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BIODEGRADATION OF CELL TYPES IN NORMAL AND BROWN MIDRIB MUTANT PEARL MILLET (*Pennisetum glaucum* (L) R Br): MICROSPECTROPHOTOMETRIC AND ELECTRON MICROSCOPIC STUDIES OF WALLS AND WALL LAYERS

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Abstract

Aromatic constituents of cell walls limit the feeding value of forages, but information is lacking on the sites and types of these constituents that retard biodegradation. A series of cell types in stems and leaf blades of a normal (N) and a brown midrib (bmr) mutant line of pearl millet (*Pennisetum glaucum* (L) R Br) were analyzed by UV absorption microspectrophotometry from freeze-dried bulk walls and from 4 μ m thick sections. Cell types were evaluated by scanning and transmission electron microscopy for rumen microbial degradation. Generally, N cell walls had a higher absorbance, hypsochromatically shifted λ_{max} , and a higher absorbance ratio (280:320 nm) compared with bmr walls. Spectra suggested that ester-linked phenolic acids were present in lignified and non-lignified walls of both plant lines. Results are consistent with previous information that N cell types have substantially higher concentrations of ester-linked *p*-coumaric acid and more condensed aromatics than bmr walls. Pronounced variations occurred within regions of some cell types, including the middle lamella region vs. secondary layers of sclerenchyma cells and the outer vs. inner regions of epidermal cells. Biodegradable cell walls, such as stem parenchyma and epidermis and parenchyma bundle sheaths of leaf blades, showed less absorbance than heavily lignified walls. For some walls, UV spectra related well with variations in digestibility, while for other cell walls further work is needed to relate spectra to biodegradation.

Key Words: Lignin, phenolic acid esters, plant cell types, microspectrophotometry, electron microscopy, biodegradation.

Introduction

Biodegradation of plants is important for ruminant production and offers the possibility of alternative energy supplies. Improved utilization of the plant cell walls will increase feeding efficiency and reduce production costs for supplying energy. Aromatics within the plant cell wall limit the utilization of this potential source of energy for fermentation. These aromatics exist within the cell wall as condensed, non-esterified polymeric phenylpropanoid units, often called "core" lignin (Kuc and Nelson, 1964), while *p*-coumaric and ferulic acids are ester-linked to arabinoxylans of the cell wall (Hartley and Ford, 1989). Ether linkages, which constitute stronger bonds than esters, also exist in grasses (Kondo et al., 1991). In wheat, ferulic acid is ester-linked to carbohydrates and also ether-linked to condensed aromatics, thus forming a recalcitrant bridge across multiple cell wall components (Iiyama et al., 1990).

Removing or altering the aromatics within the plant cell wall is one strategy to improve utilization of plant biomass. To this end, the brown midrib (bmr) mutations of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L Moench), and pearl millet (*Pennisetum glaucum* (L) R Br) result in reduction and alteration of aromatic constituents in cell walls and in improved biodegradation compared to their normal (N) counterparts (see review by Cherney et al., 1991). Ester-linked phenolic acids are lower in concentration in bmr mutants, and alterations in the structure and composition of the "core" lignin have been reported between N and bmr plants (Kuc and Nelson, 1964; Akin et al., 1986b; and Morrison et al., 1993).

Thorstensson et al. (1992) recently reported that both rate and extent of digestion of neutral detergent fiber were greater in bmr plants. Microscopic studies of cell type digestion of sorghum and pearl millet have indicated that non-lignified, digestible tissues, such as parenchyma, were more readily degraded in the bmr plants, while heavily lignified structures, such as vascular xylem, were poorly degraded in both N and bmr plants (Akin et al., 1986a; Akin et al., 1991).

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Therefore, it is important to characterize the aromatics within specific cell types to gain a full understanding of the relationship between aromatics and biodegradation.

Ultraviolet absorption microspectrophotometry has recently been used to characterize phenolic constituents and their distribution within cell types (He and Terashima, 1991; Goto et al., 1992; and Akin and Rigsby, 1992) and further has provided information on the more condensed and ester-linked phenolics (Akin and Hartley, 1992a,b). The objective of this study is to characterize the aromatic constituents of walls in a series of cell types from N and bmr pearl millet leaf and stem using UV absorption microspectrophotometry and to evaluate digestion of specific cell walls using electron microscopy.

Materials and Methods

Plant samples

Plants of Normal (N) pearl millet 5848 and the brown midrib (bmr) mutant 5778 were grown in Tifton, Georgia, in well-managed plots as described (Akin et al., 1991). Four-week old, vegetative plants were harvested for analyses, and a series of studies has been carried out to define characteristics of these plants related to biodegradation. Results of these studies are summarized as follows: bmr was lower in lignin and higher in *in vitro* digestibility (Akin et al., 1991, 1993), midvein and stem parenchyma of the bmr were sites where modification resulted in the greatest apparent improvement in digestion (Akin et al., 1991), bmr rinds were more digestible and weaker in textural strength than N rinds (Akin et al., 1993), cell types from bmr stems had lower concentrations of alkali-extractable *p*-coumaric acid (Hartley et al., 1992), and ether-linked phenolic acids were lower in bmr cell types and UV absorption microspectroscopy of walls from stem cell types suggested a less condensed nature for bmr lignins (Morrison et al., 1993).

Cell types of stems

Stem internodes near the lower part of the plant (6th to 8th internode from the apex) were excised for study in order to evaluate the older, more lignified regions. From 4 plants for each plant type, the pith was manually removed from the rind, and pith was then manually separated into parenchyma and vascular bundles. These three components (parenchyma, vascular bundles, and rind) were freeze-dried and ground, then extracted with a series of alcoholic solvents, and the cell walls collected as described (Hartley et al., 1992). Cells were observed under the microscope and representative regions were chosen from each of the cell types for analysis by microspectrophotometry. Individual cell types from the rind were broken from the matrix by grinding, and as a result, two major cell types could be identified with light microscopy. To be sure of the origin of the cells,

a segment of N rind was teased apart under a stereo-microscope, and the vascular bundle strands and the "in between cells" evaluated by light microscopy and by histochemistry with the lignin stain acid phloroglucinol (Jensen, 1962). From this evaluation, rectangular cells, which appeared to be sclerenchyma, were easily identified in the ground sample, while the "layered" fragments appeared to be cells associated with vascular bundles. Therefore, walls of parenchyma and vascular bundles from the pith and sclerenchyma and vascular bundles from the rind could each be analyzed by microspectrophotometry.

