

THE DIGESTION OF CORN AND SORGHUM SILAGE  
AS OBSERVED BY SCANNING ELECTRON MICROSCOPY

by

MARI L. THOUVENELLE

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Major Professor

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TABLE OF CONTENTS

	Page
INTRODUCTION AND LITERATURE REVIEW . . . . .	1
MATERIALS AND METHODS . . . . .	7
RESULTS AND DISCUSSION . . . . .	9
CONCLUSION . . . . .	40
LITERATURE CITED . . . . .	41

## ACKNOWLEDGMENTS

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## INTRODUCTION AND LITERATURE REVIEW

Prediction of forage quality has been a subject of great concern for researchers. Various chemical methods have been utilized. The Weende system of proximate analysis was developed in the mid 1800s and is still the most widely used chemical method for forage evaluation. In recent years a more comprehensive technique for fiber fractionation has been developed (Van Soest, 1967; Goering and Van Soest, 1970). In vitro studies of ruminal forage digestion predominantly follow the two-stage fermentation scheme devised by Tilley and Terry (1963).

There are inherent disadvantages associated with these techniques. The sodium hydroxide digestion step of the Weende system causes a major portion of the lignin in a sample to become solubilized. The lignin as a result is calculated as a component of the nitrogen-free extract. The major drawback of the Van Soest analysis appears to be the time required for analysis and errors which appear when separating protein from lignin. These errors are generated by heating and drying the sample (Van Soest, 1964). In vitro fermentation may give inaccurate results due to its closed system which results in accumulation of metabolic end products (Shelton and Reid, 1960). Van Soest (1967) attributed higher digestibility of in vitro samples to the absence of endogenous excretions which are a normal occurrence in the animal.

Silage evaluation presents additional complications. Volatile fatty acids produced during ensiling must be considered

when determining digestibility. If the loss of the volatile constituents is not accounted for it will result in an apparent depression of the digestibility of the herbage during ensiling (Harris and Raymond, 1963). Schmid et al. (1975) found differences in cell wall digestibility between corn silage and sorghum silage to be directly related to the presence of starch in the cell wall residues. Simikins and Baumgardt (1963) and Marten et al. (1975) found several chemical methods generally used in prediction of forage quality to be inadequate and inconsistent estimators for silage quality.

Researchers have begun to utilize alternate methods to provide better interpretation of forage quality and digestibility. Regal (1960) used light microscopy to determine the plant cellular components which passed through the ruminant digestive system virtually undigested. These tissues were vascular tissue, cutinized epidermis and sclerenchyma. Shenk and Elliot (1971) employed histological examination of alfalfa progeny selected for high and low six-hour in vitro dry matter disappearance (IVDMD) to reveal visually the relationship of cellular components to digestibility. Monson and Burton (1972) showed IVDMD % to increase with the number of cut surfaces in forage leaves. In vitro digestion patterns are essentially identical on various species of fresh forage leaves however, rate of digestion varies considerably (Hanna, Monson and Burton, 1973). Anatomical structures and/or their organization within a specific plant are a factor in determining the rate of microbial digestion (Monson, Powell and Burton, 1972).

The scanning electron microscope (SEM) is an alternate and more advantageous approach to further clarify the mode of digestive attack in various feedstuffs. SEM allows observation in situ, by providing a three-dimensional image at high magnification with a resolution of up to 200 A. The concept of stereoviewing of micrographs is a valuable asset in resolving the internal structural organization and relationships of various plant tissues and organs as well (Howell, 1975).

Considerable research has been conducted on the structural changes and enzymatic hydrolysis patterns of sorghum grain starch and endosperm (Davis and Harbers, 1974; Sullins and Rooney, 1974; Harbers, 1975). Researchers have recently begun to examine forages and their degradation by ruminants. Akin and Burdick (1973) examined several warm-season grasses by light and electron microscopy. They pointed out microanatomical differences and site of lignification as factors limiting forage digestibility. Plant tissues differ in the rate of digestion by rumen microbes. Mesophyll and phloem cell walls are more rapidly digested than other plant tissues in forages (Akin et al., 1973; Akin and Amos, 1975). Digestibility of a specific forage is also dependent on the ability of the bacteria to form physical interrelationships with the plant tissue. These relationships are directly dependent on the inherent structural characteristics of the plant tissue (Akin et al., 1974). Akin et al. (1975) identified the anatomical plant structures which appear in neutral detergent fiber (NDF) and acid detergent (ADF) confirming Van Soest's scheme of fiber analysis.

In vivo studies of forage digestion have been virtually non-existent until Brazle (1976) described by SEM the in vivo digestion of alfalfa hay, brome and tall fescue. Vascular tissue and cutinized abaxial leaf epidermis were left as end products of ruminal degradation (Brazle, 1976; Brazle and Harbers, 1977).

Forage quality is depressed further by the presence of structural inhibitors to digestion of grasses (Deinum, 1973). Digestibility is decreased by three units for each 1% of silica present (Van Soest, 1968; Goering and Van Soest, 1970). Microbial digestion of certain plant epidermal surfaces is inhibited by silica-containing structures (Brazle, 1976). Determination of silica has been accomplished by differing methods. Early work with silica utilized spodograms for analysis. Ponnaiya (1951) employed a variation of the technique described by Uber (1940) for preparation of spodograms. He described two types of silica deposition in the leaf epidermis of sorghum. Lanning et al. (1958), working with leaf blades and sheaths of corn and sorghum, used Ponnaiya's method to reveal patterns, particle sizes and subepidermal deposition of silica. He also reported the chemical form of silica by x-ray diffraction.

The development of a new means of x-ray analysis has expanded the applications and analytical capabilities of SEM. Elemental composition of the sample can be qualitatively determined and its dispersion identified by distribution images. The major advantage is the examination of the sample in its natural state, i.e., not requiring destruction of the sample

in the elemental analysis. Previously, x-ray analysis was obtained with an electron probe microanalyzer using a wavelength dispersive spectrometer. Several limitations of this method when used in conjunction with SEM were fundamental in the refinement and preferential use of the energy dispersive (ED) spectrometers (Marshall, 1975). The principles and details of its design and operating features have been described in several publications (Russ, 1972; Yakowitz, 1974; Goldstein and Yakowitz, 1975; Edax, 1975). The basic principle of ED x-ray analysis involves the collection of the characteristic x-ray photons generated by the sample in the electron-irradiated region via a silicon detector combined with a multichannel analyzer. Each channel is calibrated for a convenient energy, eg. 20 eV, to make it possible to divide the x-ray spectrum into energy groupings. The individual element can then be identified by Moseley's law which relates the energy peak of the detected x-ray to the atomic number of a specific element.

X-ray distribution images are obtained by setting the x-ray analyzer for the element of interest. The spatial arrangement of the element is shown on the display tube of the SEM as a brightened dot wherever its corresponding x-ray was detected. This technique will supply only qualitative information about the elemental concentration but, is an excellent means of revealing the distribution (Edax, 1975).

The effects of ensiling and ruminal digestion of corn and sorghum silage were observed by SEM in this study to provide a resolution to their differing digestibilities. Energy

dispersive x-ray analyses were performed on samples to determine the role of silica in the digestive pattern of each silage.

