



Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: <http://www.elsevier.com/locate/jas>

Phytolith radiocarbon dating in archaeological and paleoecological research: a case study of phytoliths from modern Neotropical plants and a review of the previous dating evidence

Dolores R. Piperno ^{a, b, *}^a Smithsonian Tropical Research Institute, Balboa, Panama^b Department of Anthropology, Smithsonian National Museum of Natural History, Washington, DC, USA

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Carbon 14 phytolith dating
Modern plants
Archaeological and paleoecological
phytolith dates

ABSTRACT

Recent carbon-14 studies of phytoliths from modern plants collected from extra-tropical regions of the world have yielded dates that are too old by several hundred to thousands of years. These findings have prompted questions about the suitability of phytolith-derived carbon for dating in archaeological and paleo-environmental research. In this paper, phytolith ¹⁴C ages are determined from a number of modern Neotropical plant taxa collected between 1964 and 2013. The specimens studied are maize (*Zea mays* L.), two squash species (*Cucurbita ecuadorensis* H.C.Cutler & Whitaker and *C. ficifolia* Bouche), and two trees common in Neotropical forest, *Hirtella americana* L. and *Socratea durissima* (Oersted) H. Uendl. They represent families, genera, and species that are well-represented in Neotropical archaeological and paleoecological sediments. Every phytolith sample returned a post-bomb ¹⁴C phytolith age reflecting collection after 1955, with the exception of a herbarium specimen that was treated with chemicals containing radiocarbon-dead carbon. The phytolith dates do not indicate a source of extraneous old or young carbon occurring on the surfaces or inside of phytoliths that bias their ages. Such findings are also reflected by previous phytolith ¹⁴C studies of ancient Neotropical sites. Possible reasons for the differences in results between these and other studies and varying interpretations of soil and sediment phytolith ¹⁴C analyses by different investigators are discussed.

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1. Introduction

¹⁴C dates derived directly from the organic carbon inside of phytoliths recovered from archaeological and paleoecological sites have become an important source of chronological information in Neotropical research. Applications of phytolith dating followed from work originally undertaken in North America that demonstrated the promise of the technique (e.g., Wilding, 1967; Wilding et al., 1967; Kelly et al., 1991; Mulholland and Prior, 1993; see Piperno, 2006 for a review). Results from a variety of Neotropical depositional contexts, including archaeological sites, lake sediments, deep sea cores, and terrestrial soils indicated that phytolith ¹⁴C determinations generally were internally consistent, in stratigraphic order, and in good agreement with ¹⁴C determinations

made on other types of associated materials such as shell and charcoal (e.g., Piperno and Becker, 1996; Piperno and Stothert, 2003; McMichael et al., 2012). No dates that appeared anomalously old were obtained. Where phytolith dates and those on associated material such as from lake and deep sea sediments diverged, the phytolith dates often appeared to be more acceptable when taphonomic and sediment formation/mixing issues along with other age estimates were considered (Piperno, 2006:126–129; further discussion below).

During the past five years, ¹⁴C determinations on phytoliths removed from recently harvested grass samples from North America, France, and Australia have returned inexplicably old ages ranging from about 2000 BP to 8000 BP (Sullivan and Parr, 2008; Santos et al., 2010). Recently harvested Chinese rice and millets also returned phytolith ages from a few hundred to 1000 years too old, depending on combustion temperature (Yin et al., 2014). These findings have led to considerable discussion of whether ¹⁴C phytolith study provides accurate dates and if not, why not (Sullivan

* Smithsonian Tropical Research Institute, Balboa, Panama.
E-mail address: pipernod@si.edu.

and Parr, 2008, 2012; Santos et al., 2010, 2012a,b; Hodson, 2012; Yin et al., 2014). The discourse involves critiques and reviews of phytolith preparation techniques and procedures at radiocarbon facilities currently in practice for phytolith ^{14}C study, evaluating the likelihood they will remove extraneous carbon that may bias phytolith dates (Boaretto, 2009; Santos et al., 2010, 2012a; Corbineau et al., 2013; Sullivan and Parr, 2012; Yin et al., 2014). Some investigators question the original source of phytolith carbon itself, suggesting that rather than being predominantly derived through photosynthesis from atmospheric CO_2 during plant growth, and thus being in equilibrium with the atmospheric ^{14}C content upon plant death as assumed in ^{14}C analysis, older soil organic matter (SOM) has entered plants via the root system and become incorporated onto or within phytoliths, skewing their ages (see discussions in Hodson, 2012; Santos et al., 2010, 2012a,b; Sullivan and Parr, 2012). As mentioned, in the Neotropics ^{14}C dates on phytoliths from various ancient sedimentary contexts have not appeared to have a too-old bias (see review in Piperno, 2006:125–131). This paper further evaluates these issues through the analysis of phytolith age in modern Neotropical crop and other plants with known times of collection during the past 50 years, when the percent modern carbon in the specimens should reflect the onset of thermonuclear testing in the 1950's.