Pith and rind segments of these internodes from two plants each of N and bmr lines were also cross sectioned to evaluate the cell types. Rind segments that were incubated with rumen microorganisms were also evaluated for analysis by UV absorption microspectrophotometry. Sections about 3 mm long were fixed in 4% buffered glutaraldehyde and embedded in JB-4 Plus medium as described (Akin and Rigsby, 1992). Four- μ m thick sections were cut using a microtome and mounted on a quartz slide in glycerol with a quartz cover slip.

Transmission electron microscopy was used to evaluate the ultrastructural aspects of digestion of these cell types from rind and pith. Pith and rind segments (2 mm X 3 mm) were excised from stems and included in pre-reduced anaerobically sterile medium prepared in Hungate tubes for incubation with rumen microorganisms as described (Akin et al., 1992). Rumen fluid from steers maintained at the University of Georgia Dairy Cattle Center was strained through cheesecloth and inoculated (0.2 ml per incubation tube) into the tubes through septa. These inoculated tubes were incubated at 39°C for 124 h to provide an extended digestion time for evaluation of wall degradation. Uninoculated tubes were incubated overnight to provide control samples. After incubation, plant segments were fixed in 4% glutaraldehyde for several hours and then rinsed in 0.1 M cacodylate buffer and stored overnight in buffer. Samples were dehydrated 10 min each in 50%, 70%, 90%, and 100% ethanol and then infiltrated for 2 h each with 1:2, 1:1, 2:1 Spurr epoxy plastic: 100% ethanol. Samples were then embedded in fresh 100% Spurr plastic and cured at 70°C for 72 h. Thin sections were cut using a diamond knife. Metaxylem, xylem, and sclerenchyma cell walls were evaluated in control and digested segments of two N and two bmr stems.

Cell types of leaf blades

Cross sections about three-mm long from the oldest leaves of N and bmr plants were chosen. Three leaves were chosen from each line. The *in vitro* digestibility of leaf blades from similar plants of the same replicates used in the present study had been determined (Akin et al., 1991) to be 75% and 83% for N and bmr leaf blades, respectively. Sections included two large vascular bundles within the lamina portion of the leaf, and the large midvein was

Table 1

UV Absorption Microspectrophotometry of Ground Cell Wall Residues of Normal (N) and Brown Midrib (bmr) Stems of Pearl Millet

Cell type	Pearl millet line*		ca 280 nm		ca 320 nm		Ratio a/b
			λ_{max}	A(a)	λ_{max}	A(b)	
Pith		(No. sites)					
Parenchyma	N 5848	(10)	290.2 ± 1.1 (s) ^a	0.731 ± 0.363 ^a	312.8 ± 2.5 ^a	0.790 ± 0.386 ^a	0.92 ± 0.03 ^a
	bmr 5778	(8)	291.0 ± 2.8 (s) ^a	0.578 ± 0.538 ^a	319.3 ± 3.4 ^b	0.652 ± 0.644 ^a	0.90 ± 0.06 ^a
Vascular bundle rind	N 5848	(6)	285.0 ± 5.2 ^a	1.714 ± 0.647 ^a	313.2 ± 4.7 (s) ^a	1.678 ± 0.708 ^a	1.05 ± 0.01 ^a
	bmr 5778	(6)	290.0 ± 2.8 ^b	1.673 ± 0.512 ^a	317.7 ± 1.5 ^b	1.779 ± 0.513 ^a	0.94 ± 0.03 ^b
Rind							
Sclerenchyma	N 5848	(6)	290.3 ± 3.7 (s) ^a	1.173 ± 0.170 ^a	314.3 ± 2.0 ^a	1.261 ± 0.200 ^a	0.93 ± 0.02 ^a
	bmr 5778	(6)	291.7 ± 2.0 (s) ^a	1.067 ± 0.477 ^a	322.7 ± 2.1 ^b	1.259 ± 0.612 ^a	0.86 ± 0.03 ^b
Vascular bundles	N 5858	(10)	289.3 ± 3.0 ^a	1.607 ± 0.301 ^a	314.3 ± 2.0 ^a	1.684 ± 0.342 ^a	0.96 ± 0.03 ^a
	bmr 5778	(6)	289.7 ± 2.9 (s) ^a	1.534 ± 0.274 ^a	324.7 ± 2.7 ^b	1.689 ± 0.255 ^a	0.91 ± 0.03 ^b

^{a, b} Different superscripts within cell types within columns indicate differences, P ≤ 0.05.

* Number of sites indicated in parentheses.

(s) = shoulder

^{a,b} Different superscripts within cell types within columns indicate differences, $P \leq 0.05$.

* Number of sites indicated in parentheses.

(s) = shoulder

excluded. For microspectrophotometry, portions of the sections were mounted in a cryostat, and 4- μ m thick sections were cut and mounted as described above for the stem sections.

Portions of the same leaf blade sections used for microspectrophotometry were also used for in vitro digestion of cell types. These portions were placed into prerduced anaerobically sterile medium for in vitro digestion as described (Akin et al., 1992). The inoculum was from a steer fed bermudagrass hay plus supplement, and 0.4 ml of strained rumen fluid was added to each in vitro digestion tube. Leaf sections were removed after incubation at 39°C for 24 and 48 h and also from uninoculated medium as a control. For scanning electron microscopy, leaf blade sections from uninoculated medium and from inoculated medium were retrieved, rinsed in water, and fixed in 4% buffered glutaraldehyde at 8°C. Sections were post-fixed in 1.5% buffered OsO₄, dehydrated in an ethanol series, and critical point dried as described (Akin et al., 1987).

UV absorption microspectrophotometry

A Zeiss UMSP-80¹ microspectrophotometry system with Lambda Scan software was used as described (Akin and Rigsby, 1992). Transmitted illumination was provided by a high pressure xenon lamp (XBO 75 W) through a connecting grating monochromator with a bandwidth of 5 nm. A 32-X quartz lens with a final aperture diameter of 1.56 μ m, which

was delimited within about one-third of the area of a field-limiting diaphragm to reduce stray light, was positioned over selected regions of the walls of cell types in ground material or in 4- μ m thick sections. The system was standardized at 350 nm. For the embedded pith sections, the system was evaluated with standardization both on and off the plastic background, and variations in spectra were determined to be minimal with the two methods. Absorbance of transmitted UV illumination over a range of 230 to 350 nm was measured at 2 nm increments. Spectra from several regions of a cell wall were made, and the reported averages represent these several regions rather than averages of several spectra over the same site.

