Germination and Early Seedling Growth of Rare *Zamia* spp. in Organic and Inorganic Substrates: Advancing Ex Situ Conservation Horticulture

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Abstract. Improved propagation methods greatly benefit conservation of rare cycads. Appropriate substrate conditions, especially excellent root aeration, are crucial to successful cultivation of most cycads. Typical cycad substrates include substantial portions of organic materials that will decompose over time, reducing drainage and increasing water retention. In this study, two inorganic substrates, arcillite (Turface® MVP®) and coarse silica sand, and one mixed cycad substrate (with organic and inorganic components) were evaluated for germination and growth of three rare *Zamia* species: *Z. fairchildiana* L.D. Gómez, *Z. cunaria* Dressler & D.W. Stev., and *Z. aff. portoricensis* Urb. over a period of 14 months from seed sowing. Substrate type affected leaves per seedling and leaf length. These factors also varied by species as did taproot length and germination rate. There were also significant interactions between substrate and species for caudex diameter and leaf variables, likely reflecting ecological differences among the species, two of which are from rainforest habitats and one from dry forest. All three substrates performed adequately for germination, survival, and growth of *Zamia*. Turface® and possibly the silica sand likely require additional watering to improve their performance as cycad substrates.

As a result of the vulnerability of wild cycad populations to human impacts, including collection for commercial horticulture, the Cycad Specialist Group of the International Union for Conservation of Nature (IUCN) recommends concerted efforts to improve propagation and cultivation of cycads (Donaldson et al., 2003). With this conservation concern in mind, important progress has been made in cycad horticulture (Chavez and Litz, 2007; Dehgan, 1983, 1999; Dehgan and Johnson, 1983; Witte, 1977), but to date, no studies appear to have been published investigating the effects of different substrates on cycad growth and development. The objective of this study was to determine whether use of

100% inorganic substrates will lead to an improvement in germination or growth of rare *Zamia* spp. in containers.

Providing the proper balance of aeration, water retention, nutrient-holding capacity, and decomposition rate are among the key factors that must be considered in evaluating a cycad container substrate. Excellent drainage is especially crucial, because cycads often do best in “sandy gravelly” soils (Whitelock, 2002). As a result of the slow growth of cycads, the ability of a substrate to retain its physical properties over time is crucial, especially in a hot, humid environment such as south Florida that accelerates decomposition of organic materials. Drainage of organic container substrates can decline considerably over time as the substrate decomposes (Bilderback et al., 2005). Thus, it seems likely that an ideal long-term cycad substrate would be inorganic and resistant to decay or at least contain a sufficiently high proportion of inorganic materials to retain drainage even after the organic components have begun significant decomposition.

The Montgomery Botanical Center (MBC) is a 120-acre botanical garden in Miami, FL, that specializes in conservation horticulture of cycads and palms. Montgomery currently cultivates two-thirds of extant cycad species and expends considerable effort propagating and growing cycads in containers as well as in the ground. Historically, MBC has used a horticultural mix comprised of equal parts organic soil conditioner (Fafard Organic Soil Conditioner; Conrad Fafard, Inc., Agawam, MA), silica-based coarse building sand (6/20

grade; Florida Silica and Sand Company, Ft. Lauderdale, FL), and expanded clay pellets (Hydroton® 8/16 mm grade; Ökotau Easy Green GmbH, Eschborn, Germany) as a substrate for both seed germination and nursery container culture of cycads.

In the current study, two other substrates, Turface®, a calcined montmorillonite clay (Turface® MVP®; Profile Products LLC, Buffalo Grove, IL), also known as arcillite, and coarse silica sand (6/20 grade; Florida Silica and Sand Company), were selected for comparison with MBC’s horticultural mix (referred to as “cycad mix”) to evaluate the effects of inorganic substrates versus a typical mixed organic–inorganic substrate on germination and early seedling growth. Sand and Turface® have both shown considerable promise in hydroponic applications, and some horticulturists have used them successfully for growing cycads and succulents. As a result of their physical and chemical stability, these products are of interest to evaluate for their potential as long-term substrates for cycad culture.

