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OPAL PHYTOLITHS

by

ERWIN SUESS

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Approved by:

Page C. Twiss

Major Professor

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INTRODUCTION

Opal phytoliths are bodies of silica that accumulate in the epidermal cells of plants. Their occurrences within the various taxonomic units of the plant kingdom are diverse and a great variety of shapes exist. Botanists give special consideration to opal phytoliths in the study of anatomy of the grass family. In descriptions of grassland soils, the amount of opal phytoliths is of importance to agronomists. Also, micropaleontologists reported fossil opal phytoliths from Pliocene and Pleistocene terrestrial sediments.

Purpose and order of investigation

Samples of recent dust deposits collected at monthly intervals from several sites east of the Rocky Mountains have been studied as a factor of soil renewal by the Agricultural Research Service of the U.S. Department of Agriculture at Kansas State University in connection with the Department of Geology (Smith and Twiss, 1965). Some of these samples contained an abundance of opaline material of which several were opal phytoliths. The purposes of this investigation were to develop a morphological classification of phytoliths, to determine possible applications of this morphological classification to the sciences of geology and agronomy especially to assist in determination of the source areas of dust deposits. It is hoped that this investigation will reveal new and different types of future studies.

Morphological classification of opal phytoliths.--Seven dominant grass species of the Great Plains were chosen for this investigation. *Andropogon gerardi* Vitman (big bluestem); *A. scoparius* Michx. (little bluestem); *Panicum virgatum* L. (switchgrass); and *Sorghastrum nutans* (L.) Nash (indiangrass) represent more than 95 percent of the vegetation of the "tall grass" or true prairie vegetation in eastern Kansas. *Bouteloua curtipendula* (Michx.) Torr (sideoats grama); *Bouteloua gracilis* (H.B.K.) Lag. x Steud. (blue grama); and *Buchloe dactyloides* (Nutt.) Engelm. (buffalograss) are the dominant species of the "short grass" or western prairie vegetation. A broad transition area occurs in east central Kansas which is the "mixed grass" region. Sampling procedures are included; x-ray and optical examination, and statistical methods are described. Finally, a morphological classification of opal phytoliths is suggested.

Applications.-- The morphological classification of opal phytoliths was used in grouping the grass regions of the dust deposits of the Great Plains. Recent and fossil soils and Pleistocene sediments were also characterized by opal phytoliths.

Discussion.-- Formation and function of opal phytoliths and limitations and extensions of their application are discussed. Solubility, x-ray examination, and the H_2O content as related to the refractive index are given special consideration.

Previous Work

The botanical literature on opal phytoliths is extensive, but each work relates the opal phytoliths to anatomy and physiology of the grass family. Netolitzky's (1929) comprehensive work is the only monograph on plant opals. It includes all known reports on opal phytoliths (Kieselkörper or silica bodies) through 1929, from Struve's dissertation (1835), "De silica in Plantis nonnullis," to Kuester's discussion (1897), to Pfeifer's (1921), Molisch's (1923) and to Borrisow's (1928) publications.

A second development in the study of silica bodies started in plant physiology with Molisch (1923). "Die pflanzliche Zellwand" by Frey-Wyssling (1959) summarized all the information about plant physiology. A third line of study was that of organic silicon compounds. Engel's investigation (1953) on rye gave some aspects of silica chemistry and an extensive bibliography.

Data on physical properties are very few. Lanning and others (1958) studied plant silica by x-ray diffraction and reported alpha-quartz and cristobalite in several samples. Beavers and Stephens (1957) reported alteration of opal to chalcedony from a paleosoil in Illinois.

Reports on classification are also rare. Metcalff's "Anatomy of the Monocotyledons" (1960) stressed the importance of silica bodies as diagnostic characteristics in taxonomy. He suggested a descriptive terminology which is used in the first part of this investigation. Prat (1948) first distinguished two types of silica bodies that are characteristic for the two subcategories of the grass family, Festucoideae and Panicoideae. He named them chloridoid and panicoid respectively.

References in Russian from Tyurin (1937), Usov (1943), Parfenova and Yarilova (1956), which are not available in translation, summarized the first investigations in agronomy. They provided information which Smithson (1956 and 1957) used in his reports on grass opals in British soils. Beavers and Stephens (1957) described occurrences of plant opal in soils and a paleosoil in Illinois. Jones and Beavers (1964) published two articles on quantitative investigations of opal phytoliths. Another investigation was reported by Witty and Knox (1964). All applications for practical purposes are based on quantitative investigations of opal phytolith content in soils.

A note in the Journal of Paleontology by Jones (1964 p. 773-775) suggested that qualitative study for "Gramineae taxonomy utilizing silicified cellular elements is not advanced." Comparative studies with reference collections was his method of gaining more qualitative information from opal phytoliths. The first report on fossil opal phytoliths was by Baker (1960) and concerned the formation of Cenozoic rocks under grass vegetation in Western Victoria, Australia.

ACKNOWLEDGMENTS

Professor P. C. Twiss, who was a committee member, major instructor, and advisor to the Master's thesis, offered many suggestions, had always time and help for numerous problems raised from the beginning to completion of the work. Professor J. R. Chelikowsky, Head of Department of Geology and Geography at Kansas State University, obtained financial help where necessary to continue the extensive preparation work in the departmental laboratory and showed interest in continuation of the investigation. Co-students helped generously in sampling procedures. To all I am grateful.

Professor O. W. Bidwell, Advisory Committee member, encouraged the study and interest in soil science by both helpful suggestions and introduction to soil development. In selection and sampling of the studied grass material, I would like to thank Professor K. L. Anderson, Department of Agronomy, and Dr. R. M. Smith, Agricultural Research Service, U.S. Department of Agriculture.

An introduction to statistical analysis in geological sciences by Professor L. F. Marcus of the Statistics Department provided the incentive for applying statistical analyses. His criticism, suggestions, and discussions in application and interpretation were most stimulating.

Special acknowledgment must go to Professor. Dr. K. J. Mueller, now Department of Paleontology, University of Bonn, Germany, for the first introduction to the study of opal phytoliths, which was done under his supervision at the Department of Geology and Paleontology at the Technical University of Berlin in 1963-1964.

DESCRIPTION OF OPAL PHYTOLITHS

Sampling and Laboratory Procedures

Opal phytoliths prepared from epidermal cells of seven grass species provided information about morphology and physical properties.

Samples.-- The seven dominant grass species were collected in late December 1964 at the Kansas Agricultural Experiment Station, Manhattan, under supervision of Dr. R. M. Smith. Mature and well-developed plants were selected, washed and dryed.

Ashing.-- Two to three selected leaves per grass species were washed in distilled water and dilute HCl to remove any surface contamination and to soften the mineralized tissue. For six hours they were ashed at 500^{o}C, then washed again in distilled water and dilute HCl. The remaining structures were permanently mounted in Cadex (n=1.55) on glass slides.

Acid treatment.-- Selected leaves and portions of the stem were washed in warm six normal HCl for 15 minutes. Addition of a solution of 50 grams CrO_3 per 100 milliliters H_2O and slight heating for 90 minutes showed the first well-preserved clean structures floating on top of the acid. These were separated, washed several times, dryed on slides, and mounted in Cadex.

Staining methods.-- Congo-red and iodo-potassium-iodide treatments were used for studying surface properties of opal phytoliths. An indicator solution of 0.5 grams congo-red in

90 milliliters H_2O and 10 milliliters alcohol was added to fresh grass opals that were prepared by the chromic acid treatment. Using iodo-potassium-iodide (35 grams potassium-iodide plus 7 grams iodine in 100 milliliters H_2O) required a previous soaking of the opaline material in concentrated sulfuric acid or in zinc chloride solution.

X-ray examination.-- Three slides with oriented material containing about 50 percent opal phytoliths and 50 percent cellulose were x-rayed. The thickness varied from a thin film to 0.004 inch and 0.02 inch. Samples #1 and #2 were run from 62^o - 1.5^o, #3 from 35^o - 1.5^o in nickel-filtered copper k alpha radiation. The setting was 40 kilovolts, 18 milliamperes. The pulse height analyzer had a level of 16 volts and a window of 11 volts. The detector voltage was 1.7 kilovolts, scale factor 200, and the time constant was two seconds. One degree divergence and scatter slits were used. The receiving slit was 0.006 inches.

Optical examination.-- Plain light and polarized light were used for optical examination. Refractive indices were determined by the immersion method. Microscopic measurements were made with micrometer ocular at 675 x magnification with an accuracy of ± .0002 millimeters.

Statistical analyses of morphology.-- The measured variables for the statistical analyses were:

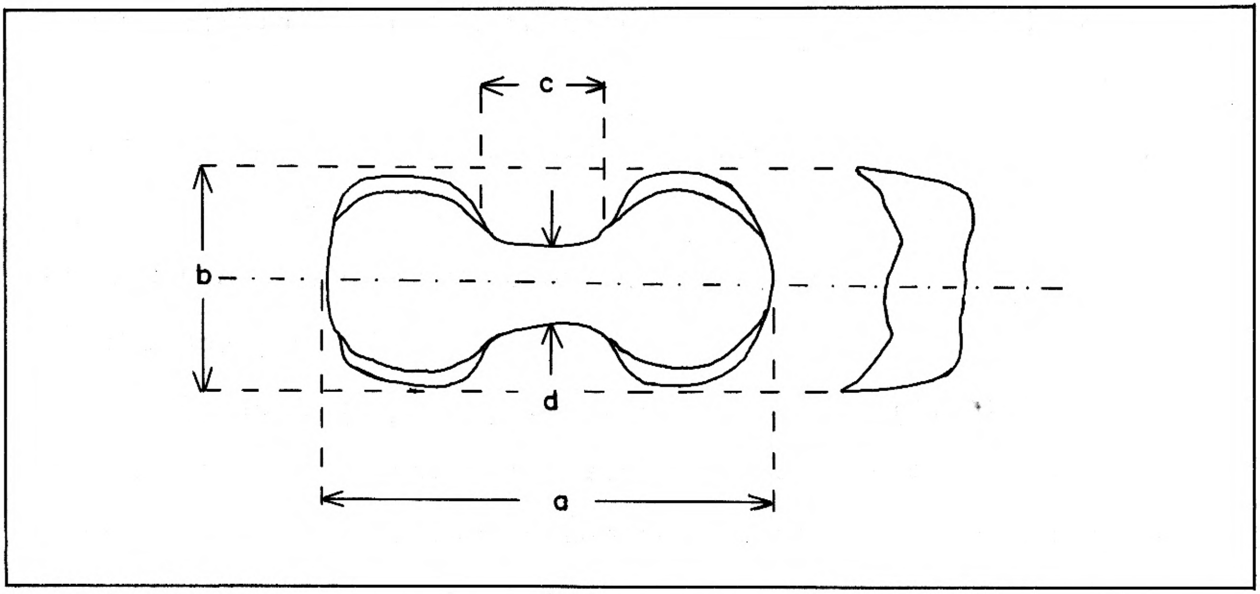


Fig. 1--Measured variables of panicoid opal phytoliths

length of opal phytoliths (a)
greatest width (b)
length of the middle portion (c)
smallest width of the middle portion (d)

Opal phytoliths from Andropogon gerardi, A. scoparius, Sorghastrum nutans, and Panicum virgatum were measured.

The analysis of Variance was applied comparing one characteristic at a time, and for multivariate analyses the Generalized Distance D^2 and Analysis of Dispersion were chosen. The computations and data are in appendix.

Results and Interpretation

X-ray analysis.-- No long range order of the silicon-oxygen tetrahedra of the opal phytoliths was observed; no sample showed indication of any crystalline substance. The background radiation from the glass slide was reduced by an amount that was proportional to the thickness of the opal cellulose mixture. The glass slide was x-rayed separately. A fourth random powder sample which was analyzed with a piece of paper as stabilizer again showed no preferred crystal planes. A test of the background radiation of the paper indicated only a reduction due to the opal cellulose material.

Optical properties.-- Under high magnification many opal phytoliths formed two Becke lines. One occurred at the contact between the medium and the silica body, whereas the second was observed about one micron inside the margin and occurred all around the silica body. This suggested a thin outer layer with a different refractive index that encloses the opal phytoliths.

The sheath had a white to yellow interference color at those portions of the opal phytoliths where the light passed through its maximum thickness. This was around the margin of the silica body. A considerably lower color was observed at the remaining surface where the light passed only through a thickness of two birefringent layers, namely at the lower and the upper surface of the opal phytolith.

The observed interference color was believed to be a result of birefringent organic fibers which were oriented with their length parallel to the surface of the opal phytoliths, thus measured indices of refraction ranging from 1.488 to 1.494 represent only one index.

Due to the curved surface of the silica bodies some portions appeared dark under polarized light representing fibers in extintion position. These fibers composing the sheath are comparable to those which are developed by organisms for protection and which are often observed around foreign bodies.

Staining qualities.-- The two specific staining methods were applied to determine the properties of the outer sheaths of the opal phytoliths. Congo-red treatment indicated after more than 20 minutes that about 80 percent of the opal material in a sample had an outer stainable layer. Some silica bodies were not altered by staining. Better results were obtained by using iodo-potassium-iodide which affected 80 percent of the opal phytoliths by producing a brown coating. (Plate I: Fig. 3 and 4)

The nature of the coating could not be identified clearly. The brown of the iodine molecules demonstrated porosity of the outer sheath. The porosity of pure cellulose is indicated by a blue color under the same test. Either the pores are too small for penetration by the iodine molecules so that they adhere to the surface and hold their (OH) group thus causing the brown color, or the pores are so big that the whole I_2 (OH) complex can penetrate the coating material without losing the (OH) group.