Results and Discussion

Stem cell types

UV absorption spectra are shown for cell types in ground stem samples of N (Fig. 1) and of bmr (Fig. 2) pearl millet. The λ_{max} , absorbance, and absorbance ratios for these cell types are listed in Table 1. For both plant types, the pith parenchyma walls had considerably lower absorbance than the other three cell types, and extremely low absorbance occurred for the bmr wall (Fig. 2). Spectral patterns for the vascular bundle were similar for this cell type from the pith and from the rind in the bmr plants, but variations occurred between these two wall types in N plants; aromatics in rind bundles had a greater absorbance near 320 nm, suggesting greater extended conjugation in these walls. Differences occurred between the N and bmr cell types in the λ_{max} as well as the ratio

¹Reference to specific products is made for identification purposes only and does not imply endorsement by the United States government.

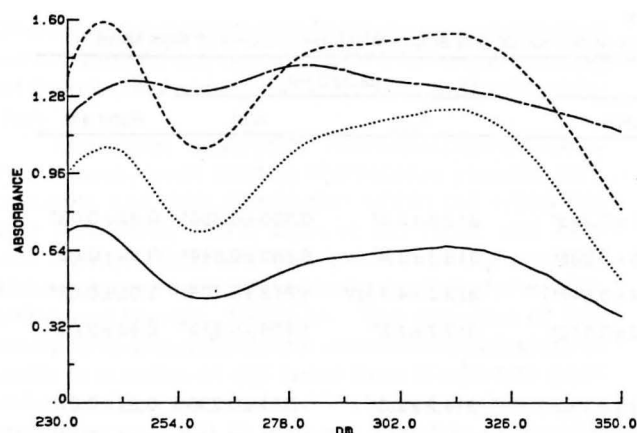


Fig. 1. UV absorption spectra of walls of ground samples of N cell types of pearl millet stem. Pith parenchyma (—), vascular bundles isolated from pith (---), sclerenchyma from rind (····), and vascular bundles from rind (- - -).

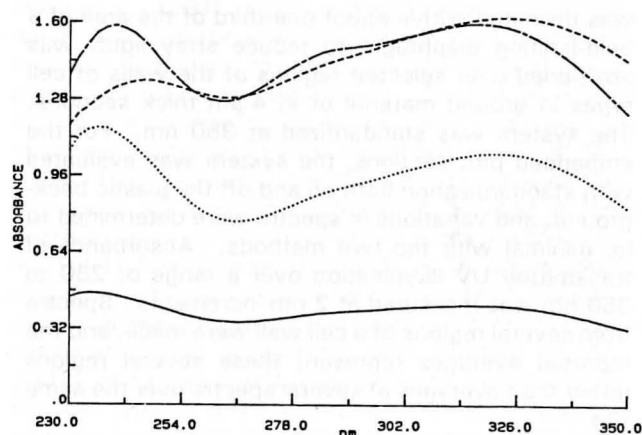


Fig. 2. UV absorption spectra of walls of ground samples of bmr cell types of pearl millet stem. Parenchyma (—), vascular bundles isolated from pith (---), sclerenchyma from rind (····), and vascular bundles from rind (- - -).

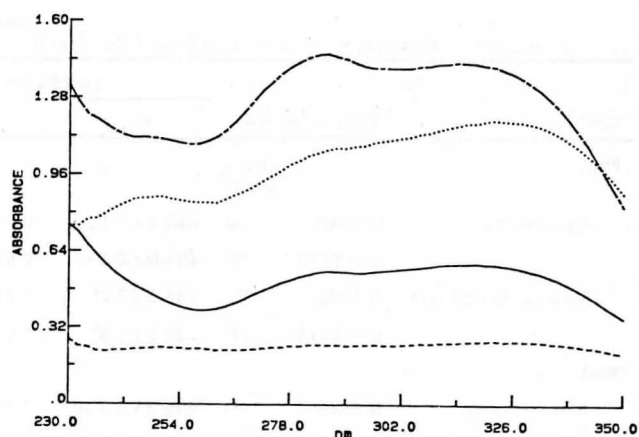


Fig. 3. UV absorption spectra of N and bmr walls of parenchyma and vascular bundles from embedded cross sections of pearl millet pith cut 4 µm thick. N parenchyma (—), bmr parenchyma (---), N vascular bundle (- - -), and bmr vascular bundle (····).

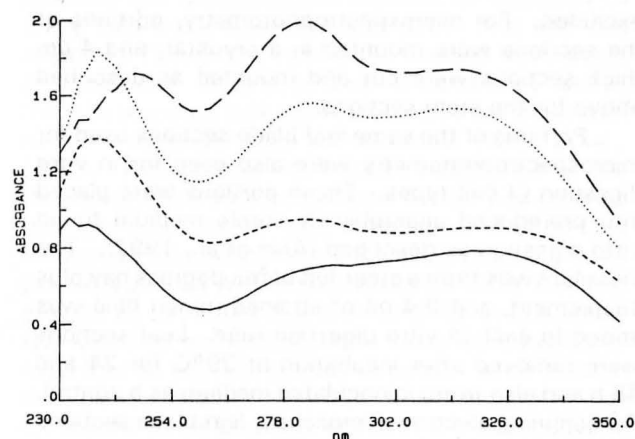


Fig. 4. UV absorption spectra of sclerenchyma cell walls of N pearl millet rind undegraded and degraded by rumen microorganisms for 124 h. Average of 3 to 5 sites for each of two sections. Undegraded secondary layer (—), undegraded middle lamella/primary layer region (····), degraded secondary layer (---), degraded middle lamella/primary layer region (- - -).

of absorbance near 280:320 nm (Table 1). Extraction with 1 M NaOH had previously shown that the N stem tissues have higher concentrations of *p*-coumaric acid but similar levels of ferulic acid to that of bmr walls (Hartley et al., 1992; Morrison et al., 1993). This characteristic was particularly evident in parenchyma, where ester-linked phenolic acids appeared to be the predominant aromatic constituent. The greater

amount of ester-linked *p*-coumaric acid was related to a lower digestibility in N vs bmr parenchyma (Morrison et al., 1993). This chemical difference could account for the spectral differences, since *p*-coumaroyl arabinoxylans absorb at 314 nm and feruloyl arabinoxylans absorb at 324 nm (Akin and Hartley, 1992a). Variations in the λ_{max} ca 320 nm between

