RUMEN DEGRADATION OF SOME FORAGES OBSERVED BY SCANNING ELECTRON MICROSCOPY

by

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This manuscript is dedicated to a friend, David McClaskey, whose courage while fighting a rare disease was a great inspiration to me. Also, to my parents, Mr. and Mrs. Clarence Brazle, for their encouragement and understanding over the years.

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GENERAL INTRODUCTION

The scanning electron microscope (SEM) provides an excellent method of viewing rumen digestion of forages. Step by step degradation can be observed. Thus, SEM becomes another tool in evaluating forages. Other methods of forage evaluation, such as proximate analysis (crude fiber), Van Soest analysis, and the *in vitro* fermentation technique go hand in hand with SEM to increase our scope of knowledge. The digestive pattern of *Bromus inermis* (smooth brome), *Festuca arundinacea* (tall fescue), *Andropogon gerardi* (big bluestem), *Andropogen scoparius* (little bluestem) and *Medicago sativa* (alfalfa) were observed in this study. Structural inhibitor's roll in digestion was clearer after viewing rumen digestion of forages.

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THE DIGESTION OF ALFALFA HAY OBSERVED BY SCANNING ELECTRON MICROSCOPY

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Summary

The scanning electron microscope was used to observe air dried Medicago sativa (alfalfa) leaves and stems before and after attack by rumen microbiota. Attack was only on the dorsal leaf surface and at random. Leaf cuticle and epidermis were sloughed after 24 hours in the rumen allowing massive digestion of the mesophyll. Only unhydrolyzed cuticle, ventral hair, and partially hydrolyzed vascular tissue remained after 48 hours. The stem's external surface was randomly attacked by sloughing of cuticle and epidermis to expose a dense matrix (cortex) that was partially hydrolyzed following 24 hours in the rumen. Fiber cells were exposed after 48 hours; 72 hours left only vascular tissue still intact, which was also found in the feces.
(Key Words: Alfalfa hay, Digestion, Rumen, Scanning Electron Microscopy).

Introduction

Both structural and non-structural inhibitors in forages are significant in digestion. Silica, a structural inhibitor, (Deinum, 1973) forms various types of phytoliths in grasses (Twiss et al., 1969) but is absent in legumes (Mowat et al., 1968). Lignin depresses the digestion of cell wall contituents

Deinum, 1973), but high alfalfa digestibility does not agree with its high lignin content. Drapala et al., (1947) and Pigden (1953) suggested that lignin's location may have more affect on digestibility than its percentage. Van Soest (1966) found that the largest fecal fiber particles had only slightly more cell wall lignin than the largest forage fiber particles.

Akin and Amos (1975), using the scanning electron microscope (SEM), concluded that rumen microbes degraded the mesophyll and phloem cell walls more rapidly than other tissue in leaves of some forages. Leaf blade microanatomy and inherent characteristics of cell walls affect digestibility by rumen microorganisms (Akin and Burdick, 1975).

SEM is an excellent method of observing microbial activity because it permits detailed observations at different magnifications of internal structure of stems and leaves. This report describes how rumen microbiota hydrolyze air-dry alfalfa stems and leaves.

Materials and Methods

Individual samples of Medicago sativa (alfalfa, hay, s-c, mature, (1), IRN L-00-071) were collected from 16 areas within one location and allowed to air dry. The leaves and stems were separated from each sample and part of each sample was ground through a 6 mm screen. Triplicate 5 g. samples were digested for 48 hours in nylon bags (Johnson, 1969) suspended in the rumens of four mature fistulated steers receiving 2.3 kg. of

ground sorghum grain (sorghum, milo, grain, (4), IRN 4-04-444) and ad libitum alfalfa and prairie hay (Native plants, Midwest, hay, s-c, mid blm, (1), IRN 1-07-956) in equal parts. Rumen fluid from these steers was used for 12, 24, 36, and 48 hours in vitro digestions (Tilley and Terry, 1963). The remaining intact leaves and stems were digested in vivo for 12, 24 hours and 24, 48, 72 hours, respectively, using nylon bags. The control of undigested entire leaves and stems was soaked in water for 48 hours. Digested and control intact leaves and stems were rinsed in distilled water after removal from bags and prepared for scanning electron microscopal observation by dehydration in an ethanol series (2 to 3 minutes each in 30%, 60%, 80%, 100%). Fecal stem residues of alfalfa hay were collected from two steers at 24 hour intervals for 2 days. These animals had been maintained on a ration of 2.3 kg. of ground grain sorghum and ad libitum alfalfa hay for three weeks. The fiberous residue was recovered on cheesecloth, then dehydrated in an ethanol series.

Cross, sagittal and longitudinal sections of alfalfa hay were prepared by freezing in liquid nitrogen and splitting with a razor blade to produce a fracture. Specimens were mounted on aluminum stubs with Delco No. 93 collodial silver (Ted Pella Co., #1603-2), coated under vacuum with 150 mm of gold palladium (Kenny Vacuum Co., Model KSE-2-A-M evaporator), observed and photographed with an Etec Autoscan scanning electron microscope at 20 KV (Davis and Harbers, 1974). Stereoscopic pairs of photomicrographs were made by rotating the vertical stage of the microscope between 5° and 7° (Howell, 1975) and interpreted using a stereo-viewer. Only single photomicrographs are shown.

Results & Discussion

Leaf

Alfalfa leaves have small ridges on the epidermal surface that would appear to be artifacts caused by air drying (Fig. 1a and 1b). Veins and hair were only on the ventral surface (Fig. 1b). The bulk of the leaf is mesophyll with only a little vascular tissue (Figs. 2a, b) at this maturity stage.

The dorsal leaf surface was randomly attacked by microbiota causing cuticle sloughing and degrading of the underlying mesophyll after 12 hours in the rumen (Fig. 3). After 24 hours in the rumen leaves varied from partially degraded mesophyll and vascular tissue (Fig. 4a) to completely degraded mesophyll with only unhydrolyzed rolled cuticle, ventral hair, and a small amount of partially degraded vascular tissue remaining (Fig. 4b). The ventral epidermis of the leaf was not hydrolyzed, a phenomenon characteristic of unmasticated leaves of Bromus inermis (smooth brome), Festuca arundinacea (tall fescue), Andropogon gerardi (big bluestem), and Andropogon scoparius (little bluestem) (Harbers et al. 1976). Since these samples were unmasticated, they may not represent normal digestion times.

The photomicrographs showed rapid mesophyll degradation. This is supported by the in vitro digestibility curve (Fig. 10). In vivo (nylon bag) digestibility of the ground alfalfa leaves ranged from 78% to 89%; 48 hours in vitro digestibility was 70.6%. Since mastication and other physical damage disrupts leaf structure, the cuticle, ventral hair, and partially hydrolyzed vascular tissue could not be identified in the feces, so could not be shown to be unhydrolyzed by post-ruminal digestion.

According to its chemical composition, cuticle should not be degraded by microbiota (Esau, 1953). This is supported by the photomicrographs.

Stem

The classic dicotyledon structures (epidermis, cortex, and vascular tissue) were easily distinguishable in stem cross section (Fig. 5b). The external surface of an alfalfa stem (Fig. 5a) has stomates randomly located. The cortex, a dense narrow band of cells under the epidermis, is clearly visible in Fig. 5b. Thin wall cells around the stem's lumen appear less structurally stable than the remainder of the stem.

Alfalfa stems exposed to rumen microbiota for 24 hours showed a sloughed cuticle (Fig. 6a), and exposed beneath the cuticle was a partially hydrolyzed dense matrix (Fig. 6b). By 48 hours the dense matrix was completely hydrolyzed, exposing the fiber cells. These cells (Fig. 7a and 7b) define the outer limit of the vascular tissue. After 72 hours, most of the vascular tissue remained (Fig. 8a) except for a small amount near the lumen. The longitudinal surface of the undegraded vascular tissue is characterized by vessel cells (Fig. 8b). Apparently, normal rumen digestion carries out maximal alfalfa stem degradation, since stem material extracted from the feces showed the same undegraded vascular tissue as that was found after 72 hours in the rumen in nylon bags (Fig. 9a and 9b).

In vivo digestion of ground stems ranged from 34% to 46%. In vitro (Tilley and Terry, 1963) digestibility averaged 38.5%. The cuticle and most of the vascular tissue remained after 72

hours. The degradable stem tissue was rapidly hydrolyzed (Fig. 10). There was no visible damage to the internal undegraded vascular tissue. The time required for alfalfa stem degradation as shown by the photomicrographs does not represent normal hydrolysis because no mastication occurred.

The amount of vascular tissue in alfalfa stems should not affect the rate or extent of digestibility of that tissue destined for digestion. But, since vascular tissue is undigestible, it will decrease percent digestibility. This agrees with Donefer et al. (1960) who found that alfalfa was more rapidly digested initially in vitro than grass although the latter had higher digestibilities after 48 hours.