Turface® is a calcined montmorillonite clay soil conditioner designed for use as an amendment or top-dressing for turf in sports fields. It is meant to hold moisture and nutrients, increase drainage, and reduce compaction. Calcined clay has long been known to be an excellent substrate for growth of experimental plants in hydroponics (Jaeger, 1981), partly because it allows easy separation of the substrate from the root system (Hiller and Koller, 1979) while also supporting good growth. Warren and Bilderback (1992) and Owen et al. (2008) found that calcined clay reduces water use and increases fertilizer efficacy in container production when used as a substrate amendment. Turface® has excellent drainage, porosity, and water-holding capacity (Table 1), is mechanically stable, and has high cation exchange capacity as a result of its montmorillonite clay makeup (Warren and Bilderback, 1992).

Coarse silica building sand is often used as a germination substrate. One grower reported exceptional growth of cycads in 100% sand (T. Broome, personal communication), including difficult *Macrozamia* species that are especially sensitive to lack of drainage (Broome, 2006). The excellent drainage and inertness of sand render it promising for cycad culture. Sand has long been used as a standalone substrate in a very broad range of hydroponic research (Hewitt, 1966).

Materials and Methods

The experiment took place in a shadehouse at MBC. Seeds from three different taxa of *Zamia*, collected in situ for cultivation in MBC’s ex situ conservation collection, were used in the experiment: *Zamia fairchildiana* seeds from Panama near the Costa Rican border, *Zamia cunaria* from two populations in Panama (Wargandí and Kuna Yala provinces), and *Zamia aff. portoricensis* from northwestern Jamaica. The later taxon is known in the horticultural trade as ‘Jamaican Giant’.

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Seeds from individual mother plants were collected separately, received a unique accession number based on the mother plant, and were sown separately for the experiment. Seeds from each accession were separated into three treatment groups (MBC cycad mix, 100% sand, and 100% Turface® MVP®) and planted in 2.6-L pots in a shadehouse (50% shade) under identical environmental conditions. The MBC cycad mix consisted of 1 coarse pine bark:1 silica sand (6/20):1 organic soil conditioner (made from pine bark):1 Hydroton® clay pellets (8/16 mm) (by volume). Each substrate had different physical properties (Table 1) and particle size distribution (Table 2). Containers were fertilized once for the duration of the experiment after natural seed abscission with 14.8 cm³ Nutricote® 18N-2.6P-6.6K (Chisso-Asahi Fertilizer Co., Ltd., Tokyo, Japan) controlled-release fertilizer with micronutrients. Pots were watered thoroughly beyond container capacity (until water drained freely from the container) three times per week (Monday, Wednesday, Friday) with automatic overhead irrigation. All containers received the same irrigation treatment.

Physical properties of the container substrates were measured after the completion of the experiment on fresh cycad mix like that used in the experiment and on the same sand and Turface® product grades as used in the experiment. Three replicates of 500-mL samples for each substrate were used for physical

property measurements. Initially dry substrates were thoroughly oven-dried in shallow pans for 20 min at 121 °C to remove any residual moisture. Initial dry weight was recorded and then water was added to thoroughly saturate the substrates so that all pore space was filled (with vigorous agitation to dislodge bubbles). Excess water was drained off and the drainage volume measured to determine the volume of air space. The saturated weight of the substrate was then measured. Percent air space was calculated as the ratio of air space to container volume. Percent water-holding capacity was calculated as the volume of water absorbed by the substrate divided by the container volume and total porosity as the sum of percent air space and percent water-holding capacity. Bulk density was calculated as the ratio of dry substrate weight to volume. Particle size distribution was measured with a set of five standard soil sieves from #5 to #120 (Hubbard Scientific Company, Northbrook, IL). Three replicate samples of each substrate (950 to 1000 mL per replicate) were shaken through the sieves and the proportional weight of substrate retained at each level was measured.