Table 2

UV Absorption Microspectrophotometry of Thin Sections of Normal and Bmr 5778 Rind Sections Undegraded and Degraded with Rumen Microorganisms for 124 h

Line	λ_{max}	A(a)	λ_{max}	A(b)	Ratio a/b
N 5847 Undegraded					
Secondary layer	289 \pm 1	0.840 \pm 0.495	320 \pm 3	0.947 \pm 0.557	0.88 \pm 0
Middle lamella/primary layer	285 \pm 1	1.615 \pm 0.313	314 \pm 0	1.575 \pm 0.277	1.03 \pm 0.02
N 5848 Degraded					
Secondary layer	283 \pm 1	0.957 \pm 0.010	317 \pm 1	0.911 \pm 0.058	1.05 \pm 0.08
Middle lamella/primary layer	283 \pm 1	1.997 \pm 0.013	315 \pm 1	1.742 \pm 0.020	1.15 \pm 0.02
Bmr 5778 Undegraded					
Secondary layer	289 \pm 1	0.910 \pm 0.438	322 \pm 3	1.037 \pm 0.482	0.87 \pm 0.02
Middle lamella/primary layer	290 \pm 0	1.515 \pm 0.622	322 \pm 3	1.271 \pm 0.151	0.92 \pm 0
Bmr 5778 Degraded					
Secondary layer	----- Not present -----				
Middle lamella/primary layer	290 \pm 0	0.726 \pm 0.310	332 \pm 0	0.776 \pm 0.314	0.93 \pm 0.02

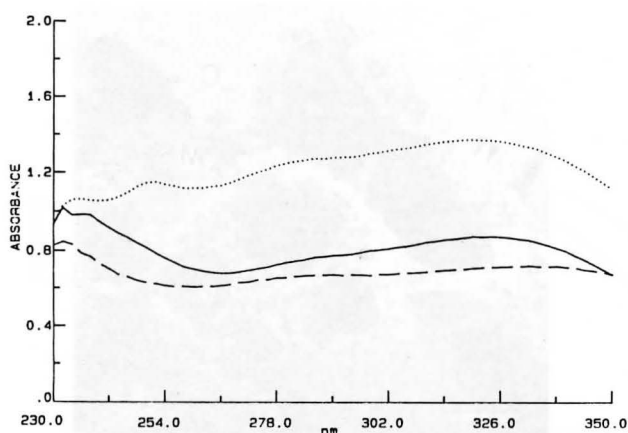


Fig. 5. UV absorption spectra of sclerenchyma cell walls of bmr pearl millet rind undegraded and degraded by rumen microorganisms for 124 h. Average of 3 to 4 sites for each of two sections. Undegraded secondary layer (—), undegraded middle lamella/primary layer (·····), and degraded middle lamella/primary layer (— — —).

N and bmr walls occurred for both parenchyma and the heavily lignified cell types.

Microspectrophotometric comparisons between ground walls of plants have a disadvantage of variations in wall thickness from site to site as well as the possibility that spectra may be derived from more than one wall. Microtomed sections are a more uniform sample for comparison, and in this respect may

provide an advantage for analysis by UV absorption. Thin sections were analyzed for parenchyma and vascular cells in pith (Fig. 3) and for secondary layers and middle lamellar regions in rind cells of N (Fig. 4) and bmr (Fig. 5) stems. For pith tissues, absorbance at λ_{max} was greater in N cells, and the ratio of absorbance (280:320 nm) in vascular walls was 1.06 and 0.90 for N and bmr walls, respectively. In sections of rinds, secondary layers of sclerenchyma cell walls were similar in λ_{max} , absorbance, and absorbance ratios between N and bmr walls (Figs. 4,5; Table 2). However, absorbance ratios were higher ($p \leq 0.05$) in N than bmr for middle lamellar regions, and the λ_{max} was hypsochromatically shifted in N walls. Extremely thin-walled cells within the rind did not provide clear spectral patterns.

Digestibilities of stem components similar to those used in the present study have been evaluated by scanning electron microscopy (Akin et al., 1991) and by gravimetric analysis (Akin et al., 1993). Bmr stem parenchyma was more digestible than N parenchyma, but the vascular bundles in piths of both plant types appeared to resist degradation (Akin et al., 1991). In the present study, transmission electron microscopy revealed bacterial pitting and erosion of the lignified bundle walls of the pith after 124 h incubation with rumen microorganisms; however, considerable vascular wall material and bundle integrity remained for both lines (not shown). Transmission electron microscopy showed that lignified cells in the undegraded rind ranged from cells with small lumina and extensive secondary layers to thin-walled, parenchyma-like cells with large lumina and relatively small secondary layers. Rumen microorganisms pitted and eroded the secondary layers to various degrees, but



Fig. 6. Transmission electron micrograph of sclerenchyma cell walls of N pearl millet rind degraded with rumen microorganisms for 124 h. Rumen bacteria have pitted and eroded the secondary layer to a minor extent. Residue consists of middle lamella/primary layer and most of the secondary layer. Bar = 1 μ m.

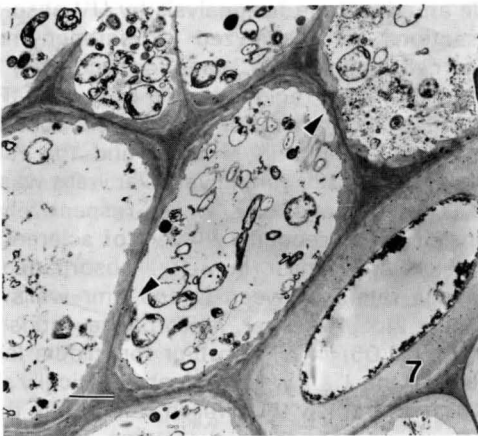


Fig. 7. Transmission electron micrograph of sclerenchyma cell walls of bmr pearl millet rind degraded with rumen microorganisms for 124 h. Rumen bacteria have substantially degraded the secondary layer, leaving only the middle lamella/primary layer in some sites (arrows). Bar = 1 μ m.

