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Short Communication

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Prophylactic Treatments of *Cycas* Stem Wounds Influence Vegetative Propagation

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Abstract

Cycad stem cuttings will develop adventitious roots if the open wound is first treated with a sealant to protect the exposed parenchyma tissue. The commercial pruning wound sealant that is often employed for this purpose is not available in many locations. We used commonly available products as a prophylactic sealant on *Cycas edentata*, *Cycas micronesica*, and *Cycas nitida* cuttings to determine efficacy for sealing the wound and enabling adventitious root formation. Success was quantified after 7 months in a sand propagation substrate. Mortality was 100% for control cuttings with no wound sealant and about 60% for cuttings with candle wax as the sealant. Cuttings that received petroleum jelly, lanolin paste, modeling clay, honeycomb wax, or commercial pruning sealant exhibited 100% survival. Success in adventitious root formation ranged from 75% to 92% among the five successful prophylactic treatments and did not differ among the species. The results indicated that four of the products we evaluated were as effective as commercial pruning sealant for treating the exposed parenchyma on *Cycas* stem cuttings and enabling asexual propagation success. The candle wax was less effective because it was brittle and cracked to expose the stem's parenchyma tissue.

Keywords
asexual propagation, cycad, *Cycas edentata*, *Cycas micronesica*, *Cycas nitida*

Introduction

Plant propagation is an important subdiscipline of horticulture and conservation biology. Standardized propagation protocols have been developed for most important horticultural species through a history of trial-and-error observations and sometimes through formal research (Davies et al., 2017). The use of stem cuttings for asexual propagation of cycad plants is a common endeavor of cycad horticulturists (Burch, 1981; Dehgan, 1983, 1999; Norstog & Nicholls, 1997; Whitelock, 2002). Despite this widespread practice among experienced cycad horticulturists, to date, there have been no replicated empirical studies aimed at improving knowledge about asexual propagation of cycads (Cascasan & Marler, 2016; Donaldson, 2003; Marler & Cruz, 2017a, 2017b; Norstog & Nicholls, 1997).

Successful propagation by cycad stem cuttings exploits the tendency of many cycad species to produce adventitious buds that develop into stems (Figure 1). Skilled cycad horticulturists remove these stems without damaging the stem of the original plant. The procedure includes the use of a sealant to cover the wounds on the original plant and the detached cutting. The reason that this sealant is used is the manoxylic design of pachycaulous cycad stems (Seward, 1917). The living parenchyma tissues that comprise the majority of a cycad stem's volume are vulnerable to desiccation and secondary problems whenever the periderm is broken (Fisher et al., 2009). The sealant serves as a synthetic periderm to prevent dehydration and exclude pathogens and herbivorous insects.

A petroleum-based pruning wound sealant is commonly used for sealing the wounds on cycad stems. This sealant was employed in the pilot study accompanying plans for *Cycas micronesica* K.D. Hill tree rescue projects from military construction sites on Guam (Marler & Cruz, 2017a, 2017b). Healthy *C. micronesica*

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stem cuttings exhibited 100% success of adventitious root formation when this sealant was used (Marler, 2018). The use of this type of petroleum-based wound sealant is a mandatory component of the work plans for treating cycad stem cuttings within the ongoing Guam salvage projects, which include the rescue of thousands of cycad specimens from military construction sites.

Our cycad germplasm research in the Philippines has included asexual propagation efforts. Unfortunately, the commercial pruning sealant product is not available in Pampanga Province where we maintain the germplasm. We have resorted to the use of alternative products to seal the wounds of our *Cycas* stem cuttings. Clarifying which of the available products is most suitable for treating *Cycas* stem cuttings would benefit cycad horticulturists throughout the world, especially in locations where a commercial pruning sealant is unavailable. Our objectives were to determine the effectiveness of eight common products for use as the wound sealant on *Cycas* stem cuttings during asexual propagation efforts. We predicted that all of the products that were malleable and adhered sufficiently to the stem surfaces would prevent tissue dehydration and enable adventitious root formation.

Methods

Mindoro-sourced *Cycas edentata* de Laub. plants, Samar-sourced *Cycas nitida* K.D.Hill & A.Lindstr. plants, and Yap-sourced *C. micronesica* plants were transplanted from a container nursery to Porac, Luzon, Philippines in June to July 2012. The germplasm collection was transplanted to Angeles City, Luzon, Philippines in June to July 2017. The trees developed numerous basal adventitious stems following the second transplantation. These 8-year-old plants were used to provide adventitious stem cuttings in June 2019.