Engel (1953) described an insoluble silicon-cellulose compound that composes the cell walls of rye. Photomicrographs

Explanation of Plate I

Opal phytoliths stained with iodo-potassium-iodide

Fig. 1 Stained panicoid opal phytolith (a) and unstained chloridoid type (b).

Fig. 2 Stained and unstained irregularly shaped opal phytoliths.

Fig. 3 Panicoid opal phytolith (top view).

Fig. 4 Same subject as in Fig. 3 (end view).

Plate I

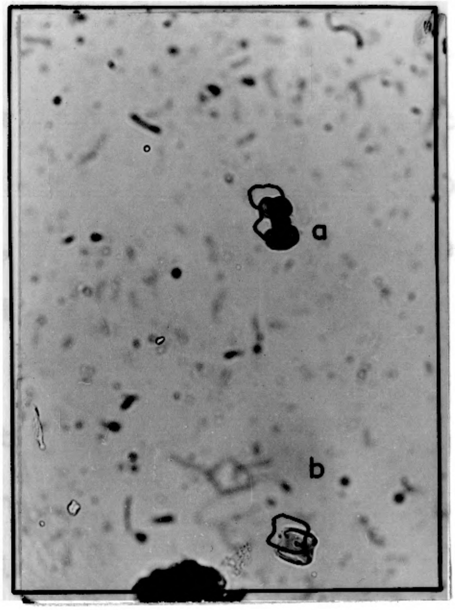


Fig. 1

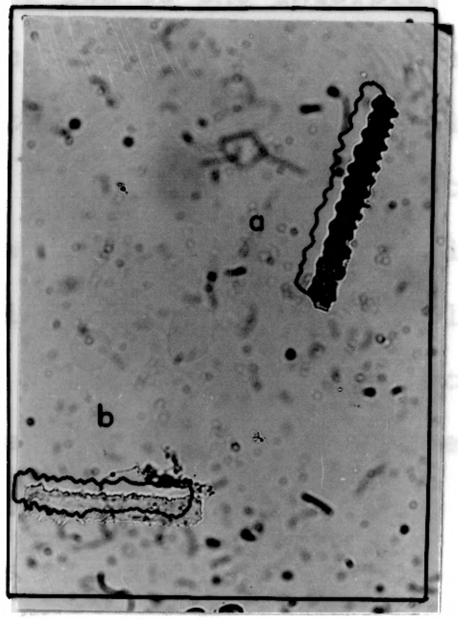


Fig. 2

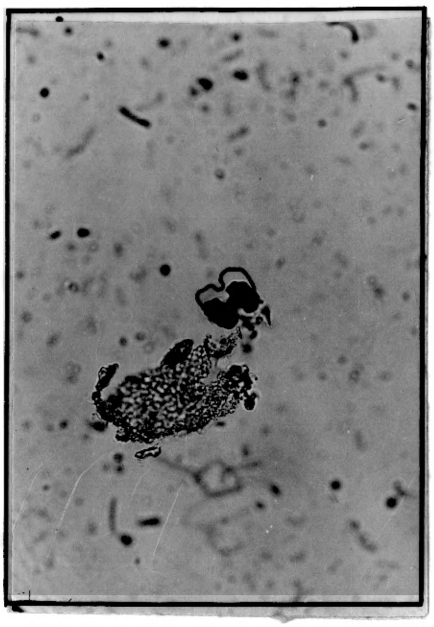


Fig. 3

Fig. 4

Scale: | | | | | | | | |
Each Division =.01 mm

of these incrustations reveal the same morphology as observed in this investigation.

Optical and staining results led to the interpretation that the opal phytoliths are enclosed in a cellulose-like outer layer. Some incrustations consisted entirely of the cellulose-like material as indicated by the absence of a second Becke line, a lower relief in Cadex, low birefringence and stainability. Details follow in the discussion of the solubility.

Analysis of Variance.-- In the Analysis of Variance the length of opal phytoliths (a), greatest width (b), the length of the middle portion (c), and the smallest width of the middle portion (d) were compared separately. A criterion for a significant difference is the ratio of the means of squares among species to those within species. The variance within samples was computed for comparative interpretation. The results are in Table I. (p. 14)

Analysis of Dispersion.-- The Analysis of Dispersion tests the hypothesis that the means of squares among species and the means of squares within species are different. If the two means are not equal the difference is indicated by the test criterion lambda differing from the value 1.

A difference to be expected among species was indicated by lambda. The probability was 0.005 as shown by comparison of the transformed test criterion to the probability of the Chi Square distribution. Transformed lambda is 29.42 whereas the

Table I.- ANALYSIS OF VARIANCE

Variable	Source	Degree of Freedom	Sum of Squares	Mean of Squares	F Ratio	Variance
a	Among	3	19026.60	6342.20	7.72	
	Within	36	29574.40	821.51		9.064
b	Among	3	1457.70	485.90	7.57	
	Within	36	2309.80	64.16		2.450
c	Among	3	962.86	320.96	24.67	
	Within	36	468.73	13.01		1.17
d	Among	3	288,47	96.16	3100	
	Within	36	1113.70	30.97		1.75

Points of significance for the F distribution are:

25% = 1.43	2.5% = 3.53
10% = 2.25	1.0% = 4.38
5% = 2.86	0.5% = 4.50

Chi Square distribution at p = 0.005 and with 12 degrees of freedom is 28.30. For calculation see Appendix 3.

Generalized Distance D^2.-- The Generalized Distance is a numerical index of the differences among species. The measured variables of each species simultaneously treated form the basis of this index. In this procedure available data from mature and young leaf samples of Andropogon gerardi were treated separately. Microscopic morphologic examination of known old and young samples from Andropogon gerardi and Sorghastrum nutans had suggested this step because considerable change in the length and width of the middle portion was observed.

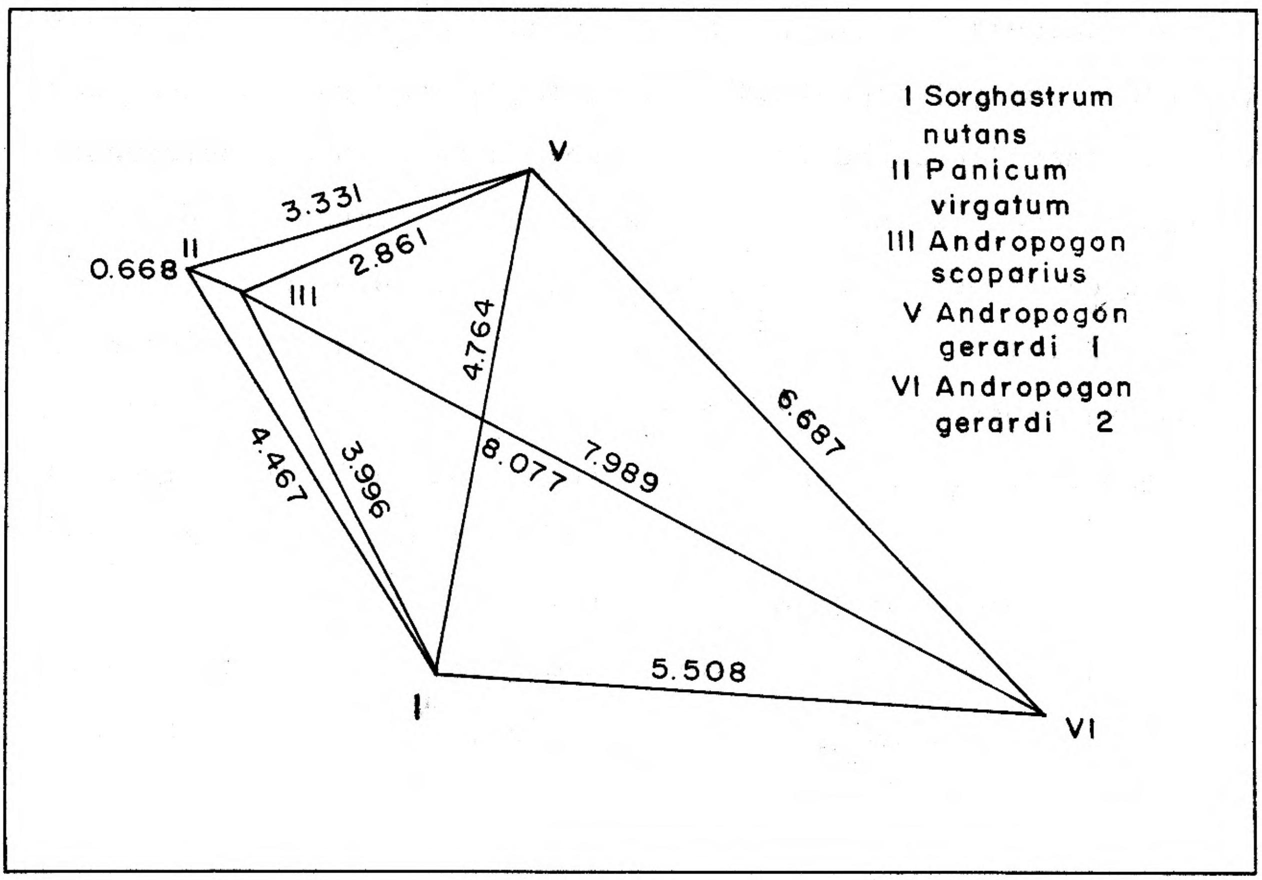


Fig. 2 Graphic Representation of the Generalized Distance

The Generalized Distances are in Table 2 and are shown graphically in Fig. 2.

Table 2.- GENERALIZED DISTANCES

Pair	D^2	D
(II-I)	19.9572	4.467
(II-III)	.4464	.668
(II-V)	11.0960	3.331
(II-VI)	65.2481	8.077
(I-III)	15.9740	3.996
(I-V)	22.7039	4.764
(I-VI)	30.3407	5.508
(III-V)	8.1852	2.861
(III-VI)	63.8291	7.989
(V-VI)	47.1574	6.862

<u>Interpretation of statistical analyses</u>.-- The Analysis of Variance of the measured characteristics partly expresses the observed fact that there is a qualitative difference in the shape of the opal phytoliths. The length (a) and width (b) measurements show that a greater than 99.5% significant difference is to be expected. But consideration of the variance within these two characteristics shows that the differences to be expected occur partly within species; the opal phytoliths of the four species have the same length and width range so that a significant difference shown by the Analysis of Variance occurs mostly within the species.

The length of the middle portion of the dumb-bells (c) is the most significantly different characteristic (24.67 compared with 4.5 at 0.5%). In this case, the variance (S = 1.17) is low which indicates that the highly significant difference occurs entirely among species.

The width of the middle portion is the measured characteristic (d). The variance within species is fairly low ($S=1.75$), but the significance of a difference among species ($F=3.10$) lies between 2.5-5.0%. The width of the middle portion is insignificant as compared to the significance of the length. This test of single variables shows that only the length of the middle portion might be used as a significantly different characteristic. However, the microscopical study of several samples from one species showed that the length of the middle portion changes with the age of the plant individual. This fact had suggested the application of multivariate analyses.

A simultaneous consideration of four characteristics is closer to the observations made by eye. All characteristics are considered at the same time by a visible impression of an object. The analysis of dispersion shows a significant difference that can be expected within the confidence interval of 99.5%. This test reveals the quantity of the difference occurring among species. It suggests a further application of determining the Generalized Distance D^2 between each of the four species.

The importance of the length of the middle portion, as indicated in the Analysis of Variance and the microscopic result of its variations within samples of one species, has led to a change in the procedure. The graphic representation (Fig. 2) shows the greatest difference between the opal phytoliths of the four mature species and those of the young plant individual. Even the difference between mature and young types of the same species is greater than the differences among species which shows the importance of the growth stage. A slight difference exists between *Panicum virgatum* and the three Andropogoneae, *Sorghastrum nutans*, *Andropogon scoparius*, and *Andropogon gerardi* 1. This can be interpreted as a tribal difference between Paniceae and Andropogoneae. A further distinction between species cannot be made because of the close similarity between *Panicum virgatum* and *Andropogon scoparius* rather than between *Andropogon scoparius* and *Sorghastrum nutans*. Here again the growth stage effect might be of greater importance. Further studies on samples of accurately-known growth stages might emphasize the limitations of distinctions between species. Another unknown factor implied is the importance of the location of the leaf on the plant stem. It is not known whether a leaf several weeks old on the base of the plant stem early in the growing season contains mature opal phytoliths. A full-length growing season of several months might be necessary for the transpiration stream to provide the plant with enough SiO_2 in solution to form opal phytoliths of mature shape. A further discussion of this question follows under botanical factors. In consideration of the listed limitations, it does not seem that a useful classification on a generic or specific level is possible.

Morphological Classification

For morphological description, Metcalff's terminology (1960) has been adapted to this study and some new terms have been introduced. Some of Metcalff's types were grouped because several shapes seemed to be simply different growth stages. Molisch in 1923 already distinguished between siliceous cell wall incrustations and massive silica bodies that occur inside special silica cells. These subdivisions were valid and easily distinguishable. Further subdivisions were of regular and irregular shapes.