2. Materials and methods

2.1. Modern plants chosen for analysis

The plants chosen for study include crops such as maize and squashes along with important woody taxa of Neotropical forest. The plants are both high phytolith producers and frequent contributors to phytolith assemblages from Neotropical archaeological and paleoecological sites and soil profiles (Table 1) (e.g., Piperno, 2006; McMichael et al., 2012; Piperno et al., in press). All specimens were mature plants and most were collected between the early 1980s and 1995; one was collected in 1964 and another from that same species in 2013. One species, *Cucurbita ecuadorensis*, was a particular focus of one of the author's previous archaeological phytolith ^{14}C (hereafter, $^{14}\text{C}_{\text{phy}}$) efforts, as a consolidated extract of its fruit rind-derived "spherical scalloped" phytoliths isolated from a sediment sample from an archaeological site in Ecuador was dated in order to further establish cultivation of the plant during the early occupation of the site (Piperno and Stothert, 2003). A tropical tree studied, *Hirtella americana*, is often represented in significant frequencies along with other members of its family, the Chrysobalanaceae, in tropical paleo-environmental records constructed from lake and other sedimentary contexts (e.g., Piperno, 2006). Like in *Cucurbita*, these species produce a particular phytolith type (in this case "spherical rugose") derived from the same plant tissue, and thus presumably subject to the same or similar carbon occlusion process during formation. Similarly, palms, represented here by *Socratea durissima*, produce a type of tissue-

specific phytolith that commonly occurs in ancient phytolith assemblages from forest. The typical life span of *H. americana* is a few hundred years while *Socratea* certainly would survive decades. To our knowledge, these are the first modern woody arboreal species providing phytolith dates. All phytoliths studied are solid pieces of silica from cell lumina (insides of cells) and not more loosely silicified products of cell wall silicification, explaining why they can be commonly found in ancient contexts (Piperno, 2006).

2.2. Processing and dating methods

In order to compare the efficacy of different oxidizing agents for organic compound removal in dating, a duplicate phytolith extraction procedure was conducted on some of the specimens, one using sulfuric acid (H_2SO_4) and the other a nitric acid/potassium chlorate mixture ($\text{HNO}_3/\text{KClO}_3$). Plant material was first washed in distilled water and then treated with a 10% solution of HCl to remove carbonates. After washing, either concentrated HNO_3 or H_2SO_4 was added and samples were placed in a boiling water bath. With the HNO_3 treatment pinches of KClO_3 were added to tubes to enhance digestion. The oxidation step occurred over several hours until digestion of plant tissue was complete. The extracted phytoliths were washed in distilled water and then treated again with new $\text{HNO}_3/\text{KClO}_3$ solution or H_2SO_4 for a few more hours to ensure that all organic material was removed from phytolith surfaces. Phytoliths were then washed in distilled water and air-dried.

All samples were dated by AMS. Treatment at the radiocarbon facility included the addition of tin to samples prior to combustion so that upon application of heat, the temperature of combustion would elevate to $1400\text{ }^\circ\text{C}$ – $1500\text{ }^\circ\text{C}$ via the exothermic reaction between tin and oxygen. It has been shown that this temperature completely melts the phytoliths and thoroughly combusts the target organic carbon within them (Piperno and Stothert, 2003).

3. Results

All of the plant specimens with the exception of *H. americana* #1 returned post-bomb ^{14}C determinations as expected, expressed in the unit percent modern carbon (pMC), indicative of their ages of collection after the beginning of thermonuclear bomb testing in the 1950s. *H. americana* #1 provided an anomalously old age of 1570–1420 cal BP. The plant was collected in 1964 at the former Albrook Air Force Base, Panama and shortly afterward was placed in the herbarium of the Smithsonian Tropical Research Institute (STRI) (accession no. 6541), where it was treated with (sprayed and/or dipped in) the then-used pesticides/fungicides lauryl pentachlorophenate and paradichlorobenzene. The two chemicals are chlorinated hydrocarbons and rich sources of radiocarbon-dead carbon from fossil fuels. Naphthalene, another product made from fossil fuels, was also placed around herbarium plants at that time. Leaf phytoliths from another *H. americana* tree (Sample #2) collected in 2013 at Fort Clayton, Panama, located just six km down

Table 1
Passport information on the plants analyzed.

| Plant specimen | Source | Collector | Collection date |
|-------------------------------|-------------------------------|--------------------------|-----------------|
| <i>Zea mays</i> | AASI | — | 1990–91 |
| <i>Cucurbita ecuadorensis</i> | Near Santo Domingo, Ecuador | O. Holm | 1995 |
| <i>Cucurbita ficifolia</i> | Chiriqui, Panama | T. Andres | 1995 |
| <i>Hirtella americana</i> #1 | Fort Clayton, Panama | J. Dwyer | 1964 |
| <i>Hirtella americana</i> #2 | Albrook, Panama | C. Galdames and I. Holst | 2013 |
| <i>Socratea durissima</i> | Barro Colorado Island, Panama | D. Piperno | 1981 |

The maize Race is Harinoso de Ocho (eight-rowed flour corn). AASI is the Agricultural Alumni Seed Improvement Station in Homestead, FL.

the road from where the 1964 sample was taken, returned a post-bomb date as expected. Clayton and Albrook have the same growing conditions (rainfall, temperature, geological substrate, etc.), indicating the dating differences aren't somehow due to environmental variability. The old ^{14}C phy age in sample #1 can then be traced to the herbarium treatment whereby the chemicals saturated plant tissue and phytoliths and were not removed during the phytolith extraction procedure. A special procedure involving a series of treatments with different solvents would have been required to attempt to remove the herbarium substances (Darden Hood, personal communication, 2015). The results on the *H. americana* #1 specimen provide a cautionary note on the use of herbarium samples for dating study.

