycad conservation. We opened wounds on mature *Cycas edentata* de Laub. stems to expose cortex and vascular tissue then installed an air layer medium to determine if adventitious roots would form. In one experiment, the peripheral vascular cylinder was exposed from 90° to 360°; in a second experiment, the use of auxin-promoting root stimulants was compared with a control group with no stimulants; and in a third experiment, the interior vascular cylinders were exposed in addition to the peripheral vascular cylinder. Every replication in every experiment developed adventitious roots beginning about 8 weeks and were ready to harvest with 10 cm roots by 14 weeks. The robust roots were about 1 cm in diameter, geotropic, and restricted to the outermost vascular cylinder exposed on the upper surface of the wounds. The number of roots and total root length per propagule increased by more than 300% as the percentage of exposed vascular tissue increased from 90° to 360°. Air layer techniques can be added to the cycad conservation toolbox, and its use may aid in conserving this threatened group of plants.

Keywords: asexual propagation; *Cycas edentata*; marcot; vegetative propagation

Academic Editors: Luigi De Bellis and Othmane Merah

Received: 1 May 2025
Revised: 7 July 2025
Accepted: 8 July 2025
Published: 9 July 2025

Citation: Marler, T.E.; Cruz, G.N. Expanding the Cycad Horticulture Toolbox: Air Layer Protocols for *Cycas* Stems. *Horticulturae* **2025**, *11*, 814. https://doi.org/10.3390/horticulturae11070814

1. Introduction

Cycads have emerged as the most threatened plant group globally [1–3]. The greatest threats to most cycad species are habitat loss and the over-collection of wild plants [4,5]. However, invasive species are increasingly becoming a critical threat, with the armored scale *Aulacaspis yasumatsui* Takagi comprising the greatest threat to many *Cycas* species [6]. Therefore, evidence-based conservation protocols are of paramount importance to mitigating the threats to species survival [4]. Efficacious propagation methods are critical for managing all endangered plant species, and nursery propagation may relieve some of the pressure from the wild harvesting of cycad plants [7]. Continued research on the development of new propagation techniques may lead to greater conservation successes.

Several books on cycad biology and conservation contain comprehensive chapters covering what is known about cycad reproduction [8–14]. Journal articles have also included overview descriptions of the current state of our knowledge concerning cycad propagation [15–17]. These publications cover seed handling and germination methods for sexual propagation and illuminate the exploitation of stem cuttings for asexual propagation. There is no mention of using air layer techniques for cycad propagation in any published materials.

The layering of plant stems is a vegetative propagation technique designed to develop adventitious roots on a stem section that remains attached to the original source plant [18]. When a horticultural rooting medium is suspended at an elevated stratum of the targeted stem section for the purpose of initiating the roots, the procedure is considered an air layer or marcot. For angiosperm species with bifacial secondary vascular cambium, the procedure includes girdling and the removal of the peripheral phloem tissue without damaging the internal woody xylem tissues. As a result, the apical leaves of the stem section that will eventually be removed as the propagule are supported by a direct connection to the existing root system of the source plant during the waiting period in which adventitious roots are formed within the air layer medium. This traditional protocol is not possible for the stems of species that do not produce bifacial secondary cambium, which generates phloem peripherally and xylem internally.

Cycads produce pachycaulous, manoxylic stems with persistent pith and cortex [11,19,20]. These stems lack bifacial secondary cambium. Cycad stem vascular tissues are embedded in parenchyma within cylinders that are positioned outside of the pith and inside the wide cortex tissues [13]. Each vascular cylinder contains mixed xylem and phloem cells. This stem construction design disallows the retention of xylem and the removal of phloem at the targeted air layer position where adventitious root formation is desired. Therefore, traditional air layer techniques cannot be used with cycads, and a custom air layer procedure will be required before the technique can be widely adopted by cycad horticulturists.

A clear need for expanding the propagation protocol toolbox for cycad conservationists exists, so our goal was to develop a technique by which to air layer an arborescent cycad stem. We predicted success due to the observance that aerial adventitious roots may form on cycad stems in wet climates when an inadvertent wound removes enough cortex tissue to expose vascular tissue to the air (Appendix A Figure A1). This occurrence of visible aerial adventitious roots on wounded cycad stems has been previously described [21,22]. We exploited an ex situ germplasm collection in which 14-year-old *Cycas edentata* de Laub. Plants were available to attempt the unprecedented air layer propagation manipulations. The *Cycas* genus has the greatest diversification rate among all cycad genera [3] and comprises 125 accepted species [23]. *Cycas edentata* is a widespread coastal species with areas of occupancy scattered among many of the Philippine islands [24]. This species is listed as endangered, with the greatest threats being habitat loss, removal of plants from habitat for landscaping, and *A. yasumatsui* herbivory [25].

Our objectives were to determine the extent of adventitious root formation in response to the percentage of the circumference of the outermost vascular cylinder that was exposed on the wounds within the air layer medium. Additionally, we determined if roots would be initiated on interior concentric vascular cylinders to augment the roots from the peripheral cylinder. Finally, we determined if adventitious root-promoting auxin applied to the wound surface would improve the success rate of the air layer technique. We hypothesized that exposing more of the surface area of the vascular cylinders within the air layer medium would lead to more root growth.