Seeds of *Zamia fairchildiana*, *Zamia cunaria*, and *Zamia* aff. *portoricensis* were sown in February of 2008 and evaluated in Apr. 2009. Sowing rates were 10 to 11 seeds/pot for *Z. aff. portoricensis* and *Z. cunaria* (with the exception of two five-seed pots and one 15-seed pot

of *Z. cunaria*) and five to six per pot for *Z. fairchildiana* (Table 3). Differences in sowing rates reflected differences in seed size and different amounts of available seeds from field collection. A separate experiment (data not shown) indicated that sowing rates of five to 11 seeds/pot did not significantly affect germination, establishment, or early growth for the three species studied. The numbers of seedlings and pots per treatment are given in Table 3. Community pots were evaluated for seed germination, and seedlings were evaluated by measuring caudex diameter, taproot length, leaf number, leaf length, and number of leaflet pairs on the largest leaf (see Fig. 1 for typical cycad seedling morphology).

Statistical analyses were carried out using JMP® 8.0.1 (SAS Institute, Inc., Cary, NC). For substrate physical properties, percentages were transformed using the standard arcsine transformation for proportions before analysis (Quinn and Keough, 2002). For each property, an analysis of variance (ANOVA) followed by a multiple comparisons test [Tukey's honestly significant difference (HSD)] was performed to distinguish among the substrates. The experiment was factorial in a completely randomized design with substrate, number of seeds sown per pot, and species being the factors. Individual seedlings, rather than pots, were treated as replicates. This was done because during the first year of growth, root development remains sparse in *Zamia*, minimizing interactions among seedlings in a given pot. Also, because the substrates were uniformly prepared, variation from pot to pot within each substrate treatment was considered negligible. Assumptions akin to this are also made implicitly when analyzing experiments when each seedling has its own pot, because there are always factors beyond the treatments that can plausibly "carry over" from one

Table 1. Physical properties of substrates used for growing *Zamia* spp.: Montgomery Botanical Center cycad mix, sand, and Turface® MVP®.^z

	Cycad mix	Sand	Turface® MVP®
Air space	28.7% a	8.7% b	16.3% c
Water-holding capacity	14.5% a	29.9% b	40.3% c
Total porosity	43.1% a	38.5% a	56.7% b
Bulk density (g·mL ⁻¹)	0.56 a	1.75 b	0.71 c

^zDifferent letters indicate significant pairwise differences between substrate means (Tukey's honestly significant difference, $\alpha = 0.05$).

Table 2. Particle size distribution of cycad mix, coarse sand (Florida Silica & Sand Company, 6/20), and Turface® MVP® used in the *Zamia* substrate experiment.^z

Sieve no.	Hole diam (mm)	Percent retained (cycad mix)	Percent retained (sand)	Percent retained (Turface® MVP®)
5 ($P < 0.0001$)	4	35.0 a	0 b	0 b
10 ($P < 0.0001$)	2	8.35 a	2.54 b	67.2 c
35 ($P < 0.0001$)	0.5	52.9 a	97.3 b	32.8 c
60 ($P < 0.0001$)	0.25	2.28 a	0.10 b	0 c
120 ($P < 0.0001$)	0.125	0.78 a	0.04 b	0 b
>120 ($P < 0.0001$)	0.063	0.68 a	0 b	0 b

^zP values in parentheses are for analyses of variance at each sieve size. Different letters indicate significant pairwise differences between substrates (Tukey's honestly significant difference, $\alpha = 0.05$).

Table 3. a) Sowing rates and number of experimental pots for each *Zamia* species: (number of pots) × (number of seeds per pot) for each species/substrate combination; and b) total seedlings and number of experimental pots for each *Zamia* species for each species/substrate combination.

