Scanning Microscopy

Volume 6 | Number 4 Article 27

9-28-1992

The Effect of Varying Environmental Conditions on Phytolith Morphometries in Two Species of Grass (Bouteloua curtipendula and Panicum virgatum)

T. B. Ball
Brigham Young University, Provo

J. D. Brotherson
Brigham Young University, Provo

Scanning Microscopy, Vol. 6, No. 4, 1992 (Pages 1163-1181) 0891-7035/92$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

THE EFFECT OF VARYING ENVIRONMENTAL CONDITIONS ON PHYTOLITH MORPHOMETRIES IN TWO SPECIES OF GRASS (*Bouteloua curtipendula* and *Panicum virgatum*)

*T.B. Ball[1] and J.D.Brotherson[2]

[1]Department of Ancient Scripture, [2]Department of Botany and Range Science
Brigham Young University, Provo, UT 84602 USA

(Received for publication April 19, 1992, and in revised form September 28, 1992)

Abstract

Solid deposits of SiO_2 (phytoliths) accumulate in many plants in specific intracellular and extracellular locations. Phytoliths have morphological characteristics unique to some taxa and therefore have taxonomic significance. Phytoliths persist and maintain their morphological integrity long after a plant has died, thus becoming a microfossil of the plant that produced them. Development of phytolith sytematics for microfossil phytoliths has traditionally followed a typological approach based on simple verbal descriptions of shape. A new method for use in phytolith sytematics is the morphometric approach which employs computer-based Image Analysis Systems to make quantified measurements of morphological parameters (size, shape, texture, etc.) which can be used as discriminators between taxa. These parameters, called morphometrics, or morphometries, are potentially important for improved phytolith sytematics. This study evaluates the effect of varying environmental conditions on 18 different phytolith morphometries relative to shape and size as a prerequisite to the further development of a morphometric based phytolith taxonomy. Results indicate that environmental conditions do indeed effect phytolith morphometries for the silica cell phytoliths produced by the two grass species considered in this study. However, the effects are not usually significant ($p \leq 0.05$). Moreover, results of discriminant analyses using the morphometric data obtained indicate that the varying environmental conditions did not hinder the potential of phytolith morphometries to discriminate between plant taxa.

Key Words: Phytoliths, morphometrics, image analysis, archaeobotany, phytolith systematics,silica in plants, computer-assisted microscopy, laser scanning microscopy, opal phytoliths, discriminant analysis.

*Address for correspondence:
Terry B. Ball
303D JSB, Brigham Young University
Provo, UT 84602 USA
Phone No: (801) 378-3357

Introduction

Phytolith Research

Monosilicic acid in the soil, created from the weathering of rocks and the dissolution of biologically deposited SiO_2 is taken up by plant roots. Following up take the acid is transported to various plant organs, where, in many taxa, some of it polymerizes to form solid silica deposits at specific intracellular and extracellular locations (Jones and Handreck, 1967; Raven, 1983; Sangster, 1970). These solid deposits of SiO_2, as well as deposits containing calcium compounds, have been given the name "phytolith", literally meaning "plant-rocks." Many plants produce phytoliths with morphological characteristics that appear unique to a given taxon, a phenomenon giving them taxonomic significance.

There has been considerable interest in phytolith research. Phytolith formation and deposition in various cereal grasses has been well documented (Blackman, 1968, 1969; Blackman and Parry, 1968; Hayward and Parry, 1973; Hodson and Sangster, 1989; Hutton and Norrish, 1974; Jones and Handreck, 1965; Kaufman *et al.*, 1972; Soni and Parry, 1973). The role of phytoliths in plant resistance to disease and insects has been investigated (De Silva and Hillis, 1980; Djamin and Pathak, 1967; Hanifa *et al.*, 1974; Jones and Handreck, 1967; Kunoh and Ishizaki, 1975; Lanning, 1966), as well as the detrimental effects phytoliths have on herbivores and humans (Baker, 1961, Baker *et al.*, 1959; Bezeau *et al.*, 1966; Forman and Sauer, 1962; Harbers *et al.*, 1981; O'Neill *et al.*, 1982; Parry and Hodson, 1982; Bhatt *et al.*, 1984).

Phytolith research has proved highly valuable to archaeobotanists. Because phytoliths are siliceous, when a plant dies, even if it is burned, buried, or ingested, its phytoliths persist and maintain their morphological integrity, becoming a microfossil of that plant. Microfossil phytoliths have been collected by archaeobotanists from such diverse environments as paleosols exposed by erosion or excavation (Piperno, 1983, 1988), ceramics and bricks made from clay upon which vegetation once grew, or to which plant fibers were added (Rands and Bargielski, paper presented at the 1986 meeting of the Society for American Archaeology) tooth tartar and coproliths of herbivores (Bryant, 1974; Armitage, 1975), and the surface of stone tools used to process plants and/or plant parts (Kamminga, 1979; Anderson, 1980).

Once collected and analyzed, microfossil phytoliths can provide researchers with significant information and insights. Microfossil phytoliths have been used for the reconstruction of paleoenvironments (Fisher *et al.,* 1987; Lewis, 1981; Robinson, 1979; Rovner, 1971; Twiss, 1987), as indicators of ancient industrial and agricultural practices (Liebowitz and Folk, 1980; Piperno, 1984; Rosen, 1992; Rosen, in press), and for tracing the origins and developments of cultigens (Piperno, 1988). Rovner (1983) in reviewing the value and advances of phytolith research, suggested that it has the potential to become a second palynology. Pearsall (1989) and Piperno (1988) point out that phytolith analysis is especially valuable to archaeobotanists at sites of study were other plant remains are absent. They further indicate that when phytoliths are used in conjunction with other plant remains, they add precision and support for any interpretations made.

Phytolith Sytematics

Pearsall (1989) indicates that one area of phytolith research especially in need of further development is phytolith systematics. Obviously, if classification keys can be developed such that plant taxa can be identified solely on the basis of their phytoliths, the keys would be exceptionally valuable as research tools.

Typological approach. Advances have been made in developing such taxonomic keys, particularly for short cell or silica cell phytoliths in grasses, i.e., those phytoliths produced in the silica cells located in grass epidermis. These keys generally attempt to use simple phytolith shapes to discriminate between plant taxa. The common shapes of phytoliths found in various taxa are grouped into descriptive classes of morphotypes such as bilobate, saddle, trapezoid, horned tower, etc. The presence and/or frequency of phytolith morphotypes in a plant are then used as discriminating characteristics for taxa identification. Hence, this has been called a typological approach. Examples of "keys" based primarily on a typological approach include Twiss *et al.* (1969) and Brown (1984)(see also Blackman, 1971; Bozarth, 1987; Mulholland and Rapp, 1989; Ollendorf *et al.*, 1988; Parry and Smithson, 1964, 1966; Piperno, 1985; Rapp, 1986; Rovner, 1971). Although such keys have been valuable to phytolith research, development of a typologically based phytolith key that is consistently diagnostic and taxa specific is still pending. Persistent obstacles to developing keys using only typological data are that to the human eye the morphotypes of phytoliths are often subjective, cannot be quantified, and appear to be polymorphic within, and redundant between many plant taxa (Rovner and Russ, 1992). Moreover, typological approaches have not generally been effective in dealing with some phytolith types, such as those produced by interstomal cells, bulliform cells, and sheet elements.

Morphometric approach. An approach that may overcome such obstacles, and which promises to enhance work already under way on phytolith sytematics development, is called the "morphometric" approach. Morphometrics can be defined as the measuring of a feature's morphological parameters (morphometries) such as size, shape, texture, orientation, etc. Rather than relying strictly on phytolith morphotypes as a basis of classification, the morphometric approach to phytolith sytematics tries to discriminate between taxa on the basis of the actual measurement of phytolith morphological parameters.

Possibly the first researchers to successfully use morphometrics in phytolith sytematics were Pearsall (1978), and Piperno (1984). They used the morphometric parameter of width of the short axis of cross-shaped phytoliths as measured with an eyepiece micrometer, in conjunction with the frequency of various morphotypes, to distinguish between phytoliths produced in corn and several wild grasses, including the closely related species, *teosinte*. Russ and Rovner (1987), in attempting to validate the findings of Pearsall and Piperno, greatly expanded the use of morphometrics for phytolith classification when they used a computer-based Image Analyses System (IAS) to calculate several previously unmeasurable morphometric parameters of maize and *teosinte* phytoliths. Computerized systems, like that used by Russ and Rovner, are able to measure accurately up to 30 morphometries of a phytolith, such as area, perimeter, convexity, solidity, volume, formfactor, aspect ratio, compactness, elongation, curl, etc. IAS software make the measurements in a matter of seconds, thus generating tremendous amounts of quantified data in relatively short periods of time. Many of the computer-measured morphological parameters could never be consistently, nor accurately measured without the use of an IAS. In their study, Russ and Rovner (1987), using morphometric data and statistical analyses, were able to distinguish between maize and *teosinte* phytoliths on the basis of several computer-measured morphological parameters. Russ and Rovner's success at using a computer-based IAS for phytolith morphometric analyses suggests a new paradigm for phytolith systematic studies. Using quantified IAS measured morphometric data, in conjunction with traditional taxonomic statistical procedures such as discriminant analysis, it may be possible to develop a more consistently diagnostic, and taxa specific sytematics for phytoliths (Rovner and Russ, 1992). Such is the impetus behind this study.

The Effect of Environment on Phytolith Morphometries

Using the morphometric approach to develop a phytolith taxonomic key will need to begin with the collection of pertinent morphometric data from reference taxa. Prerequisite to collecting valid reference data is an understanding of the effect varying environmental conditions have on phytolith morphometries. If phytolith morphometries are consistent, regardless of the environment in which the taxa producing the phytoliths grow, then researchers can feel confident that valid reference data will be obtained after sampling only a narrow spectrum of individuals. If it is found that the morphometries vary significantly between individuals of the same taxa grown under differing environmental conditions, then preparing reference data will require sampling many individuals from many environmental settings. Of particular concern is the question of whether or not phytolith morphometries for plants grown under environmental conditions which alter a plant's size and/or amount of silica uptake vary significantly. This study evaluates the effect of varying environmental conditions on the morphometries of phytoliths produced in two species of grass, *Panicum virgatum* and *Bouteloua curtipendula*.