2. Materials and Methods

The study site was an ex situ cycad germplasm garden located in Barangay Sapang Bato, Angeles City, Philippines. The research garden was established by the University of Guam to provide a location at which to conduct horticultural research on various *Cycas* species. The germplasm included numerous 14-year-old *C. edentata* plants derived from various localities throughout the Philippines and Thailand. The soil is an entisol (coarse loamy, isohyperthermic, Typic Untipsamment), which is unstructured and well-drained. Weather conditions are benign and typical of a tropical monsoon climate (Köppen–Geiger classification: Am). The plants selected for developing the new air layer protocols were single-stem specimens with stems that were 47–84 cm in height and 15–22 cm in basal diameter.

2.1. Vascular Tissue Exposure Study

A hand saw and butcher knife were used to create wounds by cutting through the bark and cortex tissues to reach the peripheral vascular cylinder on 15–18 November 2024 (Figure 1a). Each wound was created to a depth that exposed this vascular cylinder on each replication, which was clearly visible on the surface of the wounds and was about 3.5 cm. The upper and lower cuts on each wound were about 4 cm apart. The percentage of exposure of the peripheral vascular cylinder was the treatment variable. One-fourth of the circumference was removed to expose 90°, one-half of the circumference was removed to expose 180°, three-fourths was removed to expose 270°, and 100% of the circumference was removed to expose 360° of the peripheral vascular cylinder. The internal vascular cylinders and pith were undisturbed by these protocols (Figure 1b).

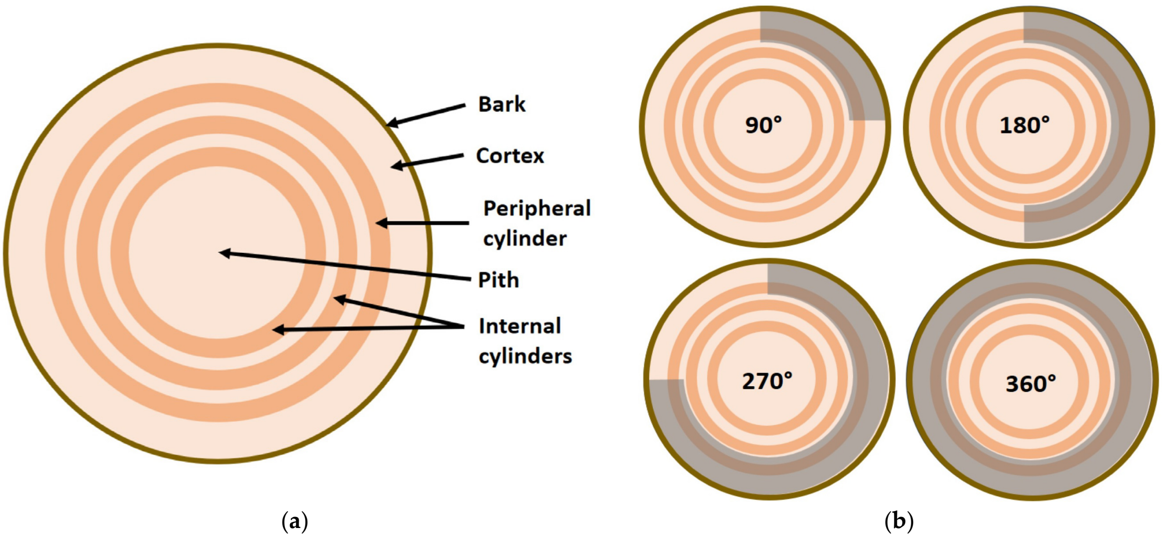

Figure 1. *Cycas edentata* stem construction including concentric vascular cylinders between persistent pith and cortex. (**a**) Air layer protocols were designed to expose the peripheral vascular cylinder tissue on wounds without influencing the internal cylinders and pith. (**b**) The shaded area depicts the tissue removed to create wounds to expose 90°, 180°, 270°, or 360° of the peripheral vascular cylinder in Study 1.

The open wound surfaces were sprayed with a 10% bleach solution, which was allowed to dry, coated with 3 mg·g^{-1} indole-3-butyric acid (IBA) administered in powder form (Brooker Chemical Corp., Chatsworth, CA, USA; Figure 2a), then completely covered with commercial pruning sealant (Spectracide, Middleton, WI, USA; Figure 2b). The sealant was given a minimum of 2 h to dry; then, the air layer medium was installed and covered with black plastic sheets (Figure 2c). A mixture of perlite, crushed pumice, and rice husks was employed. These horticultural substrates provide adequate water retention with abundant aeration. The lack of cohesion enabled subsequent bare-rooting without damaging the new roots.