secondary layers in bmr cells were clearly more degraded than in N ones (compare Figs. 6 and 7). In both lines, middle lamella and the primary layer remained intact, often with degradation up to the middle lamella/primary wall region (Fig. 7, arrows). The thin-walled cells appeared to be more degraded in bmr rinds. These microscopic observations support gravimetric results indicating a dry weight loss of 33% and 50% for N and bmr rinds, respectively, and

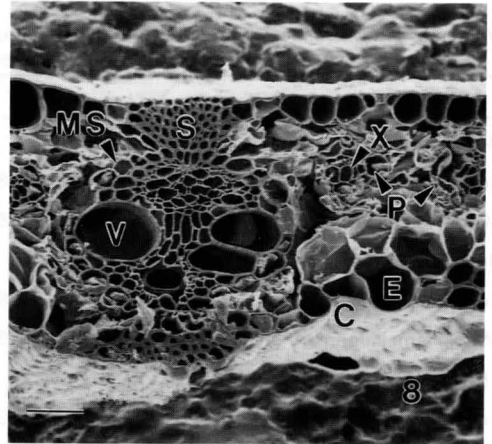


Fig. 8. Scanning electron micrograph of undegraded leaf blade cross section showing the arrangement of cell types. Epidermis (E) with the cuticle (C) covering, sclerenchyma (S), mestome sheath (MS), metaxylem vessel (V), parenchyma bundle sheath (P), and xylem of small vascular bundles (X). Bar = 50 μ m.

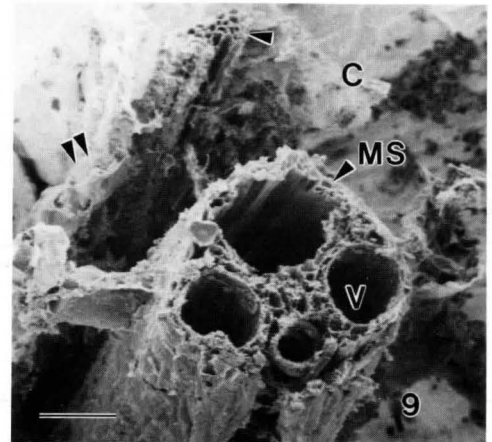


Fig. 9. Scanning electron micrograph of N leaf blade section incubated with rumen microorganisms for 48 h showing a residue of sclerenchyma (arrow), cuticle (C) with attached epidermal cells at some sites (double arrows), and large vascular bundle with mestome sheath (MS) and metaxylem vessels (V) clearly visible. Bar = 50 μ m.

further identify the sites for improved digestibility in bmr stems. In relation to our findings, Ohlde et al. (1992) reported that pitting and erosion of secondary layers of sclerenchyma cells of cereal straws contributed significantly to the digestibility of these feeds.

UV absorbance spectra did not differentiate N and bmr secondary layers in rind sclerenchyma (Figs. 4, 5; Table 2) even though bmr layers were more digestible (Figs. 6, 7). Spectral patterns for middle

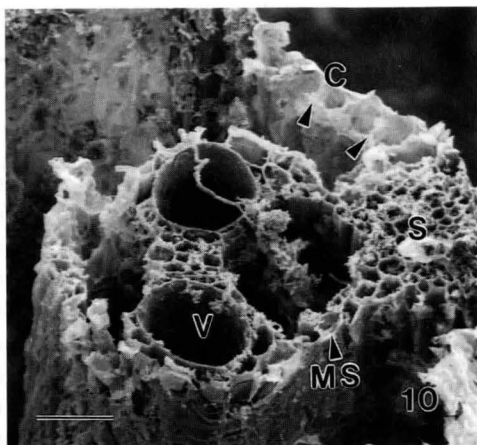


Fig. 10. Scanning electron micrograph of bmr leaf blade section incubated with rumen microorganisms for 48 h showing a residue of sclerenchyma (S), cuticle (C) with attached abaxial epidermal cells (arrows), and large vascular bundle with mestome sheath (MS) and metaxylem vessels (V) clearly visible. Bar = 50 μ m.

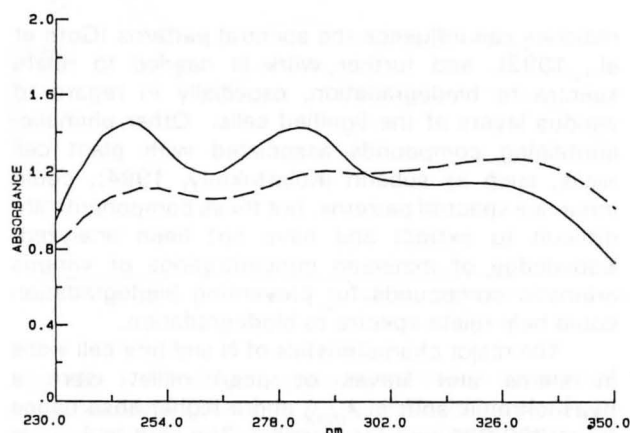


Fig. 11. UV absorption spectra of mestome sheaths from cryosectioned (4 μ m thick) N (—) and bmr (---) leaf blades of pearl millet.

lamella/primary wall regions for both N and bmr lines indicated a high aromatic concentration, but patterns were dissimilar even though these regions were not degraded in either line. The spectrum of secondary layers remaining in N rinds after microbial degradation showed a hypsochromic shift and a higher absorbance ratio compared with undegraded walls (Fig. 4; Table 2), suggesting some modification in remaining aromatic constituents. Spectra for middle lamella/primary layer regions indicated a higher absorbance at λ_{max} and higher ratio for digested sections (Table 2). The middle lamella/primary layer regions remained after

digestion of bmr rinds (Fig. 5), but UV absorption was substantially reduced and the λ_{max} showed a bathochromic shift to 332 nm (Table 2).

Leaf blade cell types

Leaf blades from plants similar to those of the present study have been observed by scanning electron microscopy for their disposition to biodegradation (Akin et al., 1991). In the present study, sections from the same segments analyzed by microspectrophotometry were incubated with rumen microorganisms and observed by microscopy for precise comparison. Tissue arrangement is shown for a control leaf (Fig. 8) and for degraded leaves from N (Fig. 9) and from bmr (Fig. 10) plants after 48 h. Results were similar to those in the previous study, showing the general integrity and recalcitrance to biodegradation of mestome sheaths and metaxylem vessels and a large part of the sclerenchyma. Epidermis and parenchyma bundle sheaths walls were degraded for the most part as shown by their removal from the residue, but some of the outer (toward the cuticle) regions of the epidermal walls remained (Fig. 10). The refractory tissues comprising the residues were similar for N and bmr leaves, indicating that rate of digestion of the more biodegradable walls was a major difference responsible for greater degradation of bmr leaves. Similarly, Thorstensson et al. (1992) reported that rate of digestion of potentially digestible cell walls was greater for bmr than N plants in some comparisons.