## MATERIALS AND METHODS

Plant specimens of known variety were obtained from plots of similar soil type and fertility. Leaves and stems of corn (IRN 3-08-153) and sorghum (IRN 3-04-468) were obtained fresh at the time of ensiling, frozen in liquid nitrogen and stored at  $-10^{\circ}$  C. Ensiled samples were obtained from upright silos after fermentation and immediately separated and prepared for scanning electron microscopy studies. Samples were cut to 10-12 mm size to accomodate the SEM.

Four ruminally fistulated steers adapted to either corn or sorghum silages for three weeks were used to study the hydrolytic patterns of ensiled stems and leaves. Leaves and stems were placed in separate nylon bags, then subjected to ruminal digestion 12, 24, 48 and 72 hours (Brazle and Harbers, 1977). Eight to ten samples of either stem or leaf were suspended in each bag. Five digestions were conducted to be confident in the results.

All samples were prepared for SEM in the same manner. Preparation consisted of initial fixation in 4% glutaraldehyde solution (10 ml of 8% glutaraldehyde in 10 ml buffer containing 7 ml of .07 M  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  and 3 ml of .07 M  $\text{KH}_2\text{PO}_4$ , pH 7.168) (Sjostrand, 1967). Specimens were dehydrated through a graded ethanol series (30 min. each in 30%, 60%, 80% and 100%) as suggested by Parsons et al. (1973). Carbon dioxide critical point drying was used to avoid exposing the leaves and stems to any surface tension forces (Cohen, 1974; Humphreys, 1975).

Pelco No. 93 colloidal silver (Ted Pella Co., #1603-2) was used to mount the samples on aluminum stubs. Specimens were then coated by vacuum evaporation (Kenny Vacuum Co., Model KSE-2A-M evaporator) with carbon followed by 10-20 nm of gold/palladium (DeNee and Walker, 1975). The samples were viewed and photographed (Polaroid PN/55 film) with an ETEC autoscan scanning electron microscope at an accelerating voltage of 10 kv. Stereoscopic pairs were taken for interpretative purposes; only single photomicrographs are presented here (Howell, 1975).

Plant tissue used for x-ray analysis of its elemental spectra was air dried and mounted on stubs with a low resistance contact cement (E. F. Fullam Co., Schenectady, N.Y.). Air drying minimized ionic translocation while the carbon cement eliminated contamination the colloidal silver cement commonly used for observing secondary images (Echlin and Saubermann, 1977). The samples were vacuum-coated with 1-2 nm of evaporated carbon and viewed in the microscope equipped with an energy-dispersive x-ray analyzer (EDAX Model 711). The instrument was used to detect relative elemental composition (Z 10 and above) and locate silicon. Qualitative analyses were made due to the inherent characteristics of the instrument and geometry of the plant specimen which prevents confidence in quantitative procedures (Yakowitz, 1974). Energy-dispersive x-ray spectrographs, silicon elemental maps and concomitant secondary images were photographed on Polaroid film.



## RESULTS AND DISCUSSION

### Fresh Samples

Corn. The adaxial surface of the corn leaf is characterized by the presence of veins, macrohair, small bristles and stomata (Fig. 1 a-b). The veins are clearly identified in parallel lines along the leaf with stomata situated between them. Macro-hairs originate from enlarged basal regions on the veins while bristles (microhair) border on either side. Waxy layers (cuticulae) form repeating patterns over the leaf surface (Lott, 1976).

Energy dispersive x-ray spectrographs of the upper epidermal surface (Fig. 2) indicate it to contain mainly silicon (Si) with minor amounts of potassium (K), calcium (Ca) and chlorine (Cl). The Si is deposited over the entire surface shown by the Si element map of the adaxial tissue (Fig. 3a) and its corresponding secondary image (Fig. 3b). The deposition of Si is not uniform but, appears to be concentrated in venous areas of the leaf with some contiguous regions devoid of Si or lower in contraction. These results concur with others that have also found small siliceous structures adjacent to the veins. Spodograms of mature corn leaf blades reveal that silica is not strictly limited to the outer epidermal surfaces but, has been found to exist as rectangular-shaped particles in the sub-epidermal tissue (Lanning, Ponnaiya and Crumpton, 1958). Micrographs show dumb-bell-shaped phytoliths in rows between veins



Figure 1. Scanning electron photomicrograph of the adaxial surface of corn leaf. a) Veins (V) are situated in a parallel pattern with stomata (→) found in the region separating the veins (40X). b) Macrohair (mh) and bristles (→) are typically seen on and bordering the veins respectively (50X).

Figure 2. A comparative energy dispersive spectrograph of the epidermal surfaces of corn leaf; abaxial/adaxial shows no significant differences in elemental composition. Silicon (Si) is present in the greatest amount with smaller amounts of magnesium (Mg), sodium (Na), chlorine (Cl), potassium (K) and calcium (Ca). Aluminum (Al) appears due to the specimen stub.

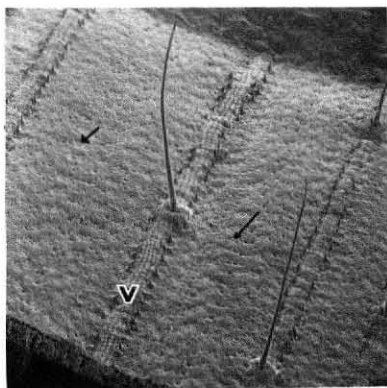
Figure 3. Adaxial surface of corn leaf. a) A silicon elemental map shows silicon to be concentrated over the veins (V) with lesser amounts in the areas lacking stomata (S) (90X). b) Corresponding secondary image (90X).

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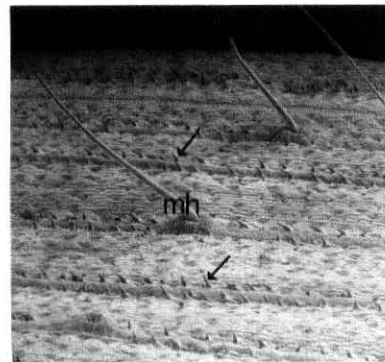
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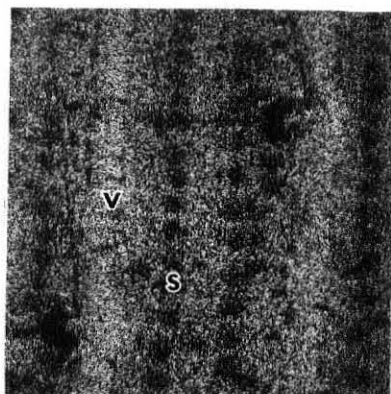
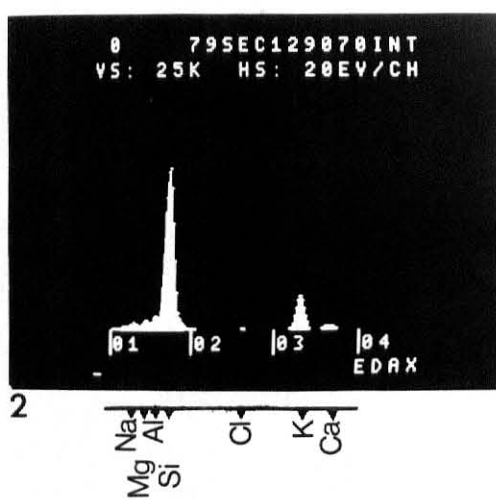
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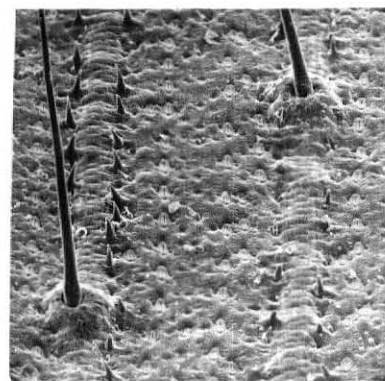
1a



1b



3a



3b

(Lott, 1976) that have been verified as silica bodies in addition to those found by Lanning's group (Lanning, personal communication).

