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SILICA AND ASH IN SEVERAL MARSH PLANTS

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ABSTRACT Ash and silica content and their depositional patterns in tissues of *Spartina alterniflora* Loisel., *Distichlis spicata* (L.) Greene, *Scirpus validus* Vahl., *Zizania aquatica* L., and *Limonium carolinianum* (Walt.) Britt. were determined. *Zizania aquatica* leaves had the highest silica content (6.0%) of any of the plant parts tested; silica making up over half of the ash. Silica in the plants was opaline in character. *Limonium carolinianum* did not accumulate silica in any great amount. However, ash content was very high in *Limonium carolinianum* and *Scirpus validus* making up over 17% of the dry weight of the leaves. X-ray diffraction showed the presence of halite (NaCl) and calcium sodium phosphate (2.4 CaO • 0.6 Na_2O • P_2O_5) in the ash of the leaves of all the plants from Mississippi except *Zizania aquatica*. Mineral deposition (silica and other minerals) in *Zizania aquatica* occurs in rows lengthwise of the leaf, and there are bowtie- and oval-shaped phytoliths. In *Distichlis spicata* leaves, mineral deposits occur in rows of elongate, serrated units. Spongy mineral deposition occurs lengthwise of the leaves of *Spartina alterniflora* and some of these deposits, including the silica, are fibrous. In *Scirpus validus* heavy deposits occur lengthwise of the leaf. Silica occurs in a sheetlike pattern and in rows of oval particles.

INTRODUCTION

Silica content of agricultural plants has been shown to be variable and to depend primarily on soil type and plant species (Jones and Handreck 1967, Lewin and Reimann 1969, Lanning and Linko 1961). Certain cultivated grasses such as rice may contain up to 20 times that of crimson clover *Trifolium incarnatum* (Russell 1961). Based on available information, monocotyledons generally have much more silica than dicotyledons (Metcalfe and Chalk 1950a, 1950b; Metcalfe 1960). Sangster (1978) suggested that the ability of plant species to absorb, transport, and deposit silica in similar aggregate formations may indicate a common origin or epicenter and subsequent migration of the species to other biogeographical realms. However, it is equally possible that the ability of a plant species to absorb and deposit silica within their tissues may have changed with evolution and migration to different regions.

Silica deposits in the aerial portion of crop plants contribute several beneficial effects. Leaves of rice are stiff and erect when silica content is high, but droop when it is low (Ishizuka 1971). Erect leaves are more photosynethetically active than drooping leaves (Ishizuka 1971); furthermore, high silica content enhances seed retention and prevents shattering, a common characteristic of grasses which complicates harvesting of seed in some species (McWilliams 1963). Lodging (bending of the culm: weak culm) of cereal plants also can be prevented by additions of silica to the soil (Mulder 1954). There is evidence that increasing the silica content of the soil (Monteith 1966) may cause a decrease in transpiration. A reduction in susceptability to fungal and insect attacks of rice and other gramineous crops has been attributed to a high silica content of the respective plants (Vlamis et al. 1958, Jones and Handreck 1967, Ishizuka 1971). Relatively high concentrations of silica in plants also are known to prevent manganese toxicity, apparently by decreasing manganese uptake (Vlamis and Williams 1967). Rice plants with a high silica content also have a low iron content (Jones and Handreck 1967). High concentration of nitrogen or phosphorous in cultivated fields causes a decrease in the concentration of silica in barley, wheat, and other crops grown in those areas (Jones and Handreck 1967). Ponnamperuma (1964) has shown that silica improves oxygen supply to the root by increasing the volume and rigidity of gas channels in the root and shoot. Other edaphic factors such as soil pH also are known to affect silica uptake by cultivated plants. If those facts hold true for wild plant species, they have important implications regarding our understanding of natural ecosystems.

Little is known about silica content or the factors affecting silica uptake in wild plant species (Chen and Lewin 1969). The same distribution patterns and factors affecting silica deposition may or may not be similar between wild and cultivated plant species. There may or may not be major differences between silica content of terrestrial and aquatic species. Does the same plant species found in different geographical areas have the same silica uptake ability? Do native grasses and other wild monocotyledons, as present evidence indicates, have more silica as a rule than native dicotyledons? Furthermore, most work on native plants has been done on terrestrial species. To our knowledge no work has been done on floating or submerged aquatic plant species. Recently, however, Lanning and Eleuterius (1978) investigated silica content and depositional patterns of *Juncus roemerianus* Scheele, a common rush (Eleuterius 1975, 1976) of salt marshes of the South Atlantic and Gulf coasts. *Juncus roemerianus* was found to contain considerable opaline silica but no α-quartz. The amount of silica in plants corresponded to the amount found in soil, and silica content increased during the growing season. Lanning (1972)

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showed that *Juncus interior* Weig and *Juncus bufonius* L., rush species of inland low areas, contained considerable silica, both opaline and α-quartz, and that silica content of *Juncus interior* increased nearly eightfold over the growing season.