Table 1
DESCRIPTION OF THE EIGHT TREATMENTS USED

NUMBER	SOIL	LIGHT	WATER
1	sandy	full	adequate
2	sandy	full	drought
3	sandy	shade	adequate
4	sandy	shade	drought
5	peat	full	adequate
6	peat	full	drought
7	peat	shade	adequate
8	peat	shade	drought

Materials and Methods

One hundred and twenty plant pots for each species of grass were planted, sixty of which contained plain commercial sphagnum peat moss, and sixty of which contained a half-and-half mixture of peat moss and sandy soil (one part fine sand to one part loam). Plants sprouted after four days, and each pot was thinned to one plant per pot. After two weeks, the plants were randomly assigned to a treatment of either adequate watering or drought watering, and to a treatment of either full sunlight, or 60% shade. This yielded eight different treatments for each species with 15 repetitions in each treatment as summarized in Table 1. These treatments were chosen so as to grow plants that would vary significantly in overall size, and silica uptake.

Following ten weeks of growing under the different environmental treatments, data were collected for statistical analysis. The tallest lamina for each plant was measured and recorded as an index of overall plant size. Leaf sections, one cm in length, were cut from the middle-most lamina of the plants, approximately one third of the way down from the leaf apex. These sections were then subjected to Energy Dispersive X-ray Analysis (EDS) on the Scanning Electron Microscope (SEM) to determine the relative amount of silica uptake in the grasses. The sections were prepared for the SEM by washing in distilled water, sonicating in acetone, sonicating again in distilled water and Teepol, and fixing in 2% gluteraldhyde. The sections were then critical point dried, after which each section was mounted on an aluminum SEM stub, and sputter coated with approximately 10 nm of gold. The EDS analyses were performed on the SEM at an accelerating potential of 20 keV, and 100X magnification, for 50 second acquisition time and a count rate of approximately 2000 cps (kept constant by adjusting the spot size). Relative values for the amount of silica accumulation in the lamina samples were obtained by recording the number of silicon X-rays (1.74 keV) counted. For this study, silicon X-ray counts were taken from the adaxial surfaces over the mid-vein on each sample.

Culm and lamina tissue from the plants in each treatment was then randomly harvested and phytoliths were extracted to be used in gathering the morphometric data. The tissue was processed for phytolith extraction following a modified version of the procedures of Kaplan and Smith (unpublished manuscript distributed at 1980 meeting of the Society for American Archaeology) as follows.

a). Plant tissue from which phytoliths were to be extracted was chopped, placed in a clean beaker, and sonicated for 10 minutes in distilled water containing a drop of Teepol which was added as a detergent and surfactant. The tissue was rinsed several times in distilled water and dried by placing the beakers in a drying oven over night at 60° C. Chromic acid was then added to the beakers at a ratio of about 40ml of acid to .5gm of dried tissue to digest the organics of the tissue, and thus yield a suspension of extracted phytoliths. The digestion reaction was hastened by heating the tissue/acid mixture at low heat for 20 minutes under a fumehood. The chromic acid was prepared by dissolving, in a 4000 ml flask, 240 gms of sodium dichromate in 2000 ml of water, and then slowly adding 1200 ml of concentrated sulfuric acid while swirling the flask in a cold water bath.

b). Following digestion, the phytoliths and acid were separated by centrifugation in 15 ml centrifuge tubes in a swinging bucket head at 1750 rpm for three to five minutes. The supernatant was removed with a pipette and discarded. The precipitate was then resuspended in distilled water, and again separated by centrifugation. Resuspension and centrifugation was repeated three times in distilled water, followed by three times in 100% ethanol, three times in 100% acetone, and then three times in 100% benzene.

c). After removing the final benzene supernatant the precipitated phytoliths were stained in suspension by adding first 0.1% solution of crystal violet lactone in benzene, followed by a few drops of a benzene saturated solution of methyl red (Dayanandan *et al.*, 1983).

d). Slides for light and laser scanning microscopy were prepared by shaking the phytolith/stain combination to resuspend the phytoliths, and then using a pipette, placing a few drops of the suspension on a clean slide and allowing to air dry. A cover slip was then mounted over the assemblage using Permount.

e). Samples for electron microscopy were prepared by placing drops of the phytolith suspension on clean cover slips that were adhered to stubs using double stick tape. After air drying, the stubs were sputter coated with 10 nm of gold, and then the cover slip grounded to the stub using silver paint.

Images of the extracted phytoliths were obtained for analysis by using transmitted laser light on a Zeiss laser scanning microscope. The images were recorded on video tape in 10 second segments via a video tape recorder connected to the scopes video output. The analyses of phytolith images were made using an Apple MacIntosh IIci computer and the "Prism" IAS software distributed by Dapple. The phytolith images recorded on the reference video tape were digitized into a computer image using the Data

Table 2
DESCRIPTION OF THE MORPHOMETRIC PARAMETERS MEASURED

Type	Morphometri	DESCRIPTION
SIZE	Area	Simple area of the feature.
	Convex Area	Area within a taut-string around the feature.
	Perimeter	Length of the feature boundary.
	Convex Perimeter	Length of a taut-string around the feature.
	Length**	Longest cord within the feature.
	Breadth	Minimum caliper diameter of the feature.
	Fiber Length	Length of the feature along its medial axis.
	Width	The minor dimension of the feature.
	Equivalent Diameter	Diameter of a circle with the same area as the feature.
	Inscribed Radius	Radius of largest circle that can be drawn in the feature.
SHAPE	Formfactor	Equals 4 x Area x pi/Perimeter^2, it is 1.0 for a perfect circle and diminishes for irregular shapes.
	Roundness	Equals 4 x Area/pi x Length^2, it is 1.0 for a perfect circle and diminishes with elongation of the feature.
	Convexity	Ratio of Convex Perimeter to Perimeter, it is 1.0 for a perfectly convex shape, diminishes if there are surface indentations.
	Solidity	Ratio of Area to Convex Area, it is 1.0 for a perfectly convex shape, diminishes if there are surface indentations.
	Compactness	Ratio of the Equivalent Diameter to the Length.
	Aspect Ratio	Equals Length/Width.
	Elongation	Equals Fiber Length/Width.
	Curl	Equals Length/Fiber Length.

** Note that the length as measured by IAS is not the same as length measured optically.

Translation DT-2255 frame grabber board. Measurements of the parameters of concern were made on sample populations of 75 phytoliths of each morphotype from plants grown in each treatment. Eighteen morphometric parameters were evaluated (Table 2).

Statistical analysis began with Tukey HSD comparison tests on plant height and silica uptake data obtained for plants grown in each environment to verify that the eight treatments used did indeed result in plant populations that differed significantly in their mean size and silica accumulation. All significance levels were determined at $p \leq 0.05$, i.e. the 95% confidence level. Next Tukey HSD comparisons were further used to test for significant differences in treatment means for each of the 18 morphometric parameters considered. Multiple regression tests of the morphometric data means on the means of the silica uptake and height data in each treatment were then conducted to evaluate for significant correlations using the following model:

(1) Morphometric Parameter = Constant + Plant height + Silica Uptake

Finally, a discriminant analysis using the 18 morphometric parameters of concern was performed on a sample population of 50 phytoliths randomly selected from all the treatments for each species, and a third species, *Zea mays,* which among others, produces bilobate and cross-body phytoliths very similar to *P. virgatum*, to see if the varying environmental conditions adversely affected the ability of the morphometric data to discriminate between similar shaped phytoliths produced by different species. The following model was used:

(2) 18 Morphometric Parameters = Constant + Species

All statistical tests were performed using Systat statistical software manufactured by Systat, Inc.

Results

All of the silica-cell phytoliths in *B. curtipendula* were of the morphotype traditionally described as "saddle-shaped" (Figs. 1-3). In *P. virgatum* four morphotypes were evident: cross-body, bilobate, trilobate, and bi-trilobate (Figs. 4-9). In *Zea mays* cross-body and bilobate phytoliths were found (Figs. 10 and 11). In this study, the morphometries of each of these morphotypes were considered separately, with one exception. Because there were many intermediate forms between cross-body and bilobate types (Figs. 12-13), more often than not making classification a subjective matter, all cross-bodies were lumped together with the bilobate phytoliths. Although the means of the measurements made for each morphotype in *P. virgatum* varied, the overall effect of the different environmental treatments on each of the four phytolith morphometries were virtually identical, and therefore, only the bilobate data is reported in this paper.