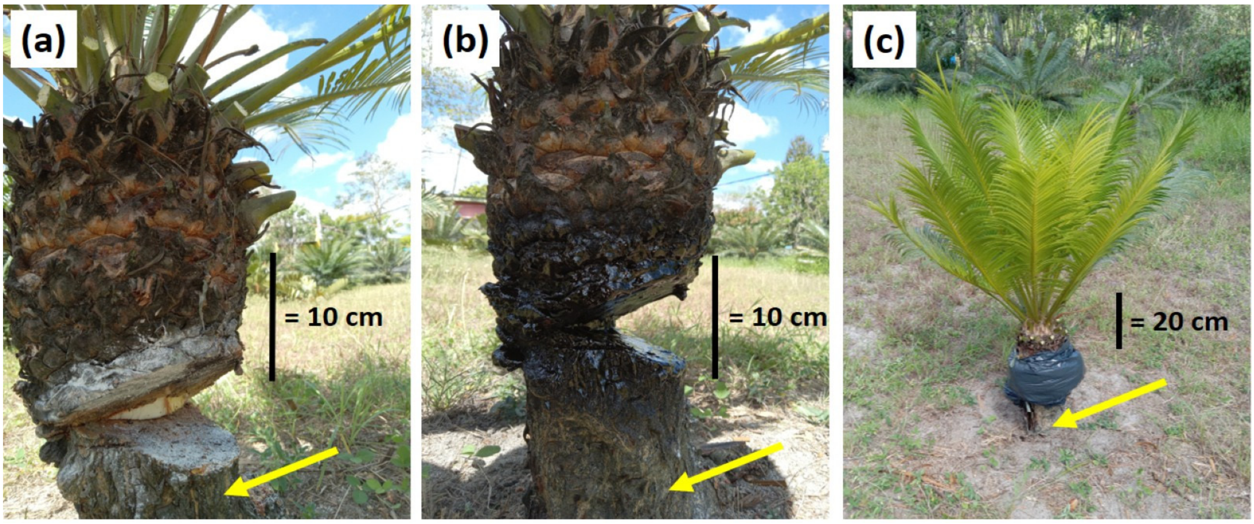

Figure 2. Air layer protocols included opening a wound that exposed vascular tissue on *Cycas edentata* stems: (**a**) auxin in powder form was applied to the wound surfaces of an air layer in Study 3 prior to sealing the wound surfaces; (**b**) pruning sealant covering entire air layer wound surfaces in Study 3 prior to installing the rooting medium; (**c**) finished air layer with wound and medium, enclosed within plastic sheet. Yellow arrows point to an unsightly stem where the bark and cortex have been damaged, leaving the stem unsightly in appearance.

The root development within two random replications per treatment level was observed by removing the medium after 6 weeks and every 2 weeks thereafter. Roots were visible by 8 weeks on most replications. The repetitive bare-rooting procedures continued until 14 weeks, when many of the roots were 10 cm in length. This study was terminated at this time.

The air layer medium was gently removed from all six replications in each vascular cylinder exposure level, and adventitious roots were counted. The length and maximum diameter of every root was measured directly. The stem diameter and length above the air layer stratum were measured. Total root length was calculated for each replication by adding the individual root lengths.

2.2. Root-Promoting Auxin Study

The need for IBA in the promotion of adventitious root initiation in air layer procedures involving *C. edentata* stems was tested in a separate study. Protocols for the 180° treatment in Study 1 were exploited for the treated replications, and a control group was added by applying no IBA. There were four replications, and the air layers were constructed on 19 November 2024. The response variables were as described in Section 2.1.

2.3. Vascular Cylinder Study

The potential for the interior vascular cylinders to add more adventitious root formation was tested in a third study. The cuts that created the air layer wounds were made similarly to the 180° treatment in Study 1, but the cuts continued to the center of the pith such that every vascular cylinder was exposed on the wound surfaces (see Figure 2a). There were four plants included in this study, and the treatments were imposed on 20 November 2024.

2.4. Statistics

A one-way analysis of variance (ANOVA) was employed to determine levels of significance in the response variables in the vascular cylinder exposure study. Parametric prerequisites were confirmed with Levene's test and a Shapiro–Wilk test. Total root length was the only response variable that failed to meet parametric prerequisites, so the Kruskal–Wallis test was employed. The statistical procedures were executed with R (WebR 0.5.0) [26]. For variables that were significant, regression analysis was exploited to explain the relationship between the percentage of vascular tissue removed and the response variable.

A two-tailed *t*-test was employed to compare the differences between the two IBA treatment levels in the IBA study. Total root length was the only response variable that failed to meet parametric prerequisites, so the Mann–Whitney *U* Test was employed.

The response variables for the vascular cylinder study were as described in Section 2.1. There were three vascular cylinders on these experimental plants, and roots that emerged from each cylinder were counted and measured. A one-way ANOVA was planned to compare the differences in root growth among the vascular cylinders. However, roots were restricted to the peripheral vascular cylinder, so the ANOVA was not possible as only one of the three cylinders contained roots.

3. Results

3.1. Vascular Tissue Exposure Study

The adventitious roots emerged from the vascular tissue that was visible on the upper surface of the air layer wounds after 8 weeks and grew in length at a rate of about 1.5 cm per week. The roots emerged from the wound surface with a similar diameter of about 1 cm, and many of the roots reached 10 cm in length by 14 weeks. Every replication in the experiment produced adventitious roots. The general appearance of the roots was similar across every replication, with no branching observed on thick, geotropic roots for the first 14 weeks (Figure 3a). Secondary lateral roots were initiated on the primary adventitious roots after about 18 weeks (Figure 3b). The lateral roots were 2–3 mm in diameter regardless of length.