Although this paper focuses on phytolith dating a few points can be made about the phytolith $^{13}\text{C}/^{12}\text{C}$ ratios (hereafter $\delta^{13}\text{C}$) obtained from the modern plants. Previous research by a number of investigators, the first being Kelly et al. (1991), found that phytoliths from C_4 plants such as grasses, including maize, and sedges are often markedly depleted in $\delta^{13}\text{C}$ compared with values found in whole plant tissue. $\delta^{13}\text{C}$ values from C_3 plants studied to date are marginally depleted (see Piperno, 2006; Carter, 2009; Webb and Longstaffe, 2010 for reviews). The reasons are still unclear, but because it is known that different compounds fractionate carbon differently, it may have to do with the specific types of carbon compounds such as lipids and fatty alcohols that may accumulate in greater quantities in phytoliths than in a homogenized sample of whole plant tissue (Smith and Anderson, 2001; Smith and White, 2004). Some studies have also shown that some temperate zone C_4 grass species are so depleted in $\delta^{13}\text{C}$ as to present confusion with phytolith isotope values from C_3 plants (Webb and Longstaffe, 2010).

In the present study (Table 2), the maize samples that were derived from a combination of glumes and cupules from cobs exhibited $\delta^{13}\text{C}$ values more characteristic of a C_4 species than maize studied previously where leaves and glumes were separately analyzed (Piperno, 2006:133). In the previous work, glume phytoliths returned a $\delta^{13}\text{C}$ of -26.1 compared with -10.9 from whole glumes, and leaf phytoliths returned a $\delta^{13}\text{C}$ of -16.2 compared with -11.3 from whole leaf tissue. Reasons for the present results are unclear but may possibly have to do with a more C_4 -like fractionation being present in cupules than in glumes that moved the values more toward whole plant tissue, and/or $\delta^{13}\text{C}$ variations in response to different growing conditions of the plants analyzed. The isotope values from *Cucurbita ecuadorensis* were characteristic of a C_3 species and similar to results obtained previously from another *Cucurbita* species, *Cucurbita ficifolia*, and other C_3 taxa

(Piperno, 2006:133). With regard to data accumulated thus far from tropical paleoecological sites, there is evidence indicating that deposition of predominantly C_3 or C_4 species is differentiated with phytolith isotope ratios, and that predominantly C_3 grass cover can be discriminated from C_4 grass cover (Piperno, 2006:133–134 and Table 6.2). However, research on additional tropical grass and other C_4 species is needed to ensure their $\delta^{13}\text{C}$ values do not overlap with C_3 species and potentially bias interpretation.

4. Discussion

4.1. Implications for phytolith ^{14}C dating

The anomalously old ^{14}C ages obtained the past few years on phytoliths from recently collected and senesced grass leaves from the mid-western USA, France, and Australia, ranging from about 1800 to 8000 ^{14}C yr. BP (Santos et al., 2010; Sullivan and Parr, 2008), have raised questions about the accuracy of phytolith dating. Santos et al. (2010) also concluded that ^{14}C phy ages obtained from a series of replicated samples of an uppermost soil horizon from Cameroon, Africa, with ranges of from 415 to 430 BP and 1740 to 2760 ^{14}C BP, were somewhat to far older than expected. However, at least some of those dates can be explained by phytolith persistence in soils for considerably longer periods than most other organic-derived matter, as even uppermost soil horizons may contain an unspecified number of phytoliths incorporated over hundreds of years and more (e.g., Fredlund and Tieszen, 1994; Bremond et al., 2004; Piperno, 2006:110; McMichael et al., 2012; Piperno et al., in press). Indeed, it is a complicating factor in the construction of “modern” phytolith assemblages (Piperno, 2006:110). ^{14}C dates on charcoal fragments ranging from 1250 BP to more than 2000 BP from the uppermost 12–20 cm of terrestrial soil in Brazil, Peru, and Ecuador (McMichael et al., 2012; Piperno et al., in press) further support the point that plant remains of that age that can survive for long periods are found in uppermost soil horizons.

Furthermore, the older age range of 1740–2760 BP obtained from the Santos et al. (2010) African soils was determined from the same phytoliths that had originally provided the younger range of 415–430 BP, after those samples were treated further for organic removal by oven baking at 500 °C and another wet oxidation step. Much lower carbon content than before resulted along with melted and possibly carbon-depleted phytoliths (Santos et al., 2010). Although Santos et al. suggested the age range difference related to removal of extraneous younger carbon from phytolith surfaces upon extended oxidation, the effect of the differential processing on the age results is unclear. In any case, as Santos et al. (2012b)

Table 2

Phytolith Carbon-14 dates from modern plants.