The present study entailed five objectives: (1) to compare silica content of grass and sedge species from wetlands of different geographical locations; (2) to investigate silica content of grass and sedge species which grow under continuously but partially submerged conditions; (3) to compare silica content of several wetland monocotyledons with at least one dicotyledon from coastal wetlands; (4) to describe the silica depositional patterns in each species; and (5) to access the analytical results for these wetland plant species in view of knowledge of silica in agricultural plants. Specifically we were to determine: (1) the types and quantity of silica, if present, in several species of marsh plants, and (2) the ash (A) and silica (Si) contents of the various parts of the plants.

The four monocotyledons studied were *Spartina alterniflora* Loisel., *Distichlis spicata* (L.) Greene, *Scirpus validus* Vahl., and *Zizania aquatica* L. *Limonium carolinianum* (Walt.) Britt. was the dicot species analyzed. *Scirpus validus* and *Zizania aquatica* (wild rice) typically grow along rivers and bayous with the lower portion of the plants submerged. *Distichlis spicata, Spartina alterniflora,* and *Limonium carolinianum* are intertidal, with *Spartina alterniflora* occupying the lowest position on the tidal plane.

METHODS AND MATERIALS

Zizania aquatica was collected from Pascagoula River in Mississippi, and *Limonium carolinianum* and *Distichlis spicata* were collected from Deer Island off the coast of Mississippi and from a marsh in Cloud County, Kansas. *Scirpus validus* also was collected from Pascagoula River, Mississippi, and from marshes near St. George, Kansas, and in Decatur County, Kansas. *Spartina alterniflora* plants were from a marsh associated with Davis Bayou in Mississippi.

Plant materials were thoroughly washed and then oven dried at 110°C. Whole plants were separated into aboveground parts, rhizomes, and roots. All plant materials were ground in a Wiley mill before analysis.

Ash and silica contents of plant materials were determined by classical gravimetric techniques. Tissue samples were ashed in platinum crucibles at approximately 500°C, and the ash was treated repeatedly with 6N hydrochloric acid to remove other mineral impurities. The silica was filtered out and ignited. Silicon dioxide content of plant materials was determined as the difference in weights before and after treatment in a platinum crucible with a few drops of 45% aqueous hydrofluoric acid (Kolthoff and Sandell 1952). Determinations were made in duplicate.

The X-ray diffraction patterns for identifying the nature of the mineral deposits in the plants were made as described by Lanning et al. (1958).

Scanning electron micrographs of mineral deposits in leaves of the plants were made with an ETEC Autoscan E-1 Electron Microscope. Tissues were ashed between microscope slides as described below. Ash was transferred to an aluminum stub covered with double-faced sticky tape by lightly pressing the stub on the glass slide; then the ash was coated with Au-Pd alloy and carbon. In some cases, tissue was ashed in a small platinum crucible and treated with 6N HCl to remove soluble minerals before it was mounted on the stub.

Spodograms were prepared by the Ponnaiya (1951) modification of the Uber (1940) method. Material to be examined was placed between microscope slides for ashing in a muffle furnace at 500°C. Ash was prepared for light microscopy by removing the upper slide, adding Canada balsam directly to the ash, and covering with a cover glass. A petrographic microscope was used to determine the nature of silica in the deposits.

RESULTS

Ash and Silica Contents of Plant Tissues

Petrographic microscope studies of plants other than *Limonium carolinianum* showed silica to be clear, colorless, and isotropic with an index of refraction of 1.45. These are properties of the mineral opal (Lanning et al. 1958). Silica content of *Limonium carolinianum* was quite low, and both petrographic microscope studies and X-ray diffraction patterns showed the presence of α-quartz. Silica in *L. carolinianum* was probably detrital quartz. There also was some detrital quartz in *Scirpus validus* leaves.