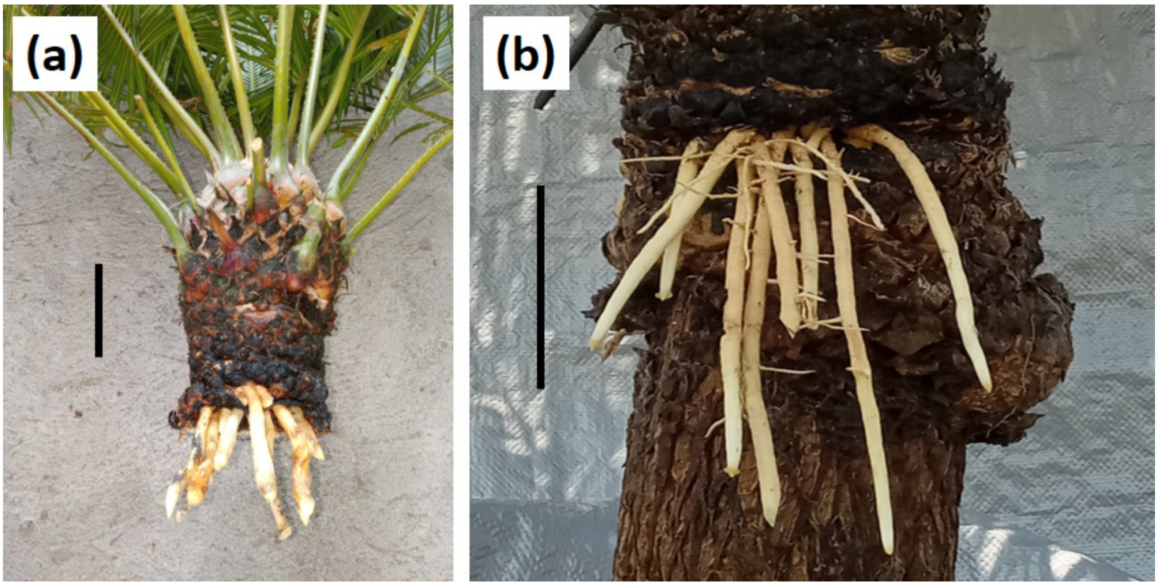

Figure 3. The appearance of adventitious roots that formed within the air layer medium on *Cycas edentata* stems: (**a**) detached air layer propagule with numerous healthy adventitious roots that formed in the 90° treatment, 14 weeks after air layer procedure; (**b**) frontal view of roots that formed in the 180° treatment on intact stem, 18 weeks after air layer procedure. Black bars are equal to 10 cm in length.

The length of the stem above the air layer did not differ among the treatment levels ($f_{3,20}$ = 0.16, p = 0.92), and the mean stem length was 48.7 ± 2.1 cm. The diameter of the stem at the stratum of the air layer did not differ among the treatment levels ($f_{3,20}$ = 0.22,

$p = 0.88$), and the mean stem diameter was 16.9 ± 0.5 cm. These results confirmed that the sizes of the resulting air layer propagules were similar among the four treatment levels. The number of roots per stem after 14 weeks was influenced by the amount of the vascular tissue that was exposed within the wounds ($f_{3,20} = 83.20$, $p < 0.01$). There were 3–6 roots on the stems with only 90° of the vascular tissue circumference exposed and 14–19 roots on the stems with 360° of the circumference exposed, and the relationship could be described with a linear function (Figure 4a).

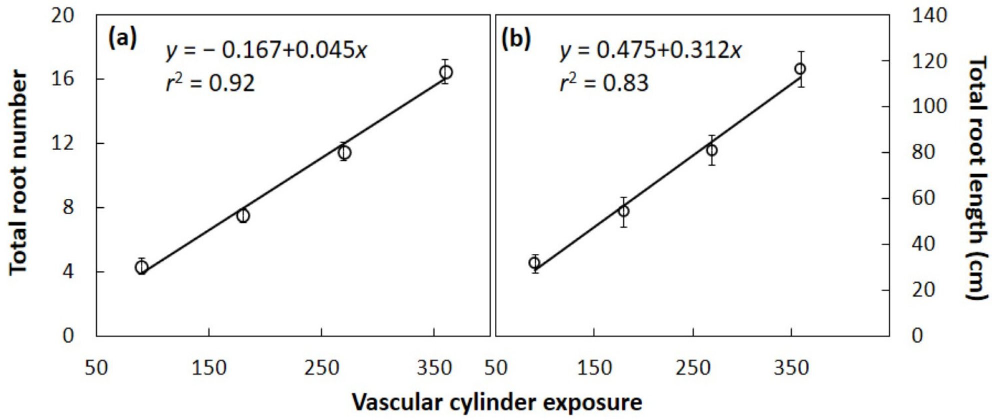

Figure 4. The influence of the exposure of vascular tissue within the air layers installed on *Cycas edentata* stems after 14 weeks of growth. Vascular cylinder exposure is defined as a percentage of circumference: (**a**) number of roots per stem; (**b**) total root length per stem. Markers are mean ± SE, $n = 6$.