The most distinguishing characteristics of the abaxial leaf epidermal surface are the presence of a greater number of stomata and absence of veins and macrohair (Metcalf, 1960) (Fig. 4a). Higher magnification of the surface reveals pronounced waxy anticlinal cell walls and detailed structures of the stomata (Fig. 4b). X-ray analysis shows similar elemental dispersion as observed in the adaxial tissue (Fig. 5a). There appear to be a few random areas of low Si concentration corresponding to intracellular spaces of the secondary image (Fig. 5b). Si forms the predominant peak in an energy dispersive spectrograph of the elemental spectra of the abaxial leaf tissue (Fig. 5c). Anatomical features of the internal leaf structure are shown in transverse section in figure 6. Starch-forming bundle sheath cells surround the vascular tissue which is connected to both epidermal surfaces by fiber cells. The mesophyll cells lie between these tissues and remain relatively undifferentiated. X-ray dispersion of Si in internal structures of the leaf were too low to map indicating a low level of Si. This does not preclude the absence of Si, only that the counts could not be differentiated from Bremsstrahlung.

The typical scattered arrangement of vascular tissue peculiar to monocotyledons can be seen by transverse section of the corn stem (Shih, 1974) (Fig. 7). A thin layer of epidermis surrounds the stem with parenchyma cells forming the matrix.

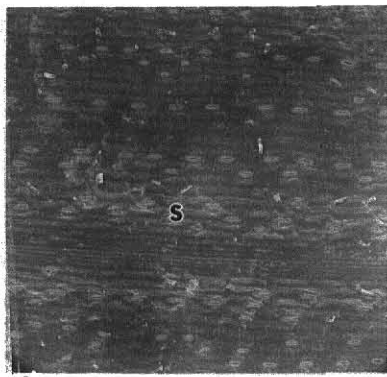




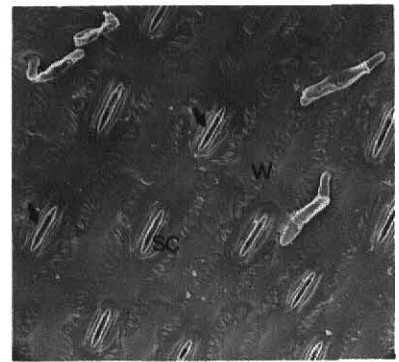
Figure 4. Abaxial surface of corn leaf. a) Characterized by a greater number of stomata (S) and the absence of veins and macrohair than adaxial surface (80X). b) At higher magnification the detail of individual stomata can be seen; guard cells (→) backed by subsidiary cells (Sc) and wavy anticlinal cell walls (W) which are very conspicuous in *Zea mays* (300X).

Figure 5. Abaxial surface of corn leaf. a) Silicon elemental dot map reveals silicon distributed widely across the surface with higher density in regions corresponding to underlying vascular tissue. Silicon is not seen in stomata (S) (90X). b) Corresponding secondary image (90X). c) Energy dispersive spectrograph indicates silicon to be the predominate element present.

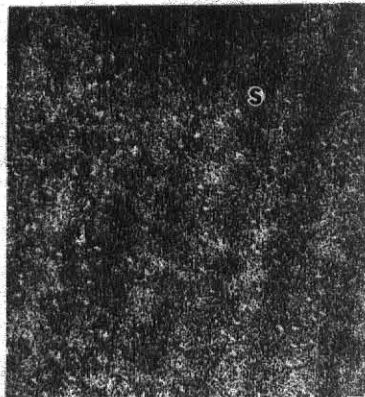
Figure 6. Transverse section of corn leaf consists of prominent veins made up of xylem which consists of two metaxylem vessels (vs), protoxylem (→), phloem (p), and fibers (f). Bundle sheath cells (b) surround the veins and are very active in starch formation (50X).



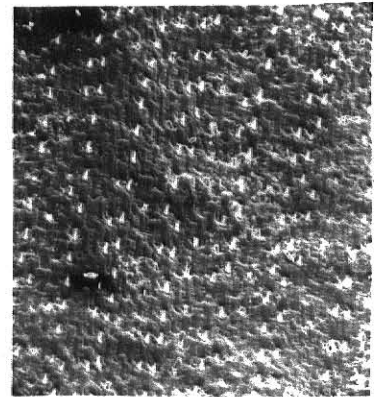
4a



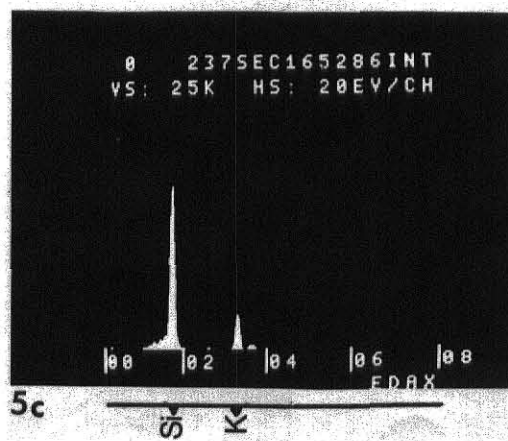
4b



5a



5b



6

Silica counts were too low (32-36 c.p.s.) preventing differentiation of silica on the epidermis or internal stem tissue (Fig. 8).

Sorghum. The adaxial surface of the sorghum leaf shows similar identifying anatomical structures previously described for the corn leaf. Veins and stomata lie in parallel fashion on the upper epidermal surface with bristles found intermittently along the veins (Fig. 9a). Silica cells, stomata and their guard cells, waxy deposits on the veins and wavy anticlinal cell walls are clearly discernable at higher magnification (Fig. 9b).

The basic distinguishing structures of the abaxial surface of sorghum are the same as delineated for corn. Stomata are more abundant and protrusion of vascular tissue is not seen (Fig. 10). A fundamental difference between the leaf epidermal topography of sorghum and corn is the absence of macrohair from both epidermises of the sorghum leaf blade.

X-ray analysis of both the upper and lower leaf tissue shows virtually identical dispersion of silica. As with corn there are some areas which exhibit a lower concentration of silica. This may be due to structural inhibition of the x-rays resulting from the varying thickness of the leaf (Fig. 11 a-b and Fig. 12 a-b).