I. Silica incrustations

Epidermal long-cell and short-cell incrustations were observed in all seven spodograms. They are elongated with a sinuous margin and showed accurately the anatomic details of the epidermal cells including stomata. The length ranged from .02 to .2 millimeter and the width from .01 to .02 millimeter.

Plate I, b; Plate IV, d_1, d_2, f.

II. Silica bodies

Unlike the long-cell incrustations which occurred in all epidermal cells, true silica bodies were restricted to special short cells. They occurred singularly, in pairs, or in rows of cells (Pl. III, Fig. 4). Two basic shapes were observed.

A) Irregular silica bodies. Their basic shape is columnar but there are several possible variations, especially in length. They occurred mostly as individual bodies.

1) Elongated with rounded corners, smooth outlines, uneven "bumby" surface.
Plate IV, b_3; Pl. III, b.

2) Elongated with sinuous outlines.
Plate IV, b_2.

3) Elongated but with acute angle on the ends.
Plate III, c; Pl. IV, c.
Length variations: .03 - .15 millimeter, width: .01 - .02.

B) Regular silica bodies. The basic shapes are cubes and dumb-bells. They occurred always in rows of cells of varying numbers.

1) Chloridoid type (cuboid). More or less cubical with rounded edges and convex or concave curved sides. Some varied with appearance with focus. The size was very consistantly .01 x .01 millimeters.
Plate II, a.

2) Panicoid type (dumb-bell shaped). Includes all variations that Metcalff (1960) proposed such as
(a) short dumb-bell shaped
(b) with narrow middle portion
(c) with wide middle portion
(d) with varying appearance with focus
(e) nodular type
The varying appearance with focus could be explained by an endview of a panicoid opal phytolith showing a keeled margin of the circular ends.
Plate I, Fig. 3 and 4.
Length variations: .01 - .02 millimeter, width consistantly .01 millimeter.
Type (a): Pl. III, a_3.
(b): Pl. III, a_2.
(c): Pl. III, a_1.
(d): Pl. III, a_1.
(e): Pl. III, e_1; e_2.

3) Alternating with the chlofidoid and panicoid type in the same rows are
(a) tall and narrow silica bodies
(b) crescent shaped silica bodies
Their length was less than .005 millimeter, the width .01 millimeter.
Type (a): Pl. II, d.
(b): Pl. II, c.

Classification of the Grass Regions

For classification of the vegetative regions only the regular silica bodies (Morphological Class. II. B) were used. The morphological description and the use as diagnostic characteristics in plant anatomy also suggested that opal phytoliths may be used in grass taxonomy.

A classification of the vegetative regions was only possible on a basis of grass taxonomy.

The characteristic "short grass" species of the western prairie are:

Bouteloua curtipendula (Michx.) Torr (sideoats grama)

Bouteloua gracilis (H.B.K) Lag. x Steud. (blue grama)

Buchloe dactyloides (Nutt.) Engelm. (buffalograss)

All three species belong to the tribe Chlorideae within the subfamily Festucoideae and contained only chloridoid (cuboid) opal phytoliths. A vegetative region entirely composed of the studied species would be clearly characterized by the chloridoid opal phytoliths.

The "true prairie" grass species are:

Andropogon gerardi Vitman (big bluestem)

Andropogon scoparius Michx. (little bluestem)

Sorghastrum nutans L. Nash (indiangrass)

Panicum virgatum L. (switchgrass)

Three characteristic "true prairie" species belong to the tribe Andropogoneae. Switchgrass which was chosen because it favors a wet environment belongs to the tribe Paniceae. The two tribes are included in the subfamily Panicoideae. All four species of the two tribes are characterized by dumb-bell shaped opal phytoliths for which Prat (1948) applied the term "panicoid."

Both chloridoid and panicoid opal phytoliths should characterize the mixed-grass region. The east and west

Explanation of Plate II

Spodograms from the Western Prairie vegetation

Fig. 1 <u>Buchloe dactyloides</u>, Buffalograss
Chloridoid opal phytoliths (a)
Silicified stomata (b)
Crescent shaped opal phyt. (c)

Fig. 2 <u>Bouteloua curtipendula</u>, Sideoats grama
Chloridoid opal phytoliths (a)

Fig. 3 <u>Bouteloua gracilis</u>, Blue grama
Chloridoid opal phytoliths (a)
Silicified stomata (b)
Tall and narrow opal phyt. (d)
Irregular opal phyt. short (e)

Plate II

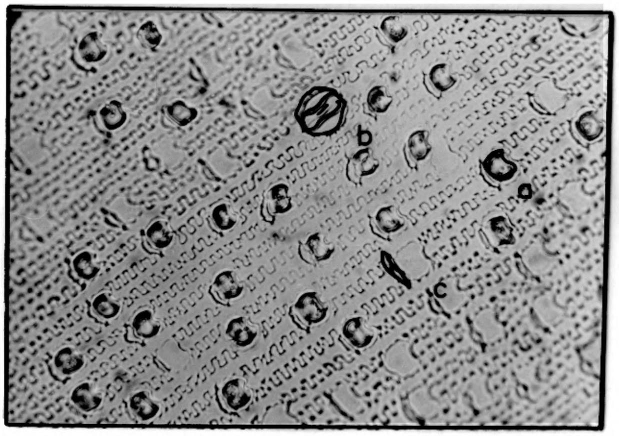


Fig. 1

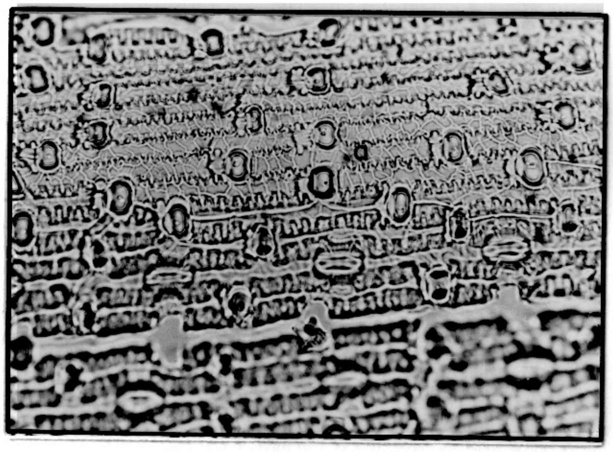


Fig. 2

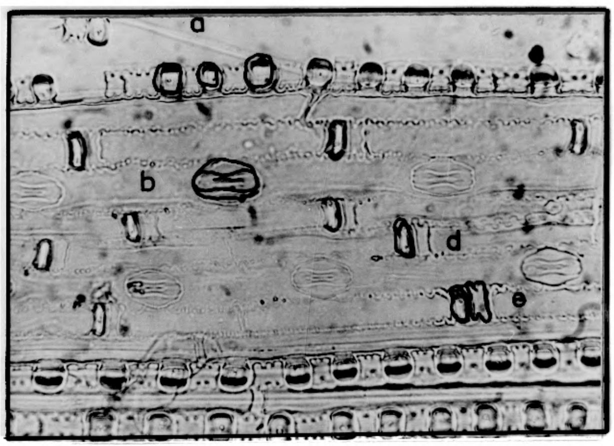


Fig. 3

Scale: | | | | | | | | |
Each Division = .ol mm

Explanation of Plate III

Spodograms from the True Prairie vegetation

Fig. 1 *Panicum virgatum*, Switchgrass
Prepared by ashing
Mature stage, panicoid (a_1)

Fig. 2 *Panicum virgatum*, Switchgrass
Prepared by acid treatment
Young stage, panicoid (a_2)

Fig. 3 *Andropogon gerardi*, Big bluestem
Young stage, panicoid (a2)
Irregular type elongated (b)
Crescent shaped (d)

Fig. 4 *Sorghastrum nutans*, Indiangrass
Mature stage, panicoid (a_1)
Young stage, panicoid (a_2)
Nodular type (e)
Nodular type sideview (e_1)

Plate III

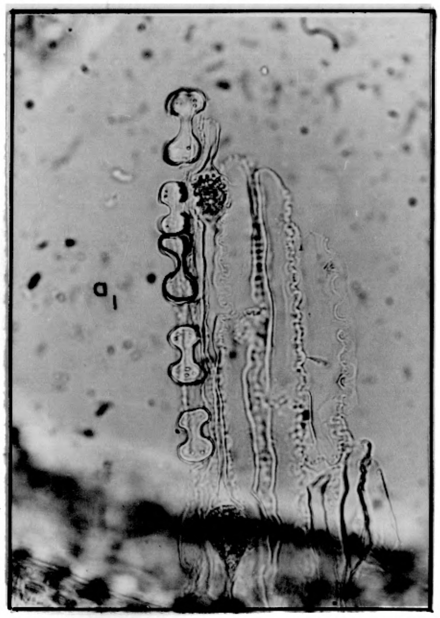


Fig. 1

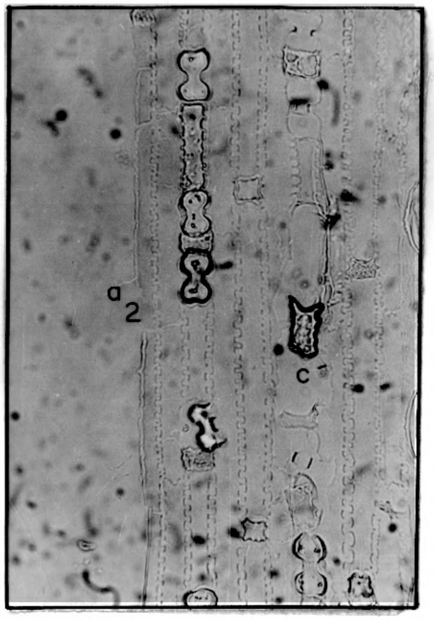


Fig. 2

Fig. 3

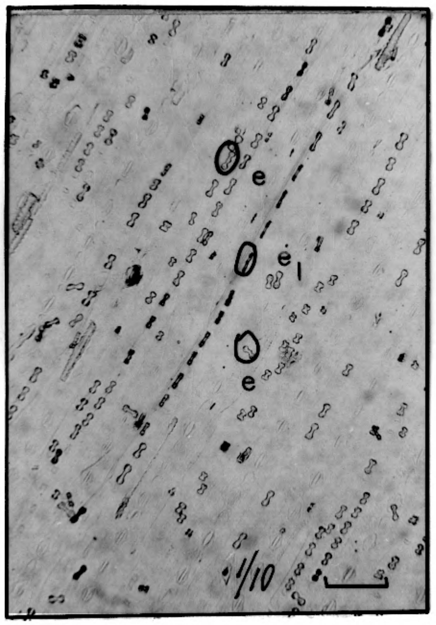


Fig. 4

Scale:
Each Division = .01 mm

Explanation of Plate IV

Irregular Silica Bodies

Fig. 1 Long-cell incrustations
Silicified stomata (f)

Fig. 2 Elongated irregular silica bodies with smooth outlines (b_3)

Fig. 3 Elongated irregular silica body
Acutely angled (c)
Smooth outlines (b_1)

Fig. 4 Elongated irregular silica bodies
Sinuous outlines (b_2)

Plate IV

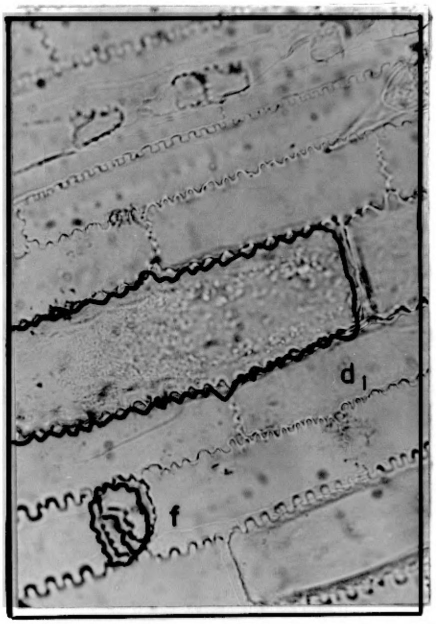


Fig. 1

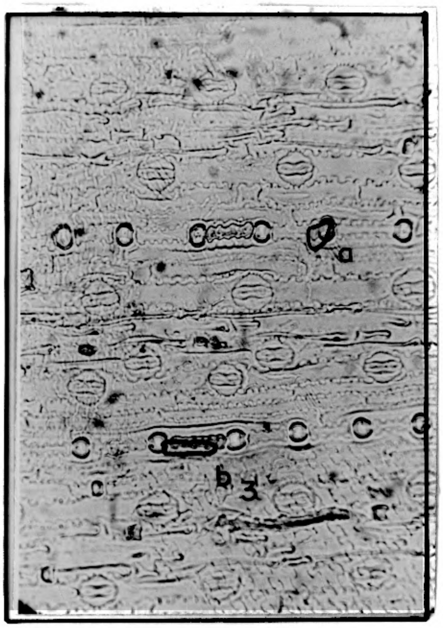


Fig. 2

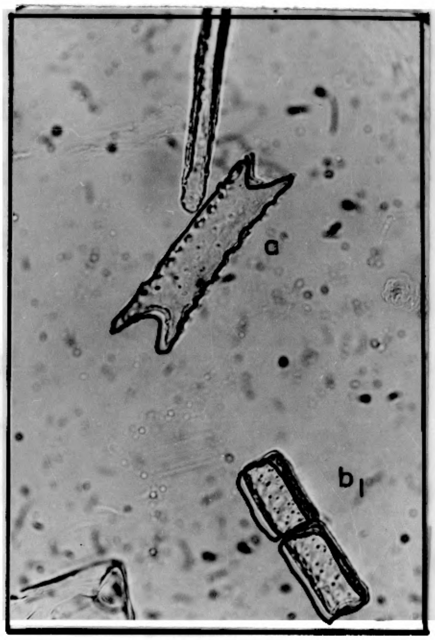


Fig. 3

Fig. 4

Scale: |||||||||
Each Division = .01 mm

boundaries are gradual and so the ratio of the two types of phytoliths should change gradually. As mentioned before, this classification is limited to a vegetative region that is composed extensively of the studied species. Other panicoid opal phytoliths are reported from the tribe Festuceae subfamily Festucoideae (Smithson 1958) and Tribsacea subfamily Panicoideae (Prat 1948). Further comparison of the occurrences of panicoid and chloridoid (dumb-bell and cuboid) opal phytoliths are necessary for a taxonomical application in botany.