| Plant | Part | Processing method | ^{14}C age BP | $^{13}\text{C}/^{12}\text{C}$ ratio | Lab no. |
|-------------------------------|--------------|-------------------|------------------------|-------------------------------------|-------------|
| <i>Zea mays</i> | Cupule/glume | Nitric acid | 103.3 ± 0.3 pMC | −11.3 _{0/00} | Beta-360361 |
| <i>Zea mays</i> | Cupule/glume | Sulfuric acid | 106.7 ± 0.3 pMC | −13.5 _{0/00} | Beta-360362 |
| <i>Cucurbita ecuadorensis</i> | Rind | Sulfuric acid | 114.2 ± 0.3 pMC | −27.0 _{0/00} | Beta-360359 |
| <i>Cucurbita ecuadorensis</i> | Rind | Nitric acid | 114.4 ± 0.4 pMC | −23.2 _{0/00} | Beta-360082 |
| <i>Cucurbita ficifolia</i> | Rind | Nitric acid | 101.8 ± 0.4 pMC | NA | Beta-368019 |
| <i>Hirtella americana</i> #1* | Leaf | Nitric acid | 1640 ± 30 | NA | Beta-360360 |
| <i>Hirtella americana</i> #2 | Leaf | Nitric acid | 100.1 ± 0.4 pMC | NA | Beta-368020 |
| <i>Socratea durissima</i> | Leaf | Nitric acid | 123.1 ± 0.5 pMC | NA | Beta-368023 |

Z. mays and *C. ecuadorensis* specimens processed in sulfuric and nitric acid are from the same plant. *STRI herbarium accession number is 6541; the two-sigma calendar year age for the sample is 1570–1510 and 1430–1420 BP. NA = some samples were too small to provide both ^{14}C results and a $\delta^{13}\text{C}$ on the sample itself. However, a value including both natural and laboratory effects was determined during ^{14}C detection and was used to calculate the true Conventional Radiocarbon age; although this value corrects to the appropriate Conventional Radiocarbon Age, it is not reported since it is not systematic with sample $\delta^{13}\text{C}$ values which do not include laboratory chemical and detector induced fractionation. The bomb curve calibration was not used to directly compare pMC results with the dates of plant collection since local environmental variability, such as fires set annually during the dry season or proximity to urban centers, can cause significant deviation from available bomb curve databases (Darden Hood, personal communication, 2015).

suggest, analyzing phytoliths from modern plants of known age is key to further understanding these issues.

There is little suggestion from the post-bomb dates reported herein from a variety of herbaceous and woody Neotropical plants collected between 1964 and 2013 from a number of different environmental settings that a source of extraneous old carbon either absorbed to phytolith surfaces or occurring inside of them is present that would significantly bias dates. The single anomalously old date from a Neotropical tree collected in 1964 can be explained by the specimen having been treated with substances containing radiocarbon-dead carbon when it was curated in the herbarium. Santos et al. (2010) note that silica surfaces have strong absorptive abilities. The results provide a cautionary note for employing herbarium specimens in radiocarbon study.

A primary question becomes, what is causing the old ^{14}C dates determined from other modern plant material studied (e.g., Sullivan and Parr, 2008; Santos et al., 2010)? Curiously, in Sullivan and Parr (2008), all phytolith dates from sub-layers of the leaf litter directly below living and recently senesced bamboo leaves yielded modern, sometimes post-bomb ages, whereas the living and recently senesced leaves provided ^{14}C ages of 3510 and 1865 BP, respectively. Santos et al. (2010) suggested the problem was a source of old carbon in phytoliths and proposed (Santos et al., 2012a) it was recalcitrant (resistant to chemical or biological degradation) dissolved soil organic matter (SOM) that entered plants via root systems during growth and then preferentially attached to the surfaces of phytoliths or entered them, skewing ages. Sullivan and Parr (2012) remarked that it was unrealistic to expect the living and recently senesced bamboo plants in their study to only have recently begun to take up ancient SOM when this phenomenon, if it occurred, little affected leaf litter phytoliths immediately below them.

With further regard to the Santos et al. (2012a) hypothesis for two different pools of carbon in phytoliths, one from photosynthesis and the other from SOM, with the latter then being sequestered by cells that are subsequently silicified, no evidence or reasons have been offered for how and why SOM would be preferentially lodged inside phytoliths. It seems unlikely and Hodson (2012) further makes the point that because so much more carbon enters plant tissue via photosynthesis than through the xylem (vascular system), SOM would have to be preferentially sequestered inside phytoliths while the abundant photosynthetic source would seemingly be required to be preferentially excluded. Furthermore, if any significant amount of old SOM were to be taken up and then not strongly partitioned inside phytoliths, it would seem that radiocarbon analysis generally would be flawed.

The origin and persistence of SOM around the world is, in itself, a complicated issue. Contributing biota, soil type, root system depth, type of vegetation cover, climate change, and other factors all contribute to the quantity and degradation properties of SOM, which can be derived from a complex mixture of biological products (e.g., Torn, et al., 2009). It has been found that tropical soils typically have faster SOM turnover rates than those in temperate biomes (e.g., Trumbore, 1993). One study showed that the most resistant soil carbon fraction in northeast Brazilian soil was only 100 years old, one-tenth that of temperate soils (Chao and Holm, 1997). Another in the Brazilian Amazon indicated that most organic carbon in the uppermost 22 cm of the soil horizon had a residence time of 10 years or less and the proportion of highly recalcitrant carbon was very minor; temperate zone soils had much longer organic carbon residence times and considerably more recalcitrant carbon (Trumbore, 1993). Therefore, even if recalcitrant SOM turns out to be a cause of some of the too-old dates from modern plants reported in other research, a similar

general phenomenon cannot be assumed to be influencing modern or ancient phytolith assemblages elsewhere in the world. Moreover, as discussed above, the SOM hypothesis seemingly doesn't explain Sullivan and Parr's results on living bamboos vs. their litter.