Transverse sections of the sorghum leaf reveal less mesophyll in comparison to that seen in corn (Fig. 13). The similarity of the arrangement of internal structures of the sorghum stem to corn is apparent by transverse section (Fig. 14). The

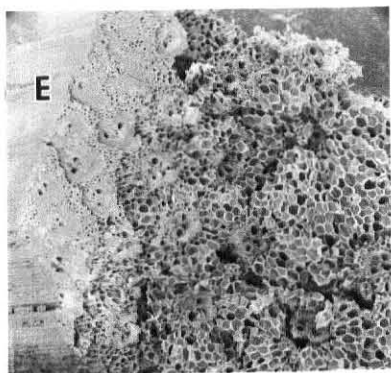


Figure 7. Corn stem in transverse section contains vascular bundles in the typical scattered arrangement. Parenchyma cells make up the matrix and a thin layer of epidermal cells (E) surrounds the entire stem (20X).

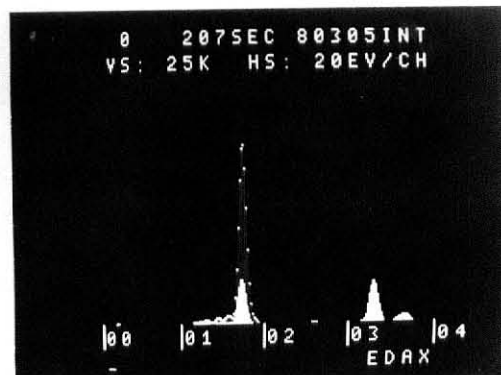
Figure 8. Energy dispersive spectrograph of corn stem comparing the elements present in the epidermal tissue (dotted graph) to the internal tissue (solid graph). Differentiation is not possible due to the very low counts per second.

Figure 9. Adaxial surface of sorghum leaf. a) General features of leaf are veins (V) and stomata (→) (100X). b) Silica bodies (Sb) lie in areas between wavy anticlinal cell walls (→). Stomata with guard cells (G) and subsidiary cells (Sc) are also recognizable features (1000X).

Figure 10. Abaxial surface of sorghum leaf appears to exhibit a greater density of stomata and absence of vascular tissue protrusion (100X).

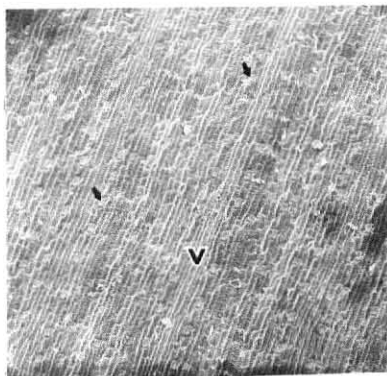


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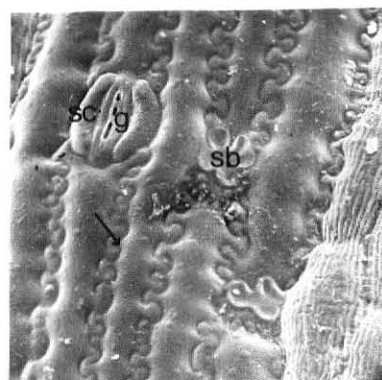


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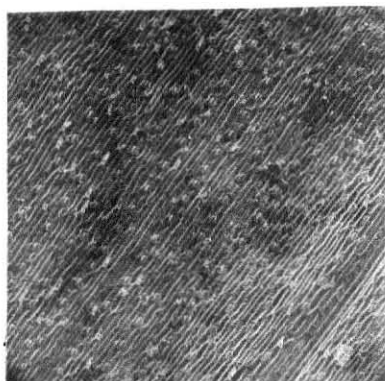
O Si K Ca



9a



9b



10



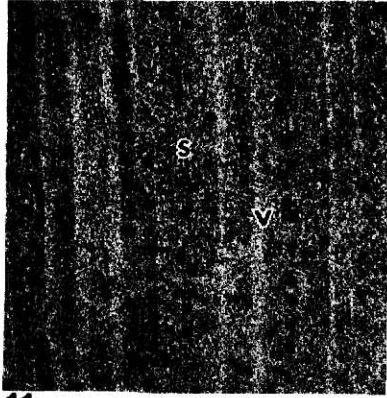
Figure 11. X-ray analysis of adaxial epidermal surface of sorghum leaf. a) Silicon elemental map shows distribution of the element to be widespread yet varying in density over veins (V) and stomata (S) (90X). b) Corresponding secondary image (90X).

Figure 12. X-ray analysis of abaxial epidermal surface of sorghum. a) Silicon elemental map indicates a similar distribution as seen in adaxial tissue (90X). b) Corresponding secondary image (90X).

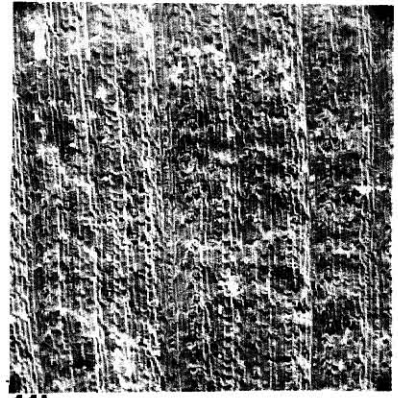
Figure 13. Transverse section of sorghum leaves reveals vascular bundles (V) surrounded by undifferentiated mesophyll (m) and prominent spongy mesophyll near the epidermis (300X).

Figure 14. Sorghum stem as seen by transverse section shows vascular bundles (v) ensheathed by fiber cells scattered through its interior with the parenchyma cells (pa) comprising the major portion of the stem. Fiber cells form the hypodermis (h) which is adjacent to the epidermis (30X).

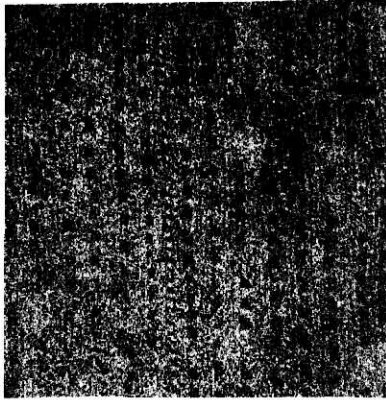




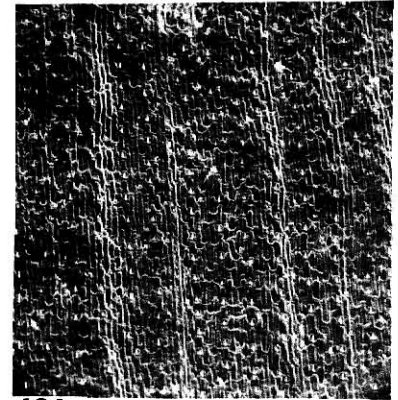
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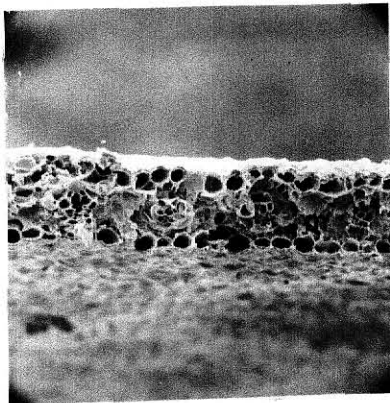
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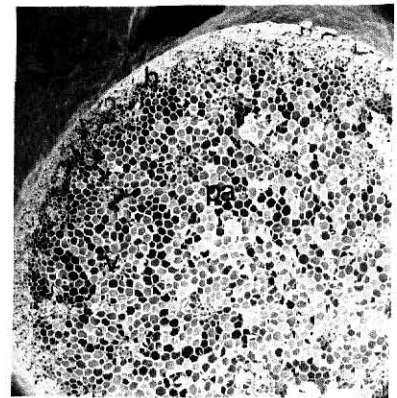
12a



12b



13



14

vascular tissue is scattered throughout while a thin epidermal layer surrounds the entire stem.