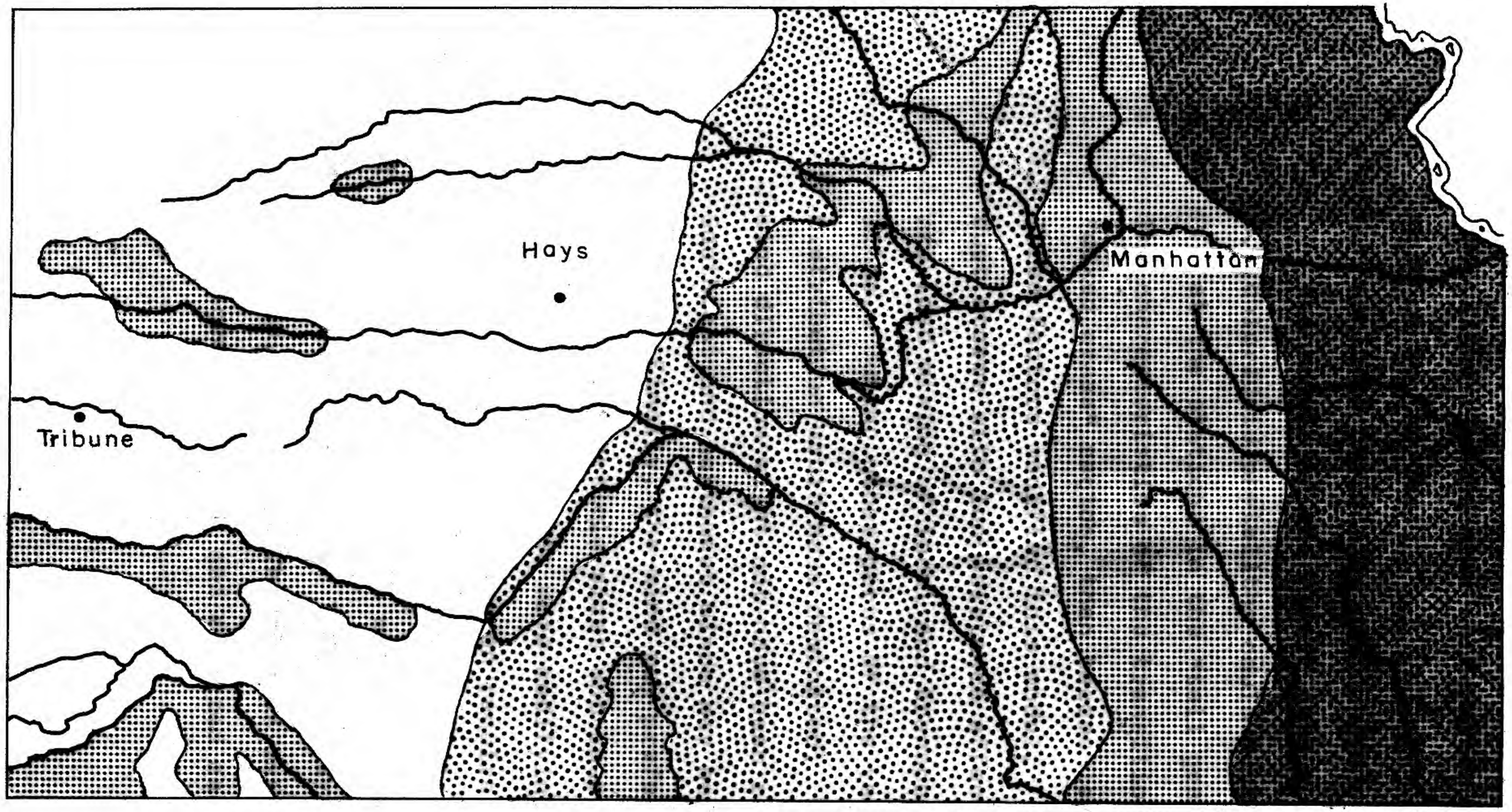
APPLICATIONS

Transportation of Dust

A model of distribution of opal phytoliths in recent dust samples has been established according to the classification of the three vegetative regions. microscopic study of dust samples collected at three locations in Kansas and one station in Missouri over the seven-month growing season showed a relationship between source and deposition area.

Hypothesis.-- The following hypothesis can be used to develop a model for the distribution of opal phytoliths in recent dust samples:

1. If all collected dust material had been of local origin, each sample should have been characterized by its local opal phytoliths, chloridoid or panicoid.



(Gates, 1936)

Short grass region — Mixed grass region — Tall grass region

Fig. 3 GRASS REGIONS OF KANSAS

Andropogon gerardi (big bluestem) shown separately.

2. In case of transportation of characteristic opal phytoliths by wind over large areas either to the east or to the west,
   a. panicoid phytoliths could have occurred in the sample of the "short grass" region, or,
   b. chloridoid phytoliths could have been found at the stations in the "tall grass" region.

   The characteristics of the transition zone, the "mixed grass" region, then became less distinguishable.
3. Three factors modified this model. The periodical dust storms with long-distance effects might have provided mixing of local and transported opal phytoliths. During other parts of the year, the local distribution would have dominated. Secondly, the wind direction might not have been restricted. Southwest and northwest winds may have had a shifting effect. Finally, the wind may preferentially transport special particle sizes and shapes.

Observations.-- To include more modifying factors and to select a final model for application required a more detailed study. Some observations and a general trend that apply to the model are shown below. Samples were available from four locations in a west-east line from Tribune, Hays, and Manhattan, Kansas, to McCredie, Missouri. The samples were taken continuously during the growing season and analyzed each month.

The April samples indicated a local influence; a very low percentage of opal phytoliths was observed at all locations. Only the Tribune sample contained a few chloridoid opal phytoliths, whereas all other samples had some irregular-shaped phytoliths. No opal was observed in the sample from McCredie.

For May, an increase of up to estimated 20% of the total sample was observed in the Tribune sample. Also an increase of the chloridoid type in the Tribune and Hays samples was found.

All June and July samples had a continuous increase of opal content (estimated 1/4 to 1/3 of total sample). More chloridoid opal phytoliths occurred in the Tribune and Hays samples. The latter sample also was characterized by some immature panicoid opal phytoliths. Manhattan had equal concentrations of the chloridoid and panicoid types. Also, in the McCredie sample a few chloridoid opal phytoliths were observed. The dominant phytolith at all locations was the irregular-shaped type.

The high percentage of opal phytoliths remained during August. Hays had a maximum, with about 50% of irregular and cuboid types. Manhattan showed local types plus a considerable amount of chloridoid phytoliths. No sample from McCredie was available.

September showed a decrease in the content of phytoliths (1/3) in all four samples. The distribution of the characteristic types remained constant. A further decrease occurred in October.

Explanation of Plate V

Opal Phytoliths from Dust Samples

Fig. 1 Sample #6641, irregular elongated silica body, collected at Manhattan, Kansas, June, 1964.

Fig. 2 Sample #7641, panicoid opal phytolith collected at Manhattan, Kansas, July, 1964.

Fig. 3 Sample #7642, chloridoid opal phytoliths collected at Tribune, Kansas, July, 1964.

Fig. 4 Sample #7646, chloridoid and panicoid (young stage) opal phytoliths, colleted at Hays, Kansas, July, 1964.

Plate V

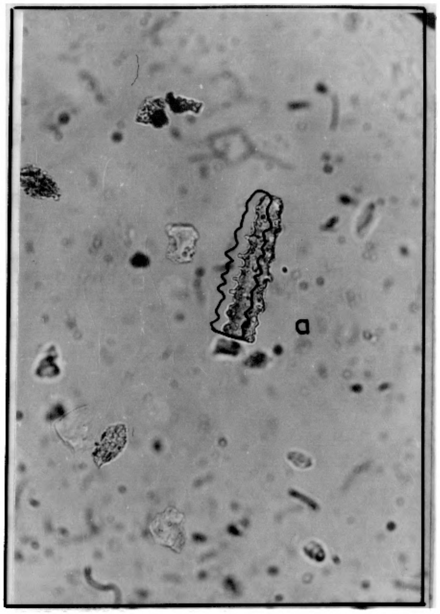
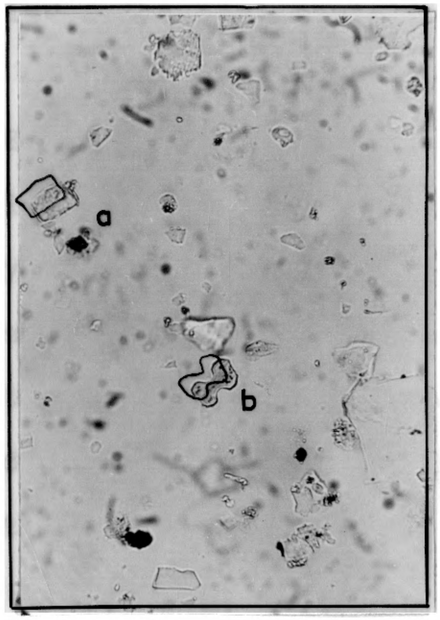


Fig. 1



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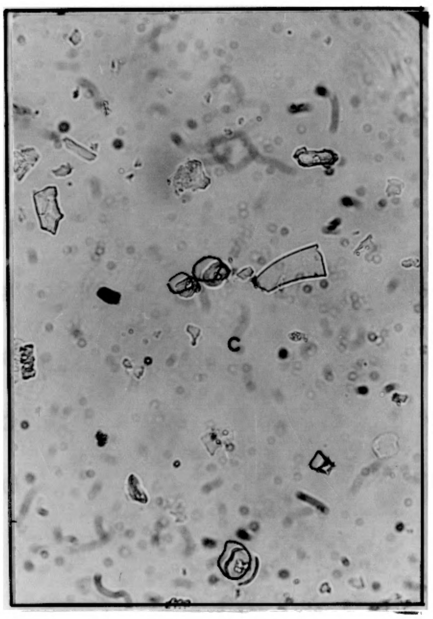

Fig. 3

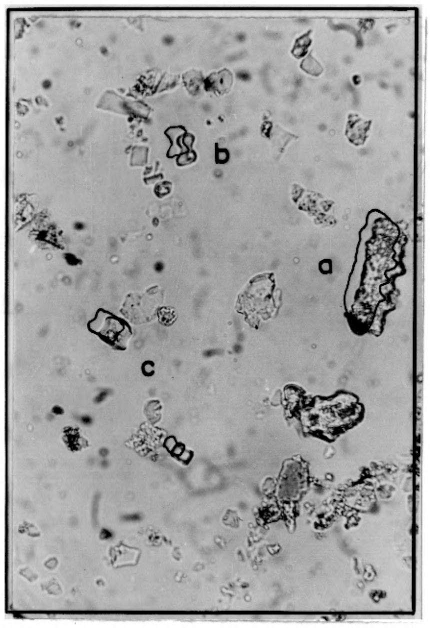

Fig. 4

Scale:
Each Division = .01 mm

Interpretation.-- The maximum content of opal phytoliths (50%) and the average content of about 10% in the dust samples indicated a selective action by wind. The opal phytolith content of the soil, which partly provides the wind-blown material, is about 1% (Jones and Beavers, 1964). However, the uppermost unconsolidated layer "fluff" of the soil profile at Manhattan, Kansas, has an opal phytolith content of about 80% (personal communication P.C. Twiss, 1965). By this, the importance of opal phytoliths in the dust composition is shown. The maximum in July and August indicates a strong relationship of the opal phytolith content to the growing season.

The influence of the local opal phytoliths on the dust deposits can be seen. At Tribune, Kansas, only chloridoid types were found; at Hays, the chloridoid type dominates, but some panicoid opal phytoliths are present. Manhattan is entirely characterized by panicoid shapes. McCredie's local composition of opal phytoliths was not known because the dominant grass species were not investigated. The location was included in the investigation to check a possible long distance effect. The variations of the relative abundance of the chloridoid type in the Manhattan and McCredie samples might indicate a long distance effect by wind transportation.

Opal Phytoliths in Soils

Samples.-- Recent soils that have been partly derived from different parent material, two unnamed paleosoils, and several Pleistocene terrestrial sediments were analyzed for phytolith content.