		<i>Zamia fairchildiana</i>	<i>Zamia</i> aff. <i>portoricensis</i>	<i>Zamia cunaria</i>
a) Sowing rates	Cycad mix	1 × 5, 1 × 6	6 × 10	4 × 10, 1 × 5
	Sand	3 × 5	6 × 10	2 × 10, 1 × 11, 1 × 15
	Turface®	2 × 5	6 × 10	3 × 10, 2 × 11, 1 × 5
b) Total seedlings and number of pots	Cycad mix	10 (2 pots)	55 (6 pots)	32 (5 pots)
	Sand	21 (4 pots)	57 (6 pots)	39 (4 pots)
	Turface®	10 (2 pots)	58 (6 pots)	42 (6 pots)

Fig. 1. *Zamia* aff. *portoricensis* seedling grown in Turface®. This individual exhibits typical seedling morphology with one mature and one developing leaf.

apparent replicate to the next (Oksanen, 2004). Also, cycad seeds are normally sown in community pots at MBC and elsewhere so it was considered more desirable to accurately recreate normal nursery practices rather than to sow each seed in an individual pot. This is a natural tradeoff between classic replication (one seed or seedling per pot) and applicability of the experiment to actual growing conditions (Oksanen, 2001).

We performed separate ANOVAs on each continuous response variable using the full factorial model (except for germination rate, in which a model without interactions was used, because the interaction model was not significant) using Type III sums of squares (partial sums of squares) to test hypotheses. For the count response data (number of leaves and leaflet pairs), an ordinal logistic model was used because of the large number of repeated values that render the ANOVA assumptions invalid. The ordinal logistic model is conservative in the case of count data (Sturman, 1999). For continuous variables, pairwise means comparisons were performed using Tukey's HSD. In addition, for the initial experiment, a separate ANOVA was performed to test for differences in germination rates and sowing rates among substrates after using the standard arcsine transformation for proportions. Where feasible, model assumptions were checked through residual plots.

Results

Air space ($P < 0.0001$), water-holding capacity ($P < 0.0001$), total porosity ($P = 0.001$), and bulk density ($P < 0.0001$) differed significantly among substrates. Tukey's HSD ($\alpha = 0.05$) detected significant pairwise differences among all substrate pairs except for total porosity, which was not detectably different between cycad mix and sand (Table 1). Particle size distribution also varied significantly among substrates at each mesh size (Table 2).

Germination rates did not vary significantly among substrates ($P = 0.163$) but did vary significantly among species ($P = 0.0056$) with the lowest germination rate for *Zamia cunaria* (80.4%, $n = 180$) in contrast to *Z. aff. portoricensis* (94.4%, $n = 163$) and *Z. fairchildiana* (94.7%, $n = 36$). Pairwise means comparisons are given in Table 4.

Caudex diameter differed significantly among species ($P < 0.001$; Fig. 2). Tukey's HSD determined that all pairwise species means were significantly different at the $\alpha = 0.05$ level (Table 4). However, there were no significant differences among substrates across species ($P = 0.10$). There was a significant species $\times$ substrate interaction ($P = 0.005$; Fig. 2).

Substrate type affected number of leaves per seedling ($P < 0.0001$) as did species ($P < 0.0001$). Furthermore, there was a significant interaction between species and substrate ($P < 0.0001$; Fig. 3).

Substrate type also affected length of longest seedling leaf ($P = 0.0093$) as did

Table 4. Significant pairwise least squares means comparisons between species using Tukey's honestly significant difference.^z

	Germination (%)	Caudex diam (mm)	Leaf length (cm)	Taproot length (cm)
<i>Zamia fairchildiana</i>	95% a	23.7 a	27.1 a	20.4 a
<i>Zamia aff. portoricensis</i>	94% a	17.5 b	14.6 b	11.4 b
<i>Zamia cunaria</i>	80% b	11.8 c	8.8 c	9.6 c

^zDifferent letters represent significant differences at the $\alpha = 0.05$ level.

Fig. 2. Means and SDs for *Zamia* seedling caudex diameter for plants grown in three different substrates.