After performing initial statistical tests, it was noted that the morphometric data obtained were not normally distributed, and did not demonstrate homogenous variance between populations. This caused us to be concerned about the validity of using multiple comparison tests on population means, like the Tukey HSD, that were designed for parametric data. Because the size of the sample populations were relatively large, the means were expected to still be well behaved, minimizing the concern of non-normal distributions. Log and square root transformations of the data were performed in order to obtain homogenous variances between populations, and all the tests were again performed on the transformed data. The results of the tests on the transformed data matched

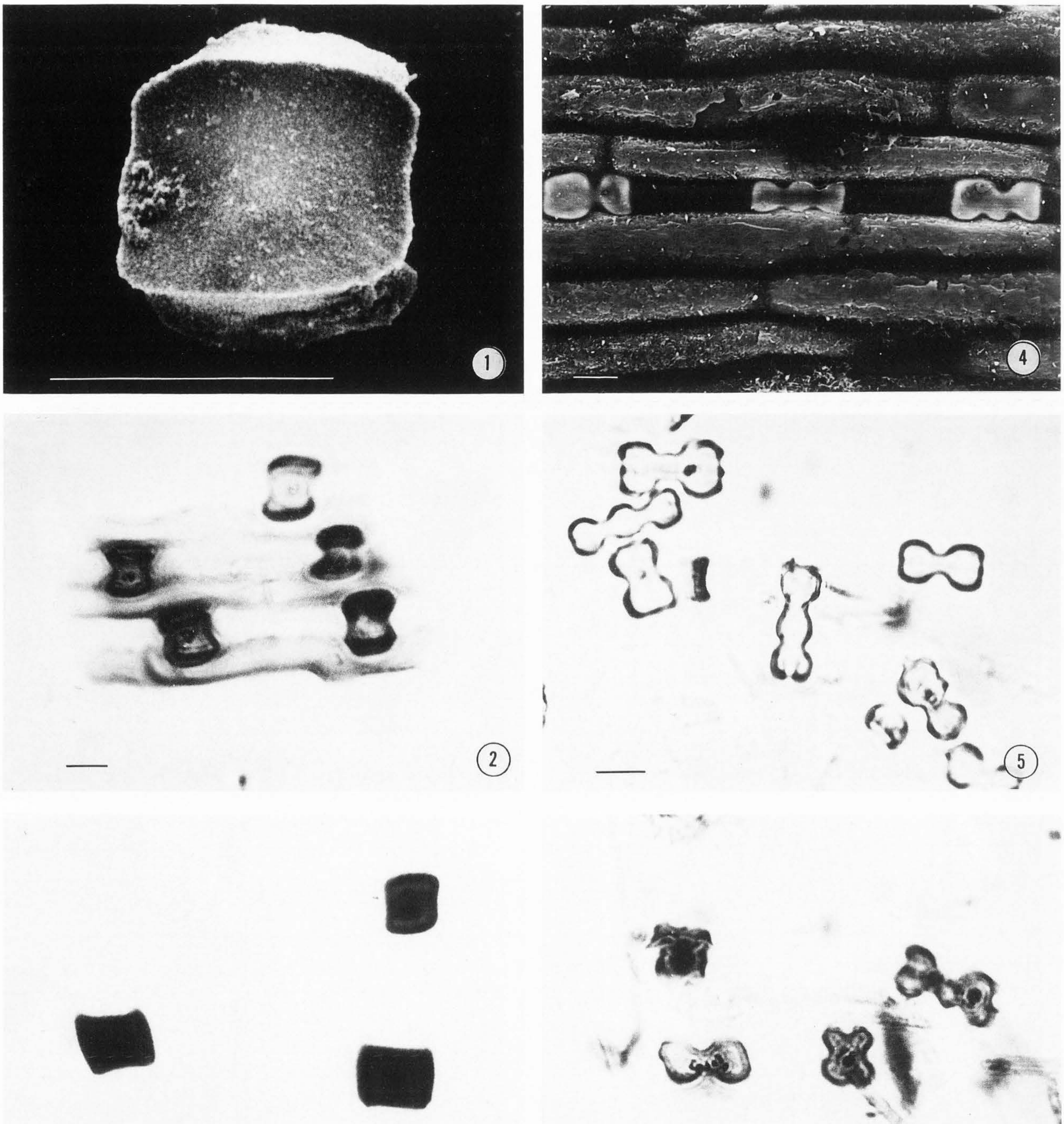

Fig. 1. Scanning Electron micrograph of saddle-shaped phytolith morphotype from *B. curtipendula*. Fig. 2. Light micrograph of silica skeleton from *B. curtipendula* illustrating the *in situ* location of saddle-shaped phytoliths. Fig. 3. Transmitted Laser Scanning micrograph of stained extracted saddle-shaped phytoliths from *B. curtipendula*. The excellent contrast and sharp edges created by this type of light facilitates the computer analysis of the image. Fig. 4. Backscattered Scanning Electron micrograph of *in situ* phytoliths from *P. virgatum* illustrating from left to right three morphotypes:bilobate, trilobate, bi-trilobate. Fig. 5. Light micrograph of extracted, unstained phytoliths from *P. virgatum*. Fig. 6. Light micrograph of extracted, stained phytoliths from *P. virgatum*. Bar = 10 μm.

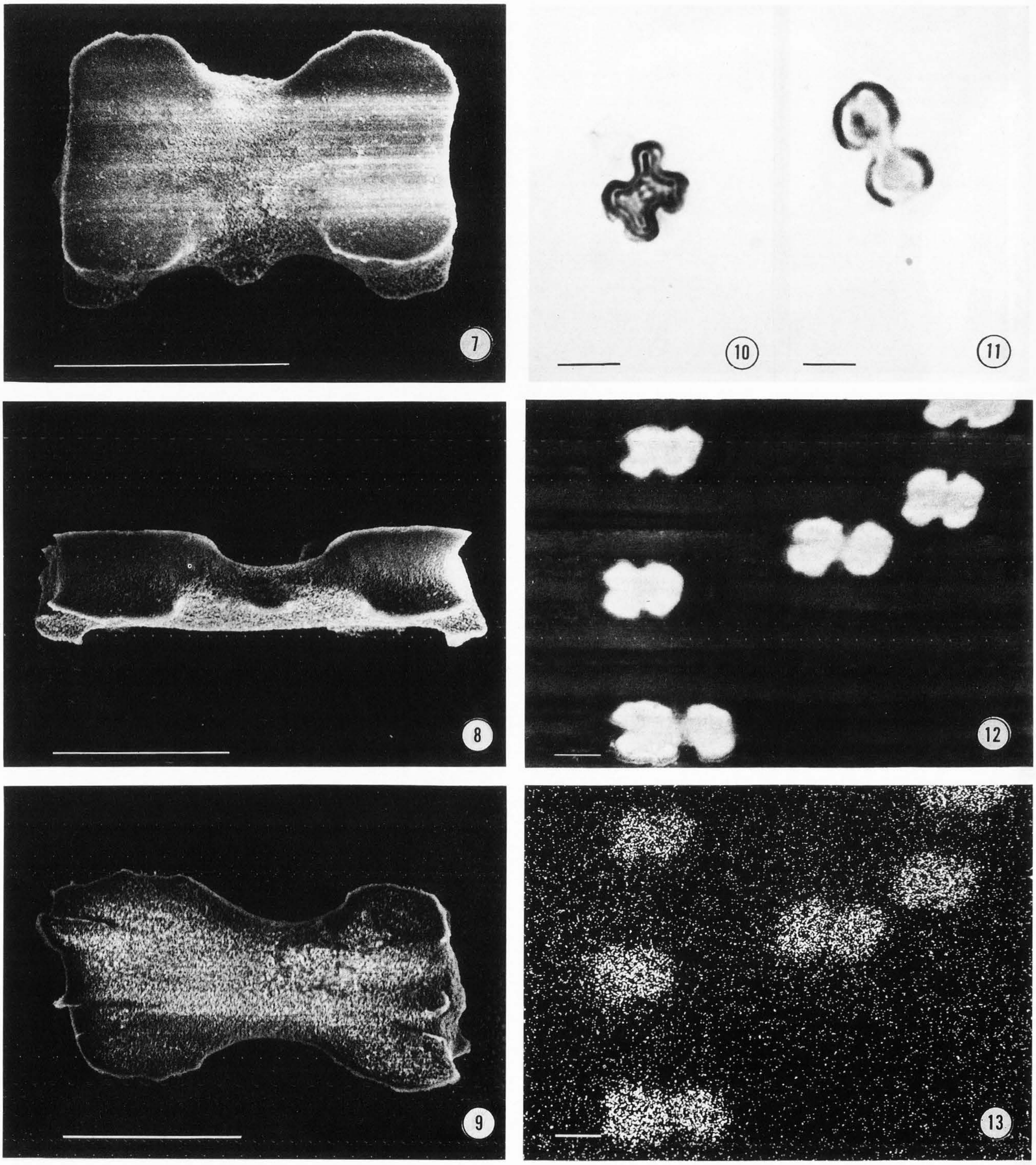

Fig. 7. Scanning Electron micrograph of bilobate phytolith from *P. virgatum*. Top view. Fig. 8. Scanning Electron micrograph of bilobate phytolith from *P. virgatum*. Side view. Fig. 9. Scanning Electron micrograph of bilobate phytolith from *P. virgatum*. Bottom view. Fig. 10. Light micrograph of cross-body phytolith from *Z. mays*. Fig. 11. Light micrograph of bilobate phytolith from *Z. mays*. Fig. 12. Backscattered Scanning Electron micrograph of *in situ* bilobate/cross-body phytoliths illustrating intermediate forms found in *P. virgatum*. The backscattered electron imaging causes the *in situ* phytoliths to stand out with excellent contrast. Fig. 13. X-ray dot map of silica location in above micrograph of *in situ* phytoliths from *P. virgatum*.
Bar = 10 μm.

those on the original tests at the 95% confidence level. Because non-transformed data are generally more meaningful, we have chosen to report them in this paper rather than the transformed data.

Effects of the treatments on plant size and silica accumulation

Results of the Tukey HSD comparison tests are summarized in Table 3 for *B. curtipendula* and Table 4 for *P. virgatum*. The tests indicate that the treatments were effective in producing plant populations that varied significantly in size (height) and amount of silica accumulation.

B. curtipendula. Soil type appears to have created the most significant differences between treatments in *B. curtipendula*. All of the means for plants grown in sandy soil treatments were significantly larger than peat soil treatment plants for both height and amount of silica accumulation. In fact all plants grown within peat soil treatments performed so poorly that none of the within peat soil treatment means for height and silica accumulation were significantly different from each other regardless of the light or water treatment applied. Such was not the case for plants grown in sandy soil.