The ash and silica contents of the tissues of the plants analyzed are given in Tables 1 and 2. Ash content of *Limonium carolinianum* leaves was very high, and low silica content indicated an unusual mineral content. X-ray diffraction patterns of the ash of leaves showed a large amount of halite (sodium chloride) and a calcium sodium phosphate (2.4 CaO · 0.6 Na_2O · P_2O_5). Ando (1958) prepared this substance by fusing CaC_2O_4 with Na_2CO_3 and H_3PO_4 for 1/2 an hour at 1,300°C, and determined its X-ray diffraction pattern to be an α-rhenanite structure. X-ray diffraction of leaves also showed the halite. Other peaks were too small for positive identification but indicated that calcium sodium phosphate was present in the leaves of *Limonium carolinianum*. X-ray diffraction also showed the presence of considerable halite and calcium sodium phosphate in the ash of *Spartina alterniflora, Distichlis spicata,* and *Scirpus validus* leaves of plants from Mississippi. However, halite was not present in *Zizania aquatica*.

Minerals tend to deposit in leaves as shown by both higher ash and silica contents. *Limonium carolinianum* differs from other plants in that it does not accumulate silica in any great amount. *Zizania aquatica* leaves have the highest silica content of any of the plant parts tested, and silica makes up half of the ash. The nodes of the culms of *Zizania*

TABLE 1.

Percentage of Ash in Marsh Plants.

Plant	Leaves	Rhizomes	Roots	Inflo-rescence	Culm	Culm Nodes
Scirpus validus St. George, Kansas	17.88			7.31		
Scirpus validus Mississippi	17.86	8.33	17.85			
Limonium carolinianum Mississippi	17.11		4.70			
Spartina alterniflora Mississippi	11.95	8.45	15.76			
Zizania aquatica Mississippi	11.71				8.88	13.72
Scirpus validus Decatur County, Kansas	10.65			5.43		
Distichlis spicata Cloud County, Kansas	10.24			9.31		
Distichlis spicata Mississippi	8.18	5.92	19.48			

TABLE 2.

Percentage of Silica in Marsh Plants.

Plant	Leaves	Rhizomes	Roots	Inflo-rescence	Culm	Culm Nodes
Scirpus validus St. George, Kansas	3.39			3.66		
Scirpus validus Mississippi	2.34	1.50	7.02			
Limonium carolinianum Mississippi	0.44		0.14			
Spartina alterniflora Mississippi	0.83	0.95	1.63			
Zizania aquatica Mississippi	6.00				2.04	5.21
Scirpus validus Decatur County, Kansas	1.39			2.49		
Distichlis spicata Cloud County, Kansas	4.55			4.24		
Distichlis spicata Mississippi	2.07	0.21	2.77			

aquatica also have a high silica content. The ash and silica values for *Spartina alterniflora, Distichlis spicata,* and *Scirpus validus* roots are all very high. Although silica content of roots is generally high, some inaccuracy may exist because of residual soil particles. Soil is difficult to wash from roots; therefore, the samples may not have been thoroughly cleaned, thus the very high values.

Scirpus validus plants grown at St. George, Kansas, had considerably more silica in the leaves than those from Mississippi, and the latter had considerably more than those from Decatur County, Kansas. *Distichlis spicata* leaves from Cloud County, Kansas, had twice as much silica as those from Mississippi. Inflorescences of Kansas plants had nearly as much or somewhat more silica than the leaves; silica in each inflorescence made up almost half of the ash.

Patterns of Ash and Silica Depositions

Electron micrographs of mineral deposition in ashed leaf tissue of *Zizania aquatica* leaves are shown in Figure 1. The deposits are in rows lengthwise of the leaves. Figure 1A shows rows of solid deposition, a row of "bowtie" phytoliths, and a row of the bowtie phytoliths joined together at the sides. Also there are oval phytoliths and open spaces that appear to be stomata. These are shown highly magnified in Figure 1B. The bowtie-shaped phytoliths are shown in Figure 1C, and the bowtie phytoliths joined together in Figure 1D.

Petrographic microscope examination of silica deposits in *Zizania aquatica* leaves shows that they occur in bowtie-shaped particles. Silica also occurs in cell walls. These silica deposits in cell walls run in strips lengthwise of the leaves, and form bands three cells wide.