Recent soils.-- The following recent soils were examined:

1. Florence cherty clay loam. A dark colored soil with a clay loam surface and various amounts of chert fragments. Part of the parent material was the Florence Limestone. The native vegetation consisted of tall and mid grasses. The sample was taken east of Highway 13 in the center of NE1/4, NE1/4, Sec. 21, T 8 S, R 7 E, Riley County, Kansas. The slope was 1-2%; the depth 5-15 centimeters.
2. Monona silt loam. A weakly developed grayish brown silty loess. Tall grasses were the vegetation on the gentler slopes. The sample was taken in the center of NW1/4, NW1/4, of Sec. 18, T 10 S, R 5 E, in Geary County, Kansas, on a 7-10% slope within 12-40 centimeters of depth.
3. Hastings silty clay loam. A dark colored soil formed from silty loess occurring on 4-8% slopes. The sample was taken in the SE corner of the NE1/4 of Sec. 18, T 10 S, R 5 E, in Geary County, Kansas, from a pasture with native tall grasses.
4. Crete silty clay loam. A dark grayish brown soil formed from fine textured loess. Native grasses occurred on a 0-1% slope. The sample was taken in the NE1/4, NE1/4, Sec. 3, T 10 S, R 5 E, in Geary County, Kansas.

Unnamed buried soil horizons.-- The following unnamed buried soil horizons were sampled:

5. A dark brown silt loam which occurred about 120 centimeters below the Monona silt loam. The buried soil represented an A_1 horizon from silty loess. The sample was taken from the location at the center of NW1/4, NW1/4, Sec. 18, T 10 S, R 5 E, Geary County, Kansas.

6. A dark brown loamy sand was taken about 100 centimeters beneath the Derby loamy sand in Geary County, Kansas. The parent material was dune sand; however, the subsoil consisted of varying amounts of chert fragments developed from the Florence limestone on top of the Blue Springs shale. The location was the SW corner of SE1/4, SW1/4, Sec. 9, T 12 S, R 5 E.

Pleistocene sediments.-- All samples of the Pleistocene sediments were taken at the Iowa point section (NE1/4, SE1/4, Sec. 6, T 2 S, R 20 E), Doniphan County, Kansas.

7. Peoria silt member. (Wisconsian Stage, Iowan Tasewellian Sub-stages) A tan, massive structureless silt. The sample was taken from the lower part of the vertical face of the quarry.

8. Loveland silt member (Illinoian Stage). Reddish massive silt, characterized by a joint system with limonite concentrations along joints. The silt

included the lower part of the Sangamon soil profile where the sample was taken.

9. Kansas till (Kansan Stage). The till matrix was clay and silt. It contained pebbles and cobbles of limestone and igneous rocks. Gray and yellow mottling were characteristic.

10. Nebraska till (Nebraskan Stage). Till with clay and silt matrix, limestone and igneous rock pebbles. The Afton soil profile was a good marker bed within the Nebraska till. The sample was taken from this characteristic buried soil profile.

Laboratory Procedures.-- Previous measurements (see p. 19-20) of opal phytoliths suggested that they should be found in the size range from 10 to 25 microns; this fraction was separated from the sample by gravity sedimentation. Data for particle size, temperature, and settling time were taken from Jackson's "soil Chemical Analysis" p. 115. Clay aggregates were dispersed with 50 milliliters of one normal Calgon solution per 1000 milliliters suspension. The settling process was repeated from 10 to 19 times according to the different concentrations of clay and organic matter of the 150-200 grams samples.

After slow and careful drying of the silt, the opal phytoliths were separated by centrifugation. A mixture of bromoform and tetrachlormethane with a density that was between 2.2325 and 2.3188 was used as heavy liquid. The density was constantly checked by density glass cubes. Lipophobic properties of the silt size particles made it necessary to add a .5% rubber solution (natural rubber in bromoform) for a good suspension. Wetting of the particles with acetone was another method to obtain a good suspension. One to one-half grams of silt in two milliliters of acetone were added to six milliliters of heavy liquid. After evaporation of the acetone (24 hours), the suspension was centrifuged for five minutes. The opal phytoliths and other light minerals were decanted, filtered, washed several times in acetone, dryed and mounted in Cadex.

Results.-- Microscopic examination of opal phytoliths from recent soils showed that the separation from other soil minerals was almost perfect. Many opal bodies from the Florence cherty clay loam were coated with clay skins. Irregular phytoliths were dominant and some panicoid were observed. The Crete, Hastings, and Monona samples contained uncoated opal phytoliths. The number of panicoid types was greater than in the Florence cherty clay loam and several had been broken at the smallest width of the middle portion. In all samples, irregular types and incrustations were dominant. One-third of the incrustations and irregular types of the samples could be partly stained by iodo-potassium-iodide. The staining occurred mostly over less than one-half of the surface area.

Monona and Derby buried soils were very similar to the recent soil samples in the amount and type of opal. Many of the irregular types were pitted. Seemingly more chloridoid types were present than in the recent samples. Some opal

Explanation of Plate VI

Opal Phytoliths from Recent Soils

Fig. 1 Panicoid opal phytolith from Monona silt loam, Geary County, Kansas.

Fig. 2 Panicoid opal phytolith from Hastings silty clay loam, Geary County, Kansas

Fig. 3 Irregularly shaped opal phytolith from Florence cherty clay loam, Riley County, Kansas.

Plate VI

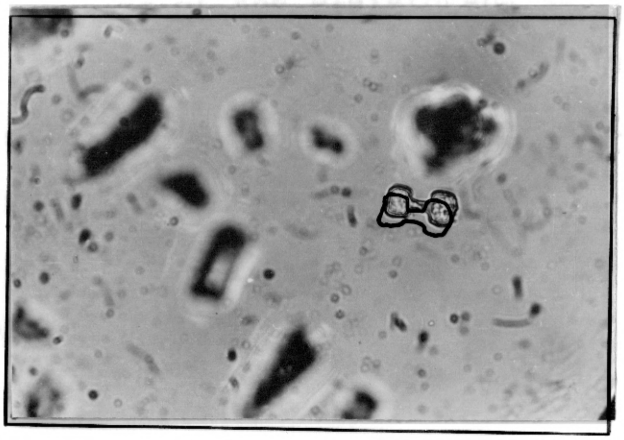

Fig. 1

Fig. 2

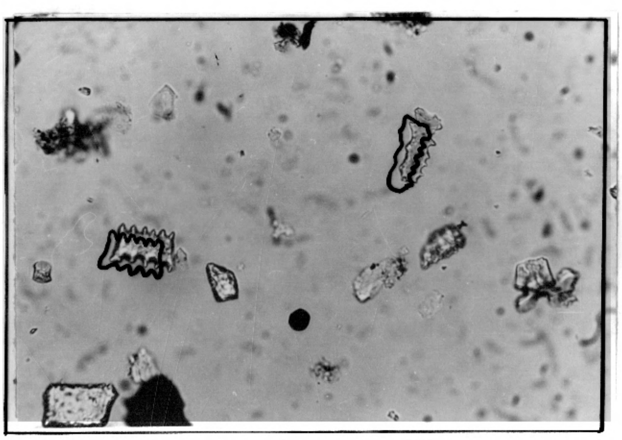

Fig. 3

Scale: ||||||||

Each Division = .01 mm

phytoliths were stained with iodo-potassium-iodide, but most remained colorless.

All four samples of the Pleistocene sediments contained opal phytoliths and most were larger than 20 microns. Pitting and other corrosion characteristics were strongly developed. Only irregular phytoliths were found. The remnants of the ancient soils, Sangamon and Afton, and the Peorian loess contained more opal phytoliths than the Kansas till sample. No staining was possible with iodo-potassium-iodide.

Optical examination was made under plain and polarized light. The phytoliths are isotropic and only one Becke line was observed. The indices of refraction were determined by the immersion method. (Table 3).

Table 3.-- Index of refraction of Opal Phytoliths

Opal Phytoliths from	Index of Refraction (± .001)
Florence cherty clay loam	1.440 - 1.449
Crete silty clay loam	1.440 - 1.448
Hastings silty clay loam	1.440 - 1.448
Monona silt loam	1.440 - 1.448
Monona buried horizon	1.448 - 1.459
Derby buried horizon	1.447 - 1.460
Peorian loess	1.465 - 1.476
Sangamon soil	1.447 - 1.461
Kansas till	1.470 - 1.478
Afton soil	1.469 - 1.476

<u>Interpretation and conclusions.</u>-- The opal phytoliths which could not be stained with iodo-potassium-iodide in the <u>recent soil</u> samples indicated that a rapid removal of the stainable

layer must have taken place after the opal phytoliths had been released from the epidermal cells of the plants. The index of refraction that ranges between 1.440 and 1.449 indicated also that the sheath had been removed. Removal by dissolution was not expected because Engel (1953) reported highly insoluble silica-cellulose compostion. So mechanical abrasion in the soil is one possible explanation.

The dominance of the relatively large irregular opal phytoliths as compared to the number of the regular types, might be due to a difference in solubility. Only the leaves were studied but another source of opal phytoliths, especially of the irregular types, could have been the stems of grasses. But all recent soil samples contained enough regular shaped opal phytoliths so that a relationship to the vegetative region could be recognized. This observation together with the determination of the grassland forest boundaries, as shown by Witty and Knox (1964), will add more qualitative details in study of grassland soils.

The depth distribution of phytoliths in soils and a catenary relationship reported by Jones and Beavers (1964) provided other suggestions which demonstrate that opal phytoliths were specific characteristics within some great soil groups. Among these soils differences occurred in the physical properties of permeability, temperature, reaction (pH value), and vegetation. The permeability affects the distribution of opal phytoliths in the soil profile. Temperature and reaction influence the solubility; and the vegetation provides the overall opal content. Opal phytoliths as influenced by these factors could be used as descriptive soil characteristics.

Soil classification.-- The new soil classification system in the United States is based on soil morphological characteristics (U.S.D.A. Soil Classification, A Comprehensive System: 7th Approximation, 1960). The grouping of the families depends on the same properties which influenced the preservation of opal phytoliths. Similar mineralogy of observed soil series characterize the family category. With respect to the opal phytolith content, the soil could be called "siliceous" if other SiO_2 compounds were present. Nonacidic and neutral soils provide better conditions for the preservation of opal phytoliths than acidic soils.

The mean temperature has some influence on a soil reaction (pH). According to Niggli (1926) and Siever (1962), frigid soils (annual mean temperature $< 47^{o}F$) would contain the most opal phytoliths. This conclusion can only be proved, if accurate values of accumulation of opaline silica per acre per year can be calculated. Witty and Knox (1964) as well as Jones and Beavers (1964) reported some data on the accumulation of silica per acre per year. These data included the kind of vegetation and parent material which make the soluble silica available.

If the higher categories (Order-Subgroup) of soils, containing opal phytoliths were established by common methods, Explanation of Plate VII

Opal Phytoliths from Buried Soils

Fig. 1 Chloridoid, panicoid and irregular shaped opal phytoliths from Derby buried soil horizon, Geary County, Kansas.

Fig. 2 Panicoid opal phytolith from Derby buried soil horizon, Geary County, Kansas.

Fig. 3 Panicoid (a), chloridoid (b), and irregularly shaped (c) opal phytoliths from Monona buried soil horizon, Geary County, Kansas.

Plate VII

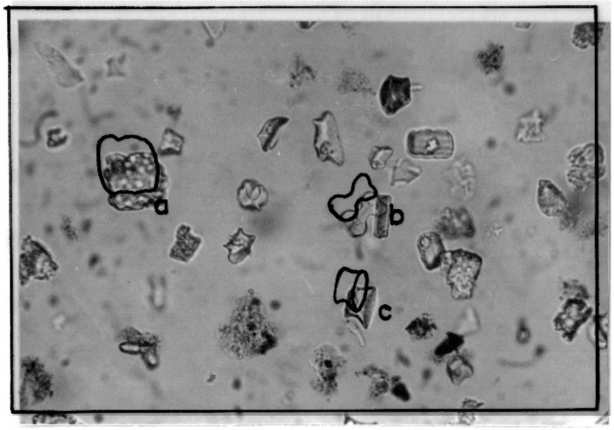

Fig. 1

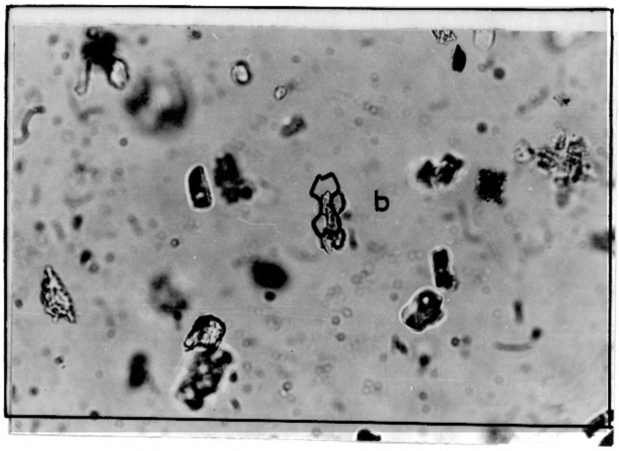

Fig. 2

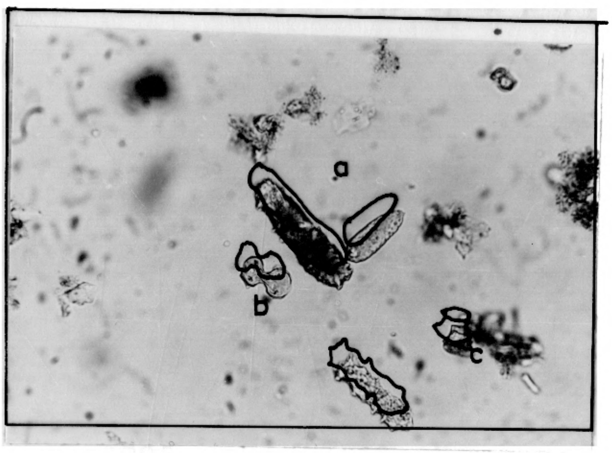

Fig. 3

Scale: |||||||||
Each Division = .01 mm

then the amount and distribution of opal phytoliths could be used for classifying the lower categories, especially the soil families. No example has been tested, but the studies of Jones and Beavers (1964) show that within the great soil groups, series occur which have similar ratios of opal phytoliths of fine silt to coarse silt size (5-20 microns/20-50 microns).