Table 3

RESULTS OF TUKEY HSD TESTS

(Means underlined with "******" are not significantly different from each other at p ≤ .05)

Species: *Bouteloua curtipendula*

Measurement: Plant Height (cm)

Treatment	6	7	8	5	2	1	4	3
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	full	shade	shade	full	full	full	shade	shade
Water	drought	adequate	drought	adequate	drought	adequate	drought	adequate
MEANS	5.01	5.88	7.15	7.46	20.31	30.41	32.09	39.66
	******	******	******	******		******	******	

Measurement: Plant Silica Uptake (total counts)

Treatment	7	6	5	8	4	2	1	3
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	full	full	shade	shade	full	full	shade
Water	adequate	drought	adequate	drought	drought	drought	adequate	adequate
MEANS	430.0	495.9	507.0	572.7	3470.3	3608.8	4249.7	4673.4
	******	******	******	******	******	******		

Measurement: Area (μm^2)

Treatment	7	8	5	6	4	3	1	2
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	adequate	drought
MEANS	89.69	94.03	100.35	104.64	112.49	119.85	126.21	128.38
					******	******	******	******
			******	******	******	******		
	******	******	******	******	******			

Measurement: Convex Area (μm^2)

Treatment	7	8	5	6	4	3	2	1
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	drought	adequate
MEANS	95.10	103.40	107.15	116.20	120.95	129.25	135.36	138.58
					******	******	******	******
				******	******	******	******	
		******	******	******	******			
	******	******	******					

Measurement: Perimeter (μm)

Treatment	7	5	8	6	4	3	2	1
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	full	shade	full	shade	shade	full	full
Water	adequate	adequate	drought	drought	drought	adequate	drought	adequate
MEANS	39.03	41.53	41.77	43.98	44.17	45.69	46.32	48.32
						******	******	******
				******	******	******	******	
			******	******	******	******		
		******	******	******	******			
	******	******	******					

Table 3 continued

Measurement:	Convex Perimeter (μm)							
Treatment	7	8	5	6	4	3	2	1
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	drought	adequate
MEANS	36.21	38.15	38.47	39.94	40.89	42.32	43.08	43.52
					*****	*****	*****	*****
				*****	*****	*****	*****	
		*****	*****	*****	*****			
	*****	*****	*****					

Measurement:	Length (μm)							
Treatment	7	8	5	6	4	3	2	1
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	drought	adequate
MEANS	13.42	14.09	14.25	14.74	15.12	15.71	15.96	16.04
				*****	*****	*****	*****	*****
		*****	*****	*****	*****			
	*****	*****	*****	*****				

Measurement:	Breadth (μm)							
Treatment	7	8	5	6	4	3	2	1
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	drought	adequate
MEANS	9.52	10.18	10.22	10.63	10.98	11.08	11.42	11.17
					*****	*****	*****	*****
				*****	*****	*****	*****	
		*****	*****	*****	*****	*****		
	*****	*****	*****					

Measurement:	Fiber Length (μm)							
Treatment	7	5	8	4	6	2	3	1
Soil	peat	peat	peat	sand	peat	sand	sand	sand
Light	shade	full	shade	shade	full	full	shade	full
Water	adequate	adequate	drought	drought	drought	drought	adequate	adequate
MEANS	15.03	16.04	16.46	17.10	17.43	17.77	17.78	19.03
					*****	*****	*****	*****
			*****	*****	*****	*****	*****	
		*****	*****	*****	*****			
	*****	*****	*****					

Measurement:	Width (μm)							
Treatment	7	8	6	5	4	3	1	2
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	drought	adequate	drought	adequate	adequate	drought
MEANS	8.32	8.35	8.67	8.78	9.29	9.38	9.76	10.00
					*****	*****	*****	*****
				*****	*****	*****		
	*****	*****	*****	*****				

Measurement:	Equivalent Diameter (μm)							
Treatment	7	8	5	6	4	3	1	2
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	adequate	drought
MEANS	10.54	10.83	11.17	11.28	11.83	12.12	12.49	12.61
					*****	*****	*****	*****
			*****	*****	*****	*****		
		*****	*****	*****	*****			
	*****	*****	*****	*****				

Table 3 continued

Measurement: Inscribed Radius (μm)

Treatment	7	8	5	6	4	3	1	2
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	shade	full	full	shade	shade	full	full
Water	adequate	drought	adequate	drought	drought	adequate	adequate	drought
MEANS	4.41	4.48	4.62	4.58	4.94	5.01	5.04	5.28
					*****	*****	*****	*****
			*****	*****	*****	*****	*****	
		*****	*****	*****	*****			
	*****	*****	*****	*****				

Measurement: Formfactor

Treatment	6	8	1	3	4	5	7	2
Soil	peat	peat	sand	sand	sand	peat	peat	sand
Light	full	shade	full	shade	shade	full	shade	full
Water	drought	drought	adequate	drought	drought	adequate	adequate	drought
MEANS	.654	.669	.679	.698	.712	.715	.725	.735
					*****	*****	*****	*****
				*****	*****	*****	*****	
			*****	*****	*****			
		*****	*****	*****				
	*****	*****	*****					

Measurement: Roundness

Treatment	6	8	3	1	4	5	7	2
Soil	peat	peat	sand	sand	sand	peat	peat	sand
Light	full	shade	shade	full	shade	full	shade	full
Water	drought	drought	adequate	adequate	drought	adequate	adequate	drought
MEANS	.590	.596	.600	.610	.618	.618	.622	.630
		*****	*****	*****	*****	*****	*****	*****
	*****	*****	*****	*****	*****	*****	*****	

Measurement: Convexity

Treatment	6	1	8	5	3	4	7	2
Soil	peat	sand	sand	peat	peat	sand	peat	sand
Light	full	full	shade	full	shade	shade	shade	full
Water	drought	adequate	drought	adequate	adequate	drought	adequate	drought
MEANS	.909	.910	.914	.926	.927	.927	.929	.931
				*****	*****	*****	*****	*****
			*****	*****	*****	*****		
	*****	*****	*****					

Measurement: Solidity

Treatment	6	1	8	3	4	5	7	2
Soil	peat	sand	sand	peat	sand	peat	peat	sand
Light	full	full	shade	full	shade	full	shade	full
Water	drought	adequate	drought	adequate	drought	adequate	adequate	drought
MEANS	.898	.911	.913	.929	.931	.934	.943	.949
				*****	*****	*****	*****	*****
		*****	*****	*****	*****			
	*****	*****	*****					

Measurement: Compactness

Treatment	6	8	3	1	4	5	7	2
Soil	peat	peat	sand	sand	sand	peat	peat	sand
Light	full	shade	full	full	shade	full	shade	full
Water	drought	drought	adequate	adequate	drought	adequate	adequate	drought
MEANS	.767	.771	.773	.780	.784	.785	.787	.793
		*****	*****	*****	*****	*****	*****	*****
	*****	*****	*****	*****	*****	*****	*****	

Table 3 continued

Measurement:	Aspect Ratio							
Treatment	4	1	8	6	5	2	7	3
Soil	sand	sand	peat	peat	peat	sand	peat	sand
Light	shade	full	shade	full	full	full	shade	shade
Water	drought	adequate	drought	drought	adequate	drought	adequate	adequate
MEANS	1.380	1.381	1.393	1.395	1.403	1.403	1.418	1.434
	******	******	******	******	******	******	******	******

Measurement:	Elongation							
Treatment	2	7	5	4	3	8	1	6
Soil	sand	peat	peat	sand	sand	peat	sand	peat
Light	full	shade	full	shade	shade	shade	full	full
Water	drought	adequate	adequate	drought	adequate	drought	adequate	drought
MEANS	1.784	1.827	1.848	1.860	1.912	1.978	1.987	2.041
					******	******	******	******
		******	******	******	******	******	******	
	******	******	******	******	******			

Measurement:	Curl							
Treatment	6	1	8	3	4	5	7	2
Soil	peat	sand	peat	sand	sand	peat	peat	sand
Light	full	full	shade	shade	shade	full	shade	full
Water	drought	adequate	drought	adequate	drought	adequate	adequate	drought
MEANS	.848	.858	.859	.886	.887	.889	.896	.900
				******	******	******	******	******
	******	******	******					

Table 4
RESULTS OF TUKEY HSD TESTS
(Means underlined with "******" are not significantly different from each other at $p \leq .05$)

Species: *Panicum virgatum*

Measurement:	Plant Height (cm)							
Treatment	6	8	5	7	2	4	1	3
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	full	shade	full	shade	full	shade	full	shade
Water	drought	drought	adequate	adequate	drought	drought	adequate	adequate
MEANS	8.85	13.98	17.52	22.05	26.98	46.11	54.00	71.99
			******	******	******		******	******
		******	******	******				
	******	******	******					

Measurement:	Plant Silica Uptake (total counts)							
Treatment	6	8	7	5	2	3	4	1
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	full	shade	shade	full	full	shade	shade	full
Water	drought	drought	adequate	adequate	drought	adequate	drought	adequate
MEANS	336.9	349.7	352.1	439.1	504.8	1471.0	1646.2	2063.4
	******	******	******	******	******		******	******

Measurement:	Area (μm^2)							
Treatment	7	5	6	8	2	3	1	4
Soil	peat	peat	peat	peat	sand	sand	sand	sand
Light	shade	full	full	shade	full	shade	full	shade
Water	adequate	adequate	drought	drought	drought	adequate	adequate	drought
MEANS	196.65	205.77	225.18	233.00	233.82	239.38	244.53	303.21
			******	******	******	******	******	
	******	******	******	******	******			

Table 4 continued

Measurement:	Convex Area (μm^2)							
Treatment	7	5	2	6	8	3	1	4
Soil	peat	peat	sand	peat	peat	sand	sand	sand
Light	shade	full	full	full	shade	shade	full	shade
Water	adequate	adequate	drought	drought	drought	adequate	adequate	drought
MEANS	227.35	243.27	259.08	264.28	271.04	278.58	287.92	352.21