Vascular tissue in alfalfa stems should contain most of the lignificated tissue in the plant. Therefore, lignin percentage would not appear to be an inhibitor to digestion, but only determine the amount of undigested residue that remains in the reticulo-rumen to be removed by the reticulo-omasum orifice.

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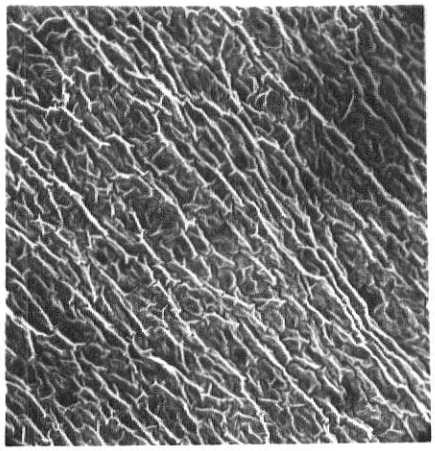
Figure 1. Scanning electron photomicrographs of alfalfa hay leaves. a) Dorsal epidermal cells and stomates form an irregular pattern with intercellular ridges, the ridges are probably artifacts of drying (113X). b) Ventral surface of a leaf characterized by veins and hair (151X).

Figure 2. Cross sections of air-dried alfalfa leaves. a) Fracture shows inside of leaf composed mainly of mesophyll (189X). b) Fracture across vein shows small amount of vascular tissue (189X).

Figure 3. Dorsal surface of alfalfa leaf following 12 hour digestion in the rumen shows (→) sloughed cuticle and beginning of hydrolysis (113X).



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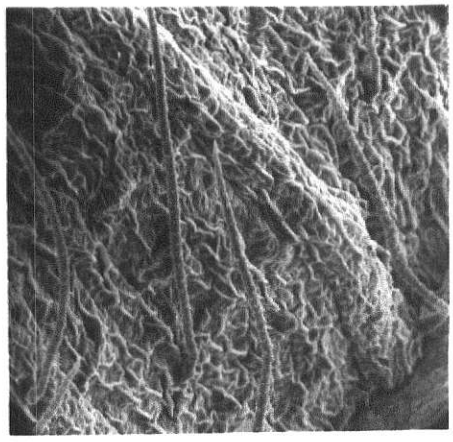

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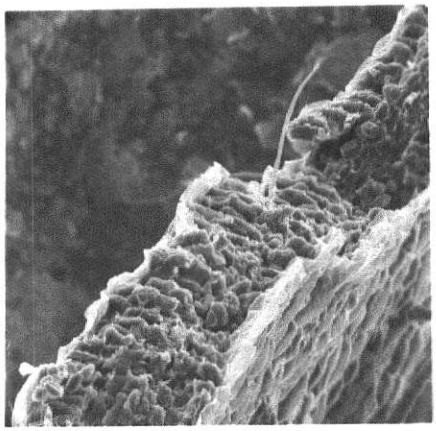

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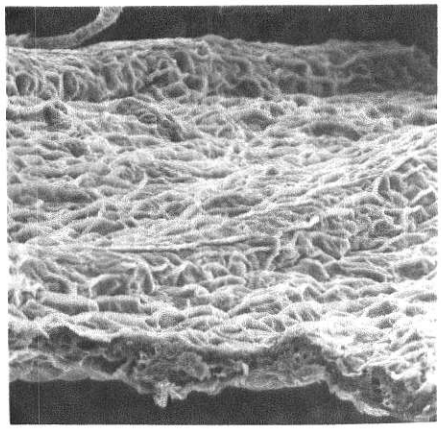

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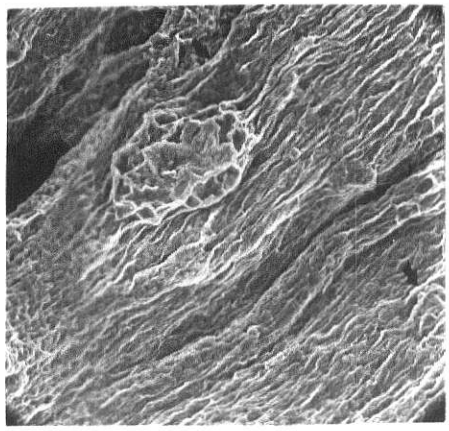

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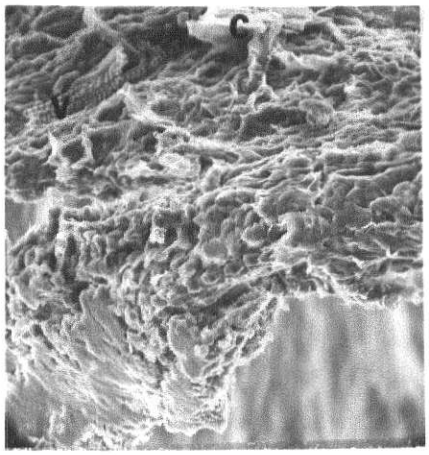
Figure 4. Scanning electron photomicrographs of alfalfa leaves after 24 hour ruminal digestion. a) dorsal cuticle (C) has been sloughed revealing partially hydrolyzed mesophyll and vascular tissue (V) (189X). b) undigested dorsal cuticle (C) and rolled into a strand, some vascular tissue (V) is still present as are ventral cuticle and hair (H) (95X).

Figure 5. Scanning electron photomicrographs of alfalfa hay stem. a) external surface shows characteristic random stomates (113X). b) cross section shows cuticle (C) and epidermis (E), cortes (CO), vascular tissue (V) and lumen (L) (32X).

Figure 6. Alfalfa stems subjected to 24 hour rumen fermentation. a) Wall of stem shows cuticle layer (→) has been partially sloughed exposing the cortex (32X). b) Higher magnification of dense cortex shows partial hydrolysis (630X).



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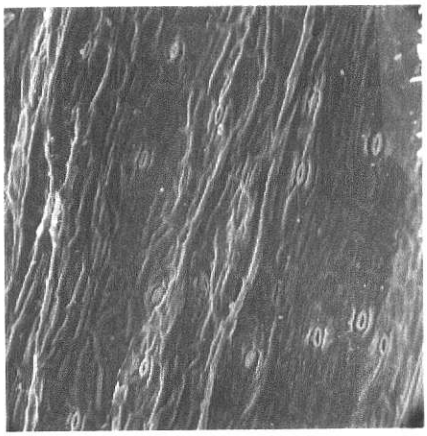

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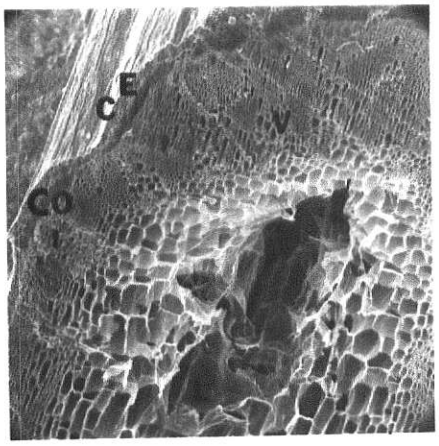

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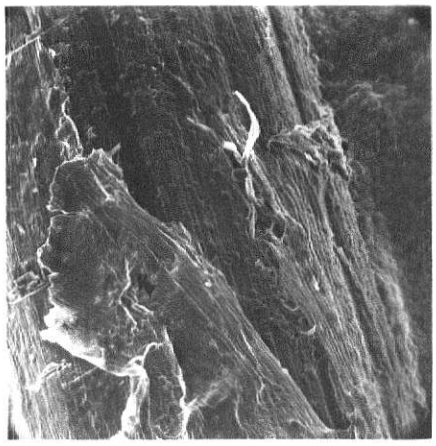

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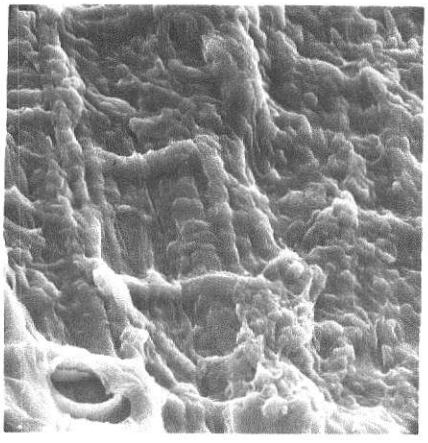

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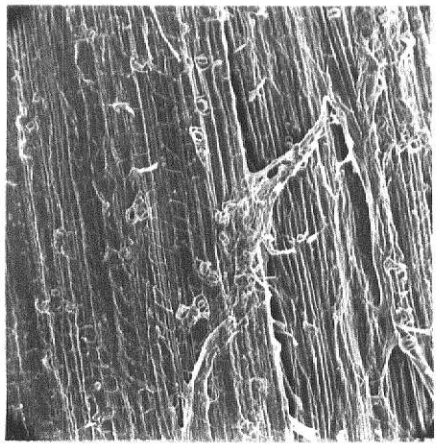
Figure 7. Alfalfa stems subjected to 48 hour rumen fermentation. a) Stem wall has been hydrolyzed to fiber cells found in the external part of the vascular tissue (69X). b) Angular view showing relationship of fiber cells to remaining vascular tissue (63X).

