horticulturae

MDPI

Communication

Fresh and Dry Weight Relations Are Predictors of *Cycas micronesica* Seed Age

Thomas E. Marler [1,*] and Christopher A. Shaw [2]

[1] Western Pacific Tropical Research Center, University of Guam, UOG Station, Mangilao, Guam 96923, USA
[2] Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC V5Z 1M9, Canada; cashawlab@gmail.com
* Correspondence: marler.uog@gmail.com

Received: 3 April 2020; Accepted: 12 May 2020; Published: 18 May 2020

Abstract: *Cycas micronesica* is a foundation species in several Micronesian islands and its seeds have been a historical source of starch for the island residents. The species has become endangered by invasive specialist insect herbivores and conservationists struggle with the inability to estimate the age of observed seeds. To inform this agenda, we evaluated numerous *Cycas micronesica* seed traits to determine if any exhibited a relationship with age and a substantial change in absolute value. Of the 30 direct and derived seed traits that we evaluated, most of them were non-linear and exhibited minimal change after about 12 months in age. The only traits that emerged as unambiguous estimators of age were the quotients derived as gametophyte fresh weight/total seed fresh weight and sarcotesta dry weight/sclerotesta dry weight. These two simple metrics can be used to accurately estimate seed age for this arborescent cycad species.

Keywords: cycad; Cycadaceae; ontogeny

1. Introduction

Fruit maturity standards are critical components of various horticulture production systems in order to maximize fresh quality, post-harvest shelf-life or processing success [1,2]. The assessment tools developed for determining fruit ripeness rely on contrasting approaches. For example, rapid chemical tests of internal tissues such as the balance between sugar and acid have been advocated [3]. Alternatively, nondestructive estimations of various traits of external tissues are used where exocarp pigments are remotely sensed as an accurate indicator of ripeness [4,5].

Maturity standards are equally important for understanding gymnosperm seed development as for angiosperm fruit development. However, limitations in knowledge presents challenges for horticulturists and conservationists. Cycads comprise an ancient group of gymnosperms. The tissues of the cycad seed are spatially separable into the sarcotesta, which is the soft external tissues of the integument, the sclerotesta, which is the hard internal tissues of the integument and the gametophyte which develops inside the sclerotesta [6]. The embryo develops inside the gametophyte structure and often matures only after abscission of the seed. Several *Cycas* L. species belong to a complex which produces seeds that float to enable oceanic dispersal [6]. Guam's *Cycas micronesica* K.D. Hill belongs to this complex [7]. The tissues that enable flotation in the seeds of these species are also spatially distinct and develop between the sclerotesta and gametophyte.

The gametophyte of this arborescent cycad species contains copious starch [8] and its use as a source of flour for human consumption is of historical, cultural and medical interest. In previous research, we have studied the changes in gametophyte secondary compounds throughout the *C. micronesica* seed aging process, which revealed that sterols decrease in concentration, increase in total pool and shift to exhibit greater glucoside pools relative to free sterol pools [9–11]. These methods were supported by

repetitive visits to label female trees as megastrobili emerged, such that determination of seed age for each harvest was unambiguous.

Cycad research is often conducted with in situ expeditions [12]. In contrast to ex situ cultivation where managers can used record-keeping to accurately define seed age, the age of seeds observed during the in situ work cannot be determined from a history of record-keeping. The ability to estimate the age of observed seeds would greatly benefit the in situ research efforts [13]. Unfortunately, the maturity of embryos cannot be estimated with external appearance for any cycad species and if harvested prior to embryo maturation the seeds need to be stored for a period prior to planting [14,15]. Horticulturists who lack an understanding of these developmental issues often kill seeds prior to germination if the seeds are planted prior to embryo maturation [15]. Moreover, the continued use of this important source of starch has been plagued by the suspected neurotoxic aspects that accompany ingestion [13,16,17]. Continued toxicology research and a potential resurgence of using this culturally important traditional food would benefit from an unambiguous method for estimating seed age.

Many cycad species are endangered, including our model species *C. micronesica* [18]. For these species, all research and management decisions should support a conservation agenda. To address these issues, we measured numerous *C. micronesica* seed traits to determine their changes with seed age. We probed the response variables for traits that exhibited a linear relationship with seed age such that no transformations of the data were needed to correct a non-linear relationship. Moreover, we screened for traits that exhibited a substantial change in absolute value to increase accuracy of the estimation. We predicted several of the traits would meet these criteria and when used in combination could enable accurate estimation of seed age.

2. Materials and Methods

2.1. Gametophyte Study

We harvested *C. micronesica* seeds from a habitat in west Guam from December 2002 until October 2004 to obtain a range in age of 6 to 26 months. This was accomplished by tagging a population of trees with emerging megastrobili in June and July 2002. The non-native armored scale *Aulacaspis yasumatsui* Takagi invaded the study site in 2005 and prevented our continued use of the tagged plants. The gametophyte was extracted from each seed and immediately weighed to obtain fresh weight. Water displacement in a graduated cylinder was used to measure gametophyte volume. Each gametophyte was dried at 75 °C for 24 h then dry weight was measured. Bulk density was derived by dividing gametophyte volume into dry weight. The quotient dry/fresh weight was also calculated.