Most recently, Yin et al. (2014) reported that modern phytoliths from rice and millets increased in ^{14}C age from a few hundred to 1000 years with increasing combustion temperatures (range: 600–1400 °C), with the age offset becoming the greatest at the highest temperature. They also concluded that two sources of carbon were present; however, in contrast to Santos et al. (2012a) both sources were hypothesized to be intrinsic to the plant, deriving from different places in cells. Solid phytoliths derived from cell lumina were considered by Yin et al. to be more resistant to combustion (they also termed this a recalcitrant fraction) and thought to contain mostly lipids and nucleic acids, whereas cell wall silicification, considered to be more labile under heat, was said to contain more mitochondria and plastids. Yin et al. suggested these differences accounted for the age patterns seen, although no data exist on whether lumina-derived phytoliths would provide older ages than those from cell wall silicification. It is noteworthy that all of the phytoliths analyzed in the present work are solid pieces of silica combusted at 1400–1500 °C, factors Yin et al. proposed would in particular make phytolith ages too-old, yet that did not happen. Soil and sediment phytolith assemblages previously dated in the Neotropics, discussed further below, and probably from elsewhere, contain a predominance of solid phytoliths with rare cell wall silicification forms, further indicating that lumina-derived phytoliths should not significantly skew phytolith dates.

Amid these confusing results and competing explanations, it still is unclear if old carbon from any source was possibly bound to phytolith surfaces and differentially removed by the various phytolith extraction procedures utilized by investigators, causing the age variations. Santos et al. (2012a) and Corbineau et al. (2013) reviewed extraction techniques utilized in phytolith stable isotope and ^{14}C study and concluded that most to all were insufficiently rigorous for extraneous carbon removal. Sullivan and Parr (2012) commented their conclusion was speculative, a point to which this author agrees, since Santos et al., did not test every procedure including the one employed herein. Sullivan and Parr also commented that overly rigorous procedures used in Santos et al. (2010) (baking at 500 °C or repeated treatment in HClO_4 and HNO_3 after initial digestion in H_2SO_4 or H_2O_2) could erode phytoliths and cause a leakage or alteration of their carbon; how this would result in skewed ages is unclear. Corbineau et al. (2013) now propose a wet extraction procedure that incorporates an oxidizing step with HNO_3 with HClO_3 used herein and in the author's previous ^{14}C phytolith work (Piperno and Stothert, 2003; Piperno, 2006; McMichael et al., 2012). A HClO_4 and HNO_3 combination used alone has been found by the present author to be an effective oxidizer for plant, soil/sediment, and dating in phytolith research (at times sediment containing high amounts of humic acids also requires treatment with KOH). It appears to adequately remove extraneous carbon from phytolith surfaces without compromising phytolith integrity (e.g., Piperno and Stothert, 2003). Future study will include comparison of the old and new Corbineau et al. (2013) procedure with that used herein.

4.2. A review of previous archaeological and paleoecological dating results

Finally, in light of current discussions regarding ^{14}C research, it is worthwhile to summarize and evaluate here again

Table 3
Phytolith and other dates from Las Vegas culture archaeological sites, Ecuador.

| ¹⁴ C yr. BP | Dendrocalibrated BP | Provenience cm beneath surface or datum |
|---|---------------------------------|---|
| <i>Phytolith dates from Vegas Site OGSE-80, stratigraphic series E–H/8–11</i> | | |
| 7170 ± 60 | 8105–8095 and 8055–7860 | G–H 8–9, 105–110 fs + cs |
| 7960 ± 60 | 9010–8610 | G10–11, 130–140, fs |
| 10,130 ± 40* | 12,120–11,560 | E8–9, 110–120, s |
| 9320 ± 250* | 11210–9900 | E8–9, 110–120, cs |
| 9080 ± 60 | 10,365–10,325 and 10,275–10,170 | E8–9, 110–120, fs + cs |
| 7990 ± 220 | 9480–8370 | E8–9, 110–120, fs |
| 9740 ± 60 | 11,225–11,095 and 10,915–10,895 | F8–9, 110–120, fs + cs |
| <i>Carbon-14 dates on other materials from OGSE-80, stratigraphic series F–H/8–11</i> | | |
| 7850 ± 100 (shell) | 8500–8110 | G–H 8–9, 90–95 |
| 7560 ± 70 (shell) | 8175–7865 | G–H 9, 95–100 |
| 8810 ± 400 (charcoal) | 11,090–8990 | F–H 8–11, 100–140 + G–H 1–5, 90–100 |
| 8580 ± 70 (shell) | 9410–8890 | G–H 8–9, 105–110 |
| 9960 ± 120 (shell) | 11,130–10,330 | G–H 8, 140 |
| <i>Carbon-14 dates on other materials from other areas of the OGSE-80 Midden</i> | | |
| 10,200 ± 100 (shell) | 11,620–10,640 | 100–110 |
| 9010 ± 200 (shell) | 10,290–8980 | EPU |
| 8010 ± 100 (shell) | 8700–8290 | EPU |
| 6700 ± 150 (human bone) | 7820–7310 | Feature 25A |
| <i>Phytolith dates from Vegas Site M5A4-67, unit 1 column sample</i> | | |
| 3810 ± 40 | 4350–4330 and 4300–4090 | 3–10, fs + cs |
| 5900 ± 40 | 6780–6650 | 3–10 ± 20–30, s |
| 5910 ± 40 | 6790–6650 | 20–30, fs + cs |
| 7250 ± 190 | 8400–7760 | 20–30, cs |
| 8070 ± 160 | 9450–8530 | 40–50, fs + cs |
| 8240 ± 170 | 9540–8650 | 50–60 + 60–70, cs |
| 10,820 ± 250 | 13,420–11,950 | 70–80, fs + cs |