****
****
****

Measurement:	Perimeter (μm)							
Treatment	7	2	5	6	8	3	1	4
Soil	peat	sand	peat	peat	peat	sand	sand	sand
Light	shade	full	full	full	shade	shade	full	shade
Water	adequate	drought	adequate	drought	drought	adequate	adequate	drought
MEANS	65.42	68.20	68.35	70.85	70.94	73.67	75.97	85.01

****
****
****

Measurement:	Convex Perimeter (μm)							
Treatment	7	5	2	6	8	3	1	4
Soil	peat	peat	sand	peat	peat	sand	sand	sand
Light	shade	full	full	full	shade	shade	full	shade
Water	adequate	adequate	drought	drought	drought	adequate	adequate	drought
MEANS	59.31	61.52	61.37	62.94	63.40	64.24	66.05	73.07

****
****
****

Measurement:	Length (μm)							
Treatment	7	2	5	3	6	8	1	4
Soil	peat	sand	peat	sand	peat	peat	sand	sand
Light	shade	full	full	shade	full	shade	full	shade
Water	adequate	drought	adequate	adequate	drought	drought	adequate	drought
MEANS	23.79	23.84	23.93	24.67	24.81	25.86	25.90	28.83

****

Measurement:	Breadth (μm)							
Treatment	7	5	6	2	8	1	3	4
Soil	peat	peat	peat	sand	peat	sand	sand	sand
Light	shade	full	full	full	shade	full	shade	shade
Water	adequate	adequate	drought	drought	drought	adequate	adequate	drought
MEANS	12.39	13.11	13.62	13.97	14.21	14.22	14.32	15.55

****
****
****

Measurement:	Fiber Length (μm)							
Treatment	7	2	5	8	6	3	1	4
Soil	peat	sand	peat	peat	peat	sand	sand	sand
Light	shade	full	full	shade	full	shade	full	shade
Water	adequate	drought	adequate	drought	drought	adequate	adequate	drought
MEANS	26.83	27.73	28.28	29.02	29.19	30.44	31.66	35.49

****
****

Table 4 continued

Measurement:	Width (μm)							
Treatment	7	5	6	2	8	1	3	4
Soil	peat	peat	peat	sand	peat	sand	sand	sand
Light	shade	full	full	full	shade	full	shade	shade
Water	adequate	adequate	drought	drought	drought	adequate	adequate	drought
MEANS	10.37	10.80	11.40	11.66	11.83	11.89	12.06	13.22
			*****	*****	*****	*****	*****	
		*****	*****					
	*****	*****						

Measurement:	Equivalent Diameter (μm)							
Treatment	7	5	2	6	8	3	1	4
Soil	peat	peat	sand	peat	peat	sand	sand	sand
Light	shade	full	full	full	shade	shade	full	shade
Water	adequate	adequate	drought	drought	drought	adequate	adequate	drought
MEANS	15.60	15.96	16.59	16.73	17.03	17.28	17.44	19.42
			*****	*****	*****	*****	*****	
		*****	*****	*****	*****			
	*****	*****	*****					

Measurement:	Inscribed Radius (μm)							
Treatment	7	5	6	2	3	8	1	4
Soil	peat	peat	peat	sand	sand	peat	sand	sand
Light	shade	full	full	full	shade	shade	full	shade
Water	adequate	adequate	drought	drought	adequate	drought	adequate	drought
MEANS	5.37	5.48	5.74	5.82	5.90	5.91	5.84	6.45
		*****	*****	*****	*****	*****	*****	
	*****	*****						

Measurement:	Formfactor							
Treatment	4	1	3	5	6	7	8	2
Soil	sand	sand	sand	peat	peat	peat	peat	sand
Light	shade	full	shade	full	full	shade	shade	full
Water	drought	adequate	adequate	adequate	drought	adequate	drought	drought
MEANS	.529	.532	.553	.559	.564	.577	.581	.596
					*****	*****	*****	*****
			*****	*****	*****	*****	*****	
		*****	*****	*****	*****			
	*****	*****	*****	*****				

Measurement:	Roundness							
Treatment	7	4	5	6	1	8	3	2
Soil	peat	sand	peat	peat	sand	peat	sand	sand
Light	shade	shade	full	full	full	shade	shade	full
Water	adequate	drought	adequate	drought	adequate	drought	adequate	drought
MEANS	.453	.472	.473	.474	.475	.492	.494	.503
		*****	*****	*****	*****	*****	*****	*****
	*****	*****	*****	*****	*****	*****	*****	

Measurement:	Convexity							
Treatment	4	1	3	5	6	8	2	7
Soil	sand	sand	sand	peat	peat	peat	sand	peat
Light	shade	full	shade	full	full	shade	full	shade
Water	drought	adequate	adequate	adequate	drought	drought	drought	adequate
MEANS	.864	.871	.874	.889	.891	.895	.902	.909
	*****	*****	*****				*****	*****
						*****	*****	
				*****	*****	*****		

Table 4 continued

Measurement:	Solidity							
Treatment	1	5	6	3	4	8	2	7
Soil	sand	peat	peat	sand	sand	peat	sand	peat
Light	full	full	full	shade	shade	shade	full	shade
Water	adequate	adequate	drought	adequate	drought	drought	drought	adequate
MEANS	.854	.855	.858	.862	.865	.866	.868	.871
	******	******	******	******	******	******	******	******

Measurement:	Compactness							
Treatment	7	5	4	6	1	8	3	2
Soil	peat	peat	sand	peat	sand	peat	sand	sand
Light	shade	full	shade	full	full	shade	shade	full
Water	adequate	adequate	drought	drought	adequate	drought	adequate	drought
MEANS	.668	.683	.684	.684	.685	.697	.699	.706
		******	******	******	******	******	******	******
	******	******	******	******	******	******		

Measurement:	Aspect Ratio							
Treatment	2	3	8	6	5	1	4	7
Soil	sand	sand	peat	peat	peat	sand	sand	peat
Light	full	shade	shade	full	full	full	shade	shade
Water	drought	adequate	drought	drought	adequate	adequate	drought	adequate
MEANS	1.718	1.769	1.771	1.839	1.841	1.849	1.867	1.949
				******	******	******	******	******
	******	******	******	******	******	******	******	

Measurement:	Elongation							
Treatment	2	8	3	6	7	5	1	4
Soil	sand	peat	sand	peat	peat	peat	sand	sand
Light	full	shade	shade	full	shade	full	full	shade
Water	drought	drought	adequate	drought	adequate	adequate	adequate	drought
MEANS	2.40	2.50	2.56	2.59	2.63	2.65	2.70	2.71
		******	******	******	******	******	******	******
	******	******	******	******				

Measurement:	Curl							
Treatment	4	1	3	5	6	8	2	7
Soil	sand	sand	sand	peat	peat	peat	sand	peat
Light	shade	full	shade	full	full	shade	full	shade
Water	drought	drought	adequate	adequate	drought	drought	drought	adequate
MEANS	.816	.820	.824	.850	.853	.857	.861	.889
	******	******	******		******	******	******	******

Plants grown in sandy soil with adequate water accumulated significantly more silica than those grown in sandy soil under drought conditions. Plants grown in sandy soil, shade, and with adequate water (treatment 3), were significantly larger, and accumulated more silica than plants in any other treatment.

<u>*P. virgatum.*</u> Some of the results found in *B. curtipendula* were paralleled in *P. virgatum* treatments. All of the treatment means for *P. virgatum* plants grown in sandy soil were significantly larger and accumulated more silica than those grown in peat soil with the exception of those plants grown in sandy soil, in full sunlight, and under drought conditions (treatment 2). Gould and Shaw (1983) describes this species as a perennial bunch grass that prefers "low prairie sites, river banks, and swale areas," i.e. moist lands; conditions antithetical to treatment 2. It appears that the combination of drought and full sunlight was detrimental for this species to the point that even when grown in the superior sandy soil it did not grow nor accumulate silica to the levels of those plants grown in peat. Plants grown in treatment 3 (sand, shade, adequate) produced significantly larger plants than any other treatment, while plants grown in treatment 1 (sand, full, adequate) produced plants with significantly greater silica accumulations than any other treatments.

<u>Effect of treatments on phytolith size morphometries</u>

After establishing that plants grown under varying environmental conditions differed significantly with respect to size and amounts of silica accumulation, the effects of the variance of those two parameters on phytolith morphometries were evaluated. Although the effects of the eight treatments on the phytolith morphometries varied from parameter to parameter, some general trends were evident. These are best interpreted by considering parameters of size and shape separately.

<u>*B. curtipendula.*</u> Phytolith size morphometries for *B. curtipendula* were largely affected by soil type. The Tukey HSD comparison tests indicate that in general plants grown in sandy soil produce larger phytoliths than those grown in peat,

though some of the treatment differences were not significant ($p \leq 0.05$). With the exception of perimeter and fiber length, none of the plants grown under sandy soil treatments produced phytoliths that differed significantly from other sandy soil treatments in size morphometries. Likewise, within the different peat soil treatments, the phytoliths showed no differences in size morphometries with the exception that occasionally treatment 6 (peat, full, drought) phytoliths were larger than those of treatment 7 (peat, shade, adequate). Such results are in agreement with the habitat preferences for *B. curtipendula* which is known to be a warm season perennial grass that prefers well drained soils. A regression of size morphometry means on plant height and silica uptake means, summarized in Table 5, indicates that of the size morphometries in *B. curtipendula* only area, width, equivalent diameter, and inscribed radius were significantly correlated with plant height ($p \leq 0.05$), and all but perimeter, and fiber length, were correlated with the amount of silica uptake. In both cases, when significant, the size of the phytolith morphometries increased with increases in plant height and plant silica uptake.

P. virgatum. The size morphometries in *P. virgatum* were also generally larger for plants grown in sandy soil treatments than for plants grown in peat soil treatments though not all comparisons were significant ($p \leq 0.05$). Two interesting exceptions occur. Plants in treatment 4 (sand, shade, drought) produced phytoliths that consistently had significantly larger size morphometries than all other treatments, while plants in treatment 2 (sand, full, drought), similar to the height and silica data, produced consistently smaller phytoliths, often significantly so, even though grown on sandy soil. As earlier noted, this may be due to low drought tolerance in *P. virgatum* which is aggravated by full direct sunlight. A regression of the size morphometry means on the height and silica uptake means indicate that, in contrast to *B. curtipendula*, none of the size morphometries are correlated with plant height and silica uptake (Table 5). Possibly any significance may have been obscured by grouping and analyzing separately the phytolith morphotypes found in *P. virgatum*. For example, comparisons of the trilobate, and bi-trilobate phytolith size morphometries indicate that they are larger than those found in bilobates. If these larger morphotypes occur more frequently in the plants that are larger, or accumulate more silica, then differences in size morphometries between treatments might reach significant proportions. Further testing using frequency data is needed.

Effect of treatments on phytolith shape morphometries

In both *B. curtipendula* and *P. virgatum* the effect of the treatments on the phytolith shape morphometries were not as readily evident as those of size. Although the Tukey HSD

Table 5
RESULTS OF REGRESSION ANALYSIS FOR
B. curtipendula and *P. virgatum*
Model: Morphometric Parameter = Constant + Plant Height + Silica Uptake
First number applies to *B. curtipendula*, second number applies to *P. virgatum* i.e. *B. curtipendula*; *P. virgatum*

TYPE	MORPHOMETRIC	R^2	EFFECTS			
			HEIGHT		SILICA	
			F	P	F	P
SIZE	AREA	0.92; 0.36	7.85; 0.26	0.038; 0.663	22.26; 1.51	0.005; 0.274
	CONVEX AREA	0.88; 0.33	4.35; 0.29	0.092; 0.614	12.89; 1.45	0.016; 0.282
	PERIMETER	0.78; 0.27	2.00; 0.21	0.216; 0.666	6.16; 1.05	0.056; 0.352
	CONVEX PERIMETER	0.86; 0.31	3.11; 0.36	0.138; 0.575	10.33; 1.44	0.024; 0.283
	LENGTH	0.88; 0.31	3.27; 0.81	0.130; 0.411	11.27; 1.90	0.020; 0.226
	BREADTH	0.81; 0.42	2.27; 0.10	0.193; 0.770	7.24; 1.46	0.043; 0.281
	FIBER LENGTH	0.65; 0.24	0.99; 0.20	0.366; 0.673	3.14; 0.93	0.137; 0.380
	WIDTH	0.92; 0.41	8.73; 0.05	0.032; 0.832	23.15; 1.23	0.005; 0.318
	EQUIVALENT	0.92; 0.36	7.24; 0.22	0.043; 0.659	21.38; 1.47	0.006; 0.282
	INSCRIBED RADIUS	0.92; 0.46	6.70; 0.23	0.049; 0.651	19.97; 1.97	0.007; 0.219
SHAPE	FORMFACTOR	0.06; 0.02	0.107; 0.04	0.757; 0.854	0.20; 0.09	0.675; 0.781
	ROUNDNESS	0.14; 0.24	0.67; 0.53	0.449; 0.500	0.82; 0.02	0.407; 0.902
	CONVEXITY	0.04; 0.13	0.03; 0.01	0.870; 0.921	0.003; 0.11	0.959; 0.757
	SOLIDITY	0.05; 0.15	0.11; 0.20	0.750; 0.671	0.19; 0.00	0.683; 0.992
	COMPACTNESS	0.15; 0.24	0.69; 0.54	0.443; 0.495	0.84; 0.02	0.402; 0.900
	ASPECT RATIO	0.05; 0.17	0.24; 0.34	0.644; 0.586	0.18; 0.11	0.686; 0.922
	ELONGATION	0.04; 0.08	0.10; 0.31	0.764; 0.601	0.15; .010	0.713; 0.768
	CURL	0.04; 0.12	0.002; 0.03	0.970; 0.866	0.03; 0.06	0.873; 0.814

Table 6
RESULTS OF DISCRIMINANT ANALYSIS F TESTS
MODEL: MORPHOMETRIC PARAMETER = CONSTANT + SPECIES
n = 150

TYPE	MORPHOMETRIC	F	P
SIZE	AREA	117.01	.000
	CONVEX AREA	136.82	.000
	PERIMETER	190.08	.000
	CONVEX PERIMETER	199.61	.000
	LENGTH	220.89	.000
	BREADTH	59.11	.000
	FIBER LENGTH	194.48	.000
	WIDTH	49.28	.000
	EQUIVALENT	143.88	.000
	INSCRIBED RADIUS	33.41	.000
SHAPE	FORMFACTOR	121.72	.000
	ROUNDNESS	85.73	.000
	CONVEXITY	73.85	.000
	SOLIDITY	84.68	.000
	COMPACTNESS	87.93	.000
	ASPECT RATIO	112.74	.000
	ELONGATION	98.93	.000
	CURL	35.03	.000

tests indicate that some of the treatments produced significantly different ($p \leq 0.05$) results with respect to the various shape morphometries in both species, none of the effects were consistently diagnostic i.e. no obvious trends were observed. Regression tests for both species indicate that none of the shape morphometries are significantly correlated ($p \leq 0.05$) with either plant height or amount of silica accumulation. In other words, the shape morphometries are generally consistent regardless of plant height or amount of silica accumulation.

Phytolith morphometries and discriminant analysis

Results of discriminant analyses using phytolith morphometric data to distinguish between phytoliths extracted from *B. curtipendula, P. virgatum,* and *Zea mays* are found in Tables 6-8. The F and P values for each measurement (Table 6) indicate that all of the morphometries considered in this study varied significantly ($p \leq 0.05$) between species. Although stepwise discriminant analysis could have been used to eliminate some of the morphometric variables, all were included in the model used for discriminant analysis in this study. Sample data on ten individual phytoliths selected from each species illustrating how the discriminant analysis calculates a probability for each phytolith belonging to each species, and then uses that probability to predict its most likely population are found in Table 7. Table 8 summarizes the predictions made for the phytoliths from the three species used in this test. As indicated, the discriminant analysis correctly identified 100% of the phytoliths belonging to *B. curtipendula*. The saddle-shaped silica cell phytoliths of *B. curtipendula* are

Table 7
DISCRIMINANT ANALYSIS
SPECIES PROBABILITIES
(species 1 = *B. curtipendula*; species 2 = *P. virgatum*; species 3 = *Z. mays*)

ACTUAL SPECIES	PROBABILITY			PREDICTED SPECIES
	1	2	3	
1	1.00	0	0	1
1	1.00	0	0	1
1	.618	0	.382	1
1	1.00	0	0	1
1	.991	0	.009	1
1	.973	0	.027	1
1	1.00	0	0	1
1	1.00	0	0	1
1	1.00	0	0	1
1	1.00	0	0	1
2	0	.999	.001	2
2	0	.923	.077	2
2	0	.773	.227	2
2	0	.249	.751	3
2	0	.083	.917	3
2	0	.990	.010	2
2	0	1.00	0	2
2	0	1.00	0	2
2	0	.999	.001	2
2	0	1.00	0	2
3	0	.020	.980	3
3	0	.021	.979	3
3	.198	.001	.801	3
3	0	.003	.997	3
3	0	.596	.404	2
3	0	.007	.993	3
3	0	.010	.989	3
3	.002	0	.998	3
3	0	.025	.975	3
3	.002	0	.998	3

Table 8
DISCRIMINANT ANALYSIS
SUMMARY OF PREDICTION DATA

ACTUAL SPECIES	PREDICTIONS			TOTALS
	1	2	3	
1	50	0	0	50
2	0	46	4	50
3	1	7	42	50
TOTALS	51	53	46	150

readily distinguished from the bilobates of the other two species by human eye, so this result was not surprising, and can easily be duplicated using traditional typological methodology. The ability to distinguish between bilobate phytoliths of *P. virgatum* and *Z. mays* using discriminant analyses of phytolith morphometries is more impressive. Bilobate phytoliths from these two species are nearly identical, and extremely difficult, if not impossible, to distinguish from each other using typological methods. Based on the 18 morphometric parameters used in this study, discriminant analysis correctly identify 92% of the *P. virgatum* phytoliths, confusing only 8% with *Z. mays*. For *Z. mays* 84% of the phytoliths were correctly identified, confusing 14% with *P. virgatum,* and 2% with *B. curtipendula*. Although 84% correct identification may not be satisfying for some, it should be noted that if the means of the phytolith morphometries for the sample were used in the discriminant function, the sample as a whole would be correctly identified 100% of the time. In an archaeological setting, one rarely extracts a phytolith assemblage in which he/she is confident that all the individuals are from the same species. In this case, each individual phytolith would need to be evaluated separately using reference discriminant functions created from indigenous vegetation and known cultigens. The resulting array of probabilities obtained from the analyses could then be used to make inferences about the identity of the taxa that contributed to the assemblage extracted from the excavation.

Conclusions

For the two species of grass considered in this study, phytolith morphometries appear to be affected by varying environmental conditions though the effects are often not significant ($p \leq 0.05$). Natural populations of these two species are not likely to survive in such varied and/or adverse conditions as the peat soil treatments. If those treatments are dropped from the data, the significant effects of the environmental conditions on phytolith morphometries are further reduced. Nevertheless, because some differences were observed in phytolith morphometries taken from grass plants grown under widely varying environmental conditions, it seems advisable that when preparing reference data for phytolith systematics using the morphometric approach, one should sample as many different accessions from as many different populations as possible in order to ensure reliability and validity. Moreover, regression results suggest that when collecting reference data, shape morphometries should be given priority because they are less affected by differences in plant size and/or amount of silica accumulation than size morphometries.

Once the reference data are obtained, they can then be used as variables in discriminant analysis to create discriminant functions which can be used as a basis for phytolith sytematics and the identification of unknown phytolith populations. Computerization of the discriminant analysis facilitates the process. Not only does computerization make it easy to add additional reference data and morphometries to a discriminant function, but also enables a researcher to apply discriminant functions with relative ease to the phytolith morphometries of an unknown population. The computer can then generate printouts, with data similar to Tables 7 and 8, of the species to which the phytoliths might belong, along with the probabilities for each.

This material is based upon work supported under a National Science Foundation Graduate Fellowship.

References

Anderson PC (1980) A testimony of prehistoric tasks: diagnostic residues on stone tool working edges. *World archaeology*, 12, 181-194.

Armitage PL (1975) The extraction and identification of opal phytoliths from the teeth of ungulates. *Journal of Archaeological Science*, 2, 187-197.

Baker G (1961) Opal phytoliths and adventitious mineral particles in wheat dust. *Commonwealth Scientific and Industrial Research Organization, Australia; Mineralgraphic Investigations Technical Paper* No. 4, 3-12.

Baker G, Jones LHP, Wardrop, ID (1959) The cause of wear in sheep's teeth. *Nature*, 184, 1583-4.

Bezeau LM, Johnson A, Smoliak S (1966) Silica and protein content of mixed prairie and fescue grassland vegetation and its relationship to the incidence of silica urolithiasis. *Canadian Journal of Plant Science*, 46, 625-1.

Bhatt TS, Coombs MM, O'Neill CH (1984) Biogenic silica fibre promotes carcinogenesis in mouse skin. *International Journal of Cancer*, 34, 519-528.

Blackman E (1968) The pattern and sequence of opaline silica deposition in rye (*Secale cereale* L.). *Annals of Botany*, 32, 207-218.

Blackman E (1969) Observations on the development of the silica cells of the leaf sheath of wheat (*Triticum aestivum*). *Canadian Journal of Botany*, 47, 827-838.

Blackman E (1971) Opaline silica bodies in the range grasses of southern Alberta. *Canadian Journal of Botany*, 49, 769-81.

Blackman E, Parry DW (1968) Opaline silica deposition in rye (*Secale cereale* L.). *Annals of Botany*, 32, 199-206.

Bozarth SR (1987) Diagnostic opal phytoliths from rinds of selected *Cucurbita* species. *American Antiquity*, 52, 607-615.

Brown D (1984) Prospects and limits of a phytolith key for grasses in the central United States. *Journal of Archaeological Science*, 11, 221-243.

Bryant VM Jr. (1974) The role of coprolith analysis in Archaeology. *Texas Archaeological Society Bulletin*, 45, 1-28.

De Silva D, Hillis WE (1980) The contribution of silica to the resistance of wood to marine borers. *Holzforschung*, 34, 95-97.

Dayanandan P, Kaufman PB, Franklin CI (1983) Detection of silica in plants. *American Journal of Botany* 70, 1079-1084.

Djamin A, Pathak MD (1967) Role of silica in resistance to asiatic rice borer, *Chgilo suppressalis* (Walker), in rice varieties. *Journal of Economic Entomology*, 60, 347-351.

Fisher RF, Jenkins MJ, Fisher WF (1987) Fire and the prairie-forest mosaic of Devils Tower National Monument. *The American Midland Naturalist*, 117, 250-257.

Forman SA, Sauer F (1962) Some changes in the urine of sheep fed a hay high in silica. *Canadian Journal of Animal Science*, 42, 9-17.

Gould F W, Shaw RB (1983) *Grass Sytematics*, second ed., Texas A & M University Press, College Station 226.

Hanifa AM, Subramaniam TR, Ponnaiya BWX (1974) Role of silica in resistance to the leaf roller, *Cnaphalocrocis medinalis* Guenee, in rice. *Indian Journal of Experimental Biology*, 12, 463-465.

Harbers LH, Raiten RJ, Paulsen GM (1981) The role of plant epidermal silica as a structural inhibitor of rumen microbial digestion in steers. *Nutrition Reports International*, 24, 1057-1066.

Hayward DM, Parry DW (1973) Electron-probe microanalysis studies of silica deposition in barley (*Hordeum sativum* L.). *Annals of Botany*, 37, 579-591.

Hodson MJ, Sangster AG (1989) Silica deposition in the inflorescence bracts of wheat (*Triticum aestivum*). II. X-ray microanalysis and backscattered electron imaging. *Canadian Journal of Botany*, 67, 281-287.

Hutton JT, Norrish K (1974) Silicon content of wheat husks in relation to water transpired. *Australian Journal of Agricultural Research*, 25, 203-212.

Jones LHP, Handreck KA (1965) Studies of Silica in the oat plant. III. Uptake of silica from soils by the plant. *Plant and Soil*, 23, 79-96.

Jones LHP, Handreck KA (1967) Silica in soils plants and animals. *Advances in Agronomy*, 19, 107-149.

Kamminga J (1979) The nature of use-polish and abrasive smoothing on stone tools. In *Lithic Use-wear Analysis*, B. Hayden, ed. Academic Press, New York, 143-157.

Kaufman PB, Soni SL, Lacroix JD, Rosen JJ, Bigelow WC (1972) Electron-probe microanalysis of silicon in the epidermis of rice (*Oryza sativa* L.) internodes. *Planta*, 104, 10-17.

Kunoh H, Ishizaki H (1975) Silicon levels near penetration sites of fungi on wheat, barley, cucumber, and morning glory leaves. *Physiological Plant Pathology*, 5, 283-287.

Lanning FC (1966) Barley silica: relation of silicon in barley to disease, cold, and pest resistance. *Journal of Agriculture and Food Chemistry*, 14, 636-638.

Lewis RO (1981) Use of opal phytoliths in paleoenvironmental reconstruction. *Journal of Ethnobiology*, 1, 175-181.

Liebowitz H, Folk RL (1980) Archaeological geology of Tel Yin'am, Galilee, Israel. *Journal of Field Archaeology*, 7, 23-42.

Mulholland SC, Rapp GR Jr. (1989) Characterization of grass phytoliths for archaeological analysis. *Materials Research Bulletin*, 14, 36-39.

Ollendorf AL, Mulholland SC, Rapp G Jr. (1988) Phytolith analysis as a means of plant identification: *Arundo donax* and *Phragmites communis*. *Annals of Botany*, 61, 209-214.

O'Neill CH, Pan Q, Clarke G, Liu FS, Hodges G, Ge M, Jordon P, Chang YM, Newman R, Toulson E (1982) Silica fragments from millet bran in mucosa surrounding oesophageal tumors in patients in northern China. *The Lancet*, i(May 29), 1202-1206.

Parry DW, Smithson F (1964) Types of opaline silica depositions in the leaves of British grasses. *Annals of Botany*, N.S., 28(109), 169-85.

Parry DW, Smithson F (1966) Opaline silica in the infloresences of some British grasses and cereals. *Annals of Botany*, N.S. 30(119), 525-38.

Parry DW, Hodson MJ (1982) Silica distribution in the caryopsis and inflorescence bracts of foxtail millet (*Setaria italica*) and its possible significance in carcinogenesis. *Annals of Botany*, 49, 531-540.

Pearsall DM (1978) Phytolith analysis of archaeological soils: evidence for maize cultivation in formative Equador. *Science*, 199, 177-178.

Pearsall DM (1989) *Paleoethnobotany: A Handbook of Procedures*, Academic Press, San Diego, 311-428.

Piperno DR (1983) The Application of Phytolith Analysis to the Reconstruction of Plant Subsistence and Environments in Prehistoric Panama. Ph.D. Dissertation, Temple University.

Piperno DR (1984) A comparison and differentiation of phytoliths from maize (*Zea mays* L.) and wild grasses: Use of morphological criteria. *American Antiquity*, 49, 361-383.

Piperno DR (1985) Phytolith analysis and tropical paleo-ecology: production and taxonomic significance of siliceous forms in New World plant domesticates and wild species. *Review of Paleobotany and Palynology*, 45, 185-228.

Piperno DR (1988) *Phytoliths Analysis: an Archaeological and Geological Perspective*. Academic Press, San Diego, 110-118, 168-217.

Rapp GR Jr. (1986) Morphological classification of phytoliths, in, Plant Opal Phytolith Analysis in Archaeology and Paleoecology, edited by I. Rovner, Proceedings of the 1984 Phytolith Research Workshop, North Carolina State University, Raleigh, Occasional Papers No. 1 of the Phytolitharien, Raleigh, 33-35.

Raven JA (1983) The transport and function of silicon in plants. *Biological Reviews of the Cambridge Philosophical Society*, 58, 179-207.

Robinson RL (1979) Biosilica analysis: paleoenvironmental reconstruction of 41 LL 254. Appendix III to *An Intensive Archaeological Survey of Enchanted Rock State Natural Area*, by C. Assad and D.R. Potter. Center for Archaeological Research Survey Report 84, San Antonio.

Rosen AM (1992) Preliminary identification of silica skeletons from near eastern archaeological sites: an anatomical approach. In *Phytolith Systematics*, S. Mulholland and G. Rapp, Jr. eds., Plenum Press, New York, 129-147.

Rosen AM in press. Phytoliths as indicators of ancient irrigation farming in the prehistory of agriculture. In *New Experimental and Ethnographic Approaches*, edited by P. Anderson-Gerfaud, Paris: CNRS.

Rovner I (1971) Potential of opal phytoliths for use in paleoecological reconstruction. *Quaternary Research*, 1, 345-359.

Rovner I (1983) Plant opal phytolith analysis: major advances in archaeobotanical research, in *Advances in Archaeological Method and Theory*, edited by M. Schiffer, Academic Press, New York, 6, 225-266.