Fig. 3. Means and SDs for number of leaves per *Zamia* seedling for plants grown in three different substrates.

species ($P < 0.0001$). These factors also interacted to affect leaf length ($P < 0.001$; Fig. 4). All pairwise species comparisons were significant for species (Table 4). However, for substrate, the only significant pairwise difference was between cycad mix (mean leaf length = 17.9 cm) and Turface® (mean leaf length = 15.6 cm).

Number of leaflet pairs on the longest seedling leaf was affected by species ($P < 0.0001$) but not by substrate ($P = 0.152$). There was also a significant substrate $\times$ species interaction ($P = 0.0031$; Fig. 5).

Taproot length varied significantly by species ($P < 0.001$; Fig. 6) but not by substrate. All pairwise means comparisons were significant at the $\alpha = 0.05$ level (Table 4).

Discussion

The substrates used in this study contrasted strongly in their properties. The MBC cycad mix contains organic matter and retains moisture well between waterings, although the actual water-holding capacity is less than the other substrates (Table 1). Turface®, in contrast, dries quickly between waterings when used alone despite its relatively high water-holding capacity (Table 1) in contrast to its behavior as an amendment in organic mixes found by Owen et al. (2008) and Bilderback et al. (2005). This is likely the result of the highly porous structure of Turface® and its high proportion of coarse particles (Table 2) when used alone. This

Fig. 4. Means and SDS for length of longest *Zamia* seedling leaf for plants grown in three different substrates.

Fig. 5. Means and SDS for number of leaflet pairs of longest *Zamia* seedling leaf for plants grown in three different substrates.

Fig. 6. Means and SDS for *Zamia* seedling taproot lengths averaged across substrates.

creates a high surface area for evaporation, leading to increased air circulation in the substrate and more rapid drying. Turface® itself is free of organic matter.

Coarse silica sand retains more moisture between waterings than does Turface®, likely as a result of its lower porosity (Table

1) related to its higher proportion of finer particles (Table 2), but dries more quickly than MBC cycad mix despite the higher porosity of the cycad mix (Table 1). However, all substrates were watered according to a schedule designed for MBC cycad mix (three waterings per week, every other day

except during weekends). This may partly account for why the average number of leaves (Fig. 5) per seedling was higher for MBC cycad mix (2.59) than for sand (1.86) or Turface® (2.02). Similar patterns appeared for leaf length (17.37 cm cycad mix, 12.96 cm sand, and 12.85 cm Turface®) and number of leaflet pairs on the largest leaf (3.65 cycad mix, 2.84 sand, and 2.92 Turface®). The high proportion of coarse material in cycad mix (Table 2), which helped create a high amount of air space (Table 1), combined with long water retention was clearly beneficial for the *Zamia* species tested. A substrate that has a high proportion of coarse material but is largely inorganic so that it does not break down over the long production cycles of *Zamia*, would be ideal. However, adjustment should be made to the watering regime to ensure that it does not dry out too quickly as was the case for Turface® in this experiment.

The leaves of *Zamia fairchildiana* seedlings in Turface® were twisted down and the petioles bent. This was possibly the result of the more rapid drying of the Turface® than the sand or cycad mix, causing intermittent water stress. The tendency of Turface® to dry quickly when used as the sole substrate component was observed in a prior experiment with bromeliad cultivation (Sard, 1989). However, the number of leaves, length of the longest leaf, and number of leaflet pairs of *Z. fairchildiana* were greater in Turface® than in the other substrates (Fig. 3), suggesting that intermittent drought stress may stimulate increased leaf production and elongation without much affecting caudex diameter (Fig. 2). This response is surprising because the typical response of drought-tolerant trees to water stress is to allocate more resources to below-ground biomass and less to leaves (Markesteijn and Poorter, 2009). The same is the case for drought-tolerant grasses (Kalapos et al., 1996). Because *Z. fairchildiana* is considered to be a relatively fast-growing rainforest species (Whitelock, 2002), likely with high water requirements, its reaction to the sometimes droughty Turface® substrate seems to have been more marked than the other species. This is the case despite its markedly longer taproot (Fig. 6), that might have been expected to compensate for the droughty conditions by accessing moisture available deeper in the substrate. Overall, the unusual reaction of *Z. fairchildiana* to Turface® seems to have been counterproductive and may have contributed to its pronounced leaf curling. Increased irrigation would be especially crucial for this species when grown in Turface®.