A series of cell types from N and bmr leaf blades were compared by UV absorption microspectrophotometry (Table 3). Spectral patterns are shown for mestome sheath (Fig. 11), metaxylem vessel (Fig. 12), xylem of small vascular bundles (Fig. 13), abaxial sclerenchyma including mostly secondary layers and also regions of middle lamella/primary layers (Fig. 14), outer and inner (toward the mesophyll) walls of the abaxial epidermis at least four cells away from the sclerenchyma (Fig. 15), and parenchyma bundle sheaths (Fig. 16). Generally, N walls showed a hypsochromatic shift for the λ_{max} , a higher absorbance at λ_{max} , and a higher ratio for absorbance near 280:320 nm compared to bmr walls. For both plant types, the heavily lignified walls (e.g., mestome sheaths, metaxylem vessels, and xylem) showed a particularly high absorbance, with the exception of metaxylem vessels in bmr where absorption maxima were hardly observed over background absorbance. Spectra of N sclerenchyma walls (Fig. 14) that included the middle lamella region showed a higher absorbance than those obtained predominately from secondary layers, although the spectral patterns were similar (Table 3). This higher absorption for the middle lamella region was not observed with bmr sclerenchyma. The digestible tissues, i.e., epidermis (Fig. 15) and parenchyma bundle sheath (Fig. 16), had lower absorbance than the heavily lignified, recalcitrant cell types. The spectral pattern for the outer

Table 3
UV Absorption Microspectrophotometry of Thin Sections of Normal and Bmr 5778 Leaf Blades

Cell type	Pearl millet line	No. leaf blades sampled	ca 280 nm		ca 320 nm		Ratio a/b
			λ_{max}	A(a)	λ_{max}	A(b)	
Mestome sheath	N 5848	3	282.7 \pm 0.3 ^a	1.738 \pm 0.333	321.1 \pm 3.1 ^a	1.391 \pm 0.297	1.27 \pm 0.03 ^a
	Bmr 5778	3	288.1 \pm 1.7 ^b	1.258 \pm 0.441	326.9 \pm 1.6 ^b	1.316 \pm 0.422	0.96 \pm 0.05 ^b
Metaxylem vessel	N 5848	2	282.0 \pm 1.4	1.552 \pm 0.403	319.5 \pm 2.1	1.202 \pm 0.319	1.30 \pm 0.01 ^a
	Bmr 5778	2	286.5 \pm 3.5	0.871 \pm 0.383	326.0 \pm 8.5	0.903 \pm 0.416	0.97 \pm 0.01 ^b
Sclerenchyma (no middle lamella)	N 5848	3	288.0 \pm 1.0 ^a	1.275 \pm 0.177	321.3 \pm 3.1 ^a	1.370 \pm 0.126	0.93 \pm 0.07
	Bmr 5778	3	292.0 \pm 1.8 ^b	1.289 \pm 0.239	327.9 \pm 1.0 ^b	1.533 \pm 0.257	0.84 \pm 0.03
Sclerenchyma (plus middle lamella)	N 5848	3	287.3 \pm 1.2	1.559 \pm 0.124	320.0 \pm 2.0 ^a	1.556 \pm 0.276	0.94 \pm 0.04 ^a
	Bmr 5778	2	288.0 \pm 2.8	1.304 \pm 0.098	328.0 \pm 0.0 ^b	1.478 \pm 0.088	0.88 \pm 0.01 ^b
Epidermis	N 5848	2	285.0 \pm 1.4	0.896 \pm 0.072	322.0 \pm 7.1	0.915 \pm 0.047	0.99 \pm 0.13
	Bmr 5778	3	281.7 \pm 3.2	0.567 \pm 0.239	325.3 \pm 2.9	0.477 \pm 0.248	1.26 \pm 0.23
Xylem (small bundle)	N 5848	2	282.5 \pm 0.7	0.579 \pm 0.041	321.0 \pm 4.2	0.500 \pm 0.024	1.17 \pm 0.14
	Bmr 5778	3	283.8 \pm 0.4	0.714 \pm 0.093	324.4 \pm 1.3	0.640 \pm 0.065	1.14 \pm 0.05
Parenchyma bundle sheath	N 5848	2	281.0 \pm 0.0	1.078 \pm 0.209	324.5 \pm 3.5	0.735 \pm 0.249	1.56 \pm 0.25
	Bmr 5778	2	284.0 \pm 5.7	0.783 \pm 0.071	330.7 \pm 6.6	0.516 \pm 0.077	1.56 \pm 0.14

^{a,b} Values within columns within a cell type with different superscripts differ, $P \leq 0.05$.

region of the epidermis was similar to those of recalcitrant walls; absorbance was lower and digestion of this tissue was often incomplete (Fig. 9). A similar pattern was observed for UV absorption of abaxial epidermal walls of tall fescue (*Festuca arundinacea* Schreb) and of bermudagrass (*Cynodon dactylon* L Pers), although the λ_{max} for bermudagrass was 276 nm (Akin and Rigsby, 1992). Further studies are required to evaluate compounds, their linkages, and their response to degradative enzymes in epidermal walls. Spectra for parenchyma bundle sheaths showed extremely high absorbance near 240 nm with minor shoulders near 280 and 320 nm (Fig. 16). This pattern has not been observed in other plants (Akin and Hartley, 1992a,b; Akin and Rigsby, 1992).

The possibility that protein within the epidermal or parenchyma bundle sheath wall contributed to these unusual spectra was considered. UV absorption spectra of the aromatic amino acids (L-tryptophan, L-tyrosine, and L-phenylalanine) and the plant enzymes horseradish peroxidase and ribulose 1,5 diphosphate carboxylase were dissimilar to the ones for any of the plant walls, although absorption near 276 nm was observed with some compounds (Akin, unpublished data). Absorbance near 320 nm did not occur for any of the amino acids or protein samples investigated.