Washed coarse river sand was used as the propagation substrate. The sand was quarried adjacent to the study site in the Sacobia River and was soaked in 10% bleach solution for 30 min prior to being added to a raised bed. The bottom of the bed was lined with plastic with holes for drainage, and the plywood sides of the bench were 45 cm in height. This coarse sand was available due to aggradation events of pyroclastic fragments originally from the 1991 Mount Pinatubo eruption. This loose tephra on the east flanks of the mountain continues to be eroded from the watersheds then deposited into the floodplains below the alluvial fan on each of the major rivers.

The general appearance of the available adventitious stems was as shown in Figure 1. The range in size was similar for the three species. Length of the cuttings ranged from 5 to 8 cm, and number of leaves per cutting ranged from 2 to 6. All leaves were removed at the base of the petioles, and the wound on each cutting was trimmed with a sharp knife to result in the smallest smooth wound possible. The prepared cuttings were entirely immersed in a 10% bleach solution for 5 min, and then, the open wound was coated with commercial indole-3-butyric acid ($3\,mg \cdot g^{-1}$ in powder form). The treated cuttings were placed on a clean plastic surface to air-dry for 2 hr. The cuttings for each species were sorted by stem diameter from least to greatest so they could be grouped into four size categories based on diameter. The smallest category contained cuttings within the range 4 to 5 cm, the second category contained cuttings within the range 5 to 6 cm, the third category contained cuttings within the range 6 to 7 cm, and the largest category contained cuttings greater than 7 cm in diameter.

Figure 1. The Pachycaulous *C. micronesica* Stem Produces Adventitious Buds That Develop Into Adventitious Stems. These side stems can be removed for asexual propagation. Credit: T. Marler.

We evaluated seven products for use as an alternative to the commercial sealant to seal the wounds at the base of our *Cycas* stem cuttings. These products were selected because they were readily available in local markets.

1. Commercial pruning wound treatment (Figure 2a): This product served as our control product to which the other seven were compared.
2. Modeling clay (Figure 2b): This type of clay remains moist and is available in large department stores and art supply stores.
3. Petroleum jelly (Figure 2c): This product is used as a lubricant or an ointment for softening tissues and moisturizing skin and is available at most grocery and drug stores.
4. Candle wax (Figure 2d): Candles are available in markets throughout the world. When melted, the wax can be applied to surfaces during the few seconds before it rehardens.

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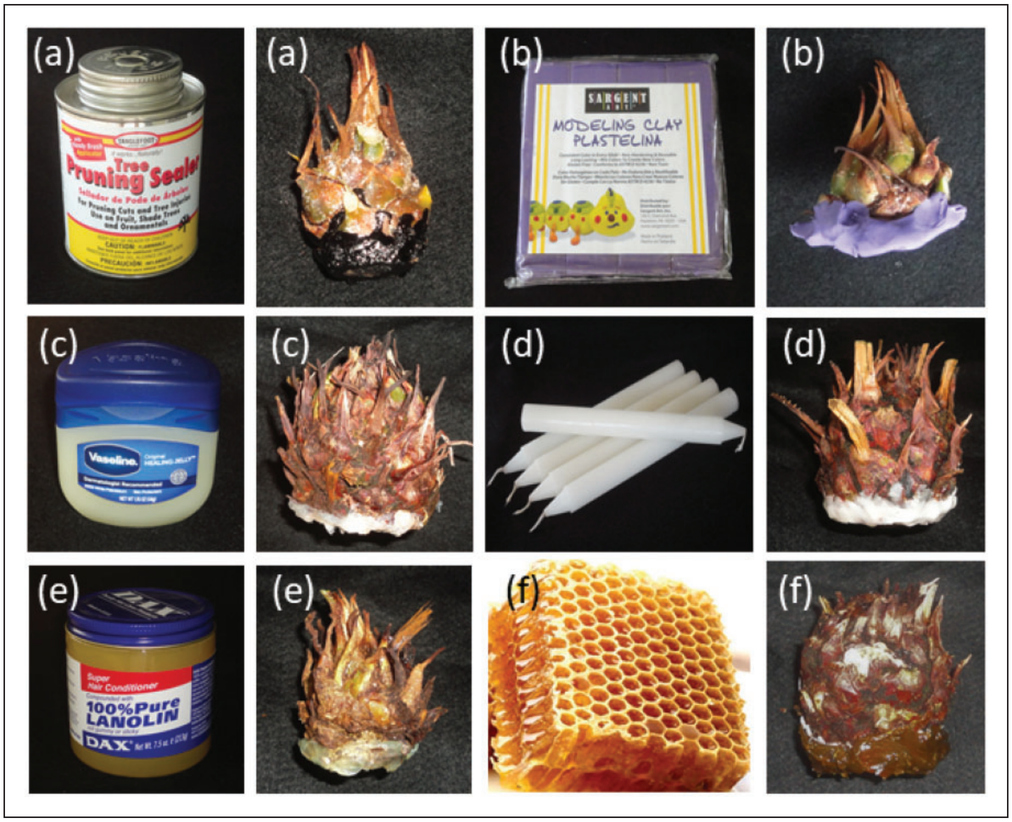