See Piperno (2006), Table 6.1a and Stothert et al. (2003) for a complete list of radiocarbon dates on non-phytolith materials from OGSE-80 and other Las Vegas occupations. EPU = excavated by earlier researchers and exact provenience unknown. For phytolith dates: fs = fine silt phytolith fraction of a sediment sample was dated, cs = coarse silt fraction, s = sand fraction was dated. * = a consolidated extract of fruit rind “spherical scalloped” phytoliths from *Cucurbita*, probably *C. ecuadorensis*, from the sand fraction of sediment combined plus an extract from the coarse silt fraction of the same sediment where the remaining *C. ecuadorensis* phytoliths occurred was used to more accurately date the appearance of domesticated-sized *Cucurbita* phytoliths in this sediment sample.

previous ¹⁴Cphy results from Neotropical archaeological and paleoecological contexts, along with a few studies undertaken by investigators in other regions of the world (see Piperno, 2006:125–131 for a more detailed discussion of Neotropical work to that point). Santos et al. (2010) previously reviewed ¹⁴Cphy studies in ancient contexts and seemed to conclude most were problematical for various reasons. In briefly commenting on the present author's ¹⁴Cphy research, Santos et al. (pg. 114) stated that in a study of the Las Vegas culture type-site OGSE 80, southwest Ecuador (Piperno and Stothert, 2003) “... one of the ¹⁴C values was matched with dates obtained on charcoal and shells” (emphasis mine). It is important to put the Las Vegas and other Neotropical dating results not mentioned by Santos et al. into their proper contexts.

Long-term, extensive excavations at Site OGSE-80 provided a robust, well-accepted chronology from ¹⁴C determinations on charcoal, shell, and human skeletons indicating the Vegas-phase occupation occurred from around 10,200 to 6600 ¹⁴C BP (e.g., Stothert, 1985, 2011 Stothert et al., 2003). A series of phytolith ¹⁴C ages were determined on sediments dominated by grass phytoliths with significant contributions from *Cucurbita*, sampled from a large, undisturbed stratigraphic series, E–H/8–11, which had produced many of the charcoal and shell dates (Table 3). The phytolith dates were internally consistent, in good stratigraphic order, and in agreement with the age ranges indicated by the other dated materials for Vegas-phase occupation; no phytolith date was too old (or young) (for further discussion see Piperno, 2006:126–129 and Table 6.1 a,b therein). It can also be noted that a phytolith date from a highly disturbed, mixed area of the site outside of the above mentioned contexts returned an age of 12,130 ¹⁴C BP and was rejected, and that previously from a disturbed area nearby that one,

a too-old charcoal date of 15,850 ¹⁴C BP was also obtained and rejected (Stothert et al., 2003).

At another nearby, contemporaneous Vegas occupation, Site M5A4-67, a series of 10 phytolith dates determined from the bottom to the top of a deep column sample ranged from 10,820 to 3810 ¹⁴C BP and were also in stratigraphic order and agreement with the age of Vegas cultural materials (Table 3) (Piperno and Stothert, 2003; Piperno, 2006:128 and Table 6.1a,b therein). With relation to the Santos et al. (2010) statement that only one phytolith date from OGSE-80 matched associated materials, archaeologists understand that because sedimentary levels of the same thickness may accumulate over varying periods of time, and phytolith deposition takes place over a number of years, it is unrealistic to expect that a shell or piece of charcoal deposited at a single moment in time will with great precision mirror the age of a collection of phytoliths (or other shell and charcoal) from a level of the same/similar thickness or depth from a same/nearby stratigraphic block.

The objectives of the phytolith dating, to examine whether “phytoliths were deposited within time periods bracketed by the shell and charcoal dates” (Piperno, 2006:128), thus adding confidence that early Holocene plant cultivation occurred, were met. Dating phytoliths from different-sized soil fractions of the same sample (fine silt, coarse silt, and sand) also interestingly showed how phytoliths of different sizes could migrate through sediment at somewhat different rates in these compressed deposits, suggesting that to obtain the most precise dates on individual plant taxa of interest, the soil fraction that contains phytoliths of that size should be analyzed (e.g., fine silt for maize phytoliths and coarse silt plus sand for *Cucurbita*). This procedure more accurately dated the appearance of domesticated *Cucurbita*, probably

Table 4
Radiocarbon dates on phytoliths and bulk sediment from paleoecological sites in the Neotropics.

| Site | Depth m b.s. | ¹⁴ C yr. B.P. Phytoliths | δ ¹³ C | ¹⁴ C yr. B.P. Bulk sediment | δ ¹³ C | Lab. No. | |
|------------------|--------------|-------------------------------------|-------------------|--|-------------------|-------------|--|
| El Valle, Panama | 3.0 | 5243 ± 65 | −27.61 | 8330 ± 150 | −26.4 | R26212/1P | |
| | 4.5 | | | 11,360 ± 130 | − | Beta-32800 | |
| | 5.0 | | | 12,100 ± 130 | | Beta-32801 | |
| | 5.45 | | | 14,180 ± 250 | −26.0 | | |
| | 6.4 | 15,470 ± 75 | −27.2 | | | R26267/1P | |
| | 7.6 | | | 19,400 ± 330 | −26.4 | | |
| | 8.2 | | | 31,800 ± 1800 | −28 | | |
| | 10.0 | | | | | R26267/2P | |
| | | 10.6 | | | >35,000 | −27.3 | |
| | | 13.6 | | | 30,700 ± 800 | −26 | |
| ODP Leg 155 | 0.91–0.93 | | | 14,360 ± 60 | −22.6 | Beta-112988 | |
| | 2.61–2.64 | | | 15,700 ± 60 | −26.9 | Beta-112989 | |
| | 6.25–7.27 | 12,902 ± 65 | −27.2 | | | R26212/5 | |
| | 7.92–7.95 | | | 23,580 ± 110 | −28.6 | Beta-112990 | |
| | 28.43–28.46 | | | 34,810 ± 350 | −29.6 | Beta-112991 | |
| | | | | | | | |

Modified from Piperno (2006), Table 6.2. See the original table for reference sources for the El Valle dates without laboratory numbers. In Leg 155, dates of 3970 ± 90 BP from a depth of 0.19 m and 32,730 ± 320 BP from 23.2 m were obtained from planktonic foraminifera.

C. ecuadorensis, phytoliths¹: (see footnote¹ for comments on a study of phytoliths in modern, diseased *Cucurbita* fruits). Moreover, in the upper level of the M5A4-67 column sample where ceramics belonging to a post-Vegas culture known as Valdivia occurred, a phytolith date of 3810 ¹⁴C BP was in accord with ¹⁴C ages previously determined from other materials at Valdivia sites. In short, there is no evidence from the dozens of directly dated phytolith samples from these or other Neotropical archaeological contexts for either a too-old or too-young age skew to phytolith dates.

With regard to paleoecological sequences, phytolith dates were obtained from lake (El Valle, Panama) and deep sea core sediments from off the coast of Brazil (ODP Leg 155), and compared with the chronology constructed from bulk sediment and other material (Table 4) (see also Piperno, 2006:128–129 and Table 6.2 therein; Piperno, 1997). At El Valle, where sediment ¹⁴C dates demonstrated dating inversions, phytolith dates from assemblages dominated by arboreal taxa, including the Chrysobalanaceae, were in stratigraphic order. Some were in good agreement with sediment ages while others were 3000 to 10,000 years younger. It is well-understood that lacustrine and other sediment types are complex mixtures of multiple organic components and open to carbon movement in and out, and for these and other reasons (e.g., bioturbation) questionable dates and dating inversions may occur in any geographic region. This is why investigators may also use other

radiometric techniques such as ²¹⁰Pb, or ¹⁴C date single components of these systems, such as pollen and macrofossils, in order to have multiple independent dating sources to compare and contrast, including from plant fossils directly used to reconstruct paleovegetation. When these more constrained dates do not closely agree with the ¹⁴C age of associated sediment they are usually considered the most reliable.

The deep sea sequence from ODP Leg 155 provides additional information on these aspects (Table 4) (Piperno, 2006:129; Piperno, 1997). Here, sediments were avoided for dating because investigators believed some would be erroneously old due to reworking from incised river channels during low sea level stands. Instead, a paleomagnetic age model anchored by AMS dates on planktonic foraminifera was constructed (Haberle and Maslin, 1999). As a comparability test, sediment samples were dated along with a sediment phytolith assemblage (Table 4). Sediment dates of 14,360 and 15,700 ¹⁴C BP from depths of 0.9–2.6 m each appeared too old by about 9000 years when compared to the age model. In contrast, a phytolith date of 12,900 ¹⁴C BP (15,984–14,493 cal BP) from 6.3 to 7.3 m on an assemblage dominated by arboreal phytoliths including the Chrysobalanaceae was a very good fit with the interpolated date for that depth derived from the age model, about 15,000 cal BP. As with the archaeological sites, there is no indication that phytolith ages are too old (or young), and phytolith utility as another independent dating source in tropical paleoecology appears strong.

It should be noted that the Neotropics is not the only place where successful ¹⁴Cphy results in ancient contexts have been reported. Boyd et al. (2001) and Lentfer and Torrence (in preparation) compared phytolith and charcoal dates from a sediment profile from Garua Island, West New Britain, and found ¹⁴Cphy determinations to be reliable and in good agreement with charcoal dates. Interestingly, ¹⁴Cphy ages initially obtained on samples that were processed and cleaned with 30% hydrogen peroxide (H₂O₂) appeared too young, but additional treatment of the same phytoliths with a stronger oxidation step using chromic acid resulted in older phytolith dates that were valid. The phytolith dates also shed new light on taphonomic and other depositional aspects of the sedimentary sequence. Madella et al. (2014) obtained phytolith dates from a site in the Sudan, Africa that were entirely consistent with ¹⁴C determinations from other site materials, further establishing presence of Near Eastern domesticated cereals along with native wild cereals there 7000 years ago.