Figure 2A is an electron micrograph of mineral deposition in ashed leaf tissue of *Distichlis spicata* leaves. Mineral deposits occur in rows of elongate, serrated units with the longer dimension lengthwise of the leaf. There are also some small rectangular particles and some irregular oval particles. Examination of acid-treated deposits with a light microscope shows silica deposition to be in rows of elongate units, and it appears to be in cell walls.

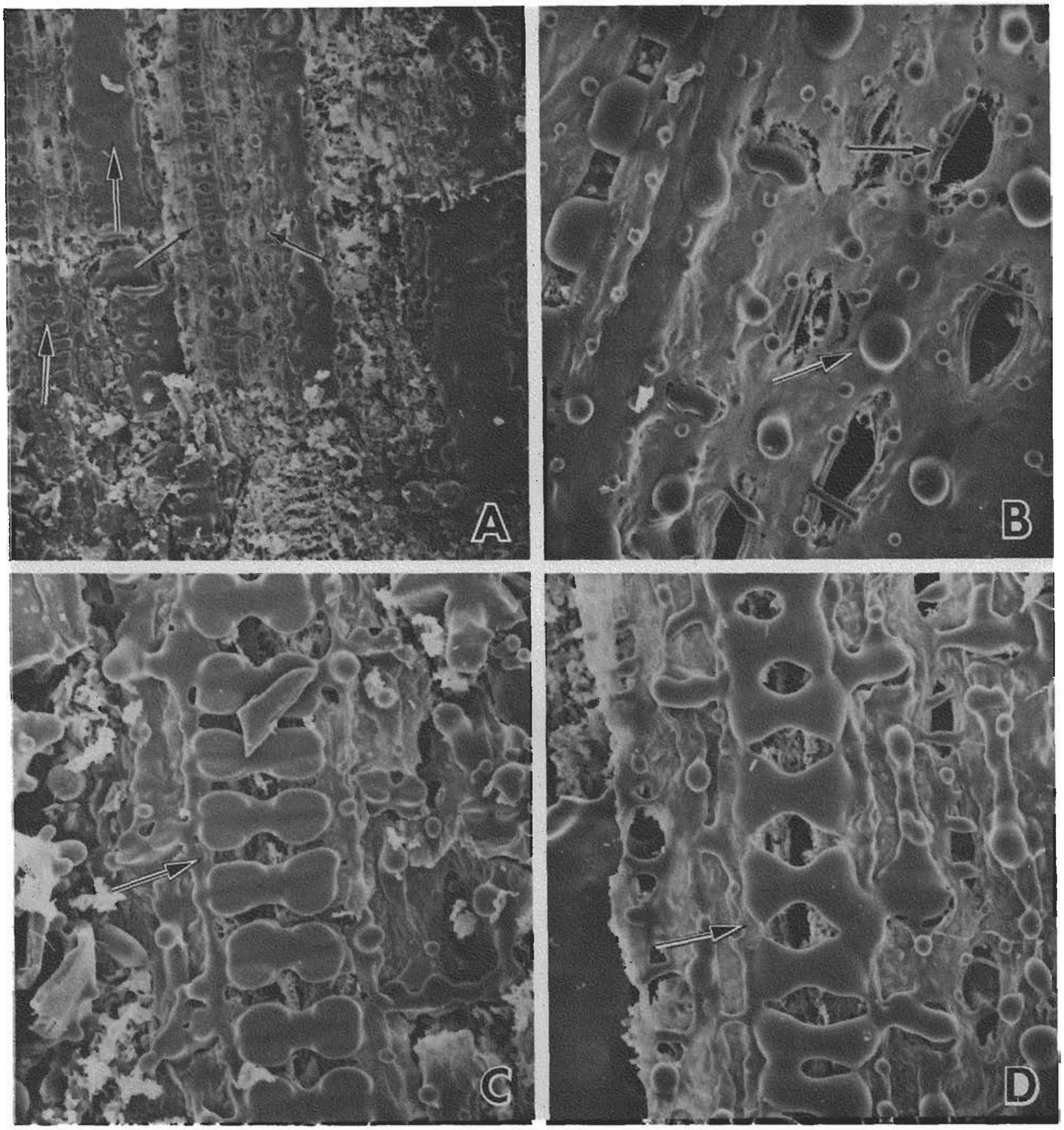

Figure 1. Scanning electron micrographs of ash deposits in *Zizania aquatica* leaves. A. Leaf surface showing all types of mineral deposition including rows of solid deposition (large arrowhead), a row of bowtie-shaped phytoliths (large arrow), a row that appears to be bowtie-shaped phytoliths joined together at the sides (small arrow), oval phytoliths (small arrowhead), and open spaces that appear to be stomata (small long arrow). × 250. B. A portion of leaf tissue with oval particles (arrowhead) and stomata (small arrow). × 1000. C. A portion of leaf showing the row of bowtie-shaped phytoliths (large arrow). × 1000. D. A portion of leaf showing the row of bowtie-shaped phytoliths joined together (large arrow). × 1000.