#### Ensiled Samples.

Corn. The major site of microbial activity is on the adaxial leaf surface where starch granules collect along the elevated surfaces overlying the vascular tissue (Fig. 15a). The bacterial action is primarily concentrated on hydrolysis of the starch with some very minor degradation of the leaf cuticle. Previous workers noted preferential ruminal digestion of starch over roughages (Burroughs et al., 1949, 1950; Williams et al., 1953), crude fiber (Kane et al., 1959) and cellulose (Abou Akkada and el-Shazly, 1958; el-Shazly et al., 1961). Physical breakage of the leaf surface is typically seen and may result from structural degradation due to bacterial action or processing for ensiling (Fig. 15b). Digestive attack on the abaxial surface is limited (Fig. 16). The leaf topography restricts the collection of starch which may cause bacteria to seek alternate digestive sites i.e., stomata.

Substantial numbers of starch granules accumulate in the parenchyma cells of the stem during ensiling (Fig. 17a). It was noted that bacteria attacked starch in preference to fibrous tissue such as parenchyma cell walls (Fig. 17b).

Sorghum. The effects of ensiling on sorghum leaf tissue follows a similar process as observed on corn. Bacterial degradation is more prominent on the upper surface. Insignificant amounts of starch granules were seen on either epidermis. Digestive



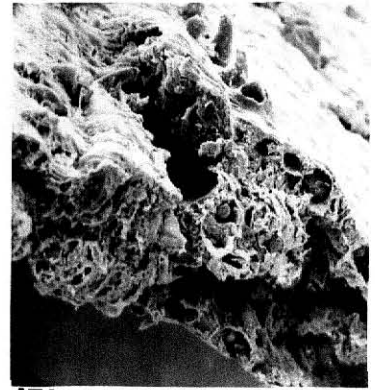
Figure 15. Ensiled corn leaf: adaxial surface. a) Starch granules collect along the veins (v) and enlarged basal regions (b) where macrohairs originate (300X). b) Physical breakage or tears appear in the venous areas (300X).

Figure 16. Ensiled corn leaf: abaxial surface. Digestive attack is minor but, is centered at the stomata (→) (200X).

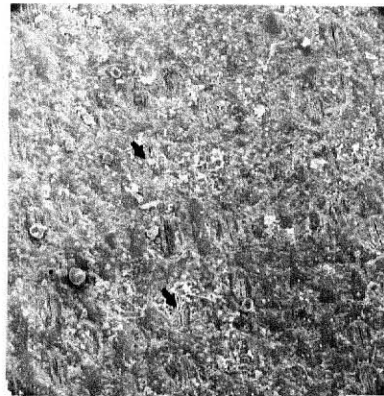
Figure 17. Ensiled corn stem. a) Large numbers of starch granules collect within the parenchyma cells of the stem tissue (200X). b) Bacteria hydrolyze the starch (s) without degrading the parenchyma cell walls (pa) (2000X).



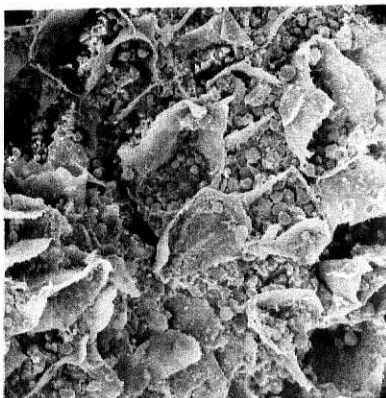
15a



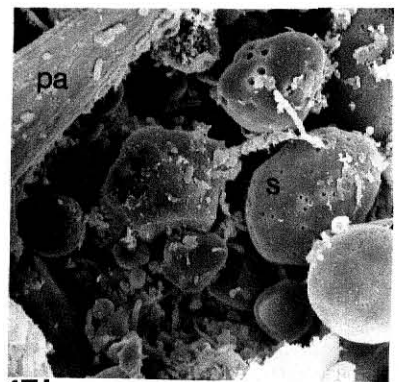
15b



16



17a



17b

activity was confined to the immediate area surrounding the stomata and possibly some erosion of the leaf cuticle (Fig. 18 a,b).

Bacterial attack on the abaxial leaf tissue is confined to the stomata (Fig. 19). Physical disruption of the leaf epidermises is evident resultant in all likelihood from preparation for ensiling.

The ensiled stem contains very few aggregations of starch in conformity with scarce amounts noted on the leaf epidermises (Fig. 20). This corroborates studies by Schmid et al. (1975) who detected starch in corn silage cell wall residues but, none in sorghum silage cell wall residues.

#### Ruminal Digestion.

Ensiled corn. There are two primary sites of digestive attack by rumen microbes evinced after subjection to ruminal action for 12 hours. The majority of this degradation can be seen by transverse section of the leaf (Fig. 21a). Mesophyll surrounding the vascular tissue is readily digested by vast numbers of bacteria (Fig. 21b). The second site of attack is centered around the venous areas of the adaxial leaf epidermis however, the extent and rate of degradation by the bacteria is more restricted at this location (Fig. 21c). The abaxial surface experiences virtually no microbial attack (Fig. 21d).

Disjunction of the epidermal surfaces from the vascular bundles becomes apparent at 24 hours of digestion (Fig. 22a). Activity by microbes located above vascular tissue on the



Figure 18. Ensiled sorghum leaf: adaxial surface.

a) Major degradative action occurs on the adaxial surface (100X). b) Very few starch granules (s) are found on the surface. Bacteria (→) are abundant even so and there is some erosion of the leaf cuticle (600X). c) Higher magnification reveals the many bacteria present particularly in and around the stomata (S) (2000X).

Figure 19. Ensiled sorghum leaf: abaxial surface.

Virtually no degradative activity is apparent (100X).

Figure 20. Ensiled sorghum stem lacks the abundance of starch granules as previously seen in the corn silage stem (50X).