The individual root length after 14 weeks did not differ among the treatment levels ($f_{3,20} = 0.07$, $p = 0.98$), and the mean root length was 72.5 ± 3.4 mm. There was a 3.7-fold difference in mean total root length among the four treatment levels, with individual replications ranging from 19.2 to 137.7 cm ($H = 19.5$, $p < 0.01$). The difference was exclusively due to the disparity in number of roots and was not defined by individual root length. The relationship between the total root length and the amount of vascular tissue exposed was described by a linear function (Figure 4b). The individual root diameter did not differ among the treatment levels ($f_{3,20} = 0.18$, $p = 0.91$), and the mean root diameter was 11.3 ± 0.3 mm.

3.2. Root-Promoting Auxin Study

The application of 3 mg·g^{-1} IBA to the wound surfaces did not influence any of the response variables in this study. The roots began to emerge from the peripheral vascular cylinder after 8 weeks and reached about 10 cm in length by 14 weeks. The ending data were collected after 15 weeks (Table 1). The propagule length above the air layer wound did not differ, and the mean stem length was 48.6 cm. Stem diameter at the stratum of the air layer was similar, and mean stem diameter was 15.3 cm. The number of adventitious roots after 15 weeks was also similar for the two treatments, and the mean root number was 8. The length of the adventitious roots did not differ between the treatments, and these roots were 72.3 mm in length. The diameter of the adventitious roots also did not differ between the treatments, and these roots were 8.1 mm in diameter. Total root length at 15 weeks was not influenced by the auxin treatment, and these air layer propagules contained 58.3 mm in total root length.

Table 1. The influence of applying 3 mg·g^{-1} indole-3-butyric acid (IBA) to stem tissue surfaces within air layers on plant growth traits. Means ± SE, *n* = 5.

Plant Trait	IBA-Treated	Control	*t*	*p*
Root length (mm)	73.0 ± 4.1	71.6 ± 4.3	0.24	0.82
Root diameter (mm)	7.8 ± 0.4	8.4 ± 0.4	1.10	0.31
Root number	10 ± 1	10 ± 1	0.32	0.76
Total root length (cm)	56.6 ± 2.7	59.9 ± 3.0	0.10 [1]	0.92
Stem diameter (cm)	15.8 ± 0.7	14.8 ± 0.7	1.07	0.32
Stem length (cm)	50.4 ± 3.1	46.8 ± 3.5	1.22	0.26

[1] *z* statistic from Mann–Whitney *U* test.

3.3. Vascular Cylinder Study

The roots on the air layers began to emerge after 8 weeks, and root growth was similar to Study 1. The ending data were measured after 14 weeks of growth. The peripheral vascular cylinder contained 100% of the adventitious roots that formed on these air layer propagules. No roots were observed on the second and third internal vascular cylinders. The outside vascular cylinders contained 9 ± 1 roots that were 71.5 ± 3.8 mm in length and 11.1 ± 0.7 mm in diameter. The total root length that emerged from the peripheral vascular cylinders was 66.9 ± 9.1 cm.

4. Discussion

Applied research is lacking in cycad biology, and more practical research is critical for cycad conservation success [4,13]. We have addressed this need by developing a highly successful protocol for developing adventitious roots on manipulated stem wounds using air layer techniques. Every replication in our three studies developed adventitious roots within the air layer medium by about 2 months. No roots were observed within the cortex, within the internal vascular cylinders, or on vascular cylinders exposed on the lower wound surface of the air layer. The utility of air layer protocols has been confirmed, and the protocol can be used to expand the conservation toolbox for cycad conservation.

The radial growth of the pachycaulous cycad stem is unique due to the absence of bifacial secondary vascular cambium, and new vascular cylinders are initiated at the base of the living cortex as stems gain diameter [13,27,28]. Therefore, at any stratum of the stem, the peripheral vascular cylinder is the youngest. Living cells are found throughout the entire cross-section of the stem, including the pith and cortex tissues, and active xylem and phloem are comingled in every vascular cylinder. The restriction of adventitious roots to the peripheral cylinder within the air layer medium may, in part, be due to the decrease in age of the vascular cylinders from the pith to the cortex.

4.1. Cycas edentata Roots

Very few studies have addressed *C. edentata* root traits. Lateral root growth in relation to stem height is extensive for *C. edentata*. Six-year-old trees that were 2 m in height were supported by root systems with a radius of more than 7 m [29]. An increase in carbon dioxide efflux from the lowest stratum of *C. edentata* stems, as compared with elevated strata, was attributed to carbon dioxide released from root respiration [30]. The roots of this arborescent cycad possess kin recognition abilities. Root dry weight and length increased when *C. edentata* seedlings were grown in competition with non-kin *Cycas* plants and decreased when grown in competition with half-siblings [31]. The results indicated increased resource acquisition behaviors when the competitor was non-kin. Adventitious root growth has been demonstrated with *C. edentata* stem cuttings [32]. Small stem cuttings were successful in adventitious root formation when the wound surfaces were protected with a malleable covering but died in the propagation bed if the wounds were left untreated [32]. Some of these *C. edentata* cuttings remained alive and healthy after seven months, even if roots had not been initiated by that time. Based on this single study and the air layer results herein, root formation in air layer medium appears to be more rapid than in detached cuttings.