Furthermore, it appears that ¹⁴Cphy studies regarded as problematic by Santos et al. (2010, 2012a) due to dating inversions or

¹ Kistler et al., 2013 (pg. 165) stated that phytoliths from the rinds of diseased wild *Cucurbita* fruits (*C. pepo* var. *texana*) were larger than those in non-diseased fruits, such that it “could potentially confound archaeological interpretations of phytolith assemblages”, as in the Piperno and Stothert, 2003 study of *Cucurbita* domestication at the Vegas sites. In fact, the Kistler et al., data show that mean (51.7 μM and 58.5 μM) and maximum phytolith size (about 104 μM) in the diseased fruits are very little different from those in healthy fruits (mean: 53 μM; maximum size about 104 μM). The diseased fruits have sizes that fall squarely in the wild *Cucurbita* category and well below the minimum size required for identification of domesticated species (Piperno and Stothert, 2003; Piperno, 2006). To support their points, Kistler et al., used a G or goodness of fit test of size distribution. Their finding of “significance” of goodness of fit has little value for identifying wild vs. domesticated taxa; it involves a few phytoliths in the diseased population within a particular sub-set of the entire size range that were marginally larger than in healthy fruits, along with a few phytoliths in the diseased population within another sub-set of the entire size range that were that much smaller than in healthy fruits (accounting for the near equivalency in mean and maximum size between the diseased and healthy fruits). The phytoliths from diseased fruits would not be classified as domesticated in archaeological assemblages (even if foragers collected mainly diseased fruits to eat) employing the well-tested and standard size identification criteria (mean and maximum size) used for all *Cucurbita* plant parts in domestication studies (fruits, seeds, phytoliths).

dates they suspected were too old (e.g., Kelly et al., 1991; Rieser et al., 2007; and McClaran and Umlauf, 2000) become credible or less convincingly problematic when taphonomic and other issues are considered. For example, Rieser et al. (2007) after experimenting with and refining extraction procedures, obtained sediment phytolith ages that were in good agreement with the optically stimulated luminescence (OSL) age control used. They concluded that phytolith "... organic carbon is suitable for ^{14}C dating and produces expected carbon ratios, but some researchers report inconsistencies in the method. The most probable cause for inconsistencies and inaccurate phytolith ages is inadequate sample pretreatment". As discussed previously, regardless of the type of material dated, dating inversions and assumed stratigraphic mixing in soil/sedimentary profiles, as reported and discussed in Kelly et al. (1991), are not unusual. Kelly et al. noted that despite the inversions all of the phytoliths dated returned ages covering the Holocene and latest Pleistocene, in accord with the independently estimated age of the deposits themselves, and they presented various lines of evidence for bioturbation and other processes accounting for dating and other interpretative complexities in the soil profiles. Unless and until these phytolith dates are shown to be erroneous due to a factor intrinsic to phytoliths themselves, questions raised about the accuracy of the dates are speculative.

Finally, findings by McClaran and Umlauf (2000) that soil ^{14}C phy ages are considerably greater than dates on directly associated SOM are to be expected given long phytolith residence times. These investigators carefully considered a number of other issues relevant to their studies in the southwest USA, discussing complexities known to adversely affect SOM dating in vegetation history research (e.g., continually incorporated new organic matter including from roots, combined with subsequent mixing and downward movement of SOM, can result in SOM ^{14}C dates considerably more recent than the originally deposited SOM and an age greater than the newest SOM). Based on these and known factors relating to phytolith taxonomy and taphonomy, McClaran and Umlauf's expectations that ^{14}C phy dates should be older than associated SOM and exhibit increasingly older ages at deeper soil depths, and that phytoliths are more sensitive indicators of historical vegetational dynamics than SOM were met. The study also highlighted how combined phytolith/SOM isotopic studies could be of utility in grassland and other vegetation history, and provided suggestions for further study.

5. Conclusions

There are clearly issues in phytolith ^{14}C and stable carbon isotope study that require further study and resolution. Prominently among them at this time are the reason(s) for anomalously old dates derived from modern plants recently studied from some regions of the world. This author suspects, as do other investigators, that at least some of the reported problems have to do with phytolith processing/cleaning procedures utilized and their varying degrees of success in removing extraneous carbon from phytolith surfaces. Where phytolith age is thought to be erroneous for some reason, investigations of different processing techniques and comparisons of results are a first step in sorting out problems. Because soils and sediments from different regions and types of depositional environments have variable organic inputs, there will likely never be a one-size-fits-all phytolith extraction/cleaning procedure for radiocarbon and isotope study. Moreover, phytoliths, like bone, sediment, charcoal, and shell have particular characteristics that require their own processing and pretreatment protocols, and sometimes dating adjustments for local or regional conditions

(e.g., for reservoir effect in marine and fresh water shells). As research progresses from region to region, an increasing number of phytolith dating applications in archaeological and paleo-environmental research can be anticipated.

Acknowledgments

Supported by a Small Grant award from the Smithsonian National Museum of Natural History, Washington and the Smithsonian Tropical Research Institute, Panama. I thank Thomas Hart for the invitation to participate in this special issue.

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