Rovner I, Russ JC (1992) Darwin and design in phytolith sytematics: Morphometric methods for mitigating redundancy. In *Phytolith Systematics*, S. Mulholland and G. Rapp, Jr. eds. Plenum Press, New York and London, 253-276.

Russ JC, Rovner I (1987) Stereological verification of *Zea* phytolith taxonomy. *Phytolitharien Newsletter*, 4, 10-18.

Sangster AG (1970) Intracellular silica deposition in immature leaves in three species of the Gramineae. *Annals of Botany*, 34, 245-257.

Soni SL, Parry DW (1973) Electron probe microanalysis of silicon deposition in the inflorescence bracts of the rice plant. (*Oryza sativa*). *American Journal of Botany*, 60, 111-116.

Twiss PC (1987) Grass-opal phytoliths as climatic indicators of the Great Plains Pleistocene, in *Quaternary Environments of Kansas*, edited by W.C. Johnson, Kansas Geological Survey Guidebook Series 5, 179-188.

Twiss PC, Suess E, Smith RM (1969) Morphological classification of grass phytoliths. *Soil Science Society of America Proceedings*, 33, 109-115.

Discussion with Reviewers

A.G. Sangster: What assumptions are being made that the methodology which employs EDX analyses for Si in a relatively minute area of the adaxial surface of the lamina is truly reflective of plant silica uptake for quantitative comparisons?

Authors: We are assuming that the silica accumulated in a small area of the lamina surface is representative of that accumulated by the plant as a whole, and that if all of the analysis parameters are kept constant, i.e., age of sample, preparation of sample, scan time, scan location, etc., then the *relative* amount of silica as determined by EDX analysis can be compared.

P.C. Twiss: Can the relative values of silicon X-rays (1.74 keV) be converted to percent silica in the lamina?

Authors: Possibly, but not easily. To obtain absolute values using EDX the specimen being analyzed must be flat, and homogenous, and the instrument used for the analysis must be carefully calibrated. There are easier ways to determine the percent of silica in the lamina such as the dry ashing technique used by Jones *et al.* (1963).

P.C.Twiss: Is there a difference in the quantity of silicon between the adaxial and abaxial surfaces of the lamina, or from different parts of the lamina?

Authors: EDX analysis may prove to be an effective tool for determining the answer to this question, but we did not address the issue in this study. Other studies reported in the literature indicate there are differences in the quantity of silicon between the adaxial and abaxial surfaces of the lamina, or from different parts of the lamina. It has been reported that usually upper leaves and apices of the internodes of grasses accumulate more silica than lower leaves and internode portions (Blackman 1968; Hayward and Parry 1973). Moreover, abaxial surfaces of leaves and inflorescence bracts of grasses have been found to accumulate more silica than adaxial surfaces (Hayward and Parry 1973; Hodson and Sangster 1988; Parry and Hodson 1982; Sangster 1970; Sangster *et al.* 1983). Dengler and Lin (1980) reported a similar preference for abaxial deposition in the spike moss *Selaginella emmeliana*, however, in the dicot *Ficus lyrata*, Davis (1987) observed heavier extracellular accumulations occur on adaxial surfaces of leaves.

A.G. Sangster: Both of the root media utilized may be lacking essential nutrients, such as N, P, or Ca in some peats, which may affect leaf cell size and thus could directly influence the phytolith size morphometries utilized in this study. Might not it be possible to produce greater size variations using enriched or fertilized media, such as might be practiced by early agrarian society, in a common temperate crop variety?

Authors: Most likely so. These are valid and important observations that need to be addressed in future studies, e.g., How do specific varying edaphic conditions affect phytolith morphometries, and which conditions create the greatest variance?

S. Mulholland: Why does sand soil type tend to produce greater plant heights, silica accumulations, and phytolith size morphometries than peat? Given that the exceptions to this trend may be related to species requirements, how do the other factors (shade, water) affect size morphometries within a particular soil type?

Authors: The sandy soil used in this study appears to have provided more essential nutrients, and was higher in silica concentration than the pure peat medium, thus plant height, silica accumulation, and phytolith size was greater in plants grown in the sandy soil. The factors of shade and water did not appear to have any consistent effect on phytolith size within soil types. Future studies might provide more conclusive data.

S. Mulholland: If sizes change with some environmental factors but shapes don't, would ratios of measurements be a more effective discriminator of plant taxa than absolute measurements? What implications does this have for construction of a phytolith classification?

Authors: Our findings indicate that ratios, such as the shape morphometries evaluated in this study, are more reliable discriminators than size morphometries, and should be given priority when preparing taxonomies. This is not to say that at some taxonomic levels size morphometries will not prove to be more effective discriminators than shape. For some closely related taxa, size may be the only discriminators. Preliminary results of a current study we are doing on three species of wheat inflorescence phytoliths appears to be such a case.

P.C. Twiss: Is there a relationship between age of plant and amount of silica and numbers of short cell and long cell silica bodies?

Authors: Some studies have addressed this issue. Lanning

Table 9
Ranges of Shape Morphometries for *B. curtipendula*, *P. virgatum*, and *Z. mays*
(species 1 = *B. curtipendula*, 2 = *P. virgatum*, and 3 = *Z. mays*)

Morpohometric	Species		
	1	2	3
Formfactor	.116-.850	.257-.684	.427-.724
Roundness	.398-.806	.249-.650	.370-.743
Convexity	.447-.963	.622-.939	.778-.913
Solidity	.635-1.00	.700-.932	.793-.947
Compactness	.631-.898	.499-.806	.608-.862
Aspect Ratio	1.43-2.09	1.30-3.24	1.85-2.20
Elongation	1.38-6.92	1.88-4.54	1.65-3.28
Curl	.351-1.00	.515-.966	.702-.909

(1960, 1961) reported increased silica accumulation with age in strawberries and sorghum, but Lanning (1966, 1966a) found higher levels silica in wheat and barley in the spring than in the fall. The timing of silica deposition in some taxa has also been reported to be a function of plant age and metabolism. Sangster and Parry (1969) found bulliform cell silicification in grasses does not occur at stages where bulliform turgor changes might affect blade development. Likewise, Sangster (1970) noted silica accumulation did not occur in long cells of grass leaves until they were fully expanded, thus providing no inhibition to young long cell expansion. Moreover, Sangster (1977) reported there is no silica deposition in photosynthetic tissue and intercostal silica cells of actively exporting leaves of *Digitaria sanguinalis*.

P.C. Twiss: What are the ranges of shapes (forms) of the short cell silica bodies in each of the three species and what, if any, problems, in systematic do these present?
Authors: The range of the shape morphometries for the silica cell phytoliths evaluated in this study are found in Table 9.

Many of the ranges overlap, a phenomenon which has two implications for phytolith sytematics: 1) Morphometrics are most useful in sytematics when they are used to evaluate populations and/or population means rather than individuals; 2) Taxonomies using many morphometries as discriminators will be more valid and reliable than those using one or few.

P.C. Twiss: Can chloridoid phytoliths be distinguished from rectangular festucoid (pooid) phytoliths which are common in C_3 grasses?
P.C. Twiss: Can chloridoid phytoliths be confused with cross-shaped panicoid phytoliths of Zea mays?
Authors: Based on the results of this study, we assume that the answer to both of the above questions is yes. Additional morphometric studies of the taxa mentioned in the questions are needed to validate the assumption.

P.C. Twiss: Should photos of discrete phytoliths be included with all morphometric studies so as to show ranges of forms?
Authors: Although photos may not be necessary in a purely morphometric approach to phytolith sytematics, they most certainly would be helpful in assisting the reader to visualize the form of the phytolith described by the data.

P.C. Twiss: What should be the next steps in morphometric studies of grass phytoliths? What direction(s) should studies of phytolith systematics head?
Authors: We would suggest that morphometric reference data needs to be gathered from which taxonomic tools such as keys and discriminant functions can be constructed. These tools can then be used in conjunction with typologic taxonomies to improve phytolith systematics. As this study indicates, because some morphometries vary with environmental conditions, when collecting reference data one should sample as many different populations as possible.

Additional References

Davis RW (1987) Ultrastructure and analytical microscopy of silicon in the leaf cuticle of *Ficus lyrata* Warb. *Botanical Gazette*, 148, 3, 318-323.

Dengler NG, Lin EY-C (1980) Electron microprobe analysis of the distribution of silicon in the leaves of *Selaginella emmeliana*. *Canadian Journal of Botany*, 58, 2459-2466.

Hodson MJ, Sangster AG (1988) Observations on the distribution of mineral elements in the leaf of wheat *(Triticum aestivum* L.), with particular reference to silicon. *Annals of Botany*, 62, 463-471.

Jones LHP, Milne AA, Wadham SM (1963) Studies of silica in the oat plant II. Distribution of the silica in the plant. *Plant and Soil*, 18, 3, 358-371.

Lanning FC (1960) Nature and distribution of silica in strawberry plants. *Proceedings of American Society for Horticultural Science*, 476, 349-358.

Lanning FC (1961) Silica and calcium in black raspberries. *Proceedings of the American Society for Horticultural Science*, 77, 367-371.

Lanning FC (1966a) Pattern of distribution: relation of silicon in wheat to disease and pest resistance. *Journal of Agricultural and Food Chemistry*, 14, 4, 350-352.

Sangster AG (1977) Characteristics of silica deposition in *Digitaria sanguinalis* (L.) Scop. (crabgrass). *Annals of Botany*, 41, 341-350.

Sangster AG, Parry DW (1969) Some factors in relation to bulliform cell silification in the grass leaf. *Annals of Botany*, 33, 315-323.

Sangster AG, Hodson MJ, Parry DW (1983) Silicon deposition and anatomical studies in the inflorescence bracts of four *Phalaris* species with their possible relevance to carcinogenesis. *New Phytologist*, 93, 1, 105-122.