4.2. The Air Layer Procedure

The layering of plant stems for the purpose of initiating new adventitious root growth is a successful protocol that is widely employed in the horticulture industry [18,33–35]. This technique exploits the ability of the new propagule to maintain access to water and nutrients from the source plant's root system while forming new adventitious roots at the manipulated location of a stem axis. The protocol is widely used for the propagation of numerous fruit tree species, most notably pome species, citrus species, and numerous tropical fruit species [36,37].

Our observations with this nascent attempt to apply the technique to a cycad stem has identified that the critical components of the procedure are few. First, a sharp blade should be used for the final cuts on the wound surfaces to ensure no sawdust or other contaminants remain. Second, the surface should be treated with a bleach or alcohol solution to reduce the chances of tissue decomposition. Cycad stems are comprised mostly of non-structural carbohydrates [13,38], causing the wounded tissues to be highly susceptible to secondary tissue necrosis if exposed to any contaminants. Third, 100% of the open wound surfaces should be covered with a malleable prophylactic covering [32] prior to installing the medium. This treatment ensures the non-sterile air layer medium cannot come into direct contact with the vulnerable stem tissues.

4.3. Broader Application for Cycads

Every cycad publication to date that has included state-of-the-art propagation methods has failed to include air layer protocols [8–17]. Our demonstration herein that air layer techniques using cycad stems were highly successful may seem peculiar in light of this lack of earlier reports. However, we note that these publications were primarily written for cycad enthusiasts, and most experienced cycad horticulturists are skilled at developing adventitious roots on detached cycad cuttings. These observations illuminate one of the applications of air layer protocols for cycad horticulture: inexperienced cycad horticulturists. Indeed, while experienced cycad horticulturists command successful propagation skills through the use of stem cuttings, novice horticulturists often fail in their initial attempts to develop adventitious roots on detached cycad stem cuttings. The common mistakes made by beginners include the use of rooting substrates with poor aeration and the over-watering of the cuttings prior to root initiation. These beginner mistakes can be avoided by using our air layer protocol instead of detached stem cuttings.

A second application of this new air layer protocol may be in conditions where the source cycad plants are in extremely poor health. Many endangered cycad populations are characterized by individual plants with severely compromised health status as a result of the chronic threats [4]. For example, *Cycas micronesica* K.D. Hill stem cuttings that were obtained from trees that suffered years of *A. yasumatusi* herbivory exhibited limited success in adventitious root formation [39]. The poor performance of stem cuttings from unhealthy plants was correlated with a severe depletion of non-structural carbohydrates and a decline in starch content relative to free sugar content after the chronic *A. yasumatsui* herbivory [38]. The ability of unhealthy cycad plants to form adventitious roots on stems may be greatly improved by using air layer protocols rather than detached stems as the propagule will benefit from the resources of the source tree throughout the root initiation period.

One prevalent propagation technique for cycad horticulturists is to detach small basal suckers from cycad stems and then place these suckers in nursery conditions to develop adventitious roots [8–17]. These common adventitious cycad stems are frequently called bulbils or offsets. Although large bulbils are easily rooted by experienced cycad horticulturists, the smallest bulbils commonly fail to develop roots. Therefore, propagators are required to wait until the bulbils reach their minimum sufficient size before removal. This requirement causes the resulting damaging wound on the source plant to be greater in size when the larger bulbil is removed. The need for larger bulbils to increase propagation success but smaller bulbils to reduce wound size presents a conundrum for cycad horticulturists. The use of air layer protocols on the smallest bulbils may enable root formation prior to removal from the source stem, providing a successful propagation protocol that allows for the smallest of wounds on the source stems when exploiting the available bulbils as propagules. Indeed, some cycad bulbils that are in contact with the soil may contain naturally pre-formed adventitious roots before they are removed from the source plant [21].

Stem cuttings require a protected nursery space that provides benign conditions in order for the cuttings to remain alive during the waiting period for adventitious roots to form [18,34]. The pinnately compound cycad leaf can be substantial in size [13,14]. For example, some of the *C. edentata* plants used for this study contained leaves greater than 1 m in length. Cycad stem cuttings are usually defoliated for propagation, but leaves may also be retained by some propagators [40]. Even if stem cuttings are entirely defoliated initially, a single *C. edentata* propagule may require 3–4 m^2 of space when new leaf growth occurs after successful root formation. Most homeowners and many commercial or botanical garden cycad nurseries do not have enough space to accommodate numerous new nursery plants of this size. A detached stem cutting may not remain viable if it is installed directly in a landscape setting rather than a nursery setting. However, an air layer propagule that is removed from the source plant with growing adventitious roots can be planted directly in the landscape (See Appendix A Figure A2). This benefit of air layers indicates that the nursery phase can be entirely avoided.

The starch-filled parenchyma tissues that comprise most of the volume of a cycad stem are susceptible to secondary problems whenever a wound on the stem surface occurs [13]. Insect herbivory or pathogen damage to the exposed cortex tissues are common problems that damage the appearance of the stem surfaces (see lower stem surfaces in Figures 2 and 3b, and Appendix A Figure A1). In lignophyte plants with bifacial secondary cambium, a peripheral stem wound is concealed and covered with new vascular tissues and bark such that the wound is ultimately not visible on the stem surface [41]. For cycads, the lack of bifacial secondary cambium disallows this wound healing process; instead, a phellogen is formed to develop phellem that constructs a corky wound periderm to conceal the damaged tissues [42]. The result may be an unsightly stem surface at the stratum of the wound for the life of the plant. Air layer procedures installed above these damaged stem surfaces can be exploited to create a large propagule that removes the unsightly wounded stratum from the plant. The newly acquired plant that was devoid of the damaged lower stem stratum would command greater horticultural value than the original source plant.

4.4. Limitations and Further Research

This study was restricted to a single Asian arborescent cycad species, yet this threatened plant group currently comprises 394 species in 10 genera and 2 families [23]. The stem construction traits that enabled our air layer success are universal defining traits among every species, so we predict that the air layer technique will be highly successful in every cycad species. This prediction needs to be confirmed.

The probability that very small bulbils can be induced to form air layer adventitious roots prior to removal from the source plant needs to be tested, as this approach will benefit valuable source plants by minimizing the resulting wound size after the removal of the bulbils. The probability that propagules derived from source plants in very poor health may be more successfully produced with air layer techniques than with detached cuttings also needs to be tested. Salvage operations designed to rescue endangered plants from constructions sites are common in cycad conservation [4,39], and these plants are often in poor health. Any protocol that can increase transplantation success rates will benefit conservation outcomes.

The root growth of most plants in heterogeneous rhizosphere conditions often leads to root proliferation within nutrient resource patches [43,44]. The air layer medium in our protocol could easily be provided with nutrient solutions as the wetting substrate rather than water. The potential for greater root growth or more rapid root elongation could be verified using these soluble fertilizer protocols. In plant salvage operations, the land owners are often in a hurry to have the endangered plant species removed from construction sites. The use of nutrient solutions to increase the speed of adventitious root growth may be one approach for shortening the air layer protocol time period.

The open surface of the stem base that is created when a cycad air layer propagule is cut from the source plants (see Figure 3a and Appendix A Figure A2a) is a likely source of added adventitious root initiation after the removal and planting of the new propagule. Indeed, these cut surfaces are consistent with the cut surfaces of detached cycad stem cuttings. Excavation of post-transplant air layer propagules after an establishment phase would yield the answers to these questions. This knowledge may improve decision-making with respect to positioning these cuts when removing the successful air layer propagules.

The ability of auxins to promote plant root growth has been demonstrated for almost a century [45]. The readily available IBA was employed for this study because it is commercially available from most horticulture supply outlets and is the most often selected auxin for promoting adventitious roots [18]. However, the auxins 1-naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) have also revealed positive activities for adventitious root initiation on plant stem cuttings [18]. The IBA dosage employed in past cycad propagation studies was in the range of 2–4 $mg \cdot g^{-1}$ [16,32,38,39,46]. To our knowledge, only one study evaluated a dose response of IBA on cycad stem cutting root formation [40]. In that study, a range of IBA concentrations from 0 to 30 $mg \cdot g^{-1}$ exhibited no differences in root initiation or growth between *Zamia furfuracea* L.f. and *Zamia integrifolia* L.f. cuttings, corroborating our results indicating that the addition of auxins to cycad stems may not be needed for adequate adventitious root formation. However, more studies using various forms of auxin and various doses may refine the technique for greater success.

5. Conclusions

We have described an unprecedented air layer procedure for use with cycad propagation that generated 100% success under the conditions of our three studies. In one study, the greater amount of circumferential exposure of the peripheral vascular cylinder within the air layer medium increased the number and length of adventitious roots. In a second study, the use of auxin-promoting root stimulants was shown to have no effect on adventitious root initiation and growth. In a third study, the addition of interior vascular cylinders to the exposed wounds within the air layer medium did not elicit adventitious roots on these interior cylinders, and all roots were restricted to the peripheral vascular cylinder. We believe that novice horticulturists will find the protocols easy to implement, leading to greater success than with detached stem cuttings. Very small stem cuttings and asexual propagation attempts using source cycad plants that are in very poor health may be two of the critical areas of application for this new propagation procedure.

Author Contributions: Conceptualization, T.E.M. and G.N.C.; methodology, T.E.M. and G.N.C.; formal analysis, T.E.M.; resources, T.E.M.; data curation, T.E.M.; writing—original draft preparation, T.E.M.; writing—review and editing, G.N.C.; project administration, T.E.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are available upon request.

Acknowledgments: We thank Gerard Sigua for providing the germplasm garden site and management resources.

Conflicts of Interest: Author Marler was affiliated with the Philippine Native Plants Conservation Society. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
IBA	Indole-3-butyric acid

Appendix A

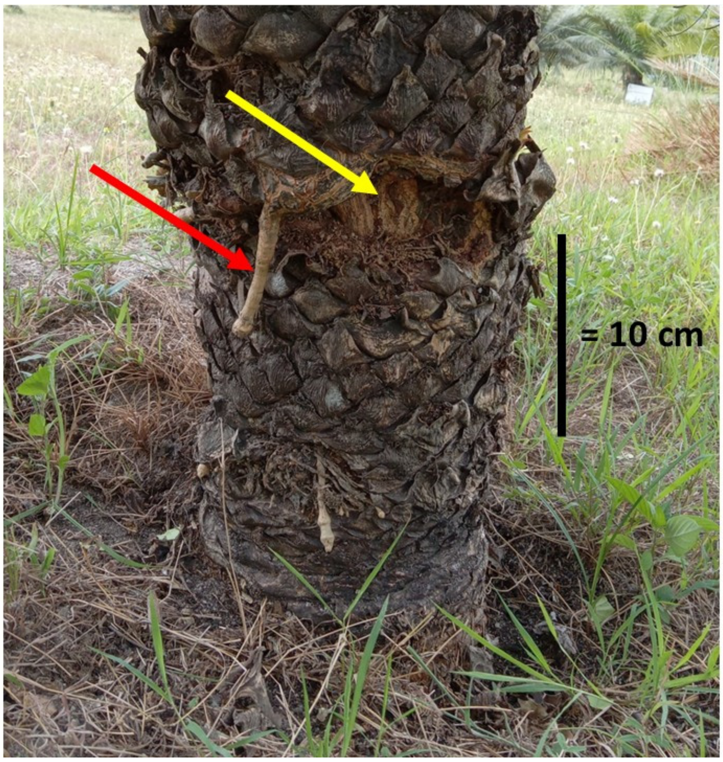

Figure A1. An adventitious root (red arrow) that has emerged following a stem wound (yellow arrow) on a *Cycas edentata* tree growing in an ex situ germplasm collection in Angeles City, Philippines. Similar photographs have been published by Deloso [40] and Pant and Das [22].

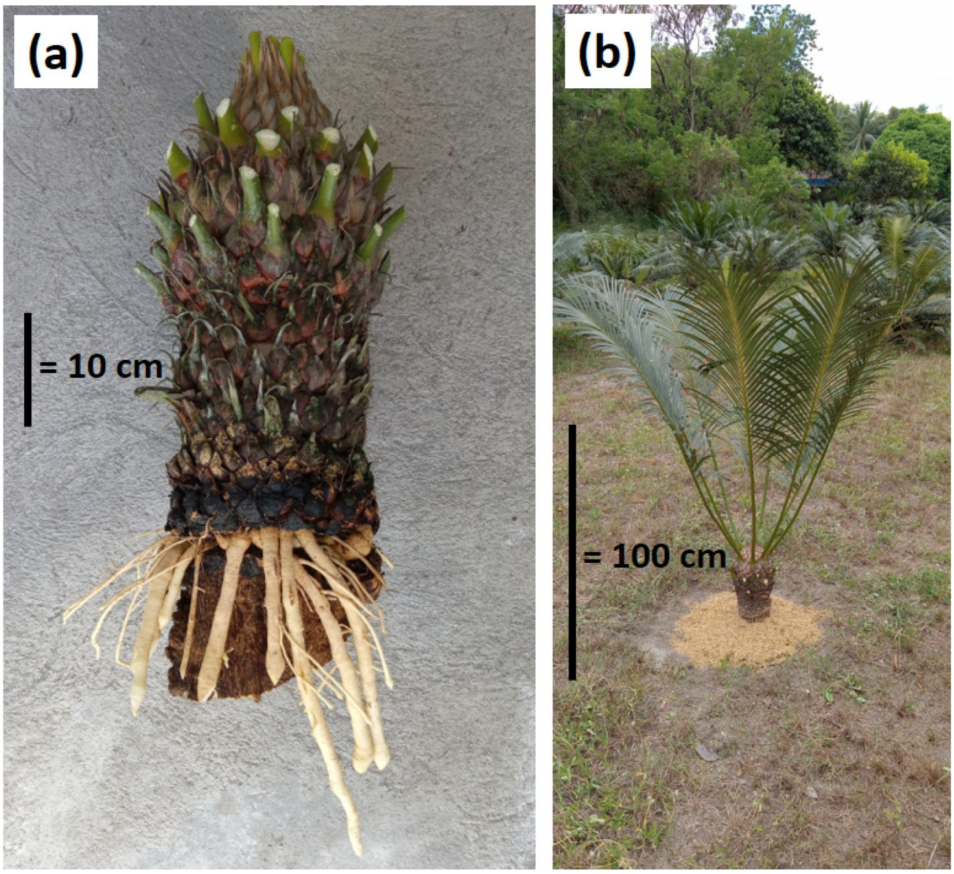

Figure A2. Example of successful air layer *Cycas edentata* propagule: (**a**) after removal from the source plant, the leaves can be removed, and the cut surface of the stem is covered with a sealant; (**b**) a large stem section with pre-formed adventitious roots within the air layer medium shown immediately after planting directly in the field without the need for a nursery phase.

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