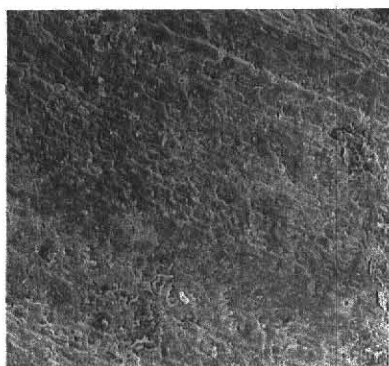
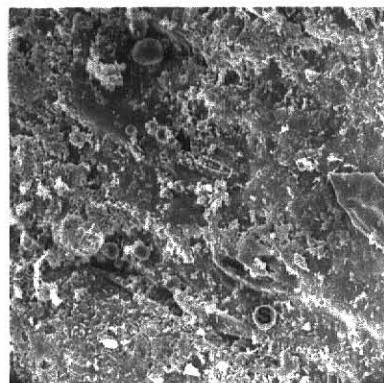
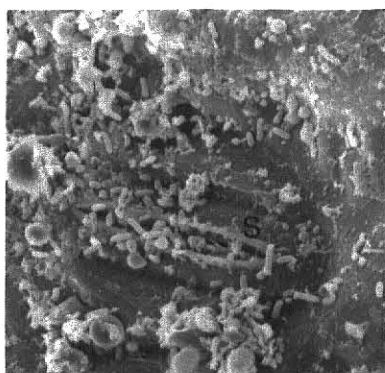
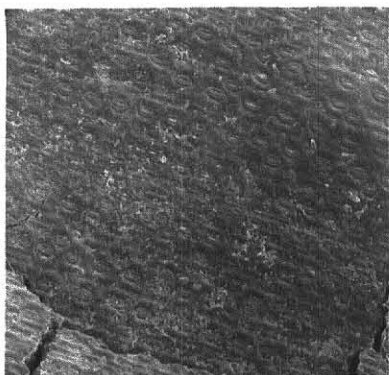
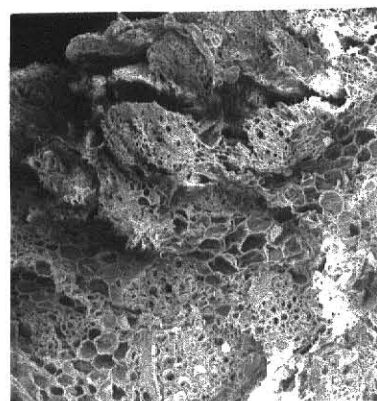
**18a****18b****18c****19****20**



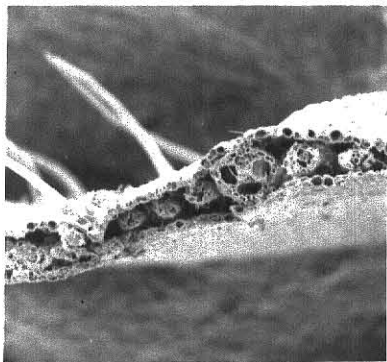
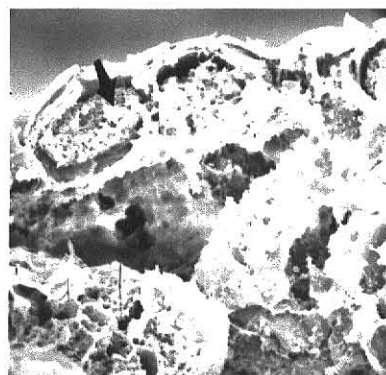
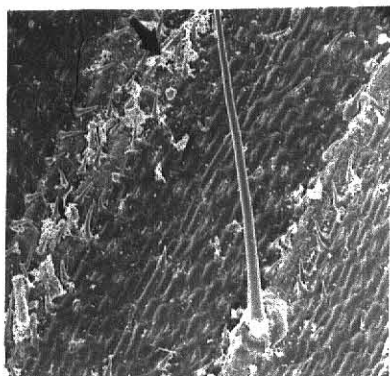
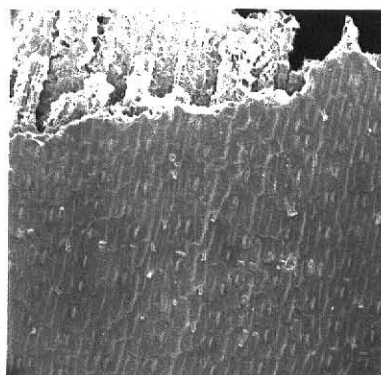
Figure 21. Ensiled corn leaf after 12 hours of ruminal digestion.

a) Initial attack is through leaf edges (100X).

b) Higher magnification reveals large numbers of bacteria (→) that are fundamental to the digestion of leaf mesophyll (1000X).

c) The second site of attack is on the adaxial epidermis where rumen microorganisms congregate along the venous tissue (→) (100X).

d) The abaxial surface shows no evidence of digestive attack (100X).

**21a****21b****21c****21d**

adaxial surface is ancillary to the weakening of the epidermeses initiated by mesophyll digestion (Fig. 22b). Abaxial tissue exhibits degradation only in regions where physical breakage of the epidermis has occurred (Fig. 23 c-d).

Extensive disruption of the leaf tissue has taken place after 48 hours of digestion within the rumen (Fig. 24a). Adaxial epidermis sustains more damage due to secondary attack at the venous regions. Both epidermal surfaces suffer substantial breakage but, adaxial breaks occur parallel, along the veins while random irregular tears are seen on the abaxial surface (Fig. 24 b-c).

Digestion is essentially completed by 72 hours. Leaf epidermeses and vascular tissue remain as end products (Fig. 24 a-c).

The ensiled corn stem shows effects of ruminal digestion after 48 hours (Fig. 25a). Parenchyma cells are progressively degraded leaving vascular bundles as digestive end products at 72 hours of ruminal digestion (Fig. 25b).

Ensiled sorghum. Rumen microbes direct digestive attack through the leaf edges digesting mesophyll while avoiding vascular bundles and epidermal surfaces (Fig. 26a). Leaf epidermeses detach from the venous tissue as noted previously in corn silage.

Photomicrographs of sorghum silage leaves digested for 12 hours within the rumen indicate that there is no substantial secondary digestive attack on either leaf surface. The adaxial surface has insignificant numbers of bacteria and protozoa



Figure 22. Ensiled corn leaf after 24 hours of ruminal digestion.

a) Epidermal surfaces detach from vascular tissue as mesophyll is digested (60X).

b) Physical breakage of the epidermises results in part from digestive activity in adaxial venous regions (V) (200X).

c) The abaxial surface experiences restricted degradation (→) (70X).

d) Digestive attack occurs only in breaks on the abaxial epidermis (1000X).

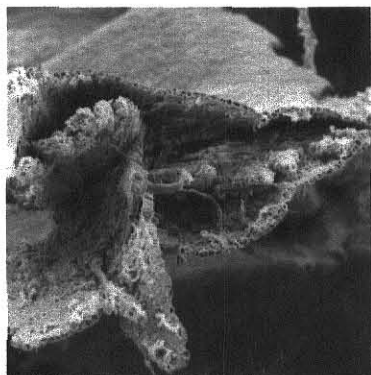
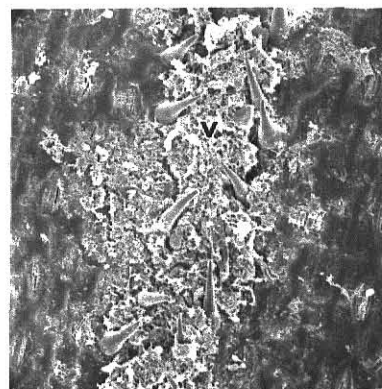
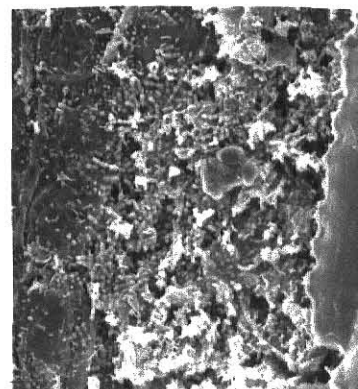
**22a****22b****22c****22d**





Figure 23. Ensiled corn leaf after 48 hours of ruminal digestion.

a) A transverse section of the digested leaf shows extensive disruption (20X).

b) The adaxial surface experiences breaks parallel to the veins (→) (20X).

c) The abaxial epidermis still lacks digestive attack although random tears (→) are apparent (50X).

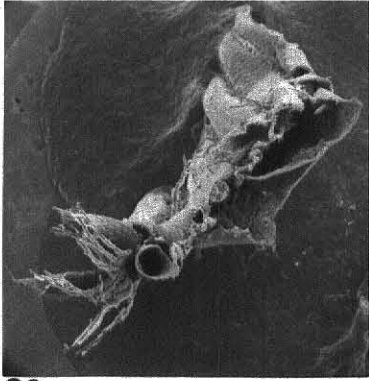
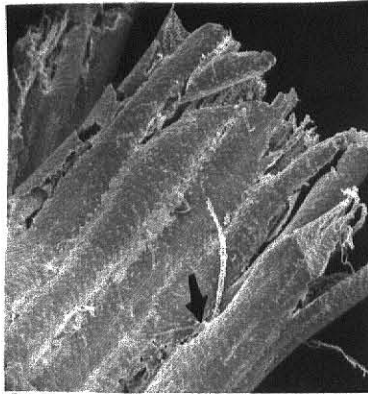
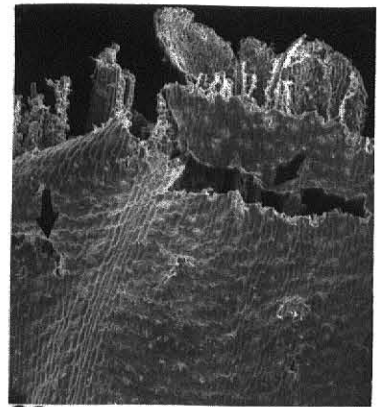
**23a****23b****23c**

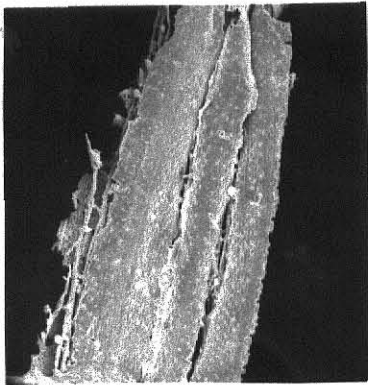


Figure 24. Ensiled corn leaf after 72 hours of ruminal digestion.

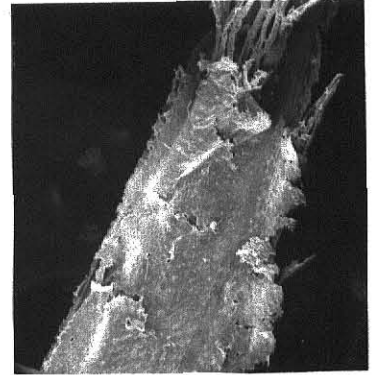
- a) Extensive breakage of the adaxial surface is evident (20X).
- b) The abaxial surface shows limited attack (20X).
- c) Vascular tissue and leaf epidermises are the end products of digestion (20X).

Figure 25. Ensiled corn stem. a) The effect of 48 hours of digestive attack is evidenced by degradation of parenchyma cells (p) that surround the vascular tissue (V) (20X).

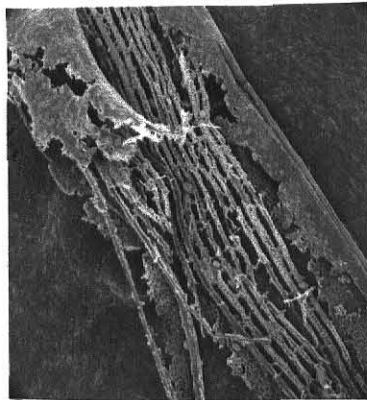
b) Digestion is essentially complete by 72 hours. Vascular bundles remain as digestive end products (20X).



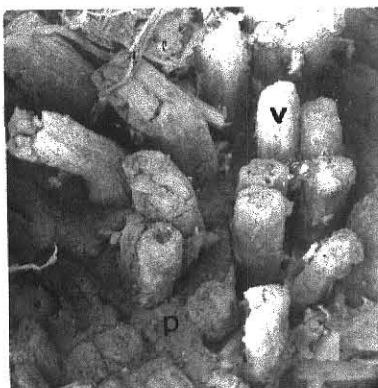
24a



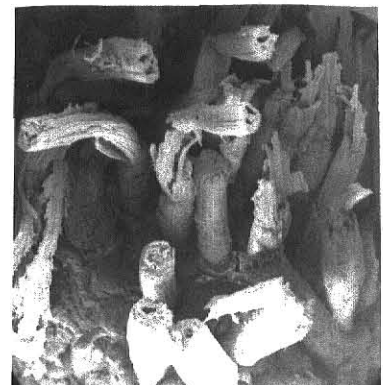
24b



24c



25a



25b

present whose activity is very limited and stationed around stomata (Fig. 26b). Digestive action on the abaxial tissue is identical to that seen adaxially (Fig. 26c).

The progression of microbial attack on ensiled sorghum leaf mesophyll occurs at a slower rate than seen in corn silage. A transverse section of the leaf shows the extent of digestion occurring by 24 hours (Fig. 27a). This reduced rate of degradation results from the limited access that rumen microbes have to the mesophyll. Both epidermal surfaces are intact making it impossible for the microbes to digest the underlying mesophyll except by entrance through the edges (Fig. 27 b-c).

Rate of digestive attack appears to increase after 48 hours as adaxial leaf epidermis weakens and random breakage occurs (Fig. 28 a-b). Bacteria are able to enter through the breaks in the surface and hydrolyze the leaf mesophyll. The abaxial epidermis is whole and intact which prevents degradation through that surface (Fig. 28c). The end products to ruminal digestion by 72 hours are vascular tissue and leaf epidermeses (Fig. 29).

Degradation of the ensiled sorghum stem follows a pattern identical to that seen in corn silage. Parenchyma cells are digested with no attack on vascular bundles by 24 hours (Fig. 30a). 72 hours of rumen digestion shows further digestion of parenchyma but, still vascular tissue remains (Fig. 30b).





Figure 26. Ensiled sorghum leaf after 12 hours of ruminal digestion.

- a) Initial attack is through leaf edges (130X).
- b) The adaxial surface experiences no digestive activity (156X).
- c) The abaxial epidermis shows no digestive activity (156X).

Figure 27. Ensiled sorghum leaf after 24 hours of ruminal digestion.

- a) The leaf mesophyll is degraded more slowly than seen in the corn silage leaf (40X).
- b) There is no microbial attack on the adaxial epidermis (20X).
- c) The abaxial tissue shows no digestive activity (20X).