Tissues that were not degraded from either N or bmr plants at times had different spectral patterns (e.g., Fig. 4, 5), indicating that recalcitrance to digestion may occur by various constituents and/or linkages between wall components. Variations in the monomeric units comprising the lignocellulosic

moieties can influence the spectral patterns (Goto et al., 1992), and further work is needed to relate spectra to biodegradation, especially in regard to various layers of the lignified cells. Other phenolic-containing compounds associated with plant cell walls, such as suberin (Kolattukudy, 1984), could influence spectral patterns, but these components are difficult to extract and have not been analyzed. Knowledge of minimum concentrations of various aromatic compounds for preventing biodegradation could help relate spectra to biodegradation.

The major characteristics of N and bmr cell walls in stems and leaves of pearl millet were a hypsochromic shift in λ_{max} and a higher absorbance ratio (280:320 nm) for N walls. The shift in λ_{max} is consistent with a higher concentration of *p*-coumaric acids in N walls (Hartley et al., 1992; Morrison et al., 1993). The higher absorbance ratio suggests that a more condensed structure for lignocellulose occurs within N tissues. He and Terashima (1991) reported that increased proportions of hydroxycinnamic acid esters added to milled lignin increased UV absorption near 320 nm and caused a bathochromic shift from near 280 to near 290 nm. Studies (Akin and Hartley, 1992b) involving sequential extraction with 1 M NaOH to remove ester-linked phenolic acids resulted in a proportionally greater reduction in absorbance near 320 nm (resulting in a higher 280:320 nm ratio) and a hypsochromic shift toward 280 nm in mestome walls. Evidence that absorbance near 320 nm corresponds to the presence of ester-linked phenolic acids is further supported from microspectrophotometric comparisons between lignified walls of alfalfa, which

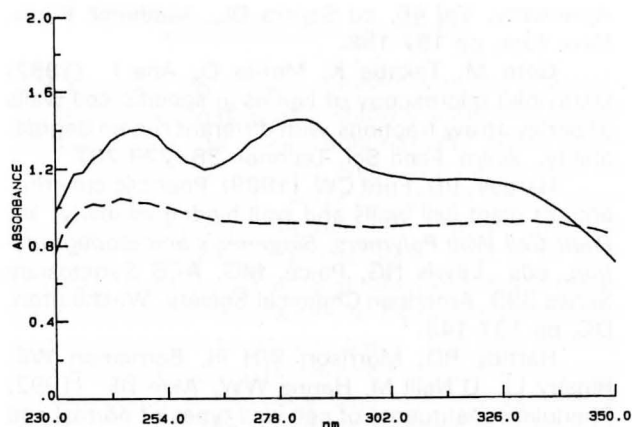


Fig. 12. UV absorption spectra of metaxylem vessels from cryosectioned (4 μ m thick) N (—) and bmr (---) leaf blades of pearl millet.

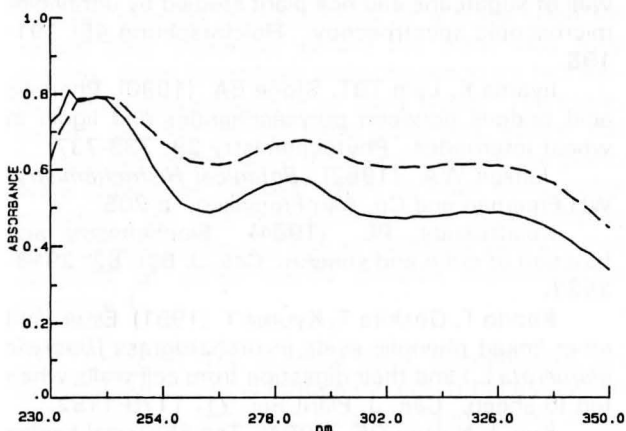


Fig. 13. UV absorption spectra of xylem vessels of small vascular bundles from cryosectioned (4 μ m thick) N (—) and bmr (---) leaf blades of pearl millet.

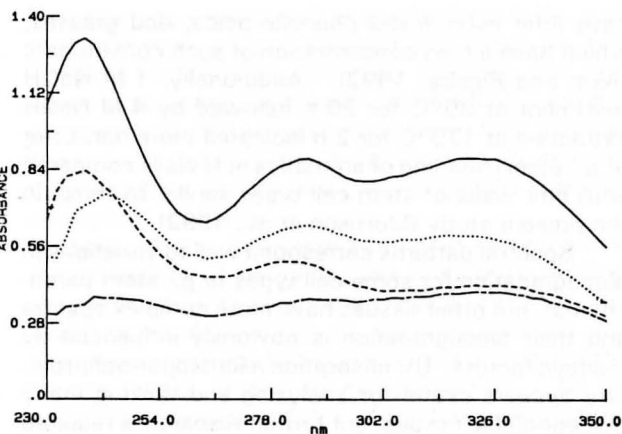
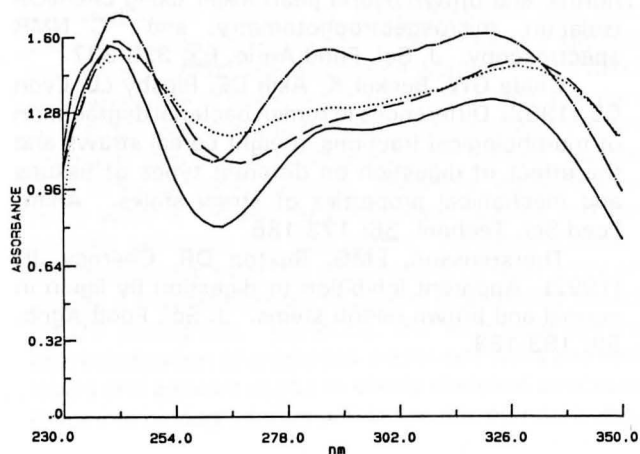


Fig. 15. UV absorption spectra of abaxial epidermal walls from cryosectioned (4 μ m thick) sections of leaf blades of pearl millet. N outer (toward cuticle) region (—), N inner (toward mesophyll) region (---), bmr outer region (····) and bmr inner region (— · — ·). Bmr inner region is from only one leaf.