2.2. Whole Seed Studies

We harvested *C. micronesica* seeds from a habitat in east Guam from March 2013 until July 2015 to obtain a range in age of 3 to 30 months. The plants were within a large plot in which we protected the trees from *A. yasumatsui* infestations with systemic imidacloprid applications. The root drench applications began in 2007 and continued every 3–4 months until 2015 when funding was terminated. Female trees were tagged as megastrobili emerged from January 2013 until June 2013. The trees were healthy and growing vigorously after 6 years of protection from non-native insect herbivores.

1. Fresh weight relations of seeds were determined by comparing gametophyte weight with whole seed weight. Seed age ranged from 3 to 30 months and included 394 seeds.
2. Dry weight relations of seeds were determined by separating seeds into sarcotesta, sclerotesta and gametophyte tissue. The flotation tissue was included with the sclerotesta category. For older seeds, the embryo was included with the gametophyte category. Seed age ranged from 7 to 24 months and included 60 seeds. To provide a check on the influence of sequentially harvesting seeds of more than one age from the same trees, a population of eight trees were set aside to supply a single seed age.

3. Seed equatorial dimension relationships were determined by cutting each seed in half then measuring the diameter of sarcotesta, sclerotesta, flotation tissue and gametophyte tissue. Total diameter of each category was determined by adding the diameters of each side. Seed age ranged from 4 to 28 months and included 210 seeds.
4. Seed longitudinal dimension relationships were determined with the same seeds by measuring the diameter of sarcotesta, sclerotesta, flotation tissue and gametophyte. Seed age ranged from 4 to 28 months and included 210 seeds.

2.3. Analyses

The seed or gametophyte traits were plotted against seed age to visualize the general shape of the scatter plots. The general linear model (Proc GLM, SAS Institute, Cary, NC, USA) was used to fit linear functions and the non-linear model (PROC NLIN) was used to fit non-linear functions to determine significance and fit of models. For the traits with more than one significant model, the model with the best fit was selected.

3. Results

The *C. micronesica* seed is separated into integuments comprised of diploid maternal tissues, gametophytes comprised of haploid tissues and diploid embryos (Figures 1 and 2). The peripheral portion of the seed is the integument comprised of the soft sarcotesta and the hard sclerotesta. The starch-rich gametophyte develops inside the flotation tissues.

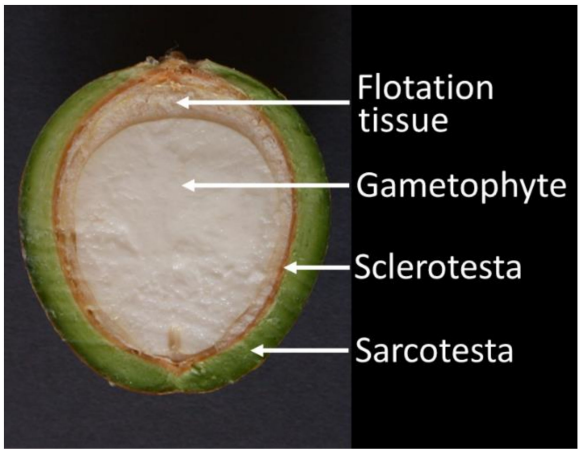

Figure 1. Eleven-month-old *Cycas micronesica* seed revealing internal structure.

The developing *C. micronesica* seed proceeds through a rapid expansion phase with little change in external color for about 6 months. With continued aging, the seed surfaces darken over time until they become bronze by 9 to 12 months in age (Figure 2). Seeds turn brown by 17 to 21 months and remain brown until dispersal, which may not occur until 30+ months. After turning brown, the external sarcotesta tissues begin to shrink and lose a turgid appearance over time.

Internal seed tissue shapes and volumes change dramatically during the first year of seed development (Figure 2). The greatest changes occur for the structures that are enclosed by the hard sclerotesta. Prior to 4 months in age, the dominant tissue is the flotation tissue but the expansion of the gametophyte tissue displaces the flotation tissue and reaches full size at 9–12 months in age. Visible embryo development occurs thereafter (seen in the 24 and 30 month seeds).

Gametophyte weight and volume relationships with seed age were non-linear and were fitted with logistic regression models (Figure 3a). The increase in volume with seed age was less than that for the weights throughout the first half of the study but the shapes of the curves were similar for all three variables for the second half of the study. Bulk density and the quotient dry/fresh weight exhibited segmented models with a linear increase with seed age until about one year, then no further change

until the end of the study (Figure 3b,c). These measured and derived variables were not of value for predicting seed age, especially after one year of age.