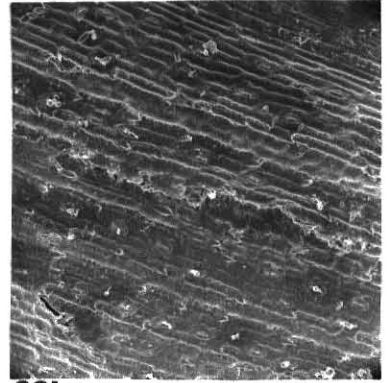
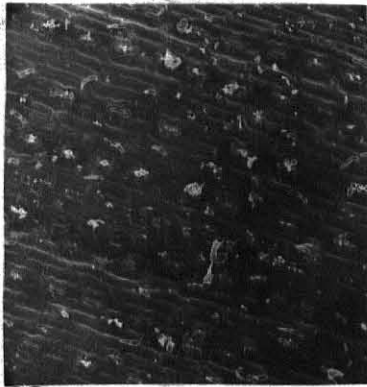
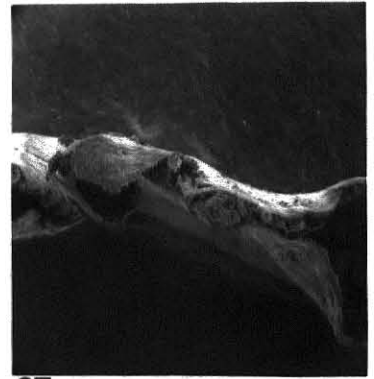
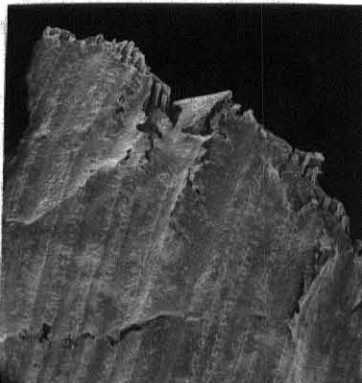
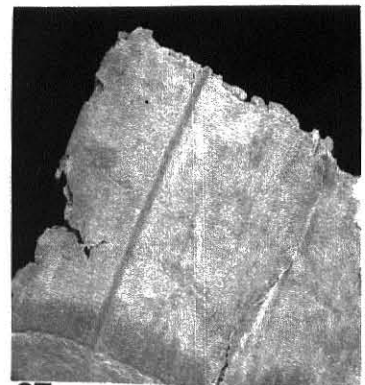
**26a****26b****26c****27a****27b****27c**



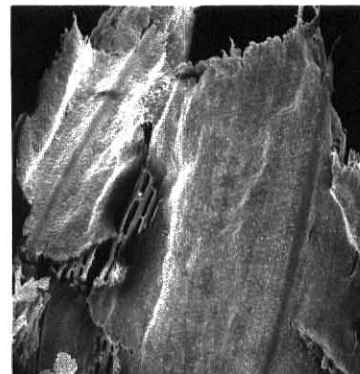
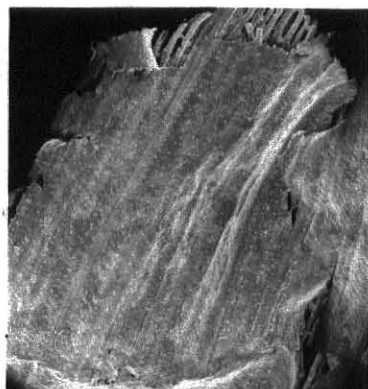
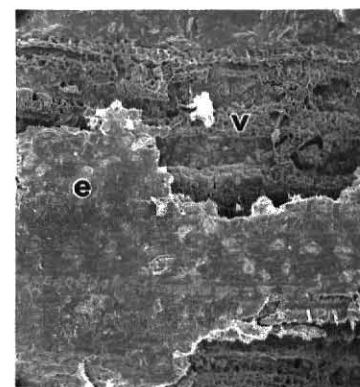
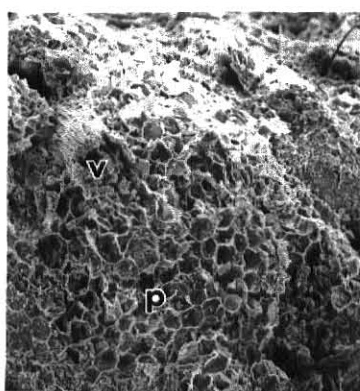
Figure 28. Ensiled sorghum leaf after 48 hours of ruminal digestion.

- a) Microbial digestion of the leaf mesophyll has progressed further causing some disruption of the epidermises (20X).
- b) Physical breakage of the adaxial surface has become more evident (20X).
- c) The abaxial tissue is still whole and intact (20X).

Figure 29. Vascular tissue (V) and leaf epidermis (e) are left as end products to ruminal digestion after 72 hours (130X).

Figure 30. Ensiled sorghum stem.

- a) Degradative action after 24 hours on parenchyma cell tissue (168X).
- b) Digestion by 72 hours shows further attack on parenchyma (p) leaving vascular tissue (v) untouched (60X).

**28a****28b****28c****29****30a****30b**

## CONCLUSION

This study indicates that there are several basic differences seen in the digestive patterns of corn and sorghum silage. A principle difference is due to the leaf topography. Venous protrusion is an inherent characteristic of the adaxial surface of the corn leaf which partially results in the higher digestibility of corn silage. It was noted that starch granules collect along the vascular tissue and are hydrolyzed rapidly which is directly related to increased digestibility values. Rumen microorganisms amass in this region during ensiling and ruminal digestion, respectively. Their resultant activity weakens the epidermis allowing access to leaf mesophyll more quickly than in sorghum silage. Greater accumulation of starch within the ensiled corn stem in comparison to sorghum silage is also a factor to consider.

The role of silica in the hydrolytic patterns of both silages appears to be inhibition of epidermal digestion. Distribution of silica in the leaves of both silages shows similar deposition, i.e., surface wide dispersion with higher concentrations over venous regions and absence from stomata.

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THE DIGESTION OF CORN AND SORGHUM SILAGE  
AS OBSERVED BY SCANNING ELECTRON MICROSCOPY

by

MARI L. THOUVENELLE

B.S., Arizona State University, 1975

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1977

The scanning electron microscope was used to observe leaves and stems of corn and sorghum silage before and after degradation in the rumen. Bacterial action due to the ensiling process was more active on the adaxial leaf surfaces in both species. Corn silage exhibited additional hydrolytic activity on starch granules that accumulated along the vascular regions of the adaxial leaf surfaces. Hydrolysis of starch was evident on each species of ensiled stem tissue but no degradation was observed on parenchyma cells or vascular tissue.

Rumen microorganisms preferentially attacked the leaf mesophyll and parenchyma cells of the stem in both silages. It was noted that the rate of mesophyll digestion was more rapid in corn silage leaves. Rumen microbes gained access more easily to the corn silage mesophyll due to more extensive breakage of the adaxial epidermis. Only leaf epidermises and vascular tissue from leaves and stems of both silages remain after 72 hours of rumen digestion.

Energy dispersive x-ray analyses revealed similar dispersion of silicon on the leaf epidermises of both corn and sorghum. Higher concentrations of silicon are seen over veins with lesser amounts in the adjacent tissue; stomata appear to be completely devoid of silicon. Differentiation of silicon in the stem epidermis and internal tissue was unobtainable due to very low counts. The role of silicon in the digestive pattern of the silages examined appears to be inhibition of initial digestion.