Figure 2. Products That Were Used for Treating Wounds on *Cycas* Stem Cuttings. (a) Commercial petroleum-based pruning sealant, *C. micronesica* cutting; (b) modeling clay, *C. micronesica* cutting; (c) petroleum jelly, *C. edentata* cutting; (d) candle wax, *C. edentata* cutting; (e) lanolin paste, *C. micronesica* cutting; and (f) honeycomb wax, *C. edentata* cutting. Inclusion of commercial products does not constitute an endorsement by the authors, their affiliations, or the journal. Credit: T. Marler.

5. Lanolin paste (Figure 2e): Lanolin is more difficult to locate than the other products, but large drug stores sell the product for hair treatment and for moisturizing highly sensitive skin.
6. Wax from honeycomb (Figure 2f): Most public markets in Asia sell raw honey contained in unprocessed honeycomb wax. We rendered the wax from the raw honeycomb by enclosing in cloth, placing in boiling water, then providing a cooldown period until the wax solidified. Because the wax floats on water, the layer of the wax was easily removed from the subtending water. We rendered the wax a second time to remove any sugars that may have been retained in the wax following the first rendering.
7. Rubber cement: This product can be found at book stores and art supply stores. We rejected the use of this product because it did not adhere to the surface of the cuttings sufficiently to seal the stem wounds.
8. Silicone caulk: Construction caulking based on silicone can be found at hardware stores. We rejected this product for the same reason as the rubber cement.

The experimental approach included seven wound treatments, comprised of one of the six acceptable products and an untreated control. There were three species and four replications positioned as blocks based on stem diameter categories described earlier. The treated cuttings were allowed to air-dry until the following day, when each cutting was inserted into the sand to a depth of 2 cm on June 27, 2019. Each of the 4 blocks contained 3 species and 7 prophylactic sealant treatments for a total of 84 cuttings.

A plexiglass roof was installed to protect the cuttings from rainfall, and shade fabric was installed on the roof to exclude 75% of incident light. The wetness of the sand surface was observed daily, and the bed was irrigated as needed. Irrigation frequency averaged two times per week.

Direct observation of each cutting to determine which stems remained alive was conducted on December 19, 2019. This was accomplished by applying pressure to the stem surface. Dead stems were soft and felt hollow when pressure was applied, but living stems remained firm and did not yield when pressure was applied to

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the stem surfaces. Cuttings that felt hollow were lifted from the sand substrate and sectioned with longitudinal cuts to confirm mortality and determine cause of death. The living cuttings were left undisturbed in the propagation bed.

The walls of the raised bed were dismantled, and the sand was washed with gentle water pressure to expose each living cutting on December 20 to 21, 2019. Adventitious rooting efficiency was evaluated by direct observation of adventitious roots and the appearance of the original stem wound surface. Each of the rooted cuttings was transplanted to an individual container and added to our production nursery. Two binary data sets were created to determine efficacy of the prophylactic treatments and enable analyses of variance. First, the influence of treatments on mortality was determined by assigning 0 to each cutting that was determined dead and assigning 1 to each cutting that remained alive. Some of these living cuttings had not developed observable roots after 7 months. Our second data set was created by assigning 0 to living cuttings with no observable roots and assigning 1 to cuttings with adventitious roots. To confirm the apparent health of the living but unrooted cuttings, we sacrificed one cutting for each species to observe the internal tissue. We used a longitudinal cut for this purpose.

The stem mortality data set was inappropriate for analysis of variance (ANOVA) due to lack of variation among the replications for most treatments. Similarly, no transformations rendered the entire rooting success data set appropriate for ANOVA because the control group exhibited no variation with 100% mortality. Therefore, we used the data from the six wound dressing products to conduct ANOVA with the General Linear Model (Proc GLM; SAS Institute, Cary, NC, USA). The analysis was a 3 Species $\times$ 6 Treatment factorial in a randomized complete block. Means separation among levels of significant factors was conducted with Tukey's honest significant difference test.

Results

Mortality was 100% for the control treatment comprised of cuttings that received no sealant on the open wound surfaces and 58% for the candle wax treatment. Viability was 100% for the remainder of the prophylactic treatments. Cause of death was dehydration for all of the control cuttings and most of the cuttings treated with candle wax. Beetle pupae were observed on top of the sand substrate adjacent to two *C. edentata* cuttings. These two cuttings received the candle wax treatment and were among the cuttings that were dead on this date. When the cuttings were lifted from the sand, beetle adults and more pupae were observed on the sand surface. The beetle was identified by T.M. as *Carpophilus mutilatus* Erichson. The vascular and pith tissues of these cuttings were intact but desiccated. The cortex and apical tissues were consumed by the grubs of this beetle. Cause of death for these two cuttings was assigned to the herbivory.

The block effect, which was related to stem diameter, was not significant for rooting success ($p = .9672$), indicating the size of the cuttings did not influence the success rate for asexual propagation. The species effect was also not significant for rooting success ($p = .5505$), indicating similar behavior for the stems of these three closely related *Cycas* species. The interaction of Species $\times$ Treatment was also not significant for rooting success ($p = .9420$), indicating the genetic differences among the species did not influence the prophylactic treatment effects.

The treatment effect was highly significant for adventitious root success ($p = .0002$). The control cuttings were a complete failure due to the mortality (not included in the ANOVA). The cuttings with candle wax treatment exhibited 17% root formation success (Table 1). The remainder of the prophylactic treatments exhibited 75% to 92% success in adventitious root formation, and the success rate did not differ among these five treatments.

Most but not all of the cuttings with adventitious roots also had one leaf that had emerged (Figure 3a and b). Althought the nonsignificant block effect indicated size of the cuttings did not influence rooting success, most of the larger cuttings exhibited extensive root growth, indicating intial root formation had occurred several months prior to the termination of the study. In contast, most of the small cuttings exhibited small root systems, indicating initial root formation had occurred in the weeks prior to the termination of the study.

The longitudinal cuts on the living but unrooted cuttings for all three species revealed turgid, healthy tissue throughout the cortex, vascular, pith, and cataphyll tissues (Figure 3C). These observations on the sacrificed cuttings indicated the prophylactic treatments were

Table 1. The Influence of Prophylactic Treatments on the Proportion of *Cycas* Stem Cuttings That Successfully Formed Adventitious Roots Following 7 Months in a Sand Propagation Bed.

Prophylactic treatment	Root formation
Candle wax	0.17 ± 0.11 a
Prune wound sealant	0.75 ± 0.13 b
Lanolin paste	0.83 ± 0.11 b
Honeycomb wax	0.83 ± 0.11 b
Modeling clay	0.92 ± 0.08 b
Petroleum jelly	0.92 ± 0.08 b

Note. Mean ± standard error, $n = 12$. Means followed by same letter are not different according to Tukey's honest significant difference test.

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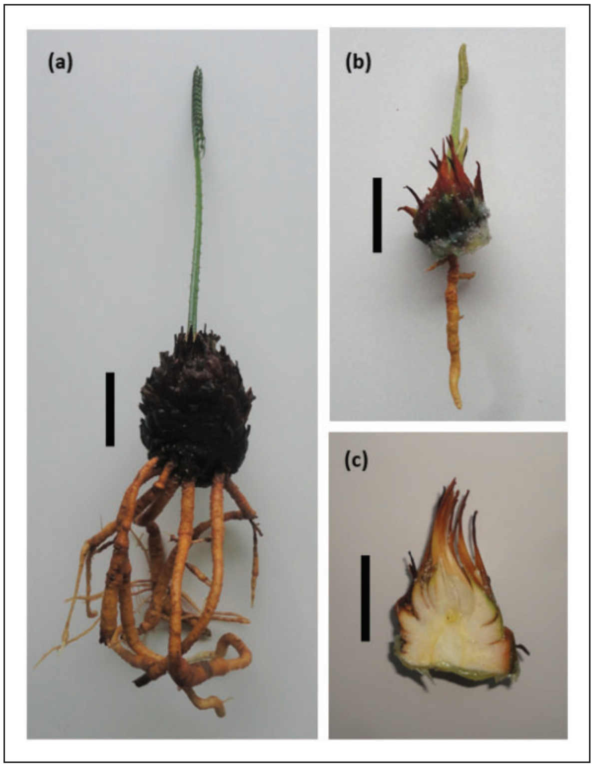

Figure 3. General Appearance of *Cycas* Cuttings Following 7 Months in a Sand Propagation Bed. (a) Large *C. edentata* cutting with commercial prune sealant showing copious root formation; (b) small *C. micronesica* cutting with lanolin paste sealant showing initial signs of root formation; and (c) medium-sized *C. micronesica* cutting with petroleum jelly sealant showing healthy stem tissues but no observable root formation after 7 months. Black bars = 5 cm. Credit: T. Marler.

highly effective in protecting the stem tissue from dehydration or attack by pathogens or insects.

Discussion

Five of the products used to seal the *Cycas* stem cuttings were effective in maintaining viability of the cuttings for 7 months. The one product that was not fully effective was the candle wax, and this was because the original seal lost integrity with cracks forming to expose the stem parenchyma tissues. The petroleum jelly, lanolin, modeling clay, and honeycomb wax effectively fostered adventitious root formation as well as the commercial prune wound sealant. The results indicated that horticulturists in regions where commercial pruning sealant is not available may use one of these products or an alternative clean, malleable product that will adhere to the stem surfaces during the propagation protocols during cycad asexual propagation efforts. Adopting these protocols may improve propagation success and quality of the propagules, which may improve economic viability of small-scale growers.

Seven months after establishment of stem cuttings in the propagation bed, up to 25% of the healthy cuttings remained devoid of observable adventitious roots, which was expected. The formation of roots on healthy cycad stem cuttings is known to require more than 1 year in some cases (Dehgan, 1999; Marler & Cruz, 2017a, 2017b).

The *C. mutilates* herbivory of the *C. edentata* stem cuttings resulted from the ineffective seal from the brittle candle wax that exposed the parenchyma tissue to ovipositioning. Herbivory of *C. micronesica* stem cuttings by *C. mutilatus* grubs has also been observed in Guam (Marler & Muniappan, 2006). This Nitidulidae beetle is widespread in the Philippines and Micronesia and feeds on numerous substrates (Gillogly, 1962). It has also been collected from male *C. micronesica* cones in Guam and Rota (Gillogly, 1962; Terry et al., 2009).

Our use of the bleach dip for each cutting and the hygiene we employed while handling the sand substrate ensured that we avoided diseases. Practitioners may consider the use of commonly available products to serve as fungicides on *Cycas* cuttings. For example, flowers of sulfur and Bordeaux mixture comprised of copper sulfate and slaked lime possess antiseptic and antifungal properties. The use of a fungicide may be warranted especially where nursery workers are unaware of hygiene protocols.

The products we evaluated for sealing the open wound of stem cuttings may also be of use during field expeditions. Application on wounds of excised organs during botanical surveys, for example, may protect the specimens from desiccation or damage during extended travel.

Implications for Conservation

Cycads comprise the most threatened group of plants worldwide (Brummitt et al., 2015; Fragniere et al., 2015). One of our model species is Red-listed as Endangered (Marler et al., 2010). Any improvement in the protocols for vegetative propagation of cycads by stem cuttings may be of crucial importance for conserving the most threatened species.

Horticulturists are ideally equipped to contribute to plant conservation research to inform adaptive management approaches (Marler, 2017). Our study is an example of how nondestructive horticultural treatments can be replicated within observational studies that include no destructive response variables. The approach can provide new knowledge to enhance horticultural successes and benefit conservation efforts.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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