Buried Horizons.-- Partly abraded opal phytoliths occur as fossils in the buried soil horizons. The index of refraction ranges between 1.447 and 1.460 and is a result of the loss of water after deposition. The few opal phytoliths which could be stained were believed to be transported down from the recent surface because the permeability of the overlying soil was very high (loamy sand). The estimated higher quamtity of chloridoid types could have indicated an ancient "short grass" vegetation. The organic content of the Monona buried horizon was considerably higher than in the recent A_{11} horizon of the Monona silt loam. This indicated a longer and intenser period of soil development.

Pleistocene sediments.-- The opal phytoliths in Pleistocene sediments indicated a depostion of the silt, till, and loess material under vegetative cover. The plant opal in the Peorian loess, however, could have been transported by wind from the source area of the loess deposits, whereas the fossil opal phytoliths from the Loveland silt and Nebraska till have been formed under local vegetative cover developed on the Sangamon and Afton soil horizons.

Explanation of Plate VIII

Opal Phytoliths from Pleistocene Sediments

Fig. 1 Irregularly shaped (a) opal phytolith from Peorian loess, note also fragment of diatom (b).

Fig. 2 Irregularly shaped opal phytolith from Loveland silt (Sangamon soil), surface is affected by dissolution.

Fig. 3 Irregularly shaped opal phytolith from Kansas till, elongated and with characteristic surface structure.

Fig. 4 Irregularly shaped opal phytolith from Nebraska till (Afton soil), elongated and with characteristic surface structure.

Plate VIII

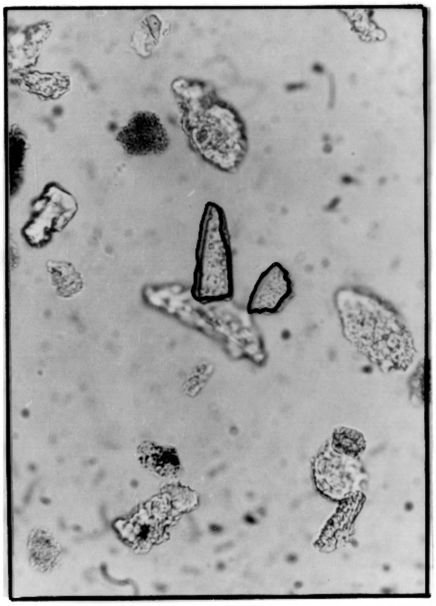

Fig. 1

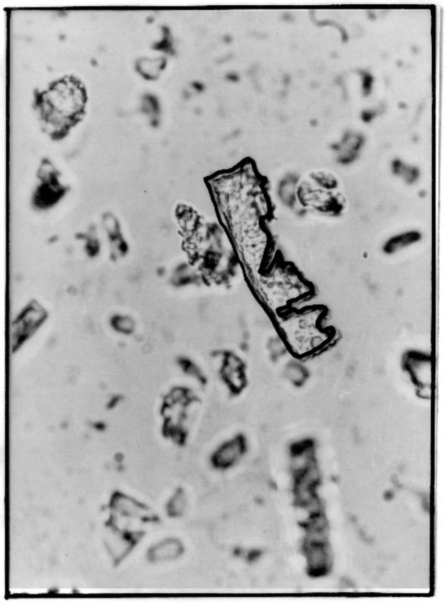

Fig. 2

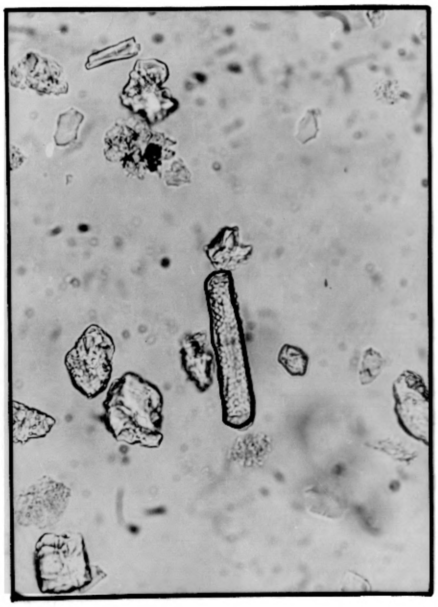

Fig. 3

Fig. 4

Scale: |||||||||
Each Division = .01 mm

The Kansas till sample was taken from a coarse silt, sand and gravel bed. Here the opal phytoliths indicated a water deposit which drained an area under vegetation rather than bare sand or sand plain in front of the retreating Kansas glaciers.

The most frequently observed size of greater than 40 microns might be due to removal of the smaller particles by dissolution. Also these larger opal phytoliths were heavily affected by solution (Plate VIII Fig. 2). Where the surface was not badly etched, a sinuous structure finer than that of recent opal phytoliths was observed; this was considered to be characteristic difference. The index of refraction indicated a further loss of water.

DISCUSSION

Formation and Function of Opal Phytoliths

The precipitation of opal in plants was described by Frey-Wyssling (1935, p. 58) as follows:

$$SiO_2 \text{ Solution} \xrightarrow{\text{Conc.}} SiO_2 \text{ Sol} \xrightarrow{\text{Prec.}} SiO_2 \text{ Gel} \xrightarrow{-H_2O} \text{Opal}$$

molecular dispers	colloid dispers	$n\ H_2O \times n\ SiO_2$	hard

This basic process of the formation of silica bodies in plant cells is accepted by all authors; however, the interpretations of the function of opal phytoliths vary considerably among workers. Some authors believe that silica bodies have no physiological function while others think they are physiologically functional in a number of ways.

After analyzing bamboo in Java, Molisch (1923) gave the first interpretation for the formation of opal phytoliths. He observed an increase in the rate of accumulation of silica in destroyed internodiae of bamboo species. He explained this as due to the transpiration stream with which the plant tried to heal the destroyed tissue. Netolitzky's summary (1929) of silica bodies showed a relationship between the abundance of silica in plants and tropical climate where the amount of available silica in solution depends on the annual mean temperature. Studies on beech leaves by Laiseca (Lanning 1960) also showed a direct relationship between accumulation of silica and transpiration water. These observations give the impression that opal phytoliths are simply accumulations of silica formed by the transpiration stream of plants and that no physiological function is served. The fact that monocotyledon families contain more species which accumulate silica than dicotyledons, supports the theory that the storage of silica might have a physiological function in lower plants.

Dicotyledons could be better adapted to the available silica in solution or could have developed selective absorption and not be faced with the problem of storing abundant silica.

Other observations made in plant growth indicate that the yield per acre of rye, oats, barley, and wheat after fertilizing with silicates has increased. Some substitution of phosphorous by silicon took place and growth deficiencies caused by a shortage of silica were reported. All experiments faced the difficulty of growing control plants completely without silica, because traces of silica changed the results.

Engel (1953) studied silica compounds in rye to show the functional importance of silicon for plant growth. He found a soluble silica-galactose substance and a highly insoluble silica-cellulose compound. The latter is considered to be identical with the described silica incrustations of this investigation and similar to the stainable and birefringent coatings of the opal phytoliths. Engel also observed different ratios of silica to galactose in these substances and concluded that different physiological functions existed.

The necessity of silica for plant growth, or, simple storage without function in growth are opposite interpretations of the role of silica in these experiments. One possible explanation encompassing both interpretations would be that the minimum amount of available mineral limits the plant growth, first stated by Justus von Liebig. The minimum amount of silicon as an essential mineral for plant growth might be very small but the available amount of soluble silica in nature is very high. This might be the reason that silicon has long been unrecognized as an essential element for plant growth.

Limitation and Extension of Applications

Dust transportation.-- The presence of silicon for plant growth might be of importance for further analysis on dust deposition. This is not only because of the possibility of recognizing the source area of dust deposits, as shown in this investigation, but more in the chemical composition of the transported silica.

If plants contain any silica-organic compounds, their use of silicon or partly decomposed silica-organic material might be satisfied by dust deposits. The dust deposits contain by far more opal phytoliths than any other known natural sediment. However, it is not known if among this opal material any silica-organic compounds were present. Unidentified amorphous aggregates might contain some of this material.

Two of the mentioned silica-organic compounds were easily soluble, which would increase their value for absorption by plants. Wind would be the only agent which could transport this material before it decomposed in place.

Recent soils.-- The different recognition of opal phytoliths as soil characteristics have been demonstrated by Beavers and Jones (1964) and by Witty and Knox (1964). As mentioned, the quantitative use was stressed rather than the qualitative. However, all studies using quantitative measurements could easily be affected by losses of opal phytoliths through solubility or other processes. Solubility as a limitation will be discussed with fossil soils, but it would also be a limitation in the suggested use of opal phytoliths in soil family classification, which has its greatest disadvantage in long laboratory procedures.

Fossil soils and Pleistocene sediments.-- Solubility optical isotropy, and the relation of the H_2O content to the index of refraction, are important in the use of fossil opal phytoliths.

As the climatic conditions might be of importance to the origin of opal phytoliths, as mentioned under Formation and Function, so is the solubility of even greater importance for the preservation of opal phytoliths. Solubilities of silica under different pH values and under different temperature conditions have been studied by many mineralogists and geochemists. The low temperature solubility of amorphous silica (this term includes opal, Krauskopf, 1956) is well known and increases linearly with increasing temperature.

The solubility as a function of the pH value has been discussed by many authors. Correns (1941) reported a steady increase in the solubility of silica with the change from pH 3 to pH 10 and from pH 3 to pH 1. However, Alexander and others (Krauskopf, 1956) found that the solubility of precipitates of monomeric silicic acid, from pH 1 to pH 9, is unchanged. Their values are proved by other workers (Siever, 1962).

If it is assumed that the opal phytoliths are precipitates of monomeric silicic acid, their solubility in soils or other sediments would depend only upon the temperature. No extreme pH values exceeding 1 and 9 are commonly present under natural conditions. If the temperature in fossil soils or other sediments that contain opal phytoliths is constantly below 25 degrees C., the solubility is between 70 and 100 parts per million

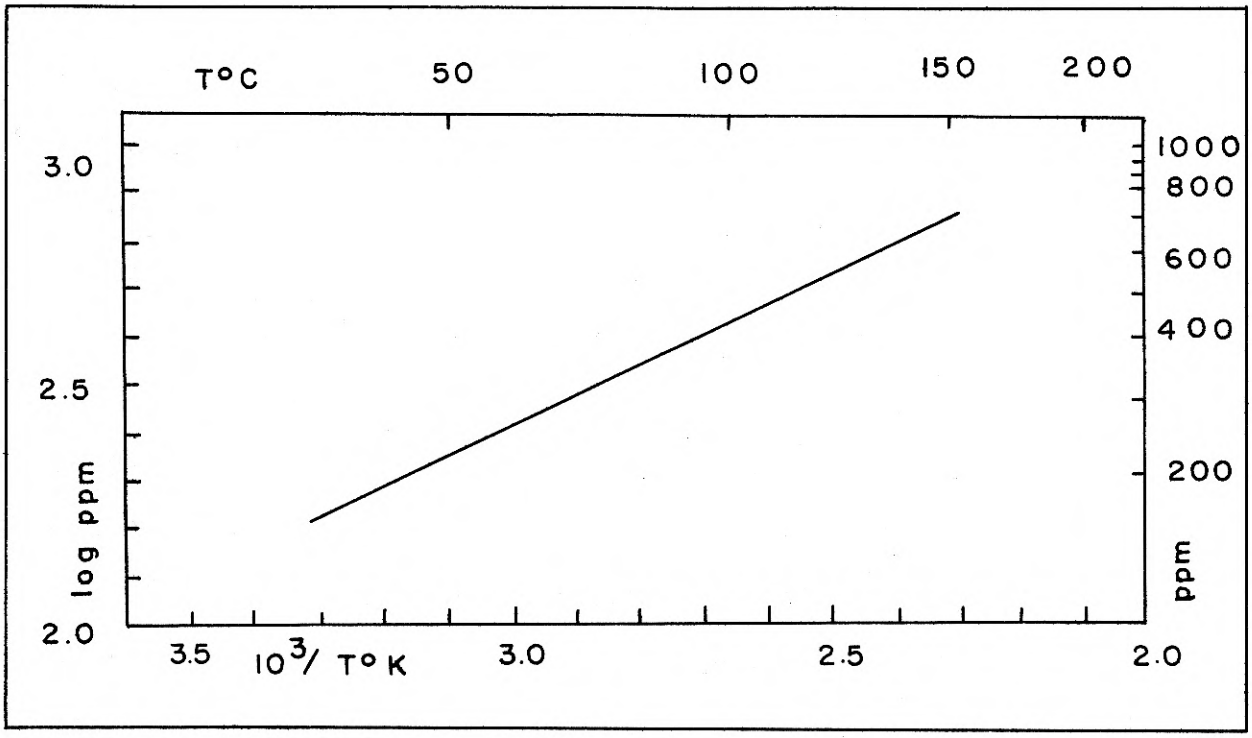

Fig. 4 The solubility of amorphous silica as a function of temperature. (R. Siever, 1962)

and the rate of solution is very slow (Krauskopf, 1956).