Heavy deposits of minerals occur in ridges lengthwise in *Spartina alterniflora* leaves. Ashed leaf tissue with a spongy deposition running continuously lengthwise of the leaf are shown in the electron micrograph of Figure 2B. Figure 2C, another electron micrograph of leaf tissue lengthwise of the leaf, shows a more fibrous character of mineral deposition. An electron micrograph of acid-treated mineral deposits shows that silica deposition is largely fibrous in character with some irregular particles (Figure 2D).

In *Scirpus validus,* deposits of minerals also occur in ridges lengthwise of the leaf, and rows of oval particles were observed with a light microscope. Silica occurs in a sheetlike

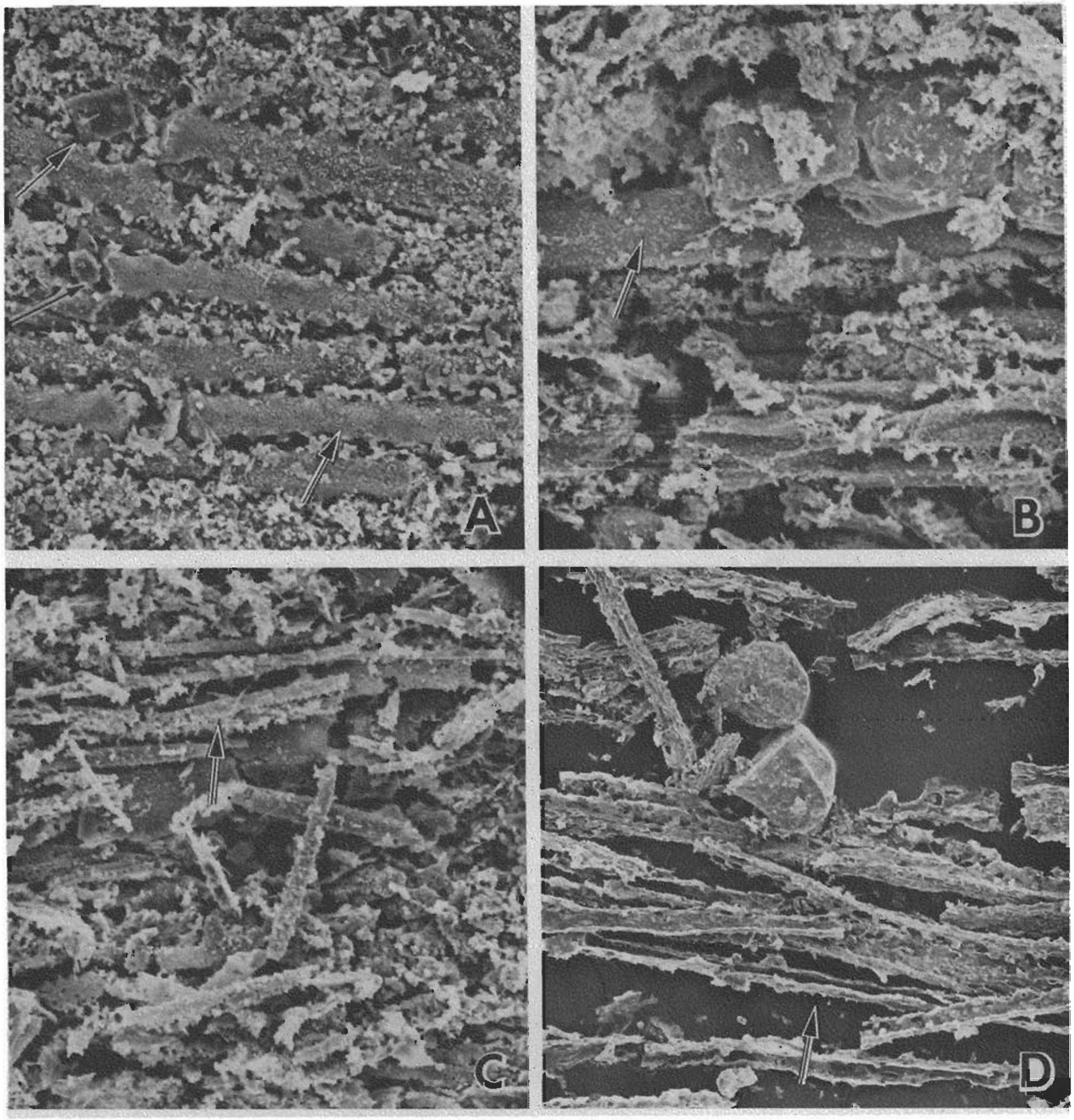

Figure 2. Scanning electron micrographs of ash deposits. A. Leaf surface showing deposits in *Distichlis spicata*. Note the rows of elongate, serrated units lengthwise of the leaf (large arrow), small rectangular particles (arrowhead), and irregular oval particles (small arrow). X 1000. B. Ash deposit from *Spartina alterniflora* leaf. Note the spongy deposition running continuously lengthwise of the leaf (large arrow). X 1000. C. Electron micrograph of *Spartina alterniflora* leaf. Note the fibrous character of mineral deposition (large arrow). X 1000. D. Silica in *Spartina alterniflora* leaf. Specimen was acid washed to remove other minerals. Note the fibrous character of the deposits (large arrow). X 125.

pattern and in rows of oval particles.

Limonium carolinianum leaves do not show a distinct pattern of mineral deposition other than heavier deposits in the veins.

DISCUSSION

Scirpus validus plants grown at St. George, Kansas, had considerably more silica in the leaves than those from Mississippi, and the latter had considerably more than those grown in Decatur County, Kansas. *Distichlis spicata* plants grown in Cloud County, Kansas, had twice as much silica in the leaves as those from Mississippi. These differences may be due to the amount of available silica in soils at the different locations. However, within a species genetic differences (local adaptation) in uptake capability may exist. Resolution of this question may be obtained by growing plants of the same species collected from widely separated localities in a common environment. Jones and Handreck (1965, 1969) contend that silica uptake is passive, but Barber and Shone (1966) obtained evidence that uptake of silica may be metabolic. Thus, environmental differences may account for variation between species.