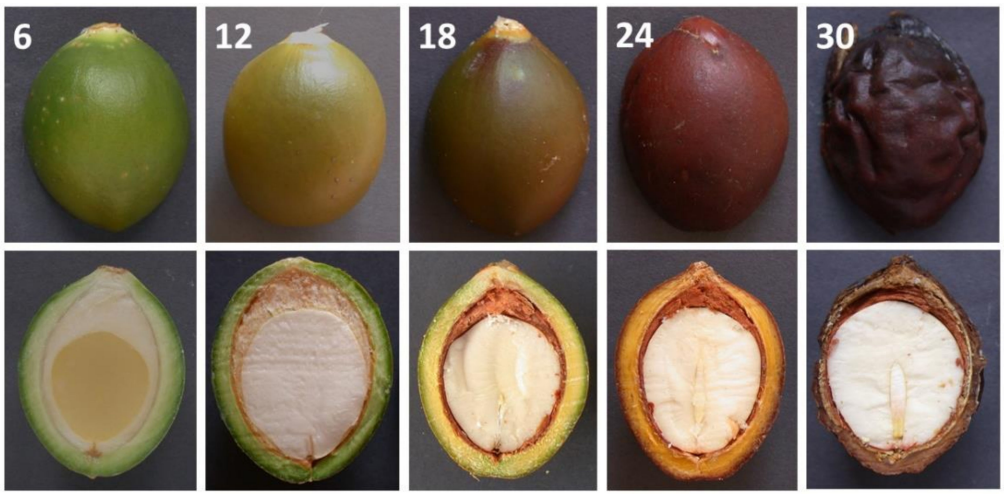

Figure 2. General appearance of *Cycas micronesica* seeds from 6 to 30 months in age (numbers in upper panel).

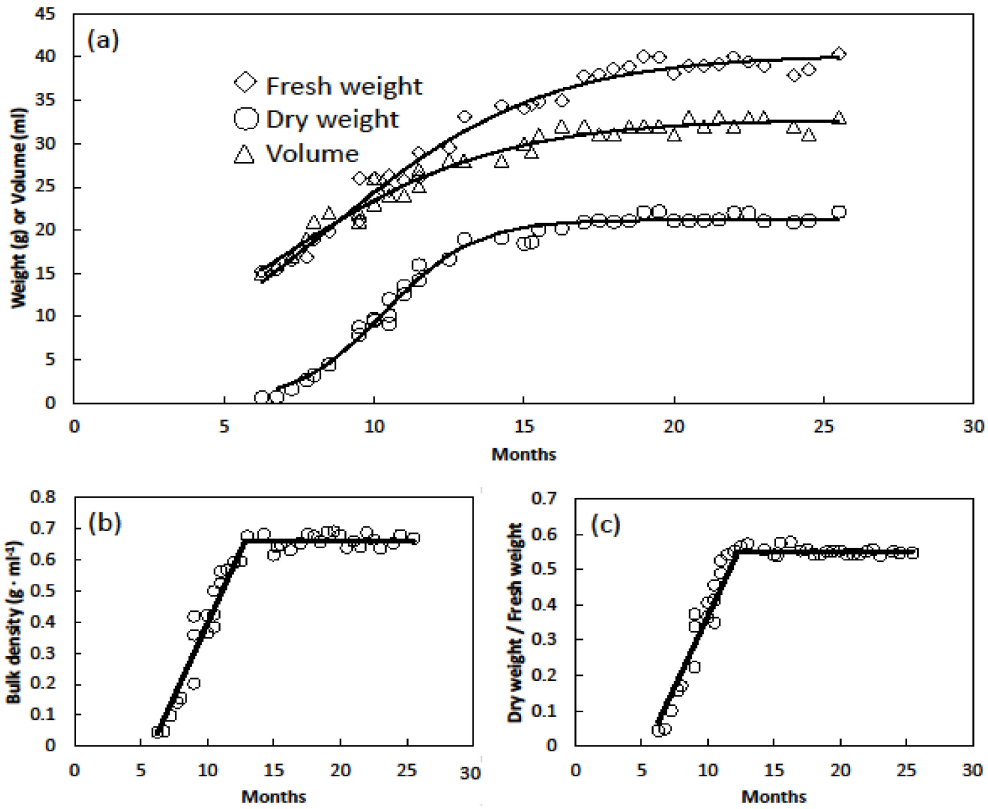

Figure 3. Weight and volume relationships of *Cycas micronesica* seed gametophytes from 6 to 26 months in age. (**a**) Fresh weight: $y = 40.313/(1 + \exp(2.382 - 0.280x))$, $p \leq 0.001$, $r^2 = 0.98$, dry weight: $y = 21.175/(1 + \exp(7.046 - 0.680x))$, $p \leq 0.001$, $r^2 = 0.98$ or volume: $y = 32.856/(1 + \exp(1.848 - 0.276x))$, $p \leq 0.001$, $r^2 = 0.97$; (**b**) Bulk density: if x = 6.2–12.9 then $y = -0.55 + 0.09x$, $p \leq 0.\ 001$, $r^2 = 0.92$, if x = 12.9–25.5 then y = 0.66; (**c**) The quotient dry/fresh weight: if x = 6.2–12.3 then $y = -0.43 + 0.08x$, $p \leq 0.001$, $r^2 = 0.89$, if x = 12.3–25.5 then y = 0.55.

Seed fresh weights exhibited non-linear relationships with seed age. Total seed weight increased rapidly until about one year and declined after about 20 months (Figure 4a). A highly significant quadratic model adequately described the curve, although the model underestimated fresh weight for the oldest seeds. Gametophyte fresh weight increased until about one year, then exhibited little change for the duration of the study. A quadratic model was also adequate for describing this curve. The fresh weight quotient gametophyte/total seed exhibited a highly significant linear relationship with seed age (Figure 4b). The rapid decline in total fresh weight and the meager decline in gametophyte fresh weight indicated a decline in relative water content of integument tissues after about 20 months. The absolute fresh weights were not of value for predicting seed age but the quotient created by dividing whole seed fresh weight into gametophyte fresh weight was valuable as a linear predictor of seed age.

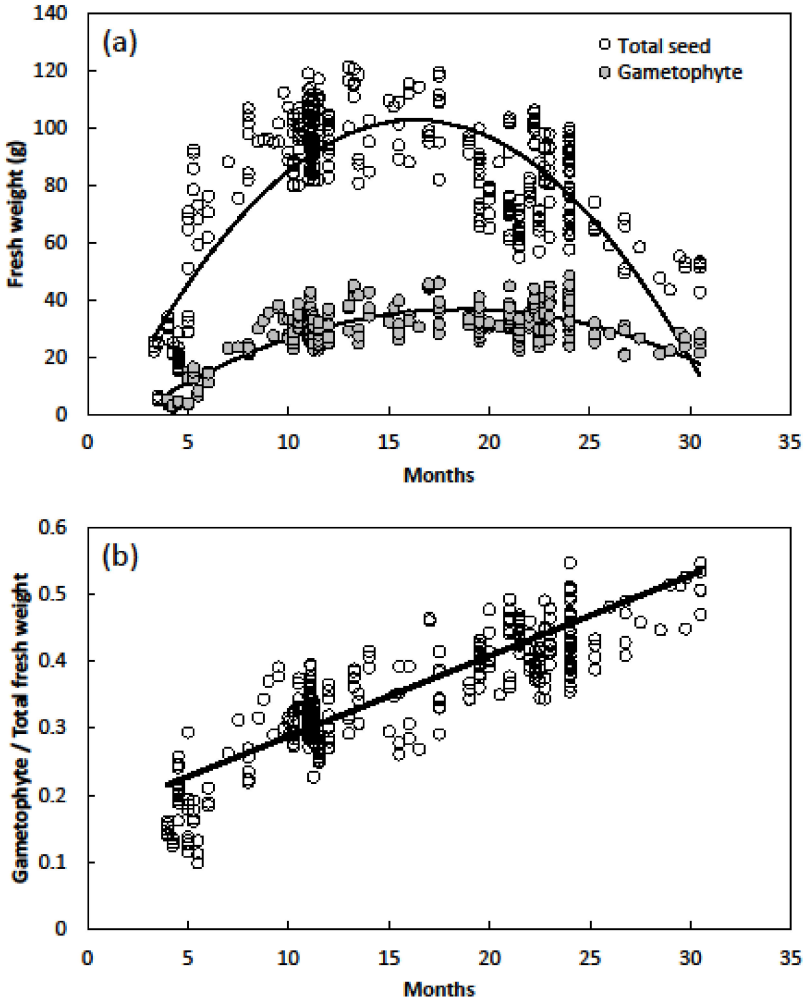

Figure 4. Fresh weight of *Cycas micronesica* seeds from 3 to 30 months in age. (**a**) Total seed weight, open circles: $y = -16.23 + 14.55x - 0.45x^2$, $p \leq 0.001$, $r^2 = 0.73$, gametophyte weight, shaded circles: $y = -10.72 + 5.09x - 0.14x^2$. $p \leq 0.001$, $r^2 = 0.66$; (**b**) The quotient gametophyte/total seed: $y = 0.17 + 0.01x$, $p \leq 0.001$, $r^2 = 0.75$.

Dry weight of the various seed tissue categories exhibited highly contrasting relationships with seed age. A non-linear increase in absolute dry weight of gametophyte tissue contrasted with the linear increase in dry weight of sarcotesta and sclerotesta tissue (Figure 5a). The gametophyte tissue dominated the relative dry weight of these seeds after about one year in age. The eight trees used to supply a single seed age generated data that did not differ from the trends of the other trees. The quotients defined as gametophyte/sarcotesta and gametophyte/sclerotesta exhibited segmented models because gametophyte dry weight was non-linear and the other tissue categories were linear as

seeds increased in age (Figure 5b). In contrast, the quotient defined by sarcotesta/sclerotesta exhibited a linear pattern throughout the entire seed age range (Figure 5b). Most of these dry weight traits of the *C. micronesica* seeds were not of value for predicting seed age but the quotient sarcotesta/sclerotesta was our second linear variable that was valuable for estimating seed age.

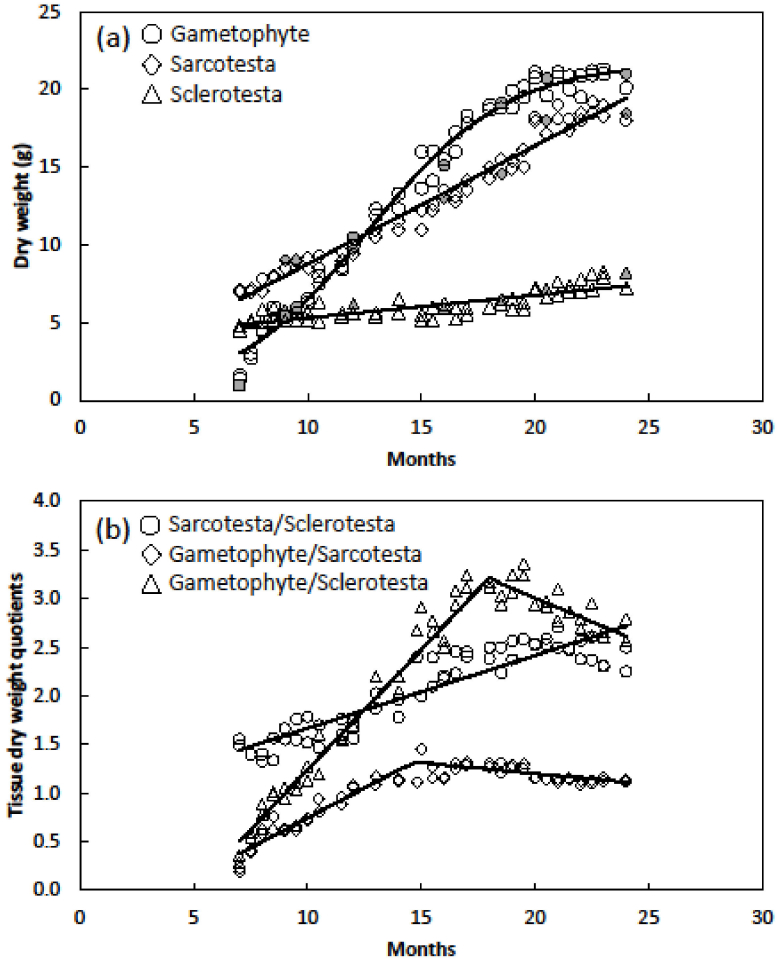

Figure 5. Dry weight of *Cycas micronesica* seeds from 7 to 24 months in age. Open symbols repetitively sampled trees, shaded symbols single harvest trees. (**a**) Absolute dry weight. Gametophyte: $y = 21.770/(1+\exp(4.088-0.324x))$, $p \leq 0.001$, $r^2 = 0.99$, Sarcotesta: $y = 1.15 + 0.76x$, $p \leq 0.001$, $r^2 = 0.97$; Sclerotesta: $y = 3.83 + 0.15x$, $p \leq 0.001$, $r^2 = 0.68$ (**b**) Quotients derived by dividing one tissue into a second tissue. Sarcotesta/Sclerotesta: $y = 0.917 + 0.075x$, $p \leq 0.001$; $r^2 = 0.83$, Gametophyte/Sarcotesta: if x = 7–15 then $y = -0.485 + 0.123x$, $p \leq 0.001$, $r^2 = 0.90$, if x = 15–24 then $y = 1.641–0.022x$, $p \leq 0.001$, $r^2 = 0.68$; Gametophyte/Sclerotesta: if x = 7–18 then $y = -1.228 + 0.247x$, $p \leq 0.001$, $r^2 = 0.97$, if x = 18–24 then $y = 4.966 - 0.098x$, $p \leq 0.001$, $r^2 = 0.77$.

Equatorial diameter of the seed tissue categories exhibited differing relationships with seed age. The absolute diameter of gametophyte tissue increased linearly until about 8 months, then did not change thereafter (Figure 6a). Sarcotesta diameter also increased linearly until about 9 months, then exhibited a slow linear decrease for the duration of the study. The diameter of flotation tissue could not be fitted with any function. It increased sharply until about 5 months, decreased sharply until 7 months, then remained relatively unchanged thereafter. The decrease from 5 to 7 months was due to the displacement by the expanding gametophyte tissue. The diameter of sclerotesta tissue did not exhibit much change in seeds between 3 and 30 months old. The proportions of total seed equatorial diameter occupied by the tissue categories were similar to the absolute diameter measurements for flotation and sclerotesta tissue (Figure 6b). A non-linear increase in relative gametophyte diameter

occurred despite the lack of absolute diameter growth for gametophytes. This increase was caused by a concomitant decrease in absolute and relative sarcotesta diameter. These diameter traits of the equatorial zone of *C. micronesica* seeds were not of value for predicting seed age.

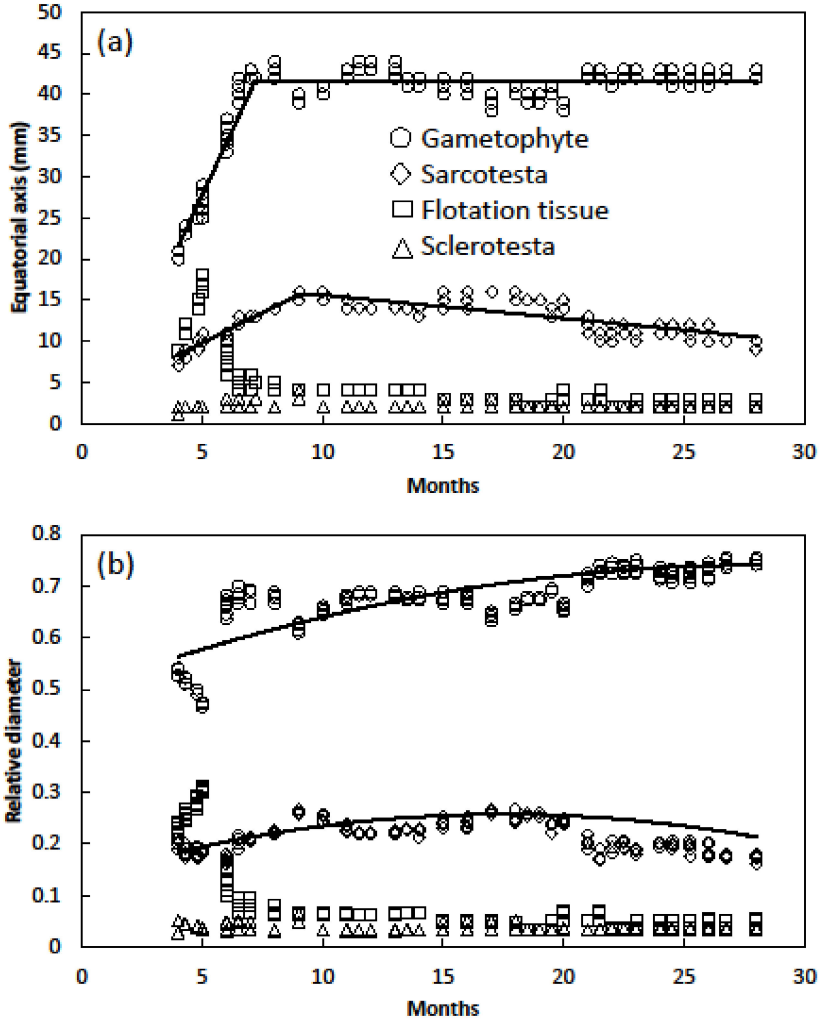

Figure 6. Equatorial diameter of *Cycas micronesica* seed tissue categories from 4 to 28 months in age. (**a**) Absolute diameter. Gametophyte: if x = 4–7 then y = −3.71 + 6.30x, $p \leq 0.001$, $r^2 = 0.96$, if x = 9–28 then y = 41.59; Sarcotesta: if x = 4–9 then y = 2.34 + 1.48x, $p \leq 0.001$, $r^2 = 0.97$, if x = 9–28, then y = 18.47 − 0.29x, $p \leq 0.001$, $r^2 = 0.77$; (**b**) The proportion of equatorial diameter. Gametophyte: $y = 0.50 + 0.02x - 0.0003x^2$, $p \leq 0.001$; $r^2 = 0.62$, Sarcotesta: $y = 0.14 + 0.14x + 0.0004x^2$, $p \leq 0.001$, $r^2 = 0.59$.

Seed longitudinal dimension relationships were highly contrasting among the four tissue categories. Absolute increase in diameter of gametophyte was similar to that of the equatorial measurements but the change was more gradual over time. Therefore, the non-linear function could be fitted with a highly significant logistic regression model (Figure 7a). The changes in flotation tissue diameter were also smooth and gradual for the longitudinal measurements and could be fitted with a quadratic model. In contrast, the sarcotesta dimensions were not smooth. Sarcotesta diameter increased until about 9 months, decreased until about 11 months, then remained relatively unchanged for the duration of the study. Sclerotesta diameter was minimal and stable for the duration of the study. The proportions of total longitudinal diameter comprised by each seed tissue category were similar to the absolute diameter measurements for all four tissue categories (Figure 7b). These non-linear traits of the longitudinal dimensions of *C. micronesica* seeds were not of value for predicting seed age.

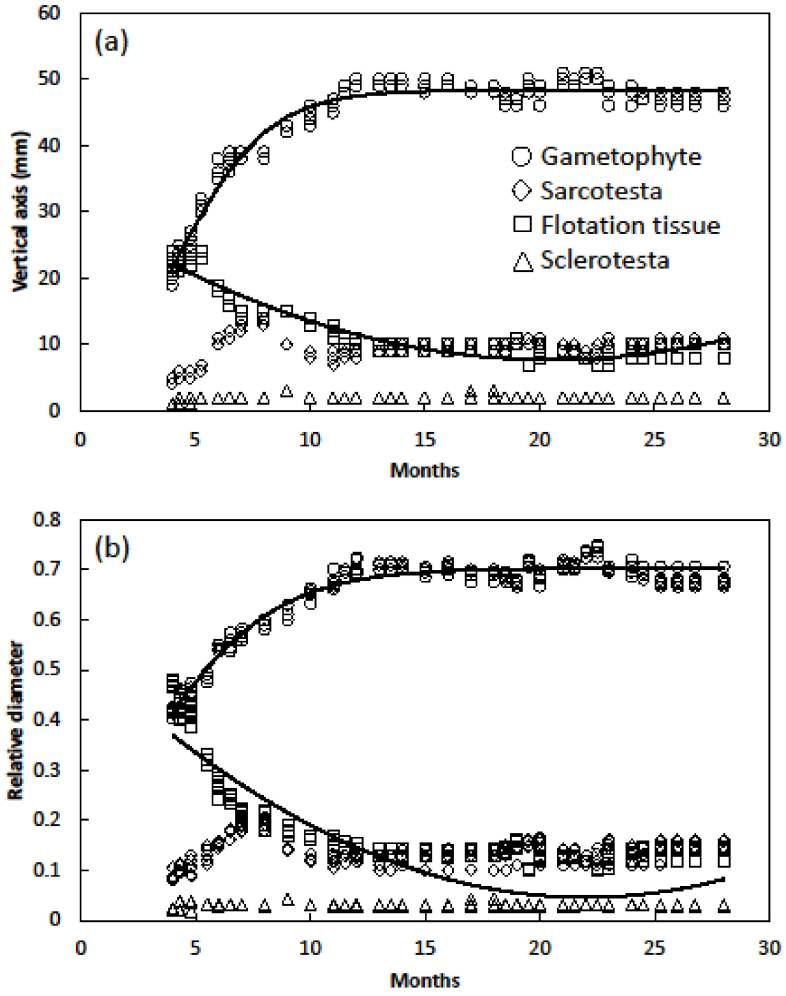

Figure 7. Longitudinal dimensions of *Cycas micronesica* seed tissue categories from 4 to 28 months in age. (**a**) Absolute diameter. Gametophyte: $y = 48.331/(1 + \exp(2.301 - 0.522x))$, $p \leq 0.001$, $r^2 = 0.96$; Flotation tissue: $y = 29.99 - 2.17x + 0.0.05x^2$, $p \leq 0.001$, $r^2 = 0.91$; (**b**) The proportion of longitudinal dimension. Gametophyte: $y = 0.703/(1 + \exp(1.124 - 0.373x))$, $p \leq 0.001$; $r^2 = 0.95$; Flotation tissue: $y = 0.53 - 0.04x + 0.001x^2$, $p \leq 0.001$, $r^2 = 0.83$.

4. Discussion

We assessed 30 direct and derived seed traits for their value in estimating *C. micronesica* seed age. We expected several of these traits to be useful such that they could be combined to accurately estimate seed age. Unfortunately, this detailed approach revealed only two derived traits that accurately estimated seed age with a linear relationship, the dry weight quotient defined by sarcotesta/sclerotesta and the fresh weight quotient defined by gametophyte/total seed. The fresh weight quotient exhibited an amplitude throughout our seed age range of 2.5-fold and a r^2 of 0.75, while the dry weight quotient exhibited less amplitude (1.9-fold) but better fit ($r^2 = 0.83$). Our results indicated these two traits can be used to estimate age of seeds from *C. micronesica* trees, including the period that exhibits brown seeds which can be 17 to 30+ months in age. This new knowledge may improve conservation and research activities involving this endangered species [18].

Cycad horticulturists employ an after-ripening period following most seed harvests to allow the embryo time to become fully mature prior to planting [15]. Several seeds are cut open periodically during this storage period to visually determine extent of embryo development. The addition of our metrics during these sampling procedures would refine the methods by being able to estimate seed age from the fresh and dry weight quotients. This information could be used to determine how long to wait for a subsequent sampling of the seed batch for the purpose of observing embryo maturation.

The ability to estimate seed age is also of crucial importance in cycad seed research [13]. Cultured plants in gardens and ex situ collections managed by professional conservationists can use frequent observations and record-keeping to accurately define seed age. In contrast, tagging female trees with records of megastrobili emergence dates is not possible for in situ research where localities are visited infrequently. Similarly, for some *circa situm* conservation settings, the professional conservationists cannot depend on the laypersons who manage the germplasm to accurately record phenological data. Identifying an accurate method for estimating cycad seed age, such as our method, is of paramount importance for these settings. To our knowledge, this is the first study for any cycad species designed to determine accurate estimators of seed age.

We have demonstrated that *C. micronesica* seed age is closely correlated to the presence of steryl glucosides—the younger the seed, the higher these glucosides [10]. This observation has been related by us and others to the neurological disorder ALS-parkinsonism dementia complex (ALS-PDC) amongst the Chamorro population of Guam who once consumed the gametophytes as a food source [10,11]. For this reason, the potential use of younger seeds by this population prior to the first observations of the disease is of potential relevance.

The increases in sarcotesta and sclerotesta dry weight exhibited linear relationships with age and the sarcotesta dry weight tripled from 7 to 24 months (Figure 5a). We considered these metrics as age estimators; however, they were of limited value because the ultimate mature dry weight of the seed's sarcotesta and sclerotesta would need to be known before being able to accurately apply the age estimation to an immature seed. Indeed, general mature seed size is contrasting among Guam's localities [19].

An unusual behavior of *Cycas* trees is the persistence of ovules that are not fertilized. The timing of pollination based on olfactory signals occurs at about 1 month after megastrobilus emergence for *C. micronesica* [20]. We do not know the timing of fertilization but the increases in seed diameter for pollinated ovules begins at about 2 months after emergence [20]. The naked un-fertilized ovules that do not grow may be retained for the life of the sporophylls that comprise the megastrobilus and can be easily identified by their homogeneous 10–11 mm diameter [21]. Their external color also tracks that of fertilized developing seeds within the same megastrobilus.

We have been most interested in the developmental stage defined by a brown external color. This is the stage at which seeds have been historically harvested for the traditional source of gametophyte starch in the human diet. While our fresh and dry weight metrics are useful for estimating seed age throughout this long period of the seed's ontogeny, the use of contemporary color measurement instruments such as colorimeters and spectrophotometers may also be of value for the same purpose. Further research is required to validate these methods.

The period 8–12 months appears to be an important transition period in *C. micronesica* seed development. Some of our seed traits either declined in growth rate or reached a plateau during these months. For example, gametophyte fresh weight, bulk density or diameter were useless for estimating seed age and any attempt to use them for this purpose would be equivocal. However, the observations indicate that a greater research focus on this age in the ontogeny of *C. micronesica* seeds may provide useful information about the functional aspect of various seed tissues.

The female megastrobilus for members of the Cycadaceae differs from that of the Zamiaceae in that individual sporophylls radiate individually from the stem apex in a loose assemblage [6]. The approach we have pioneered herein can be used with any of the members of the Cycadaceae where naked ovules and seeds are easily observed on the individual sporophylls. A survey of more *Cycas* species will determine if the fresh and dry weight quotients we have correlated with seed age are applicable to the entire family.

Author Contributions: Conceptualization, T.E.M. and C.A.S.; methodology, T.E.M.; software, T.E.M.; formal analysis, T.E.M.; investigation, T.E.M.; resources, T.E.M. and C.A.S.; writing—original draft preparation, T.E.M.; writing—review and editing, C.A.S.; project administration, T.E.M.; funding acquisition, T.E.M. and C.A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by United States Forest Service Cooperative Agreement number 09-DG-11052021-173 and 13-DG-11052021-210.

Acknowledgments: We thank Nirmala Dongol, Gil Cruz and Frankie Matanane for field support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rieger, M. *Introduction to Fruit Crops*; CRC Press: Boca Raton, FL, USA, 2006.
2. Prasad, K.; Jacob, S.; Siddiqui, M.W. Fruit maturity, harvesting, and quality standards. In *Fruit Maturity, Harvesting, and Quality Standards*; Siddiqui, M.W., Ed.; Academic Press: Waltham, MA, USA, 2018; pp. 41–69.
3. Cox, J. *From Vines to Wines*; Storey Books: North Adams, MA, USA, 1999.
4. Gasic, K.; Reighard, G.L.; Windham, J.; Ognjanov, M. Relationships between fruit maturity at harvest and fruit quality in peach. *Acta Hortic.* **2015**, *1084*, 643–648. [CrossRef]
5. Vinson, E.L.; Woods, F.M.; Kemble, J.M.; Perkins-Veazie, P.; Davis, A.; Kessler, J. Use of external indicators to predict maturity of mini-watermelon fruit. *HortScience* **2010**, *45*, 1034–1037. [CrossRef]
6. Norstog, K.J.; Nicholls, T.J. *The Biology of the Cycads*; Cornell University Press: Ithaca, NY, USA, 1997.
7. Hill, K.D. The *Cycas rumphii* complex (Cycadaceae) in New Guinea and the western Pacific. *Aust. Syst. Bot.* **1994**, *7*, 543–567. [CrossRef]
8. Marler, T.E.; Cruz, G.N. Source and sink relations mediate depletion of intrinsic cycad seed carbohydrates by *Aulacaspis yasumatsui* infestation. *HortScience* **2019**, *54*, 1712–1717. [CrossRef]
9. Marler, T.E.; Lee, V.; Shaw, C.A. Spatial variation of steryl glucosides in *Cycas micronesica* plants: Within- and among-plant sampling procedures. *HortScience* **2005**, *40*, 1607–1611. [CrossRef]
10. Marler, T.E.; Lee, V.; Chung, J.; Shaw, C.A. Steryl glucoside concentration declines with *Cycas micronesica* seed age. *Funct. Plant Biol.* **2006**, *33*, 857–862. [CrossRef]
11. Marler, T.E.; Shaw, C.A. Free and glycosylated sterol bioaccumulation in developing *Cycas micronesica* seeds. *Food Chem.* **2009**, *115*, 615–619. [CrossRef] [PubMed]
12. Marler, T.E.; Lindström, A.J. Scouting the Philippines for *Cycas*. *Mem. N. Y. Bot. Gard.* **2018**, *117*, 519–528.
13. Marler, T.E.; Lee, V.; Shaw, C.A. Cycad toxins and neurological diseases in Guam: Defining theoretical and experimental standards for correlating human disease with environmental toxins. *HortScience* **2005**, *40*, 1598–1606. [CrossRef]
14. Whitelock, L.M. *The Cycads*; Timber Press: Portland, OR, USA, 2002.
15. Jones, D.L. *Cycads of the World*; Smithsonian Institution Press: Washington, DC, USA, 1993.
16. Kurland, L.T. Amyotrophic lateral sclerosis and Parkinson's disease complex on Guam linked to an environmental toxin. *Trends Neurosci.* **1988**, *11*, 51–53. [CrossRef]
17. Mabry, T.J. Selected topics from forty years of natural products research: Betalains to flavonoids, antiviral proteins, and neurotoxic nonprotein amino acids. *J. Nat. Prod.* **2001**, *64*, 1596–1604. [CrossRef] [PubMed]
18. Marler, T.; Haynes, J.; Lindstrom, A. *Cycas micronesica*. In *The IUCN Red List of Threatened Species*; IUCN: Gland, Switzerland, 2010. [CrossRef]
19. Cibrian-Jaramillo, A.; Daly, A.C.; Brenner, E.; DeSalle, R.; Marler, T.E. When North and South don't mix: Genetic connectivity of a recently endangered oceanic cycad, *Cycas micronesica*, in Guam using EST-microsatellites. *Mol. Ecol.* **2010**, *19*, 2364–2379. [CrossRef]
20. Marler, T.E.; Dongol, N. Models to describe *Cycas micronesica* leaf and strobili development. *HortScience* **2011**, *46*, 1333–1337. [CrossRef]
21. Marler, T.E.; Niklas, K. Reproductive effort and success of *Cycas micronesica* K.D. Hill are affected by habitat. *Int. J. Plant Sci.* **2011**, *172*, 700–706. [CrossRef]