The fact that recent soils do not contain the same number of morphological varieties of opal phytoliths as observed in plants might also suggest that some phytoliths are composed of a partly soluble silica-organic substance. For application of phytoliths in Pleistocene stratigraphy, only the "true" opals with a slow rate of dissolution are useful as the phytoliths in Peorian loess, Loveland silt, and Kansas and Nebraska till demonstrate. But the rate of dissolution cannot be extrapolated into recent geologic time and can hardly be used as an absolute time criterion because the conditions for solution are extremely variable.

Opal as a disordered hydrated form of silica (Siever, 1962) has no characteristic X-ray diffraction pattern. Christobalite properties were reported by Lanning (1960) and chalcedony was observed by Beavers and Stephens (1957). Alteration into alpha-quartz was interpreted by Lanning (1960) as being due to the ashing procedure. The birefringence and the index of refraction observed in this investigation on fresh grass opals are properties of christobalite (n Omega crist. = 1.492, n epsilon crist. = 1.487, delta = .003; Observed values: n = 1.494-1.488, delta = .001-.002). However, the absence of any characteristic X-ray pattern, the stainability, and the decrease of the high index of refraction as well as of the observed birefringence in all soil samples suggested that the outer part of the phytolith is a silica-cellulose compound. To what degree alterations and inversions could take place to change the compostion of the phytoliths to a cristobalite or silica-cellulose compound was not investigated. This is considered to be worthy of further investigation.

The relationship between H_2O content and index of refraction as shown by data from Winchell and Winchell (1951) made a determination of the H_2O content of opal phytoliths possible. The water content as indicated by the refractive index is dependent on the age of the sediment (Fig. 5). Whether the increase of the refractive index was directly related to the absolute age of the opal phytoliths was not

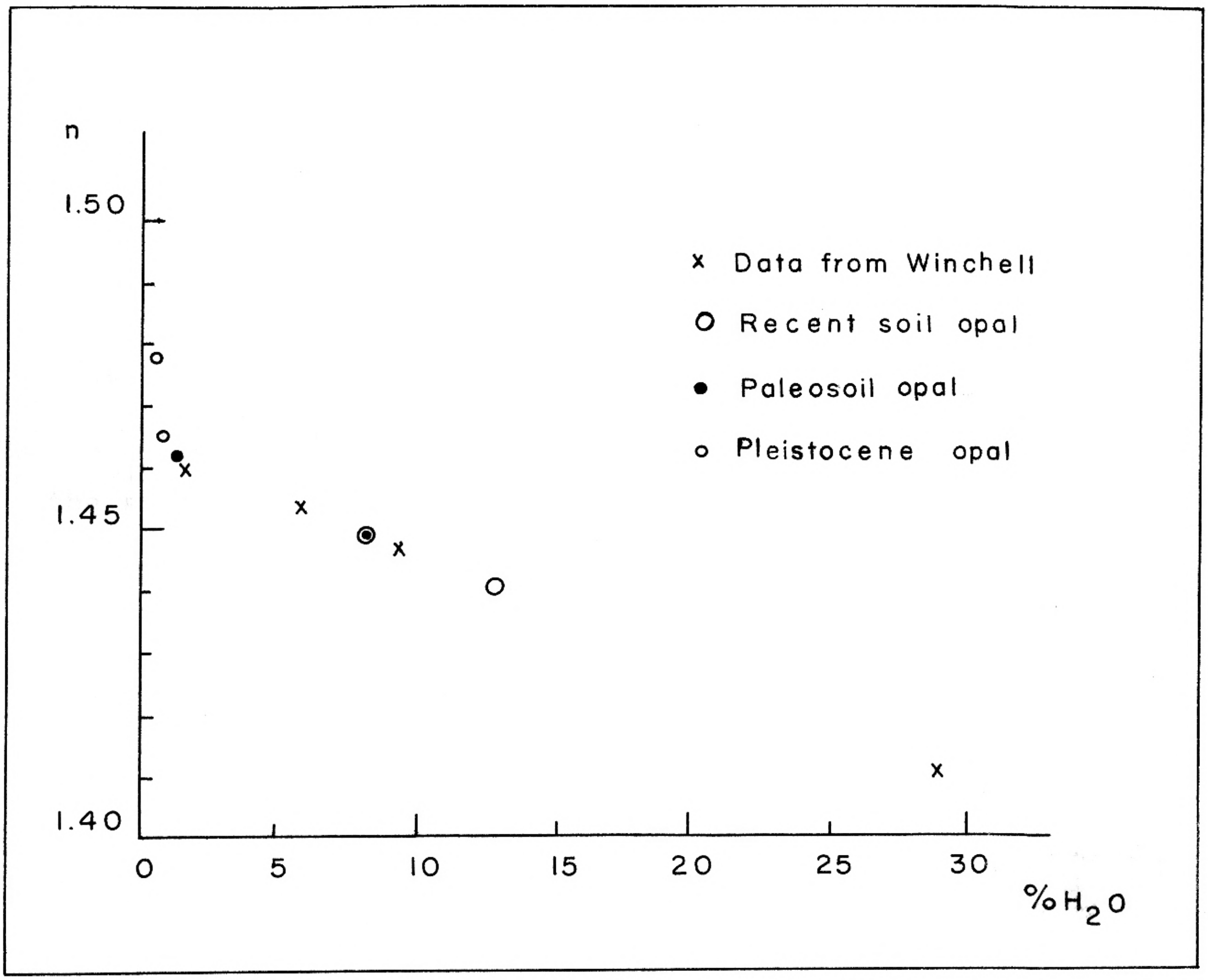

Fig. 5 Water content and refractive index of opal phytoliths from Recent soils, Buried horizons, and Pleistocene sediments.

sufficiently analyzed. This relationship might be a problem for further work to improve the usage of opal phytoliths in Pleistocene stratigraphy.

SUMMARY

Opal phytoliths have been prepared from grass leaves by ashing and acid treatment, described according to their physical properties, and classified on morphological differences. By using this classification characteristic shapes could be proposed for the different vegetative regions of the Great Plains.

The use of opal phytoliths in the determination of the source areas of recent dust deposits has been proposed. Characterizing recent and buried soil profiles and recognizing Pleistocene sediments as being deposited under a vegetative cover have been shown by examples.

The index of refraction is one characteristic used to distinguish between fresh grass opals from those in recent and buried soils and from Pleistocene opal phytoliths. Other properties such as birefringence and stainability are interpreted as being indications that at least part of the phytoliths may be composed of a silica-cellulose organic compound.

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APPENDIX

Appendix I

<u>Length-width measurements of regular opal phytoliths</u>.--

Each unit = .00019 millimeter.

Column I				Column II				Column III				Column IV				Column V				Column VI			
Sorghastrum nutans				*Panicum virgatum*				*Andropogon scoparius*				*Andropogon gerardi*				*Andropogon gerardi* I				*Andropogon gerardi* 2			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
156	63	30	37	120	69	18	27	121	68	17	29	133	67	34	27	69	74	15	39	97	72	15	32
144	70	27	30	170	78	37	25	128	63	23	28	95	67	15	35	97	77	12	39	95	67	15	35
158	56	30	31	134	75	23	30	108	69	25	30	97	72	25	32	71	80	11	45	133	67	34	27
132	73	25	34	119	70	23	32	138	69	29	30	75	87	18	45	97	77	16	30	107	61	20	22
131	67	28	28	131	69	18	26	110	86	22	33	78	85	12	49	94	80	16	42	130	78	27	29
130	70	25	35	137	79	20	33	127	83	27	36	126	80	16	42	126	65	25	15	82	79	14	43
168	62	48	35	137	82	24	31	167	74	38	27	97	77	16	30	78	85	12	48	135	65	50	30
127	44	28	22	112	74	21	26	131	67	28	32	71	80	11	45	75	87	18	45	113	60	28	28
154	53	27	23	154	81	33	42	141	71	21	28	97	77	12	39	80	76	20	42	98	67	23	25
135	48	37	24	152	75	33	26	130	63	28	27	107	61	20	22	79	74	20	37	88	67	20	36

Data for computation.--

	Column I	Column II	Column III	Column IV	Column V	Column VI
$\Sigma(a) =$	1436	1366	1301	876	866	1078
$\Sigma(b) =$	606	752	713	753	775	683
$\Sigma(c) =$	306	250	258	169	165	246
$\Sigma(d) =$	300	282	300	365	382	307
$\Sigma(a)^2 =$	207973	189480	171793	99016	77682	119538
$\Sigma(b)^2 =$	37533	56758	51375	57335	60405	47011
$\Sigma(c)^2 =$	9782	6670	6950	3251	2895	7144
$\Sigma(d)^2 =$	9237	7840	9076	14001	15418	9757
$\Sigma(ab) =$	86828	103178	92603	59491	66515	73422
$\Sigma(ac) =$	44400	35105	34167	17341	14652	28128
$\Sigma(ad) =$	43237	38052	38832	34673	31819	32502
$\Sigma(bc) =$	18297	18955	18407	12485	12637	16606
$\Sigma(bd) =$	18495	21064	21533	28068	30059	21176
$\Sigma(cd) =$	9177	6905	7721	5864	6041	7327

Appendix 2

Analysis of Variance

Data Columns I-IV
(Miller & Kahn, 1962,
p. 178-179.)

Computational formula.--

Source	Degree of Freedom (DF)	Sum of Squares (SS)	Mean of Squares (MS)	F-Ratio
Among	k-1	$\Sigma_i \frac{(a_{ij})^2}{n} - C$	$\frac{SS}{DF}$	$\frac{\text{MS among}}{\text{MS within}}$
Within	k(n-1)	$\Sigma_i \Sigma_j a_{ij}^2 - \Sigma_i (\Sigma_j a_{ij})^2$		
Total	kn-1	$\Sigma_i \Sigma_j a_{ij}^2 - C$		

Definitions.--

Σa_{ij} = Sum per species

$\Sigma_i \Sigma_j a_{ij}$ = Total sum of Species

(1.)= $\Sigma_i \Sigma_j a_{ij}^2$ = Sum of squares of species

(2.)= $\frac{\Sigma(\Sigma a_{ij})^2}{n} = \frac{\text{Sum of the squared sums of species}}{\text{Number of samples per species}}$

(C) = $\frac{(\Sigma_i \Sigma_j a_{ij})^2}{kn}$ $\frac{\text{Squared total sum of species}}{\text{Number of variables number of samples}}$

Sum of squares among species $(SS)_A$ = (2.) - (C)

Sum of squares within species $(SS)_W$ = (1.) - (2.)

Sum of squares total $(SS)_T$ = (1.) - (C)

Computations.--

Length of opal phytoliths (a)		Width of opal phytoliths (b)	
(2.)	= 638,688.04	(2.)	= 200,690.70
(1.)	= 668,262.46	(1.)	= 203,000.50
(C)	= 610,661.45	(C)	= 199,233.20

Length of middle portion (c)		Width of middle portion (d)	
(2.)	= 25,095.52	(2.)	= 39,132.53
(1.)	= 25,563.66	(1.)	= 40,246.25
(C)	= 24,132.66	(C)	= 38,844.06

Results: Table 1.- ANALYSIS OF VARIANCE, p. 14

APPENDIX 3

Analysis of Dispersion

(Data Columns I-IV)
(Miller & Kahn, 1962,
p. 249-258)

Analysis of dispersion is a multivariate analysis. The matrix which leads to the unbiased estimates of the variances and covariances is denoted by W , where the vertical bars indicate the determinant of the matrix, and W indicates the source of the variables as being within species. A second matrix leading to unbiased estimates of the variances and covariances only if the null hypothesis of equal means is true, is denoted by Q .