Figure 8. Alfalfa stems subjected to 72 hour rumen fermentation. a) cross section shows only vascular tissue remaining with some digestion (→) of that tissue surrounding the lumen (32X). b) Longitudinal surface shows unhydrolyzed vessel cells characterized by border pits (320X).

Figure 9. Scanning electron photomicrograph of alfalfa stems from fecal residue. Cross section of stem shows hydrolysis exposing vessel cells of vascular tissue (113X). b) Longitudinal segment of stem shows vessel cells with some border pit membranes intact (630X).



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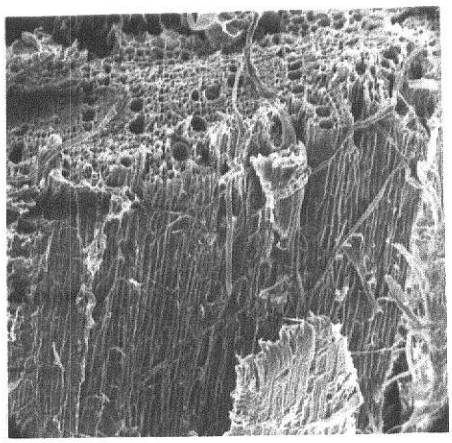

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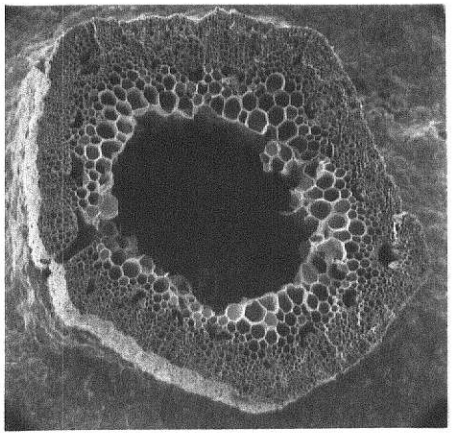

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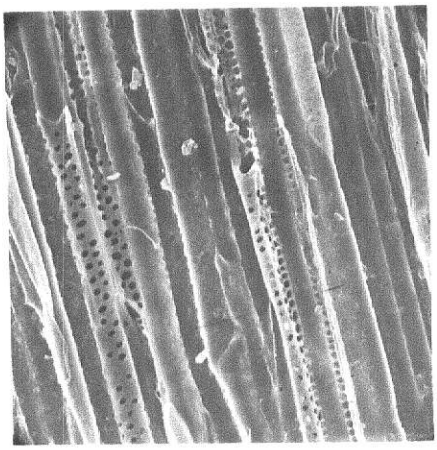

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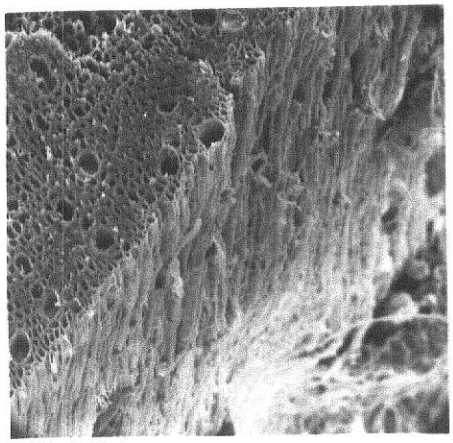

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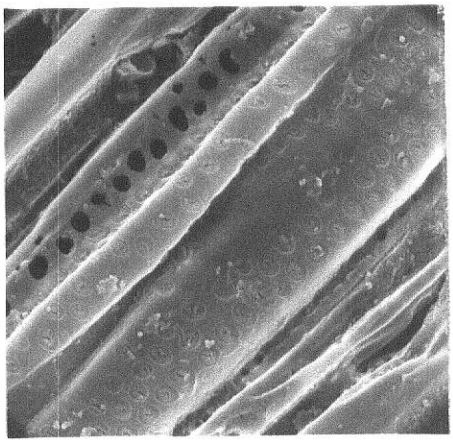

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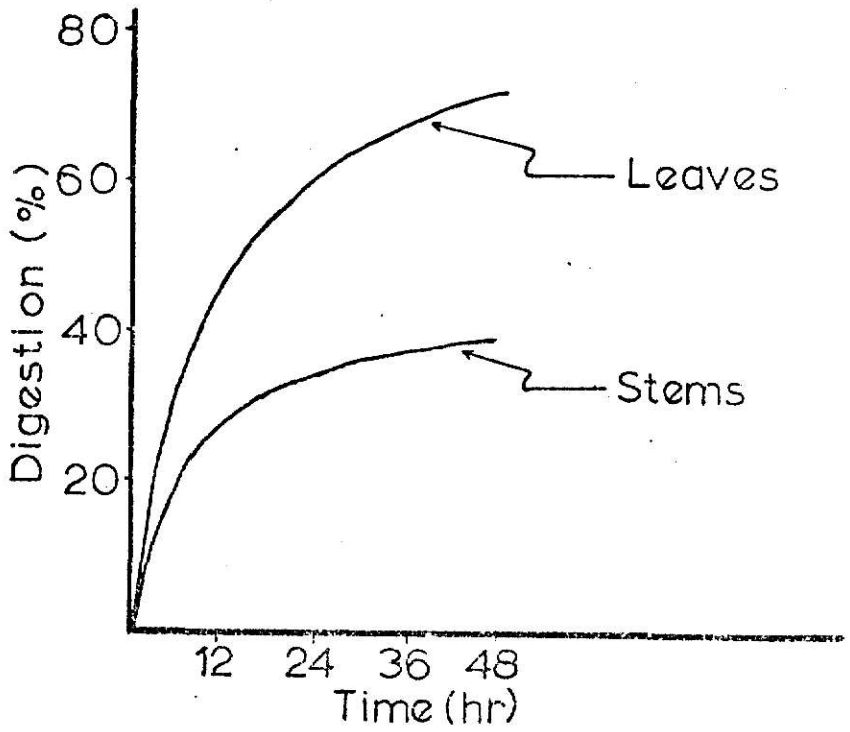
Figure 10. In vitro digestibility curve for alfalfa stems and leaves.



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MICROBIAL DEGRADATION OF SMOOTH BROME AND TALL FESCUE OBSERVED BY SCANNING ELECTRON MICROSCOPY

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Summary

The scanning electron microscope was used to observe Bromus inermis (smooth brome) and Festuca arundinacea (tall fescue) before and after degradation in the rumen. Microbiota attacked the dorsal surfaces of unmasticated leaves of both species. Dorsal cell degradation was random in smooth brome but limited in tall fescue to systematic hydrolysis of certain cells located between underlying vascular bundles. All underlying mesophyll in brome and that directly under vulnerable cells in fescue was rapidly hydrolyzed. Microbiota degraded fescue mesophyll slower, nearer vascular bundles and finally attacked material surrounding those tissues. Only vascular tissue, cutinized ventral epidermis and partially digested, or sloughed dorsal cuticle and epidermis remained after 72 hour fermentation. Rate of digestion and indirectly intake, was likely related to structural inhibition on the dorsal leaf surface and to the speed the cuticle is sloughed and microbiota by-pass other structural inhibitors such as trichomes and phytoliths. (Key Words: Brome, Fescue, Digestion, Rumen, Scanning Electron Microscopy).

Introduction

Silica was classified as a structural inhibitor by Deinum (1973). Siliceous epidermal cells (which contain phytoliths) in most grasses are elongate and short (Twiss et al. 1969).

Short cells are nearly equidimensional and the shape of the phytoliths in cells may not correspond to the shape of the enclosing cell (Metcalfe, 1960). Smooth brome and tall fescue are characterized by phytoliths of the taxonomic group Festucoid (eight different shapes mainly circular) (Metcalfe, 1960; Prat, 1936). Elongate phytoliths are not characteristic of any single grass family, but may be found in all grasses (Twiss, et al., 1969).

Lignin depresses the digestion of cell wall constituents (Deinum, 1973), although high alfalfa digestibility does not agree with its high lignin content. Drapala et al. (1947) and Pigden (1953) suggested that the location of lignin may affect digestibility more than actual lignin percentage. Van Soest (1973) found that smooth brome was 64% cell walls; tall fescue, 54%. The percent of the cell wall that was lignin and hemicellose was the same; however, smooth brome cell wall consisted of 4% more cellulose.

Microanatomy of plants may be studied in great detail using the scanning electron microscope (SEM) (Akin, et al.1973). Rumen microorganisms degraded mesophyll and phloem cell walls more rapidly than other tissue in the leaves of same forages (Akin and Amos, 1975; Akin and Burdick, 1975).

Using the SEM, we studied the effect of rumen microorganisms of smooth brome and tall fescue.

Materials and Methods

Leaf samples of *Bromus inermis* (brome) and *Festuca arundinacea* (fescue) were collected from two locations during the first week in November. Part of the leaves, while still fresh and intact, were digested in triplicate for 24, 48 and 72 hours in nylon bags (Johnson, 1969) in four mature fistulated steers receiving 2.3 kg. of ground sorghum grain (sorghum, milo, grain, (4), IRN 4-04-444) and *ad libitum* intake of prairie hay (native plants, midwest, hay, s-c, mid blm, (1), IRN 1-07-956). The control, undigested leaves, was soaked in water for 48 hours. Digested and control intact leaves were rinsed in distilled water and prepared for SEM observation by dehydration in an ethanol series (2 to 3 min. each in 30%, 60%, 80%, 100%).

The remainder of the leaves were air dried and ground through a 1 mm screen in preparation for proximate analysis and *in vitro* digestion (Tilley and Terry, 1963). Triplicate samples of .5 g. of ground leaves were digested *in vitro* for 12, 24, 36, and 48 hours and 2nd order regression equations used to plot digestibility curves.

Cross and longitudinal sections of brome and fescue leaves were prepared by freezing in liquid nitrogen and fracturing with a razor blade. Delco No. 93 colloidal silver (Ted Pella Co., #1603-2) was used to mount specimens of leaves on aluminum stubs. The specimens were coated with 150 mm of gold palladium under vacuum (Kenny Vacuum Co., Model KSE-2-A-M evaporator) and viewed with an Etec Autoscan scanning electron

microscope at 20 KV (Davis and Harbers, 1974). Polaroid PN/55 film was used to produce photomicrographs. Only single photomicrographs are shown here, however, stereoscopic pairs were taken for interpretative purposes by rotating the verticle scope stage between 5^{o} and 7^{o} (Howell, 1975).

Results and Discussion

The dorsal leaf surface (Fig. 1a) of fescue was characterized by two types of cells forming a longitudinal pattern. Stomates were located on ventral surface veins (Fig. 1b). The dorsal surface (Fig. 2a) of brome leaves had a row of trichomes directly external to the vascular tissue. On the ventral surface, trichomes were in rows on the vein, and surrounded by stomates and phytoliths. Cross sections of fescue leaves (Figs. 4a and b) show more vascular tissue and fiber cells than brome leaves, but brome leaves show more mesophyll (Fig. 2c).

Rumen microbes attacked only the dorsal surface of both grasses. Fescue leaves were attacked systematically by sloughing the cuticle and degrading the mesophyll in the intervein area of the leaf (Fig. 3a and b). After 24 hours in the rumen the intervein mesophyll was degraded and the microbiota worked laterally toward the vascular tissue (Fig. 4a and b) partially degrading the fiber cells by 48 hours (Fig. 8). Fiber cells were degraded by 72 hours (Fig. 11) leaving unhydrolyzed vascular tissue, ventral cuticle and epidermis, and a longitudinal strip of dorsal cuticle and epidermis directly external to the vascular tissue (Fig. 7 and 10). Akin et al.

(1973) found similar results after 72 hours digestion of fescue. The dorsal surface of brome leaves were degraded at random. The only areas not attacked were the rows of trichomes and surrounding cells. The cuticle was sloughed (Fig. 5) and the mesophyll partially degraded. Other brome leaves showed complete mesophyll degradation after 24 hours in the rumen (Fig. 6). The mesophyll was completely hydrolyzed in all samples by 48 hours (Fig. 9) leaving intact vascular tissue, and ventral epidermis and cuticle. No further degradation occurred when exposed for 72 hours (Fig. 12). The hydrolysis patterns of brome and fescue we observed do not include mastication effects.

Proximate analyses of our brome and fescue leaves (table 1) were similar except fescue had more ash.

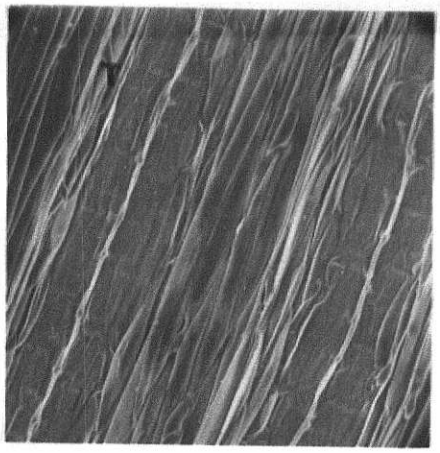
In vitro digestibility (Fig. 13) of brome during the first 12 hours of fermentation was higher than fescue ($P<.05$), but 48 hours digestibility of both species were similar. This agrees with the SEM observation. Brome had more dorsal surface vulnerable to microbial attack, so, it had faster initial hydrolysis. Only about 50% of the dorsal fescue leaves appeared vulnerable, reducing initial digestion, and possibly accounting for part of the lower voluntary intake of fescue. About 90% of the dorsal brome leaves appeared vulnerable.

Parts of fescue leaf dorsal surface may not be vulnerable to microbial degradation because of local structural inhibitors. The higher ash content in fescue leaves may partially represent silica in the undegraded epidermis.

More rapid digestion has been positively correlated with intake (Deinum, 1973; Barnes, 1966; Chenost et al. 1970). Therefore, epidermal inhibitors will have to be removed or reduced by plant breeding or processing to increase initial digestion. The epidermal inhibitors may be more important to animal intake and performance than grass's lignin content. Lignin content may have been over emphasized in forage evaluation. Lignin may only determine the end point of digestion, and the quantity of residue to be removed from the reticulo-rumen, therefore, only indirectly related to feed intake.

Figure 1. Scanning electron photomicrographs of fescue leaves. a) Dorsal surface with parallel pattern of cell type characterized by trichomes (T) on cells directly above the vascular tissue (113X). b) Ventral surface characterized by stomate (S) located on vein area (113X).

Figure 2. Scanning electron photomicrographs of brome leaves. a) Dorsal surface of leaf characterized by a narrow band of cells with a row of trichomes (T) above the vascular tissue (113X). b) Ventral surface characterized by trichomes (T) stomates (S) and phytoliths (P) (76X). c) Cross sections show mostly mesophyll (M) with a small amount of vascular tissue (V) in each vein region (189X).



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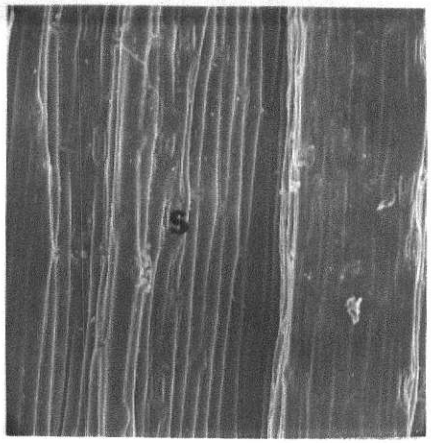

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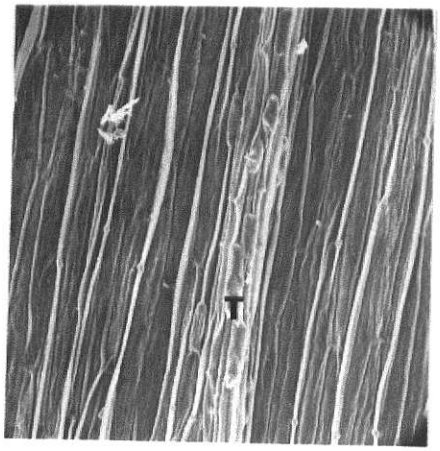

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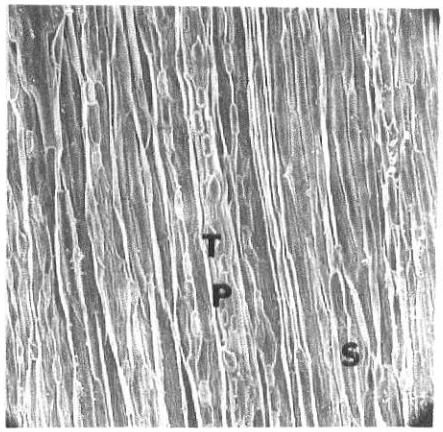

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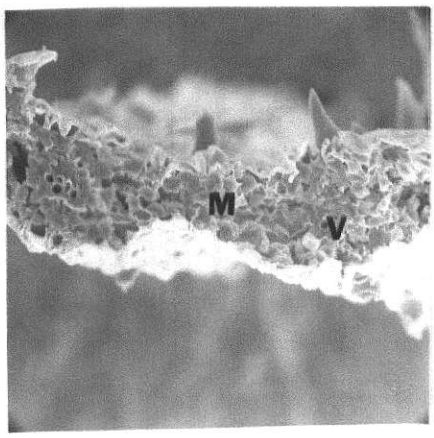

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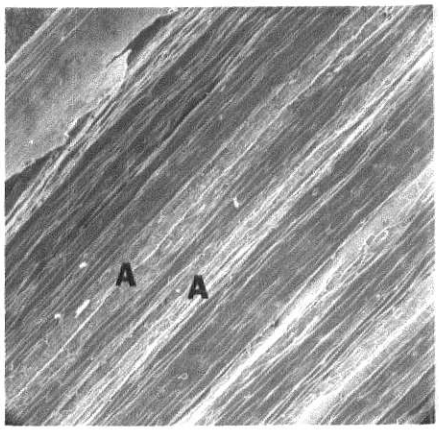
Figure 3. Dorsal surface of fescue leaves following 24 hour digestion in the rumen. a) Surface shows parallel microbiota attack on alternate groups of cells (A) (38X). b) Higher magnification of dorsal surface shows a group of cells between the vascular tissue being attacked (A) and the group of cells over the vascular tissue not being degraded (B) (88X).

Figure 4. Cross sections of fescue leaves following 24 hour digestion in the rumen. a) Partial degrading of the mesophyll that lies between the vein (M) (88X). b) Higher magnification of the vein area shows same hydrolysis of mesophyll (M) and fiber cells (F) surrounding the vascular tissue (V) (151X).

Figure 5. Dorsal surface of brome leaf following 24 hour digestion in the rumen. Dorsal cuticle (C) has been sloughed revealing partially hydrolyzed mesophyll (B) (113X).

Figure 6. Cross section of brome leaf following 24 hour digestion in the rumen shows vascular tissue (V) and undegraded ventral epidermis (E) with a row of undegraded trichomes on the dorsal surface (T) (189X).



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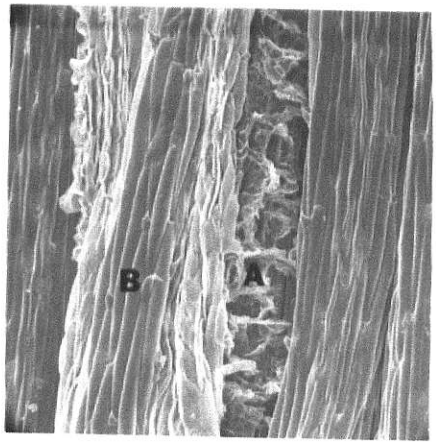

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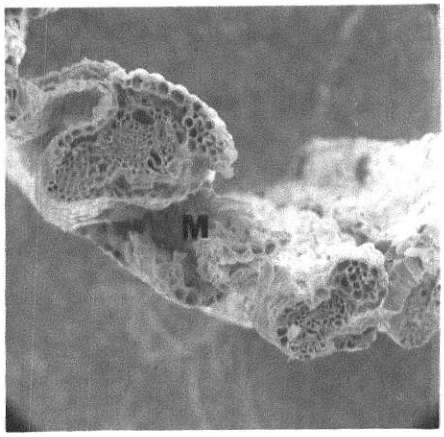

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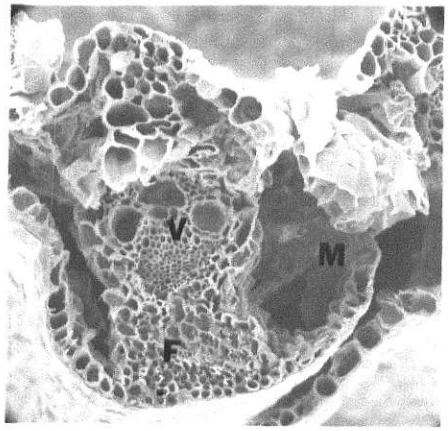

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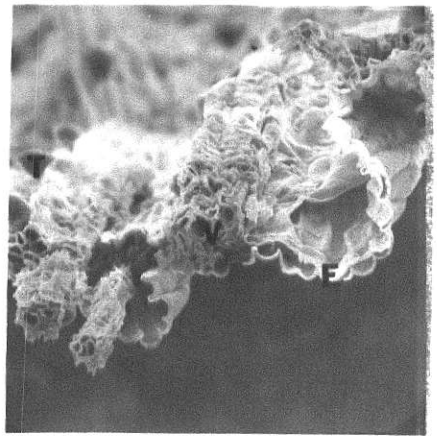

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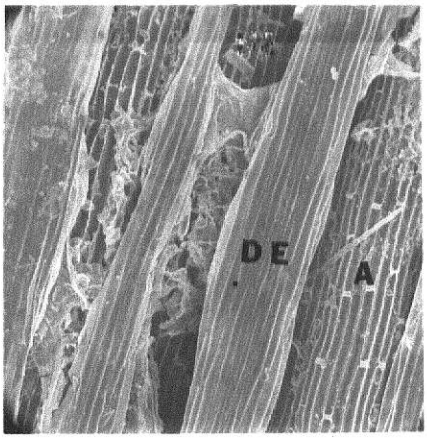
Figure 7. Dorsal surface of fescue leaves following 48 hour digestion in the rumen. a) Mesophyll has been degraded from areas between veins (A) leaving the ventral cuticle unhydrolyzed. The dorsal epidermis (DE) where trichomes were present was undegraded (63X). b) Undegraded ventral epidermis characterized by stomates (S) (76X).

Figure 8. Cross section of fescue leaf following 48 hour digestion in the rumen. All that remained was undegraded vascular tissue (V) attached to ventral epidermis (E) by fiber cells. The fiber cells attach the dorsal epidermis (DE) with the trichomes to the vascular tissue (95X).

Figure 9. Dorsal surface of brome leaf following 48 hour digestion in the rumen shows mainly undegraded vascular tissue (V) and cuticle (C). The dark area in the middle of the photomicrographs was caused by the sample breaking apart on mounting. The sample was held together by the ventral cuticle (88X).



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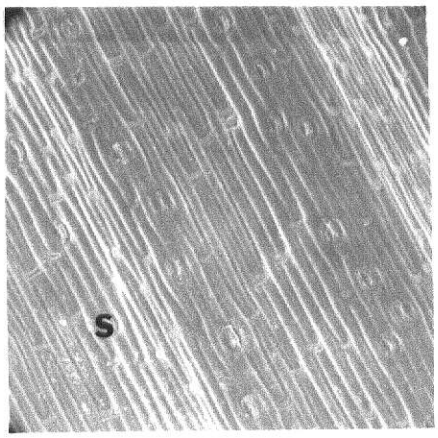

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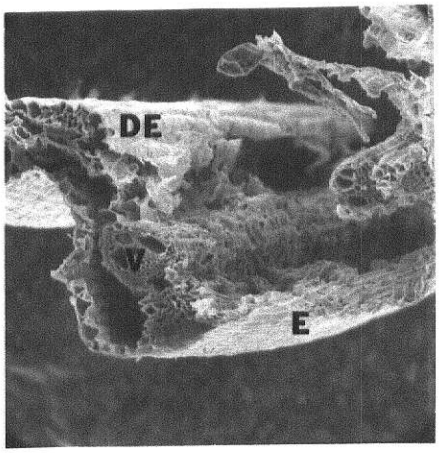

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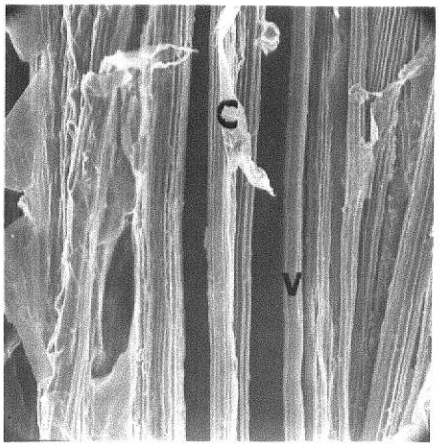

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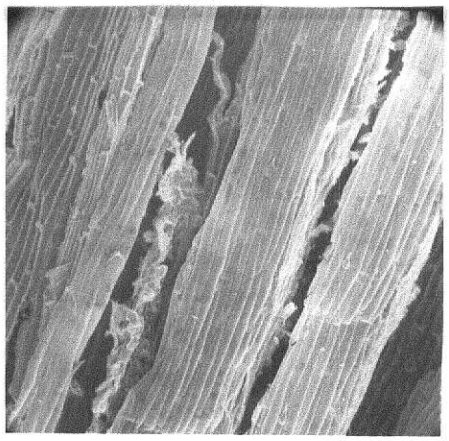
Figure 10. Dorsal surface of fescue leaf following 72 hour digestion in the rumen shows no change in amount of degradation from 48 hours (63X).

Figure 11. Cross section of fescue leaf following 72 hours in the rumen shows degrading of the fiber cells that had attached the dorsal (DE) and ventral (E) epidermis to the vascular tissue (V) (88X).

Figure 12. Dorsal surface of brome leaf following 72 hour digestion in the rumen. Only vascular tissue (V) and cuticle (C) remains undegraded. The sample was held together by the ventral cuticle and broke on mounting (44X).



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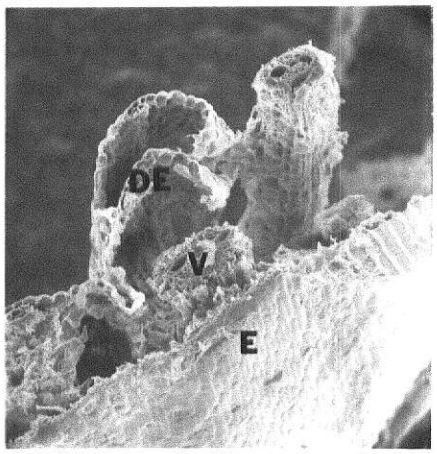

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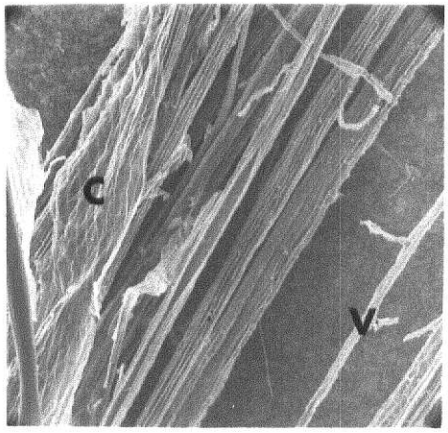

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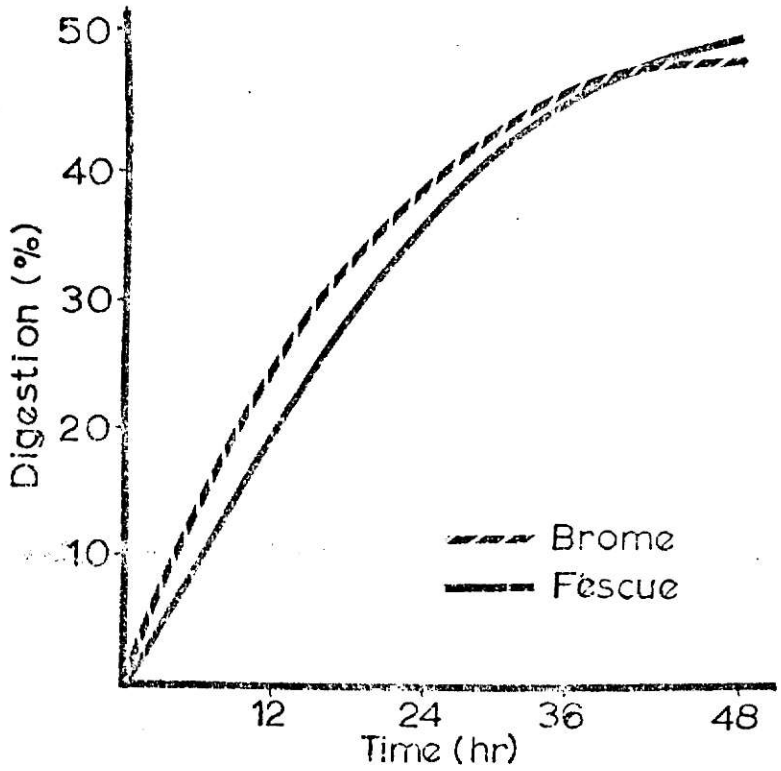
Table 1. Proximate analysis of smooth brome and tall fescue leaves.

	% Ether Extract	% Crude Fiber	% Crude Protein	% Ash	% NEF
Fescue	3.11	22.98	16.53	12.51	44.87
Brome	3.97	25.39	16.08	9.42	45.14

Figure 13. In vitro digestibility curve for smooth brome and tall fescue leaves.



Smooth brome had a significantly ($P<.05$) higher digestibility for the first 12 hours.

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STRUCTURAL INHIBITORS OF BIG AND LITTLE BLUESTEM DIGESTION OBSERVED BY SCANNING ELECTRON MICROSCOPY

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Summary

Scanning electron micrographs of Andropogon gerardi (big bluestem) and Andropogon scoparius (little bluestem) showed that each surface of the leaf were characterized by phytoliths (silica bodies), trichomes, and stomates. Rumen microbiota attacked unmasticated leaves on the dorsal surface, with varying degrees of degradation, depending on leaf maturity and lengths of fermentation. Microbiota appear to avoid areas where phytoliths were present in the epidermis. Mastication and/or digestion does not remove the phytoliths, since they appear in the feces. The dorsal and ventral surfaces appear to have cutin of different forms, which may explain the failure of microbiota to attack the ventral surface. (Key words: Big Bluestem, Little Bluestem, Digestion, Rumen, Scanning Electron Microscopy.)

Introduction

Silica was recently classified as a structural inhibitor to digestion of grasses (Deinum, 1973). Siliceous epidermal cells in most grasses are elongate and short (Twiss et al. 1969). The short cell is nearly equidimensional but the shape of the silica bodies within may not correspond to the shape of the enclosing cell (Metcalfe, 1960). Big and little bluestem have phytoliths of the Panicoid taxonomic group (eleven different shapes mainly dumbbell) (Metcalfe, 1960; Pratt, 1936) although elongate phytoliths may also be present (Twiss et al., 1969).

Lignin, depresses cell wall digestion (Deinum, 1973) but location of lignification in the cell wall may affect digestibility more than actual lignin percent (Drapala et al. 1947; Pidgen, 1953).

Waxes, oils, resins, and crystalline salts occur as surface deposits on the aerial plants parts in the form of granules, rods, network of tubes, and more or less homogeneous layers (Esau, 1953). How these deposits affect ruminal digestion is not completely understood.

The scanning electron microscope (SEM) observes the effect of microbial degradation on the epidermis and internal leaf structures.

Akin and Burdick (1973) used the light microscope to study the microanatomy of the plants and Akin and Amos (1975) used SEM to conclude that rumen microorganisms degraded the mesophyll and phloem cell walls more rapidly than other leaf tissues in the forages studied.

We found visual indications of structural inhibitors to microbial degradation of big and little bluestem leaves.

Materials and Methods

Samples of intact leaves of Andropogon gerardi (big bluestem) and Andropogon scoparius (little bluestem) were collected June 16 and Oct. 3 at one location. Samples were placed in plastic bags, frozen in liquid nitrogen for five minutes and stored frozen. Samples were thawed and digested for 0, 24, 48 and 72 hours in nylon bags in the rumens of two mature

fistulated steers receiving 2.3 kg. of ground sorghum grain (sorghum, milo, grain, (4), IRN 4-04-444) and ad libitum prairie hay (Native plants, midwest, hay, S-C, mid blm, (1), IRN 1-07-956).

Digested and undigested intact leaves were rinsed in distilled water after removal from bags and prepared for scanning electron microscopal observation by dehydration in an ethanol series (2 to 3 minutes each in 30%, 60%, 80%, 100%). Esophageal fistulated steers grazing native grass pasture were used to collect masticated samples of bluestem prairie which were dehydrated in an ethanol series. Residues of native grass hay from fecal samples were collected from two rumen fistulated steers at 24 hour intervals for 2 days. The residue was sifted out of the feces using cheese cloth, then dehydrated in an ethanol series.

Cross sections of big and little bluestem were prepared by freezing in liquid nitrogen and splitting with a razor blade to produce a fracture rather than a cut. Specimens were amounted on aluminum stubs with Delco No. 93 colloidal silver (Ted Pella Co., #1603-2), coated under vacuum with 150 nm of gold palladium (Kenny Vacuum Co., Model K SE-2-A-Mecaporator), observed with an Etec Autoscan scanning electron microscope at 20 KV (Davis and Harbers, 1974) and photographed with Polaroid PN/55° film. Stereoscopic pairs of photomicrographs were made by rotating the vertical stage of the microscope between 5° and 8° (Howell, 1975). These pairs were viewed with a stereoviewer for interpretation. Only single photomicrographs are shown here.

Results & Discussion

Both grasses were characterized by phytoliths (silica bodies), trichomes, and stomates. (Fig. 1a and 6a) on the dorsal surface. The ventral surfaces of both grasses had phytoliths, trichomes, stomates, and cork cells (Fig. 2a and 7a). Higher magnification of both surfaces of both grasses revealed cutin deposits (Fig. 1b, 1c, 2b, and 7b).

The dorsal surface of both species was randomly attacked by rumen microbiota (Fig. 3 and 4). However, the microbiota appear to avoid attacking the areas where phytoliths are present (Fig. 4). A cross section of an Oct. 3 big bluestem leaf (Fig. 5a) shows that the vascular tissue remained intact after 72 hours of rumen microbioal activity (Fig. 5b), but the leaf mesophyll was degraded.

A cross section of undegraded little bluestem shows large air filled cell near the dorsal surface (Fig. 6b). These are not present in big bluestem. The ventral surface of little bluestem leaves shows mechanical sloughing of the cuticle and epidermis. The underlying mesophyll remained following 72 hours in the rumen (Fig. 8a). Higher magnification of this mesophyll shows no evidence of degradation, so microbial activity had apparently not occurred. The epidermis does not appear to slough in areas where phytoliths are present.

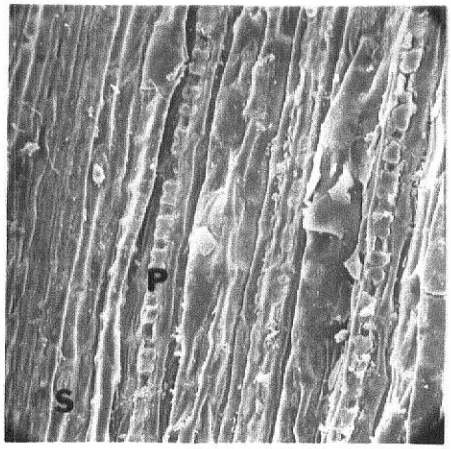
Some of the phytoliths may be mechanically removed by masitcation (Fig. 9a and b), but most remain in place. A feces

sample (Fig. 10) shows that most of the phytoliths were still in place after the complete digestion process. Because of this, phytoliths may inhibit microbial degradation of surrounding tissue.

The different forms of wax on the ventral and dorsal surface of big and little bluestem may influence microbial degradation. The wax deposits may be ester insoluble like cutin, or ester soluble (Esau, 153). The ester soluble wax may be degraded by microbiota while insoluble wax or cutin may inhibit microbes attack. Microbes attack unmasticated alfalfa, brome, fescue, big and little bluestem leaves only on the dorsal surface, indicating that the ventral cuticle maybe invulnerable to microbial degradation. The solubility of waxes deposited on or embeded in the leaf surface may have a major influence on the initial rumen digestion of forages.

Figure 1. a) Dorsal surface of big bluestem (Oct. 3) characterized by rows of phytoliths (P), trichomes (T), with a few stomates (S) (113X). b) Higher magnification of the dorsal surface shows cutin deposit (630X). c) Still higher magnification of the dorsal surface shows the shape of the cutin deposits (4410X).

Figure 2. a) Ventral surface of big bluestem (June 16) characterized by phytoliths (P), trichomes (T), cork cells (C), and stomates (S) (113X). b) Higher magnification of ventral surface shows cutin deposit (4410X).



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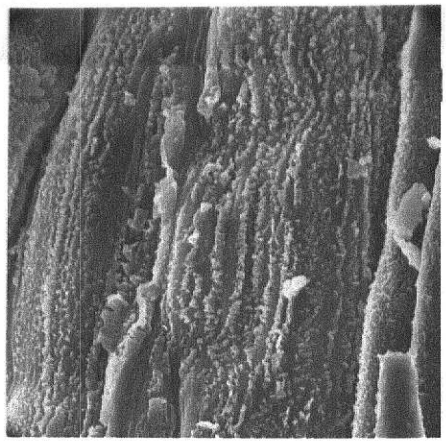

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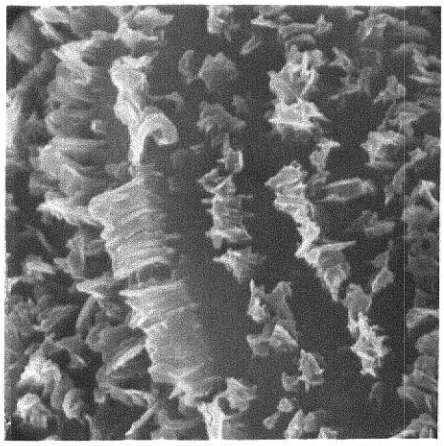

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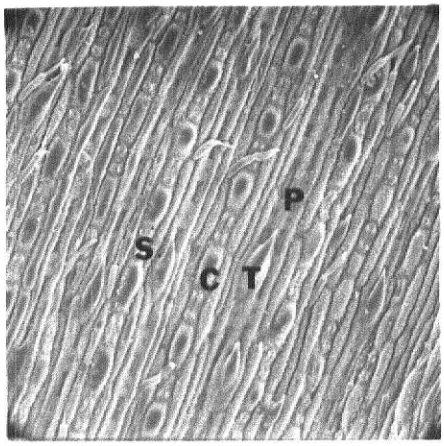

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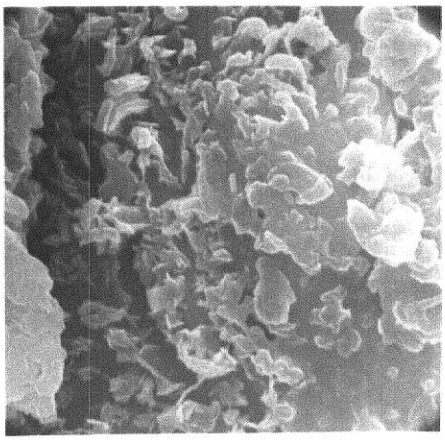
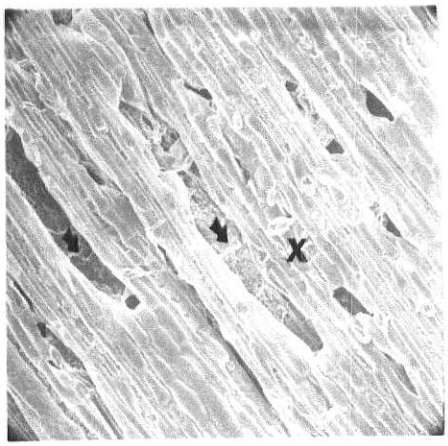


2b

Figure 3. Dorsal surface of big bluestem (June 16) following 24 hours of digestion in the rumen shows random microbiota attack (→) and some protozoa that are present on the leaf (X) (63X).

Figure 4. Dorsal surface of big bluestem (Oct. 3) following 72 hours of rumen digestion shows areas of the leaf surface where phytoliths were present remained undegraded (Y) and other areas of the leaf was partially degraded and the cuticle sloughed (→) (113X).

Figure 5. Cross section of big bluestem following 72 hours of rumen digestion shows only intact vascular tissue (189X).



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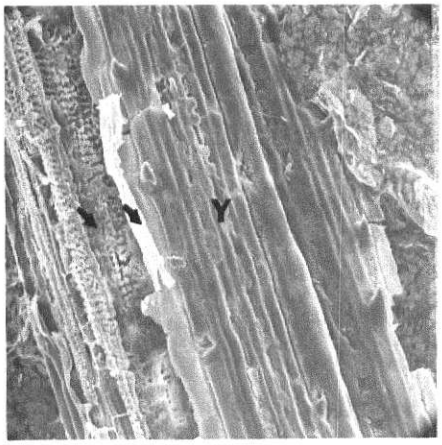

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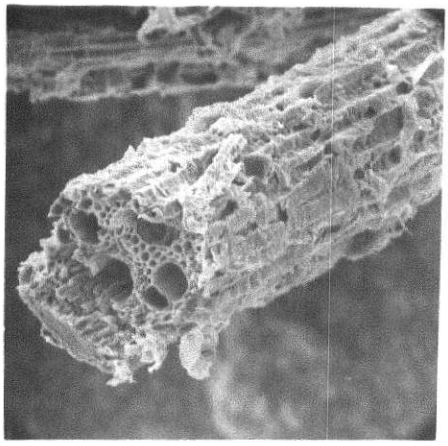

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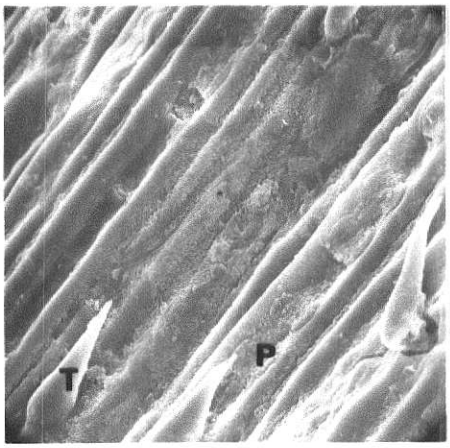
Figure 6. Dorsal surface and cross section of (Oct. 3) little bluestem. a) Dorsal surface shows phytoliths (P) and trichomes (T) (441X). b) A cross section shows vascular tissue (V) with large air filled cells in the dorsal surface (Z) (159X).

Figure 7. a) Ventral surface (June 16) little bluestem was characterized by trichomes (T) phytoliths (P), cork cells (C) and stomates (S) (113X). b) Higher magnification shows cutin deposits (4410X).

Figure 8. a) Ventral surface of (Oct. 3) little bluestem following 72 hours of rumen digestion shows sloughing of the cuticle and epidermis (CE), but partially avoiding the phytoliths (P) (113X). b) Higher magnification of areas where cuticles and epidermis was sloughed showed no degradation of mesophyll (441X).



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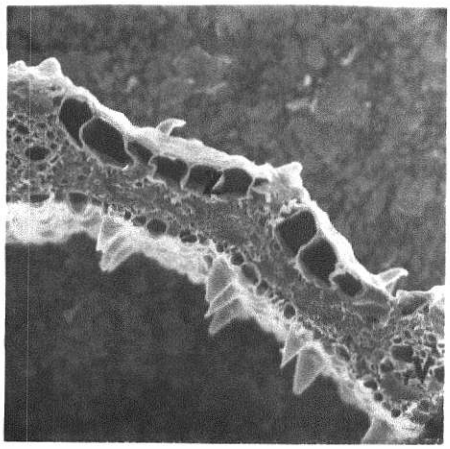

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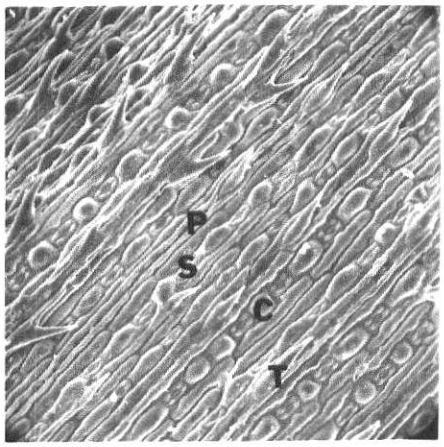

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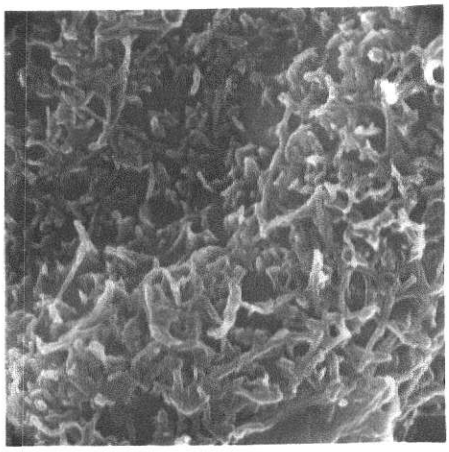
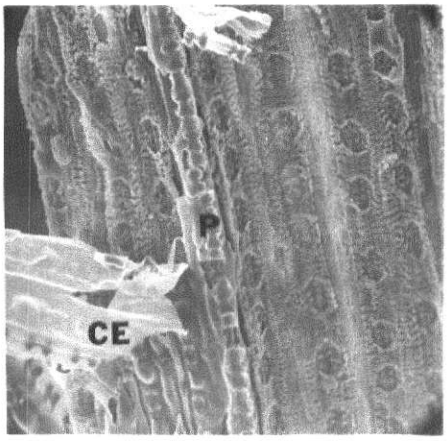
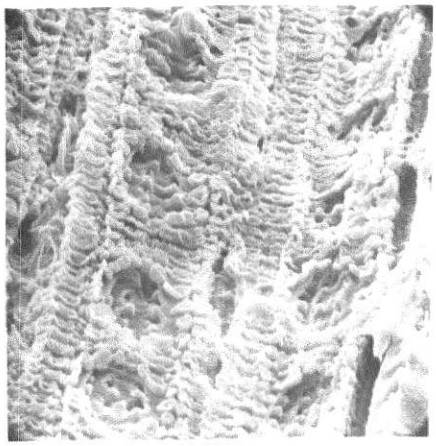


7b



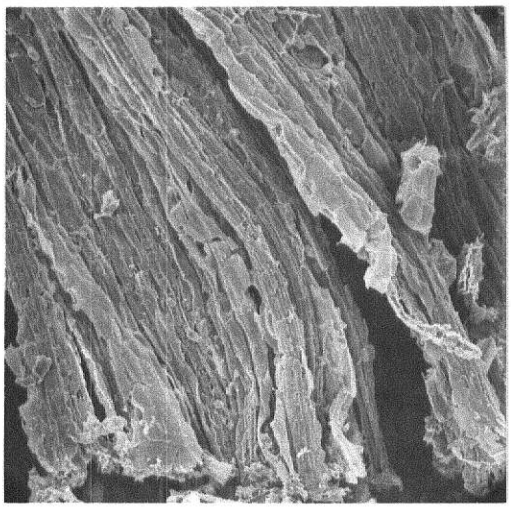
8a



8b

Figure 9. Photomicrographs of esophageal fistulated samples taken from steers grazing native grass in Oct. a) Leaf surface shows the damage of mastication to the leaf (63X). b) Leaf surface shows that some phytoliths are removed (→) by mastication while others remain intact (P) (126X).

Figure 10. Photomicrograph of feces sample from steer eating Sept. cut native grass hay shows intact phytoliths (126X).



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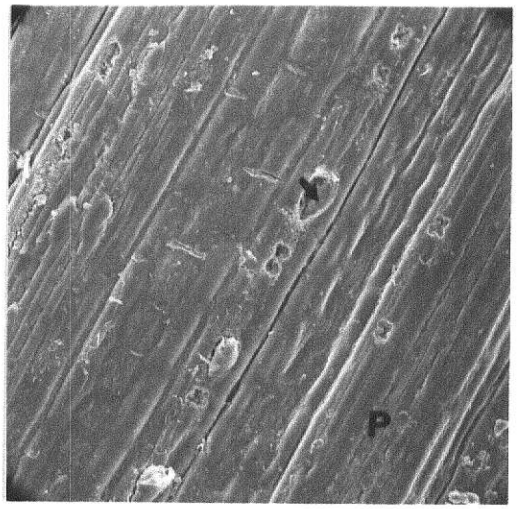
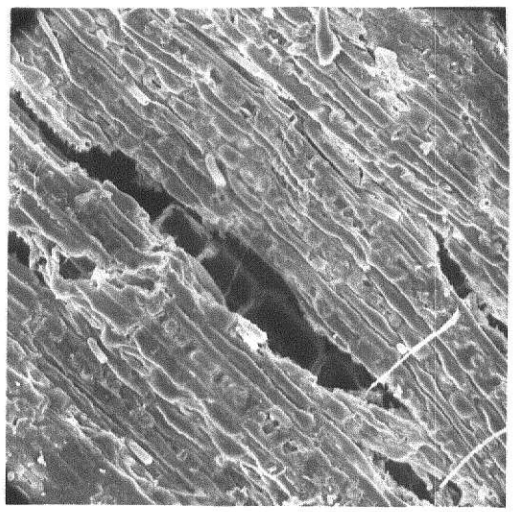


9b



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RUMEN DEGRADATION OF SOME FORAGES OBSERVED BY SCANNING ELECTRON MICROSCOPY

by

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AN ABSTRACT OF A MASTER'S THESIS

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The scanning electron microscope was used to observe fresh leaves of Bromus inermis (smooth brome), Festuca arundinacea (tall fescue), Andropogon gerardi (big bluestem), Andropogon scoparius (little bluestem), and air dried leaves and stems of Medicago sativa (alfalfa) before and after degradation in the rumen. Microbiota attacked only the dorsal surfaces of unmasticated leaves of all species. Dorsal cell degradation was random in smooth brome and alfalfa leaves, but restricted to certain cells in tall fescue, big bluestem, and little bluestem. Alfalfa stem was attacked at random by microbiota. Alfalfa and brome leaves were more rapidly degraded than fescue, big bluestem and little bluestem. Vascular tissue, cutinized ventral epidermis and some dorsal epidermis remained after fermentation. Undegraded dorsal cells were characterized by trichomes and phytoliths. After 72 hour rumen digestion, only intact vascular tissue remained in alfalfa stems. Dorsal and ventral epidermal inhibitors appeared to reduce initial digestion, thus the amount of vascular tissue only determines the end point of digestion.