Attention should be focused on the fact that wild rice (*Zizania aquatica*), a close relative taxonomically to cultivated rice (*Oryza satina*), had the highest silica content of the plant species studied. This is an interesting discovery since highly bred cultivated rice also has the ability to accumulate large quantities of silica. Furthermore, silica of *Zizania aquatica* is deposited in a very unusual pattern.

Spartina alterniflora had a relatively low silica content. Although this plant is often found with the lower portion submerged in water and is generally found on muddy shores, the collections analyzed in this study were from a very sandy area. The rectangular silica deposits in the leaf ridges of *Spartina alterniflora* may aid in reducing transpiration. Transpiration presumably is lowered by increasing thickness and compactness of silica cellulose membranes in the epidermal cells (Yoshida et al. 1959). Leaves of *Spartina alterniflora* during periods of very high temperatures become rolled. Rectangular silica deposits also occur in *Distichlis spicata* linking these two grass species. The bowtie-or dumbbell-shaped silica deposits in *Zizania aquatica* are an entirely new type of structure, with no parallel in cultivated rice.

The low silica content of *Limonium carolinianum*, a dicotyledon, is consistent with previous evidence in that the monocotyledon analyzed had consistently higher silica contents.

This preliminary study on grasses, sedges, and a dicot species, and previous work on the rush *Juncus roemerianus*, suggests that at least some of the information determined for cultivated plants also holds true for wetland species. However, each of the species investigated had peculiar depositional characteristics and various quantities of silica. Further study of wetland species is needed before there can be any clear understanding or definite statements relating our work to the agriculture literature and to that pertaining to upland species.

In closing, we point out that the continued release of domestic sewage into estuarine waters is very likely to raise the nitrogen and phosphorous concentrations of wetlands soils. High nitrogen and phosphorous concentrations are known to decrease silica content of domestic plants as shown above with corresponding detrimental effects: weakened plants, reduced photosynthesis, and increased fungal and insect attacks. In view of these facts and possibilities, it is timely and prudent to carry out work that would lead to a better understanding of the role of silica in wetland and aquatic plant species.

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