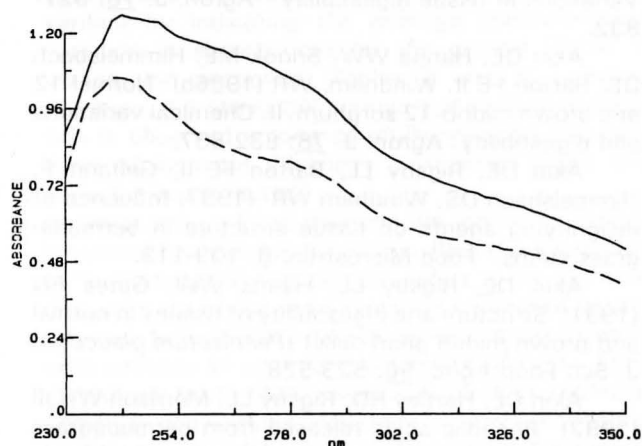


Fig. 16. UV absorption spectra of walls of the parenchyma bundle sheaths from cryosectioned (4 μ m thick) N (—) and bmr (---) leaf blades of pearl millet.

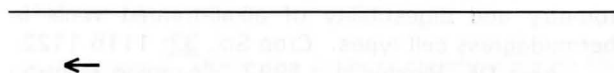


Fig. 14. UV absorption spectra of sclerenchyma from cryosectioned (4 μ m thick) N and bmr leaf blades of pearl millet. N wall with aperture on secondary layer (—), N wall with aperture predominantly on the middle lamella and primary layer and not on the secondary layer (---), bmr wall with aperture on secondary layer (····), bmr wall with aperture predominantly on the middle lamella and primary layer and not on the secondary layer (— · — ·).

have little ester-linked phenolic acids, and grasses, which have a high concentration of such constituents (Akin and Rigsby, 1992). Additionally, 1 M NaOH treatment at 25°C for 20 h followed by 4 M NaOH extraction at 170°C for 2 h indicated more non-ester (e.g., ether) bonding of aromatics in N walls compared with bmr walls of stem cell types similar to those in the present study (Morrison et al., 1993).

Spectral patterns correspond well to variations in biodegradation for some cell types (e.g., stem parenchyma), but other tissues have more complex spectra and their biodegradation is obviously influenced by multiple factors. UV absorption microspectrophotometry appears useful for analyzing and ranking many biodegradable tissues, but further research is required to fully explain the UV absorption spectral patterns in other tissues.

References

- Akin DE, Hanna WW, Rigsby LL (1986a) Normal-12 and brown midrib-12 sorghum. I. Variations in tissue digestibility. *Agron. J.* **78**: 827-832.
- Akin DE, Hanna WW, Snook ME, Himmelsbach DS, Barton FE II, Windham WR (1986b) Normal-12 and brown midrib-12 sorghum. II. Chemical variations and digestibility. *Agron. J.* **78**: 832-837.
- Akin DE, Rigsby LL, Barton FE II, Gelfand P, Himmelsbach DS, Windham WR (1987) Influence of delignifying agents on tissue structure in bermudagrass stems. *Food Microstruc.* **6**: 103-113.
- Akin DE, Rigsby LL, Hanna WW, Gates RN (1991) Structure and digestibility of tissues in normal and brown midrib pearl millet (*Pennisetum glaucum*). *J. Sci. Food Agric.* **56**: 523-528.
- Akin DE, Hartley RD, Rigsby LL, Morrison WH III (1992) Phenolic acids released from bermudagrass (*Cynodon dactylon*) by sequential sodium hydroxide treatment in relation to biodegradation of cell types. *J. Sci. Food Agric.* **58**: 207-214.
- Akin DE, Hartley RD (1992a) UV absorption microspectrophotometry and digestibility of cell types of bermudagrass internodes at different stages of maturity. *J. Sci. Food Agric.* **59**: 437-447.
- Akin DE, Hartley RD (1992b) Microspectrophotometry and digestibility of alkali-treated walls in bermudagrass cell types. *Crop Sci.* **32**: 1116-1122.
- Akin DE, Rigsby LL (1992) Scanning electron microscopy and ultraviolet absorption microspectrophotometry of plant cell types of different biodegradabilities. *Food Struc.* **11**: 259-271.
- Akin DE, Himmelsbach DS, Carl RT, Hanna WW, Barton FE II (1993) Mid-infrared microspectroscopy to assess lignin in plant tissues related to digestibility. *Agron. J.* **85**: 171-175.
- Cherney JH, Cherney DJR, Akin DE, Axtell JD (1991) Potential of brown-midrib, low-lignin mutants for improving forage quality. In: *Advances in Agronomy*, Vol 46, ed Sparks DL, Academic Press, New York, pp 157-198.
- Goto M, Takabe K, Morita O, Abe I (1992) Ultraviolet microscopy of lignins in specific cell walls of barley straw fractions with different rumen degradability. *Anim. Feed Sci. Technol.* **36**: 229-237.
- Hartley RD, Ford CW (1989) Phenolic constituents of plant cell walls and wall biodegradability. In: *Plant Cell Wall Polymers, Biogenesis and Biodegradation*, eds Lewis NG, Paice, MG, ACS Symposium Series 399, American Chemical Society, Washington, DC, pp 137-145.
- Hartley RD, Morrison WH III, Borneman WS, Rigsby LL, O'Neill M, Hanna WW, Akin DE (1992) Phenolic constituents of cell wall types of normal and brown midrib mutants of pearl millet (*Pennisetum glaucum* (L) R Br) in relation to wall biodegradability. *J. Sci. Food Agric.* **59**: 211-216.
- He L, Terashima N (1991) Formation and structure of lignin in monocotyledons IV. Deposition process and structural diversity of the lignin in the cell wall of sugarcane and rice plant studied by ultraviolet microscopic spectroscopy. *Holzforschung* **45**: 191-198.
- Iiyama K, Lam TBT, Stone BA (1990) Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochemistry* **29**: 733-737.
- Jensen WA (1962) *Botanical Histochemistry*. WH Freeman and Co, San Francisco, p 205.
- Kolattukudy, PE (1984) Biochemistry and function of cutin and suberin. *Can. J. Bot.* **62**: 2918-2933.
- Kondo T, Ohshita T, Kyuma T (1991) Ester- and ether-linked phenolic acids in orchardgrass (*Dactylis glomerata* L.) and their digestion from cell walls when fed to sheep. *Can. J. Plant Sci.* **71**: 1179-1182.
- Kuc J, Nelson OE (1964) The abnormal lignins produced by the brown-midrib mutants of maize I. The brown-midrib-1 mutant. *Arch. Biochem. Biophysics* **105**: 103-113.
- Morrison WH III, Akin DE, