RUMINAL DIGESTION OF FORAGE SORGHUM STEMS
OBSERVED BY LIGHT, FLUORESCENCE AND SCANNING
ELECTRON MICROSCOPY

by

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_____
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INTRODUCTION

Raising food at a profit has been an age old challenge for agriculturists. Food production can either be for direct consumption by humans, or indirect where feedstuffs are first fed to livestock, then humans consume the meat product. One strategy for achieving the profit goal in agriculture is cooperation between animal nutritionists and agronomists. Usually, agronomists desire a plant that is high yielding, requires minimum capital expense, and is insect and disease resistant, while animal nutritionists seek feedstuffs that are highly digestible and palatable. Unfortunately, these plant characteristics are usually in conflict.

Past, and current methods of determining plant quality have been of a chemical or biological nature, i.e. proximate and Van Soest analyses, _in vitro_ dry matter disappearance or live animal performance. Within the last fifteen years however, microscopy, especially transmission and scanning electron microscopy have become important for correlating plant anatomy with pattern and extent of digestion, and determining which plant structures are digested and which are passed on to the feces.

Comparisons of corn and forage sorghum silages using various methodologies have generally resulted in the conclusion that sorghum is inferior. In addition, sorghum cultivars show differences in nutritive properties when fed to livestock. These differences are attributed to variation in plant chemical composition. Limited research has been conducted with cultivated forages using various forms of microscopy to observe how digestion progresses in plant tissues and to predict forage utilization from that information. This research was designed to help rectify this information void.

LITERATURE REVIEW

Scanning electron microscopy (SEM) has been used extensively to observe plant anatomy, and to estimate forage quality and digestibility in native grasses but to only a limited extent in cultivated forages. One of the first accounts of using SEM to study digestibility was by Akin et al. (1973). Leaves from Kentucky 31 tall fescue and Coastal bermudagrass were digested by _in vitro_ rumen fermentation and then microanatomical differences were related to differences in degradation. Brazle et al. (1979) were the first to use SEM to observe _in vivo_ digestion of alfalfa hay, brome and tall fescue. Harbers and Thouvenelle (1980) compared leaf and stem digestion of corn and sorghum silages _in vivo_ using SEM. No studies have been reported using SEM to observe digestibility differences among sorghum cultivars.
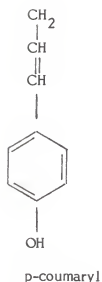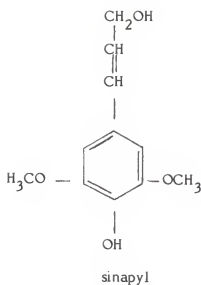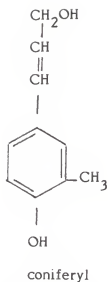
Forage sorghums are popular because of their adaptablity to different climates and their high dry matter yield. Their best stage of maturity for harvest is still being disputed. Harvesting at a later maturity stage can provide a higher yield but will usually result in questionable nutritional quality (Owen, 1967).

Sorghum cultivars differ in plant composition, anatomy and feeding value. Early work by Willaman (1919) indicated that the most significant change as sorghum matures is the increase in dry matter because of the increase in nitrogren-free extract (starch in young plants and sucrose in older plants). Schertz and Roseno (1977) observed differences in stem morphology due to variation in subepidermal cell size, shape, and cell wall thickness of the rind tissues. Those differences were thought to influence stalk stiffness and possibly, the digestibility of stems. Stems can constitute 50% of the total dry matter in sorghum plants (Eilrich, 1964). Bolsen et al. (1983) and Dickerson et al. (1985) indicated that grain sorghum silage was superior to forage sorghum silages for animal performance.

Stem tissues of sorghums are composed of parenchyma cells, vascular bundles, and the rind (sclerenchyma, vascular bundles, and epidermis). Parenchymal cells differ in size depending on location. Centrally located cells are large and thin-walled, with intercellular spaces at their angular junctions while cells adjacent to the epidermis are small, thick-walled, and lignified. Differences also occur between vascular bundle types. In the parenchyma, bundles are scattered thoughout but peripheral bundles are closer together, more numerous, and different anatomically (Hayward, 1938). Artschwager (1948) noted that transformation of parenchyma into pith sometimes can be followed by complete cellular breakdown leaving central vascular bundles suspended in air.

Digestibility of stem tissues depends on the stems maturity and lignification. Lignification increases with maturity (Mowat et al., 1969; Kamstra et al., 1958). As a stem matures, lignification starts mostly around the vascular bundles, then spreads into parenchyma adjacent to those vascular bundles (Drapala et al., 1947; Pidgen, 1953). Cellulose, the main component of cell walls, is highly digestible by rumen bacteria but when associated with lignin, its digestibility may be inhibited (Patton, 1935; Maynard, 1937; and Van Soest, 1973). Hghly lignified vascular tissues, sclerenchyma, and epidermal tissues are left as residues of grass and silage digestion (Akin, 1982b; Harbers and Thouvenelle, 1980).

Lignin, a polymer of phenylpropanoid units (Sarkanen and Ludwig, 1971), varies in type, site and extent within plant tissues. The different types of lignified tissues influences the amount of microbial degradation of plants (Akin, 1982a). The phenylpropanoid units of lignin are of three types: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol (see illustration on next page). These alcohols appear to occur as their respective glycosides. The glycoside of sinapyl alcohol is syrignin. Thus, lignins are classified a coniferyl, sinapyl and p-coumaryl (Schubert, 1965).

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CH_2OH
CH
$\|$
CH
$|$

[coniferyl structure: benzene ring with CH_3 and OH]

CH_2OH
CH
$\|$
CH
$|$

H_3CO — [benzene ring] — OCH_3
OH

CH_2
CH
$\|$
CH
$|$

[p-coumaryl structure: benzene ring with OH]

coniferyl   sinapyl   p-coumaryl

Stains for localizing lignin precursor alcohols are ammonium hydroxide (coniferyl) using fluorescence microscopy, chlorine-sulphite (syringyl) and acid phloroglucinol (p-coumaryl) with light microscopy (Harris, 1976; Sarkanen and Ludgwig, 1971). Ferulic acid, a close relative of coniferyl alcohol, may serve as a precursor to coniferyl lignin (Schubert, 1965). Ferulic acid-positive tissues (coniferyl lignin) were present in xylem parenchyma, phloem, central parenchyma and chlorenchyma of mature stems along with sclerenchyma and epidermis of immature stems of native ryegrass (Lolium termulentum) cell walls. These tissues were susceptible to digestion (Harris, 1976). Chlorine sulphite-positive tissues (syringyl lignin) are present in the more mature parenchymal cells and its presence can inhibit cell wall digestibility (Akin et al., 1977). Acid phloroglucinol-lignin is present in the epidermis, sclerenchyma ring, and vascular tissues and is resistant to rumen microbial attack (Akin, 1982a, Akin, 1982b)

The major soluble carbohydrates found in plant tissues are glucose, fructose, sucrose, starch, and fructosans (Norton, 1982). Collier (1884) found that some sugar concentrations in sorghum stems vary with maturity and location. In young plants, reducing sugars such as (glucose) are in greater concentration than sucrose (non-reducing). Reducing sugars decease and sucrose increases until the plant is in full

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bloom. The decrease in reducing sugars occurs because of conversion of glucose to non-reducing sugars and starch (Collier, 1884). After physiological maturity, sucrose is stored in the stem until frost (Urich, 1962). Upper internodes are highest in sucrose while glucose predominates in the lower internodes. Total sugars and sucrose were found in highest concentrations in the middle internodes of the sorghum plants (Willaman, 1919). Starch is the most abundant carbohydrate in the upper internodes (Ventre et al., 1937). Starch deposits will vary with cultivars. Some cultivars will only have starch surrounding the vascular bundles while others will also accumulate starch in the parenchyma between the bundles (Artschwager, 1948).

SEM techniques were used in this study to evaluate forage sorghum cultivars. SEM allowed observation of digestive patterns and specific tissue utilization with great depth of field and high magnifications. Light and fluorescent microscopy allowed observation of plant tissue arrangement and, with specific stains, the site and types of lignified tissues.

The objectives of this study were:

(A)   to observe _in_ _vivo_ digestion differences between sorghum cultivars, maturity, and tissue location;

(B)   to determine location of starch and lignin types;

(C)   to relate location of starch and lignin to digestive patterns.

MATERIALS AND METHODS

A preliminary study was conducted as described by Harbers and Thouvenelle, (1980) to compare digestibilities of corn and sorghum silage stems. Stems were picked at random from large upright silos. The corn cultivar name was unknown. Sorghum cultivars were Rox Orange, Warner 2-way, and Dekalb FS-25a^{+}. Stems were digested in nylon bags for 4, 8, 12, 16, 20, 24, or 48 hours. Problems in properly identifying stem tissue led to the study delineated below.

In the main experiment, three different cultivars of forage sorghum Sorghum bicolor (L. Moench M) were harvested at two stages of maturity (milk and mature) to observe microbial digestion pattern differences among cultivars. The cultivars were a full-season non-heading type (Funks G-1990), a mid-season heavy-heading cultivar (Acco 351), and a late-season moderate-heading sorghum, (Dekalb FS-25E). The cultivars will be represented in the rest of the manuscript as 1990, 351, and 25E. The milk stage (MI), was defined as the point at which the seed produces a milky substance when pinched. The mature stage (MA) was defined as the point at which maximum dry weight of the total plant had been reached. Harvest dates for Funks 1990 and Dekalb 25E were 102 and 127 days post emergence while Acco 351 was harvested 99 and 127 days post emergence.

Stalks were selected at random when test plots reached desired maturity. Stems were cut in to provide cross-sections approximately 1 - 2 cm in length within the first two internodes of the top and within the fourth and sixth internodes of the bottom portion of the stem. Samples were immediately sown into nylon bags and sealed approximately near the middle of 19 l experimental laboratory silos that contained the same cultivar. Silos were incubated for 90 days at 30 C. Samples were then removed and frozen until time of digestion. In addition, fresh whole stems were frozen at

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harvest for light microscopy.

Nylon bag *in vivo* digestion studies were made using two 500 kg Holstein steers fitted with ruminal cannulae. The animals were fed 4.5 kg supplement and 13.6 kg of silage (as fed basis) twice daily. Water was available *ad libitum.* The steers were on this ration for two weeks before they were used.

Stems were thawed, placed in individual nylon bags and sown on a thin nylon rope, then emersed in the rumens of the steers for 12, 18, 24, 36, or 48 hours. Upon removal, stems were washed with water and fixed with 4% glutaldehyde solution (10 ml 25% aqueous glutaldehyde solution, (EM grade); 36.5 ml .07 M Na_2HPO_4 and 16 ml .07 M KH_2PO_4) (Sjostrand, 1967).

Digested and control (non-digested) stems were dehydrated in a graduated ethanol series of 30%, 50%, 70%, 80%, 90% and 100%. Problems were encountered in the critical point drying process due to the thickness and compactness of the upper internodes. Best sample preparation resulted when control stems and digested upper internodes were dehydrated for 8 hours each in 30%, 70%, and 90% ethanol and for 16 hours in 50%, 80%, and 100% ethanol. After digestion, lower internodes were less intact and required less dehydration time. They were dehydrated 4 hours each in 30%, 50%, 80%, 90%, and 16 hours in 70% and 100% ethanol. Dehydration was carried out in tightly sealed containers to prevent ethanol from absorbing water, a factor found to be critical.

Samples were dried by using the carbon dioxide critical point drying method. The drier's soak/flush periods were altered depending on samples. Top internodes were usually soaked about two hours, processed through the regular flush cycle (4 minutes flush, 4 minutes soak) four times, then soaked again for 2 hours. If there were indications that ethanol was still present after 8 hours, samples were soaked in the chamber overnight. The same procedure was followed for digested bottom internodes, but those samples were usually dry before 8 hours.

Specimens were mounted on aluminum stubs with Pelco No. 16053 colloidal graphite. Larger stems were mounted on glass cover slips that were previously fixed to the stubs. Samples were then coated by vacuum evaporation with carbon followed by gold palladium. An ETEC Autoscan scanning electron microscope was used to observe and photograph the individual samples. Photographs were taken using Poloroid P/N 55 film at accelerating voltage of 5 or 10 Kv.

Fresh frozen stems were thin sectioned using a rotary meat slicer set at 3-6 mm. These slices were saturated a drop at a time with stain for several minutes, then were observed. Potassium iodide stain (1 g I, 2 g KI, and 300 ml of distilled water) was used to identify starch. Lignin types were identified by acid phloroglucinol (18%-20% HCl, saturated with 1 g phloroglucinol, after 24 hours filtered into dropper bottle) and chlorine-sulphite (Chlorox and concentrated HCl to pH 1; treat tissues for 5 minutes and pour off excess; drop on cold, fresh 2% Na_2SO_4; reaction takes few seconds to a minute but after 15 minutes the reaction will fade) using light microscopy. Ferulic acid was visualized by raising the pH (.07M K_3PO_4 at pH 10) and observing (color) with a fluorescence microscope. Tripotassium phosphate (Fulcher and Wong, 1982) was used as the ferulic indicator instead of ammonium hyroxide (Harris, 1976). The microscope's filter combination consisted of an exciter filter at 365 nm and barrier filter > 418 nm. These lignin stains identified p-coumaryl, syringyl, and coniferyl (ferulic) groups respectively.

RESULTS AND DISCUSSION

Harbers and Thouvenelle, (1980) showed that ensiled corn stems were more readily digested than ensiled sorghum stems. After 48 hours of _in vivo_ digestion, only vascular tissue remained in corn stems while sorghum stems only had minor parenchymal digestion. They postulated that the difference was due partially to greater lignification in the sorghum stems.

My preliminary study did not agree with Harbers and Thouvenelle (1980). Corn was found to be less readily digested than sorghum and there were differences in tissue disappearance patterns between sorghum cultivars. In corn, after 48 hours of _in vivo_ rumen digestion, (Fig. 1) parenchymal cells adjacent to the vascular bundles had disappeared, while the remaining parenchyma was left intact. Differences between sorghum cultivars after 48 hours of digestion seem to relate to which portion of the stem (rind vs center parenchyma) was being observed. Rox Orange (Fig. 2) showed the same pattern of digestion as corn with parenchymal tissue adjacent to the vascular bundle having disappeared. Warner sorghum (Fig. 3) appeared more readily utilized than corn because of the larger portion of parenchymal cells removed. In DeKalb FS-25a^{+} cultivars, the rind portion of the stem was still intact after 48 hours (Fig. 4). From these observations, it can be stated that this variation can occur in digestibility between cultivars of the same species and that this variation may be partially due to location of the observed sample within the stem.

The observations were made on stems removed from silage samples, thus cultivars might have been compared using differing stem portions (rind vs cortex). It is almost impossible to determine the origin of a stem sample from whole plant silage. Stem samples could also be misidentified as part of the leaf midrib or even part of the panicle. Since digestibility as observed by SEM may depend on the origin of the

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DIGESTION OF CORN AND SORGHUM STEM CULTIVARS

Fig. 1   Parenchymal disappearance adjacent to the vascular bundles (arrows) in corn (unknown variety) after 48 hours of ruminal digestion. (Bar, 100 μm)

Fig. 2   Rox Orange sorghum after 48 hours of ruminal digestion shows parenchymal disappearance (arrows) adjacent to the vascular bundle. (Bar, 100 μm)

Fig. 3   Warner, a sorghum cultivar after 48 hours of digestion shows an increased removal of parenchymal tissues (arrows). Anaerobic fungi are present on the lignified vascular bundles. (Bar, 100 μm)

Fig. 4   After 48 hours of digestion. The rind portion of Dekalb FS 25a[+] stem shows no major tissue disappearance. The rind consists of the epidermis (E), sclerenchyma (S), and lignified vascular bundles (V). Anaerobic fungi are present on lignified tissues. (Bar, 100 μm)
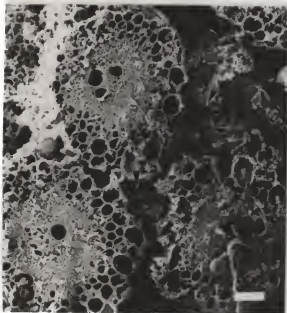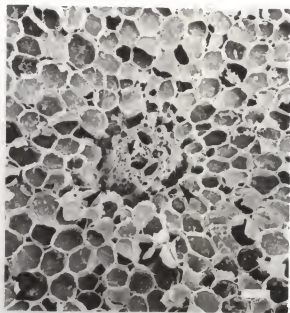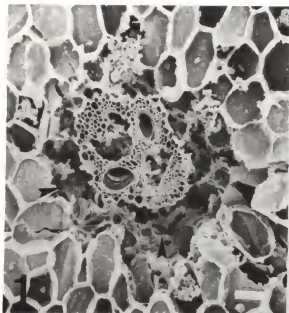
sample, a second study was conducted using whole transverse slices from known portions of stems to observe apparent digestive pattern differences among cultivars and between maturities, and to determine digestibility differences between upper and lower internodes with the aid of SEM.

All sorghum stems appear to have the same tissue arrangement; the arrangement peculiar to monocotyledons. The rind (epidermis, sclerenchyma cells and vascular bundles) surrounds the center of the stem (parenchyma cells and scattered vascular bundles). Differences arise between upper and lower internodes because of cell size and compactness.

The lower internodes of all cultivars and maturities had the same pattern of disappearance. Parenchymal disappearance occured from the central portion of the stem to the sclerenchymal rind. Actual extent of utilization could not be determined from the SEM photographs because in some samples during the critical point drying process, parenchyma tissues shrank and separated into clusters. Measuring the depth of parenchymal utilization is almost impossible. The disappearance of tissues may not necessarily mean that they were utilized. In most lower internode sample bags, numerous vascular bundles with attached parenchyma were left as residues. The disappearance of tissues could result from microbial utilization of the parenchymal tissues, leaving vascular and other tissue loose. Then with rumen movement, tissues could be removed, but not necesarily utilized.

Anatomical structure of the lower internode is seen in Figure 5. Numerous vascular bundles are dispursed within dense sclerenchymal cells; fewer vascular bundles are found in areas of large parenchymal cells. By 12 hours of _in vivo_ digestion, parenchymal disappearance was evident at the cut surface of the cortex (Fig. 6). A substantial loss of parenchymal tissues occurs during 18 hours of digestion (Fig. 7). Vascular bundles and attached parenchyma cells were removed from the major portion of the stem (Fig. 8) after 24 hours of digestion. Partial degradation of

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OBSERVATIONS OF SORGHUM LOWER INTERNODE DIGESTION

Fig. 5   General structure of lower internode sorghum stems. The center of the stem consists of vascular bundles (V) and parenchyma (P); the rind consists of the sclerenchyma (S), vascular bundles (V), and the epidermis (not shown). Sclerenchyma cells are dense and compact compared to parenchyma cells. (Bar, 100 µm)

Fig. 6   Initial parenchymal disappearance (arrows) occurs in the lower internodes after 12 hours of digestion. (Bar, 100 µm)

Fig. 7   After 18 hours of ruminal fermentation, parenchymal disappearance (arrows) seems to be most evident in the center of the stem. (Bar, 100 µm)

Fig. 8   Between 18 and 24 hours of digestion, tissue removal allows residual vascular bundles with attached parenchyma to fall to the bottom of the nylon bags. The attached parenchymal cells (P) may or may not be digested (arrows). (Bar, 100 µm)
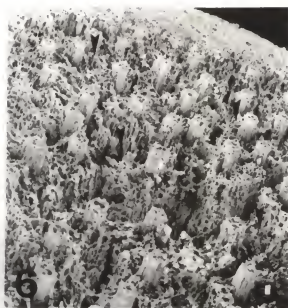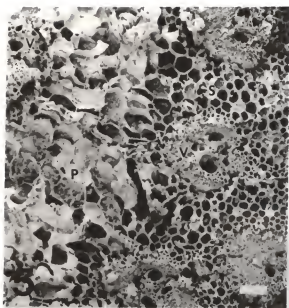
parenchymal cells adjacent to vascular bundles can occur on vascular bundle residues. Parenchymal loss continues toward the periphery by 24 hours (Fig. 9) leaving the rind undigested after 48 hours (Fig. 10). Akin et al. (1977) and Akin et al. (1984) noted that upper internodes were usually more readily digested than lower internodes. That is contradictory to sorghum stem digestion in our study. Anaerobic fungi were usually present on lignified tissues such as the rind or vascular tissue (Fig. 11) but appear to digest very little of these tissues.

The upper internode stems presented a different digestive pattern than the lower internodes even though they were anatomically similar. Differences also occurred in digestion patterns between non-heading and heading cultivars. In the non-heading type, the upper internode consisted mainly of the whorl instead of the true stem.

In the MI stage of 25E (a heading cultivar) tissues remained intact after ensiling (Fig. 12), except for the peripheral sclerenchyma tissues as seen at a higher magnification (Fig. 13). In contrast, the MA stage showed no tissue hydrolysis during ensiling (Fig. 14 and 15). The same tissue disappearance pattern occurred in both maturities. After 24 hours of digestion, forages of both maturities (as indicated by Fig. 16), showed peripheral sclerenchymal tissue disappearance, along with disappearance of phloem in the vascular bundles. Additional time did not change the tissue disappearance pattern (Fig. 17 and 18). At a higher magnification, (Fig 19) microbial activity was observed on lignified tissues after 48 hours of ruminal fermentation.

Peripheral sclerenchymal hydrolysis occurred from ensiling 351 at the MI stage (Fig. 20). Tissues in the MA stage from the same cultivar remained intact during ensiling (Fig. 21). Ruminal digestion of 351 MI for 18 hours removed additional sclerenchymal and phloem tissues compared to ensiled non-digested tissues (Fig. 22). After 48 hours' digestion of MI stage (Fig. 23), the sclerenchymal tissues adjacent to

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Fig. 9   The center of the stem is removed within 24 hours of digestion, leaving peripheral parenchymal tissues (P) and vascular bundles (V) attached to the main portion of the stem. No tissue disappearance occurs on the rind (R). (Bar, 100 μm)

Fig. 10   Components of the rind (R) remain intact after 48 hours of digestion while most central tissues have been removed. (Bar, 100 μm)

Fig. 11   After 36 hours of digestion, anaerobic fungi (F) can be present on the lignified portions of the stem. (Bar, 100 μm)
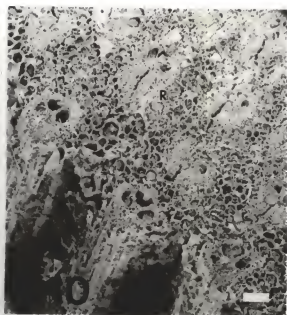
OBSERVATIONS OF DIGESTION IN THE UPPER INTERNODE OF CULTIVAR 25E

Fig. 12   Part of peripheral sclerenchymal tissues (S) disappeared from upper internodes of cultivar 25E MI during ensiling. All other tissues, parenchyma (P) and vascular bundles (V) remained intact. (Bar, 100 μm)

Fig. 13   Note the line of the sclerenchymal disappearance (arrows) near the outer layers of 25E MI during ensiling. (Bar, 100μm)

Fig. 14   Ensiling 25E MA upper internode did not cause tissue disappearance. (Bar, 100 μm)

Fig. 15   Individual cells near edge of 25E MA, show no tissue removal during ensiling. (Bar, 100 μm)
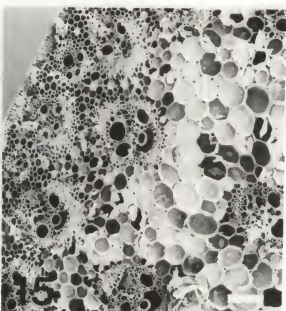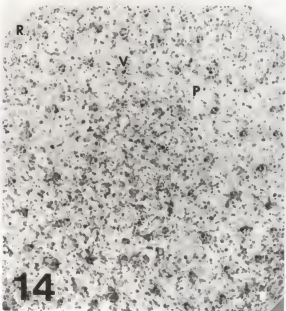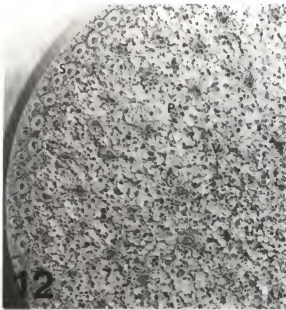
Fig. 16   Digesting 25E MI for 24 hours resulted in removal of phloem from the vascular bundles (arrows) and additional peripheral sclerenchymal disappearance (arrows). (Bar, 100 μm)

Fig. 17   Digesting 25E MI for 48 hours caused little, if any, additional tissue disappearance compared with 24 hour digestion. (Bar, 100 μm)

Fig. 18   Digesting of 25E MA for 36 hours caused similar peripherial sclerenchymal disappearance (arrows) as 25E MI digested for 48 hours. Ruminal microbes cover most of the stem tissues. (Bar, 100 μm)

Fig. 19   25E MI after 48 hours of ruminal fermentation shows peripheral sclerenchymal tissue disappearance (arrow) and anaerobic fungi (arrow) present on vascular bundles. (Bar, 100 μm)
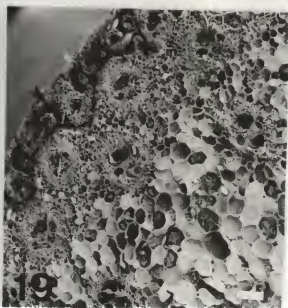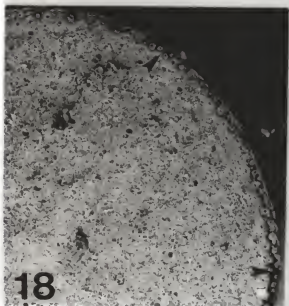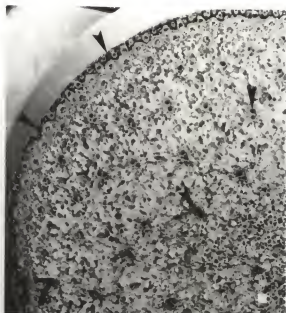
DIGESTION OF THE UPPER INTERNODES OF CULTIVAR 351

Fig. 20   Ensiling the cultivar 351 MI will cause peripheral sclerenchymal (arrows) disappearance but not as pronounced as in 25E MI. (Bar, 100 μm)

Fig. 21   View of 351 MA shows no disappearance of surface tissues due to the ensiling process. (Bar, 100 μm)

Fig. 22   Digesting 351 MI for 18 hours shows the same peripheral sclerenchymal tissue removal and phloem disappearance (arrow) as 25E. The outer layers of the sclerenchyma cells are removed while the inner layers remain intact. (Bar, 100 μm)

Fig. 23   After 48 hours of digesting 351 MI, peripheral sclerenchyma is removed from the rind leaving the epidermis and vascular bundles unattached at the cut surface. Numerous fungi (arrows) are present on lignified tissues. (Bar, 100 μm)
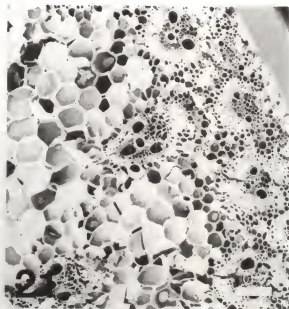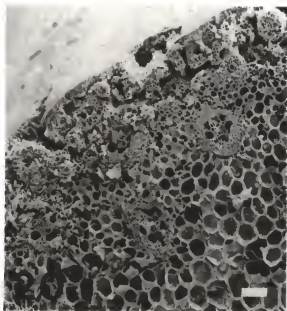
the epidermis had been removed, leaving anaerobic fungi attacking the epidermis and vascular bundles. The center of the stem remained intact. No substantial additional disappearance occurred after 48 hours of digestion in the MA stage (Fig. 24) although microbial activity was present (Fig. 25).

The non-heading cultivar 1990, had newly formed leaves arranged concentrically around the central axis. The tissue disappearance pattern was similar for the two maturities but there were differences in the extent of disappearance. Mesophyll tissues were partially hydrolyzed by silage bacteria (Fig. 26). Epidermal and vascular tissues were not degraded after 18 hours in the rumen and only partial digestion of the midrib was observed at either maturity (Fig. 27 and 28). Girder fibers also remained intact at the MA stage. Figure 29 indicates that immature vascular bundles can be utilized by rumen microbes. By 48 hours of rumen digestion, differences due to maturity became apparent. At the MI stage, residues consisted of a single epidermal layer and vascular bundles (Fig. 30) while at the MA stage the tissues remaining included a portion of the midrib, epidermal layers, and vascular tissues (Fig. 31).

Localization of lignin was similar among cultivars. There were, however, differences in lignin concentration and location of lignin types between upper and lower internodes, and between rind and center portions of the stem.

Acid phloroglucinol-positive lignin type was found predominately in the vascular bundles of the center and tissues of the rind in all cultivars and maturities. Heaviest concentration of lignin was in the peripheral vascular bundles, but the amount differed between upper and lower internodes (Fig. 32 and 33). The multiple shades of red probably indicates an immature lignin while a single shade of red indicates maturity. In the lower internodes of all cultivars, there is infusion of lignin into adjoining cellulosic cell walls. Acid phloroglucinol-positive lignin in the center portion of the stem is localized within the vascular bundles (Fig. 34).

Chlorine sulphite-positve tissues can be two colors depending on the character

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Fig. 24   Tissue removal does not occur with increased maturity of 351 after 48 hours of digestion. (Bar, 100 µm)

Fig. 25   Masses of bacteria (arrows) are present on parenchymal tissues after 48 hours of digestion. Phloem tissues from vascular bundles have been removed and the bacterial activity have initiated parenchymal cell wall degradation. (Bar, 100 µm)
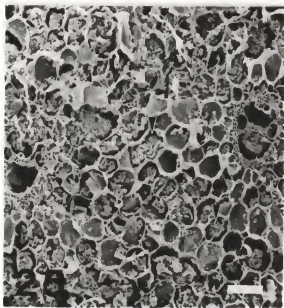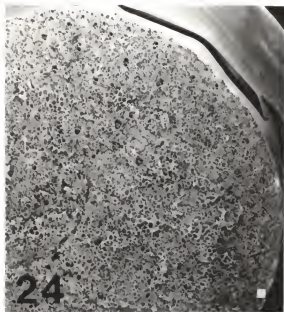
TISSUE DISAPPEARANCE PATTERN IN THE CULTIVAR 1990

Fig. 26   Ensiling the whorl of cultivar 1990 causes partial mesophyll disappearance (arrows) while the midrib (M) and other leaf (L) components remain intact. (Bar, 100 μm)

Fig. 27   Extensive mesophyll and midrib tissue (arrows) removal is evident in 1990 MI after 18 hours of digestion. (Bar, 100 μm)

Fig. 28   Tissue disappearance pattern (arrows) is similar in 1990 MA to 1990 MI after 18 hours of digestion. Mesophyll and partial midrib tissues are removed leaving the epidermis, vascular bundles, and girder fibers (G) intact. (Bar, 100 μm)
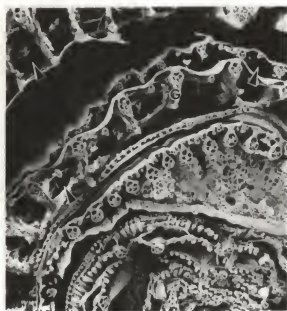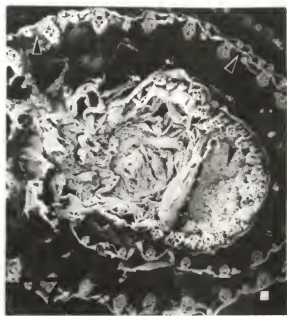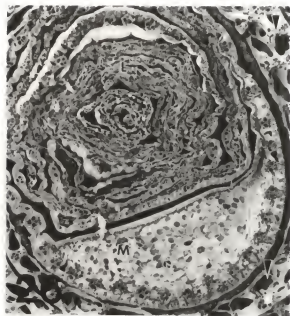
Fig. 29   View of a vascular bundle after 24 hours of digestion from an immature stem of 1990, showing sieve tubes (T) as partial remnants of digestion. (Bar, 100 μm)

Fig. 30   Sample of 1990 MI digested for 48 hours showing only epidermis (E) and cross tubes (C) intact. Vascular bundles may (arrows) or may not (V) be dislodged by digestion. (Bar, 100 μm)

Fig. 31   Photomicrograph of cultivar 1990 after 48 hours of digestion shows remnants of vascular bundles (V), partial midrib (M) remaining intact, and disrupted epidermal tissues (E). (Bar, 100 μm)
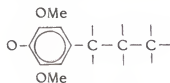
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PATTERN OF ACID PHLOROGLUCINOL STAIN

Fig. 32   Light micrograph of peripheral vascular bundles from MI sorghum stem (upper internode) indicates a difference in amount of stain retained by the p-coumaryl type lignin, suggesting an immature lignin because of the lighter red color compared with the center of the bundle.

Fig. 33   Light micrograph showing lignification of the peripheral vascular bundles in the lower internodes with some lignification of the sclerenchymal tissues (arrows).
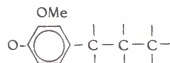
Fig. 34   Demonstration of lignification of the central vascular bundles of both the upper and lower internodes in all cultivars at both maturites.

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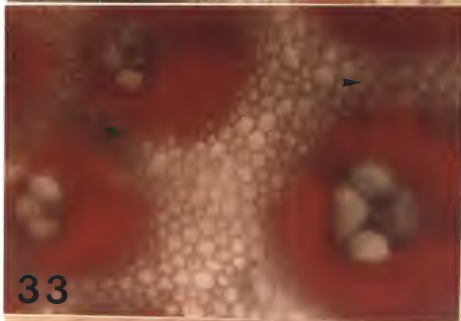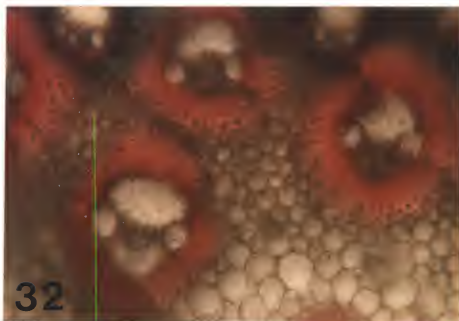
of the nucleus group (Sarkanen and Ludwig, 1971). A red color represents a syringyl nucleus and a brown color, a guaiacyl nucleus.

$$O-\bigcirc\!\!\!\!\!-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}- \quad\quad\quad O-\bigcirc\!\!\!\!\!-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-$$

OMe (top and bottom, left structure); OMe (top, right structure)

Syringyl nucleus   Guaiacyl nucleus

Both nucleus types represent a lignin that is resistant to microbial attack. In 25E MI, syringyl groups were present in central vascular bundles of the lower internode (Fig. 35). Guaiacyl groups were present in the rind of lower internodes (Fig. 36) and throughout the tissues of the upper internodes (Fig. 37 and 38). No lignification was present in the walls of lower internode parenchymal cells. Akin et al. (1984) found a similar pattern when staining the lower internode of _Panicum antidotale_ in which 97% of the tissues were digested after 48 hours.

The presence of a green fluorescent color after increasing the pH indicates ferulic acid (Harris, 1976), a lignin precursor that is apparently not inhibitory to rumen microbial digestion (Mahmoudzadeh et al., 1985). Center parenchymal cells walls fluoresce green (Fig. 39) in the lower internodes but parenchymal cells near the stem periphery and vascular bundles fluoresce blue (Fig. 40), indicating lignin. No parenchymal or sclerenchymal tissues of the upper internodes fluoresced green (Fig. 41 and 42).

Variation in the location and concentration of starch in stems did not correspond with apparent digestion patterns. Starch, indicated by iodine staining, was absent in cultivar 1990 (Fig. 43). It was present in 351 but only in the rind (Fig. 44). Location and concentration of starch varied in 25E. It was found in the rind of the lower internode MI stage (Fig 45), the rind and around the vascular bundles of the lower internode MA stage (Fig. 46), and throughout the upper internode of both maturities (Fig. 47).

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## CHLORINE SULFITE-POSITIVE LIGNIN PATTERNS

Fig. 35   Syringyl nucleus type lignin (red color) is present in the parenchymal vascular bundles of 25E MI. Lignification is not found with the parenchyma cells of the lower internodes.

Fig. 36   Rind tissues of the lower internode indicates a guaiacyl type lignin (brown color) in the sclerenchymal cell walls and vascular bundle tissues.

35

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Fig. 37   Parenchyma and vascular bundle tissues of the upper internodes of all cultivars suggests guaiacyl lignin content (brown color).

Fig. 38   The rind potion of the upper internodes of all cultivars appear to contain guaiacyl type lignin (brown color).

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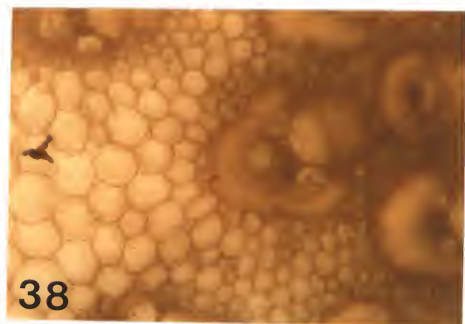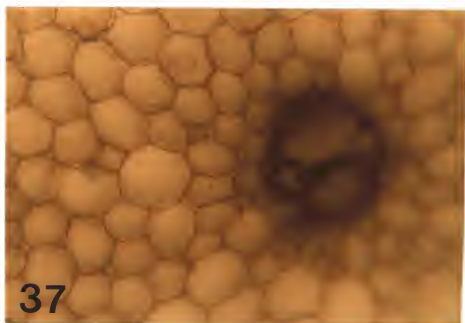
FERULIC ACID LOCATION

Fig. 39   Fluorescent micrograph of sorghum lower internode indicates ferulic acid (green color) is in parenchyma, phloem, and xylem tissues.

Fig. 40   Lignification (blue color) becomes evident towards the peripheral tissues. Parenchymal cells (left side) fluoresce green and sclerenchyma and vascular bundles show a blue color (right side).
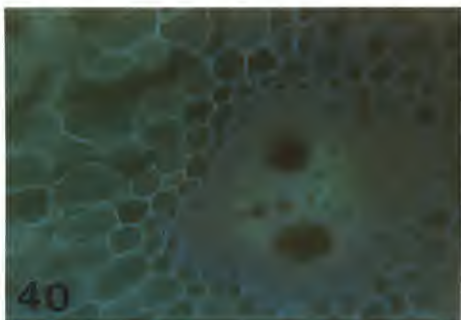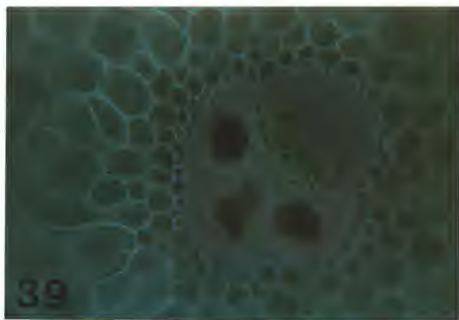
Fig. 41   Center tissues of the upper internodes show lignin (blue color) but not ferulic acid except for the phloem and xylem tissues of the vascular bundles (green color).

Fig. 42   The rind (outer portion of cut sample) of the upper internode shows ferulic acid in the vascular bundle tissues. The blue color in parenchymal tissues is indicative of lignin.
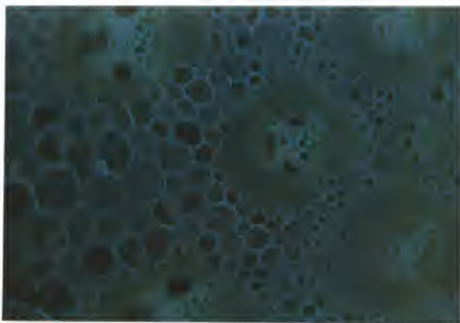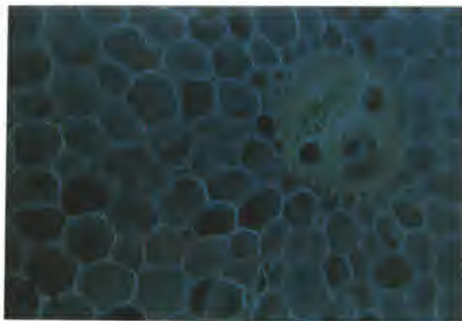
STARCH LOCATION

Fig. 43   Light micrograph of the lower internode of cultivar 1990 after being stained
with KI shows starch was absent.

Fig. 44   Starch (arrows) in cultivar 351 was found in the rind portion of the stem.

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Fig. 45   Starch content (arrows) was limited to the outer periphery of the lower internode of 25E MI.

Fig. 46   Mature 25E lower internodes show starch around the vascular bundles as well as in the rind of the stem.

Fig. 47   Light micrograph shows the presence of starch throughout the upper internode of 25E at both maturity stages.
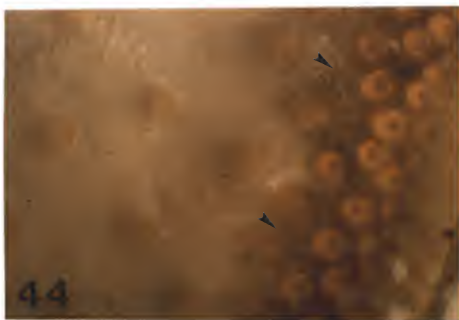
45

SUMMARY

Similar patterns of tissue disappearance were observed between cultivars but lower internodes indicated more tissue disappearance than upper internodes. The presence of acid phloroglucinol and chlorine sulphite-positive tissues appeared to correspond consistently to non-digested tissues, while the presence of ferulic acid, as lignin precursors corresponded with digested tissues. No consistent relationship was found between starch location and tissue disappearance. Degradation of sclerenchyma in the upper internodes cannot be explained by the presence of starch or lignin.

Scanning electron microscopy photographs can be misleading because tissue disappearance does not necessarily mean digestion by the rumen microbes. Many sloughed off vascular bundles with attached parenchymal cells were found as residues in the nylon bags. Further research needs to be done on the actual residues of in vivo digestion by either observing fecal residues or residues remaining in the nylon bag. The mode of tissue digestion could be further examined by using transmission electron microscopy.

The reason for sclerenchymal tissue hydrolysis in the upper internodes is unknown but one possibility might be the soluble sugar content of those tissues rather than starch accumulation. Future work might be oriented toward identifying and localizing these soluble sugars.

According to this study, bottom internodes were more readily utilized than upper internodes. Digesting each internode of the stem in vivo in nylon bags and observing the pattern of tissue disappearance and conducting digestion trials comparing plant parts could provide information for possible new harvesting methods that would maximize sorghum utilization by animals. It appears that the lower portion of the plant would be more valuable as a feed source but the influence of leaves and

grain content must also be considered.

Lignin plays an important role in stem digestion. Defining the concentration and location of the different types of lignin could be an important tool for plant breeders wishing to increase forage utlization.

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RUMINAL DIGESTION OF FORAGE SORGHUM STEMS
OBSERVED BY LIGHT, FLUORESCENCE AND SCANNING
ELECTRON MICROSCOPY

by

RUTH ANN SCHWEITZER

B. S., Kansas State University, 1976

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

ABSTRACT

Three forage sorghum cultivar silages were observed microscopically to determine plant structures that limit ruminal microbial digestion. Upper and lower internodes of the stems were harvested at milk (MI) and mature (MA) stages, cut into 1-2 cm lengths, ensiled for 90 days in experimental silos, digested _in vivo_ using nylon bags, and observed by scanning electron microscopy (SEM) for anatomical characteristics and specific tissue degradation. Cultivars represented were a full-season, non-heading cultivar (Funks G-1990); a mid-season, heavy-heading sorghum (Acco 351); and a late-season, moderate-heading type (Dekalb FS-25E). Cultivars were designated 1990, 351, and 25E respectively. Light and fluorescence microscopy were used on fresh-forage stems to localize starch and lignin.

Scanning microscopy observations showed that ensiling initiated the degradation process. Peripheral sclerenchymal tissues in the upper internodes of 25E MI and 351 MI were partially hydrolyzed but remained intact in 25E MA and 351 MA. Dekalb 1990, different anatomically, in the upper internode showed partially hydrolyzed mesophyll in the outer leaf formation. All lower internodes characteristically showed disappearance of parenchymal tissues around the vascular bundles.

In vivo digestive patterns (48 hour fermentations) varied between upper and lower internodes. Removed tissues corresponded with the location of ferulic acid and the absence of chlorine sulphite and acid phloroglucinol-positive tissues. Location of starch was not related to tissue removal patterns as observed by SEM. Upper internode tissues degraded in cultivars 351 MI, 25E MI, and 25E MA included sclerenchyma and phloem. Phloem tissues were the only tissues degraded to any extent in 351 MA. Cultivar 1990 followed the tissue disappearance pattern of a monocotyledon leaf; mesophyll was removed first, followed by phloem disappearance,

and rupturing of the epidermis. Disappearance of tissues was most extensive at the MI stage, and included partial removal of vascular tissues. The bottom internodes, regardless of cultivar, had the same pattern of tissue disappearance. Parenchymal tissues around the vascular bundles were removed first, followed by inner parenchyma, leaving a residue of free vascular bundles. Parenchymal cells were probably partially hydrolyzed by microbial activity, then movements of the rumen caused the removal of vascular residues. Vascular bundles attached to the rind portion of the stem remained intact. Anaerobic fungi were present on lignified tissues after 36 hours of digestion.

These studies show that lower internodes of these cultivars had similar digestive patterns and were generally more readily digestible than top internodes. Tissues containing ferulic acid were readily digestible while chlorine sulphite and acid phloroglucinol-positive tissues remained intact. No correlation was observed between digestive patterns and starch location or concentration.