For most growth variables, the three species included in this study reacted differently to the different substrates. *Zamia aff. portoricensis* had larger diameter caudices in Turface® than in the other substrates, whereas the other two species did not (Fig. 2). This may be related to differences in rainfall in their respective habitats with *Z. cunaria* and *Z. fairchildiana* coming from rainforest areas

(Whitelock, 2002) and *Z. aff. portoricensis* from a tropical dry forest habitat (M. Calonje, personal observations, 2008). Because Turface® was the most drought-prone substrate used in the study, *Z. aff. portoricensis* was likely the best adapted to it. The leaf variables bore this out because *Z. aff. portoricensis* had slightly fewer and smaller leaves in Turface® and sand than in cycad mix (Figs. 3, 4, and 5). This shows its ability to plastically reduce its leaf area and increase its caudex size under drought conditions. Allocation of greater resources to underground storage tissue rather than leaf area is one of several important adaptations to drought because this reduces the overall surface area to volume ratio of the plant in addition to reducing exposure to desiccating air and hence reduces water loss (Mauseth, 2000). Such a response is characteristic of drought-tolerant trees and grasses (Kalapos et al., 1996; Markesteijn and Poorter, 2009).

In contrast with *Z. fairchildiana* and *Z. aff. portoricensis*, *Z. cunaria* showed little response to the three substrates (Figs. 2–5). *Zamia cunaria* normally bears only one to three leaves in the wild, whereas *Z. fairchildiana* bears many and *Z. aff. portoricensis* bears two to five per caudex (Whitelock, 2002). Thus, it is not surprising that *Z. cunaria* exhibits little plasticity in response to substrate because it bears only a few leaves even in a lush rainforest habitat, suggesting that its ability to opportunistically take advantage of different conditions is limited. Correspondingly, its ability to plastically reduce its resources in response to drier conditions also appears limited.

It is likely that a more frequent watering schedule or placing a reservoir of water under the inorganic substrates would have improved the growth of *Zamia* in these substrates, especially in Turface®. Turface® can be used successfully to grow very drought-sensitive plants if a water reservoir is provided to prevent drying out (C. Husby, personal observations). Because the substrate is inorganic, anaerobic decomposition of the substrate under saturated conditions does not occur, making a reservoir feasible as in hydroponics. In addition, it is likely that relative behavior of plants growing in the three substrates will vary over time, because the organic components of the MBC cycad mix decompose over time (Bilderback et al.,

2005), whereas the properties of the other two substrates remain constant. This may have implications not only for substrate physical properties, but also for root-rotting fungal pathogens that require high levels of moisture in the substrate. Furthermore, as the seedlings increase in size and in the extent of their root systems, their tolerance of sometimes droughty conditions in Turface® will likely increase, perhaps ameliorating the negative response to Turface® seen in *Zamia fairchildiana* seedlings. Thus, investigation of the responses of larger cycads to the three substrates is an area for future research.

The availability of the substrate components used in this study is likely to be low or nonexistent in most areas where cycads are grown worldwide. However, other inorganic materials such as pumice, perlite, other crushed porous materials, or fired clays may be used as substitutes to create substrates with similarly excellent drainage qualities to the ones used in this study. Coarse sand is likely to be available in many localities. Because cycads often grow on limestone substrates, sand with limestone can also be used with good success for many species. Peat and bark substitutes made from local organic materials (including composts and coconut coir) can substitute for the organic materials used in this study.

Optimizing substrates for cycad propagation is a critical step in ex situ conservation efforts. Predictability of optimal substrate and watering regime relative to native habitat can help increase efficient use of limited resources for these horticulturally unique living fossils.

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