The ratio of W to W+Q forms the test criterion lambda. The entries of the matrices are assembled and calculated as follows:

Entries of the matrices.--

Source	DF	Squares a^2, b^2, c^2, d^2,	Crossproducts ab, ac, ad, bc, bd, cd,
Among	3	$\frac{(\Sigma a_I)^2+(\Sigma a_{II})^2+(\Sigma a_{III})^2+\ldots}{n} - \frac{(\Sigma a_I+\Sigma a_{II}+\Sigma a_{III}+\Sigma a_{IV}+\ldots)^2}{kn}$	$\frac{(\Sigma a_I\Sigma b_I+\Sigma a_{II}\Sigma b_{II}+\Sigma a_{III}\Sigma b_{III}+\ldots)}{n} - \frac{(\Sigma a_I+\Sigma a_{II}+\Sigma a_{III}+\ldots)(\Sigma b_I+\Sigma b_I+\Sigma b_{II}\ldots)}{kn}$
Within	36	$(\Sigma a_I^2+\Sigma a_{II}^2+\Sigma a_{III}^2+\ldots) - \frac{(\Sigma a_I)^2+(\Sigma a_{II})^2+(\Sigma a_{III})^2+\ldots}{n}$	$(\Sigma a_I b_I+\Sigma a_{II} b_{II}+\Sigma a_{III} b_{III}+\ldots) - \frac{\Sigma a_I\Sigma b_I+\Sigma a_{II}\Sigma b_{II}+\Sigma a_{III}\Sigma b_{III}+\ldots}{n}$
Total	39	$(\Sigma a_I^2+\Sigma a_{II}^2+\Sigma a_{III}^2+\ldots) - \frac{(\Sigma a_I+\Sigma a_{II}+\Sigma a_{III}+\Sigma a_{IV}+\ldots)^2}{kn}$	$(\Sigma a_I b_I+\Sigma a_{II} b_{II}+\Sigma a_{III} b_{III}+\ldots) - \frac{(\Sigma a_I+\Sigma a_{II}+\Sigma a_{III}+\ldots)(\Sigma b_I+\Sigma b_{II}+\Sigma\ldots)}{kn}$

k = measured variables per species = 4

n = measurements per variable per species = 10

<u>Matrices</u> <u>and</u> <u>determinantes</u>.--

$$
\begin{array}{rcc|cc l}
A_{11} = & 19{,}027 & -3{,}064 & 4{,}091 & -2{,}897 & \\
 & -3{,}064 & 1{,}458 & -936 & 78 & = A_{12} \\
(W) = & & & & & \\
\hline
A_{21} = & 4{,}091 & -936 & 963 & -559 & \\
 & -2{,}897 & 78 & -559 & 288 & = A_{22}
\end{array}
$$

The determinant was calculated by subdividing the original matrix (W) into four submatrices A_{11}, A_{12}, A_{21}, and A_{22} as indicated and the formula was used:

$$(W) = (A_{22})\ (A_{11} - A_{12}\ A_{22}^{-1}\ A_{21})$$

$A_{22} = -35{,}137$

$$A_{22}^{-1} = \begin{vmatrix} -.008{,}195 & -.015{,}909 \\ -.015.909 & -.027{,}407 \end{vmatrix} \cdot \begin{vmatrix} 4{,}091 & -2{,}897 \\ -936 & 78 \end{vmatrix} = A_{12}$$

$$A_{22}^{-1}\ A_{12} = \begin{vmatrix} -18.642 & 27.503 \\ -39.431 & 43.961 \end{vmatrix} \cdot \begin{vmatrix} 4{,}091 & -936 \\ -2{,}897 & 78 \end{vmatrix} = A_{21}$$

$$A_{11} - A_{22}^{-1}\ A_{12}\ A_{21} = \begin{vmatrix} -141{,}455.613 & 19{,}204.146 \\ -288{,}638.268 & 40{,}335.294 \end{vmatrix}$$

$$+ \begin{vmatrix} 19{,}027 & -3{,}064 \\ -3{,}064 & 1{,}458 \end{vmatrix}$$

$$= \begin{vmatrix} 132{,}428.613 & -22{,}268.146 \\ 285{,}574.268 & -38{,}877.594 \end{vmatrix}$$

$$(A_{22})(A_{11} - A_{22}^{-1}\ A_{21}{}^{v}A_{12}) = -1{,}210{,}703{,}643.47 \quad -35{,}137$$

$$(W) = 42{,}540{,}493{,}904{,}091$$

$$\begin{array}{l} A_{11} = \\ W = \\ A_{21} = \end{array} \left[\begin{array}{cc|cc} 48,601 & 6,201 & 8,722 & -351 \\ 6,201 & 3,767 & -1,196 & 1,188 \\ \hline 8,722 & -1,196 & 1,431 & -1,509 \\ -351 & 1,188 & -1,509 & -1,402 \end{array}\right] \begin{array}{l} = A_{12} \\ \\ = A_{22} \end{array}$$

The determinant was calculated by using the formula for the submatrices: $(A_{22})(A_{11}-A_{22}^{-1}\ A_{12}\ A_{21})$

$A_{22} = -270,819$

$$A_{22}^{-1} = \begin{vmatrix} -.005176 & -.005571 \\ -.005571 & -.005283 \end{vmatrix} \cdot \begin{vmatrix} 8,722 & -351 \\ -1,196 & 1,188 \end{vmatrix} = A_{12}$$

$$A_{22}^{-1}\ A_{12} = \begin{vmatrix} -38,483 & -4.801 \\ -42.271 & -4.321 \end{vmatrix} \cdot \begin{vmatrix} 8,722 & -1,196 \\ -351 & 1,188 \end{vmatrix} = A_{21}$$

$$A_{11} = \begin{vmatrix} 48,601 & -6,201 \\ -6,201 & 3,767 \end{vmatrix} - \begin{vmatrix} 382,555,853 & -46,521.884 \\ 361,969,991 & -41,655.768 \end{vmatrix} = A_{22}^{-1}\ A_{12}\ A_{21}$$

$$(A_{22})(A_{11} - A_{22}^{-1}\ A_{21}\ A_{12}) = -903,868,073.173 \quad -270,819$$

$$= 244,784,647,715,951$$

<u>Test</u> <u>criterion</u> <u>lambda</u>.--

$$\frac{(W)}{(W + Q)} = \frac{42,540,493,904,091}{244,784,647,715,951} = .17378$$

The distribution of the test criterion lambda is related to the Chi Square distribution by the formula:

$$V = -\ m\ \ln\ \text{lambda}$$

$$m = N - \frac{p+q-1}{2}$$

N = Total number of measurements per variable = 40

p = Number of measured variables = 4

q = Degrees of freedom per species = 4-1 = 3

pq = Total degrees of freedom = 12

$$m = 39-4 = 35; \quad -\ln .17378 = .8405;$$

$$V=35 \cdot .8405 = 29.42$$

The probability indicated by the Cni Square distribution at 12 degrees of freedom and a value of 28.3 is .005.

APPENDIX 4

Generalized Distance D^2

(Datea columns I-III, V+VI)
(Miller & Kahn, 1962, p. 258-273)

The Generalized Distance D^2 considers all four variables simultaneously. The distance is a numerical index for the similarity of the four species. The variables (a), (b), (c), and (d), their sums of squares, the sums of their crosspoducts form the basis for this index.

<u>Variance-covariance</u> <u>matrix</u>.--

Computation formulae:

$$\text{lambda (aa)} = \frac{(\Sigma a_I{}^2+\Sigma a_{II}{}^2+\ldots)-\left(\frac{(\Sigma a_I)^2+(\Sigma a_{II})^2+\ldots}{n}\right)}{n_I+n_{II}+n_{III}+n_{IV}+n_{VL}-5}$$

$$\text{lambda (ab)} = \frac{(\Sigma a_I b_I + \Sigma a_{II} b_{II} + \ldots) - \left(\frac{(\Sigma a_I \Sigma b_I) + (\Sigma a_{II} \ldots)}{n}\right)}{n_I + n_{II} + n_{III} + n_V + n_{VI} - 5}$$

lambda	(aa)	=	295,822
"	(bb)	=	51,551
"	(cc)	=	53,993
"	(dd)	=	32,677
"	(ab)	=	-13,477
"	(ac)	=	90,460
"	(ad)	=	-50,688
"	(bc)	=	-8,460
"	(bd)	=	22,728
"	(cd)	=	-13,860

Formula for the correlated variables:

$$p\ (ab) = \frac{\text{lambda (ab)}}{\sqrt{\text{lambda (aa)} - \text{lambda (bb)}}}$$

Standard deviation:

$$\text{sigma} = \sqrt{\text{lambda (aa)}}$$

Pooled estimates and standard deviation:

	a	b	c	d
a	1	-.1088	+.7162	-.5155
b		1	-.1604	+.5538
c			1	-.3301
d				1
sigma	17.20	7.18	7.34	5.72

<u>Calculation of the normalized variables</u>.--

Formula: $\dfrac{\text{Sample mean - Grand mean}}{\text{Standard Deviation}}$

Column	II	I	III	V	VI
a'	.9116	1.3172	.5279	-1.9982	-.7627
b'	.3454	-1.3259	.1782	1.0167	-.2395
c'	-1.1553	.9564	-1.1826	-2.3133	2.8773
d'	-.5590	-.2622	-.2447	1.1888	-.1223

Transformation of the normalized variables;--

Transformation equations:

$$a = a'$$

$$b = b' - p_{(ab)}a$$

$$c = c' - x'_{(32)}b - x'_{(31)}a = c' - x'_{(32)}b - p_{(ac)}a$$

$$d = d' - x'_{(43)}c - x'_{(42)}b - x'_{(41)}a = d' - x'_{(43)}c - x'_{(42)}b - p_{(ad)}a$$

$$x'_{(21)} = p_{(ab)} \qquad y'_{(22)} = 1$$

$$x'_{(31)} = p_{(ac)} \qquad y'_{(32)} = p_{(bd)} - p_{(ac)}p_{(ab)}$$

$$x'_{(41)} = p_{(ad)} \qquad y'_{(42)} = p_{(cd)} - p_{(ac)}p_{(ad)}$$

$$V_{(b)} = 1 - p^2_{(ab)}$$

$$V_{(c)} = 1 - p^2_{(ac)} - x'_{(32)}y'_{(32)} \qquad x'_{(32)} = \frac{y'_{(32)}}{V_{(b)}}$$

$$y'_{(42)} = p_{(bd)} - p_{(ab)}p_{(ad)}$$

$$y'_{(43)} = p_{(cd)} - x'_{(32)}y'_{(42)} - p_{(ac)}p_{(ad)}$$

$$x'_{(43)} = y'_{(43)}(V_{(c)})^{-1}$$

$$V_{(d)} = 1 - p^2_{(ad)} - x'_{(42)}y'_{(42)} - x'_{(43)}y'_{(43)}$$

$$a^* = a = a'$$

$$.99406\ b^* = b = b' - (-.1088a)$$

$$.69299\ c^* = c = c' - (-.0829b) - (.7162a)$$

$$.60390\ d^* = d = d' - (-.4168c) - (.5006b) - (-.5155a)$$

Correlated transformed variables:

Column	II	I	III	V	VI
a	.9116	1.3172	.5279	-1.9982	-.7627
b	.4446	-1.1826	.2356	.7993	-.3225
c	-1,7713	-.0851	-1.6313	-.8149	3.3968
d	-1.0494	.9734	-.7704	-.5915	1.0618

Uncorrelated transformed variables:

Column	II	I	III	V	VI
a*	.9116	1.3172	.5279	-1.9982	-.7627
b*	.4473	-1.1897	.2370	.8041	-.3744
c*	-2.5560	-.1128	-2.3540	-1.1773	4.9016
d*	-1.7385	1.6118	-1.2757	-.9629	1.7582

Generalized Distances D^2.--

Computation formula:

$$D^2_{(I-II)} = (a^*_I - a^*_{II})^2 + (b^*_I - b^*_{II})^2 + (\ \ldots\ldots\ldots\ )^2 + \ldots$$

$$D^2_{(V-VI)} = (a^*_V - a^*_{VI})^2 + (b^*_V - b^*_{VI})^2 + \ldots\ldots$$

For graphic representation the square root is taken from D^2.

D^2(II-I) =	19.9572	D(II-I) =	4.467
(II-III) =	.4464	=	.668
(II-V) =	11.0960	=	3.331
(II-VI) =	65.2481	=	8.077
(I-III) =	15.9740	=	3.996
(I-V) =	22.7039	=	4.764
(I-VI) =	30.3407	=	5.508
(III-V) =	8.1852	=	2.861
(III-VI) =	63.8291	=	7.989
(V-VI) =	47.1574	=	6.862

Graphic representation see Fig. 2, p. 15.

OPAL PHYTOLITHS

by

ERWIN SUESS

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Opal phytoliths in leaves from _Andropogon gerardi_, _A. scoparius_, _Panicum virgatum_, _Sorghastrum nutans_, and from _Bouteloua curtipendula_, _B. gracilis_, and _Buchloe dactyloides_ have a thin silica-cellulose layer.

Two morphological types are characteristic for the prairie vegetation. The chloridoid (cuboid) type is found in the leaves of the short grass region, while dumb-bell shaped phytoliths are characteristic for the tall grass region. Panicoid is the systematic name introduced by Prat (1948). The phytoliths are amorphous and are enclosed in silica-cellulose material that has an index of refraction ranging from 1.488 to 1.494.

Both characteristic types also occur in dust samples from Tribune, Hays, and Manhattan, Kansas, and from McCredie, Missouri.

Soil samples from Monona, Hastings, Crete, and Florence soil series contain characteristic opal phytoliths of the tall grass region and irregularly shaped types. They are optically isotropic and their indices of refraction are between 1.440 and 1.447.

Two unnamed buried horizons from Geary County, Kansas, have irregularly shaped opal phytoliths and also some of the chloridoid type. They are amorphous and their refractive index ranges from 1.448 to 1.460.

Peorian loess, Loveland silt, Kansas and Nebraska till from Doniphan County, Kansas, contain opal phytoliths of irregular shape and a characteristic surface structure. They are optically isotropic and the index of refraction ranges from 1.465 to 1.478.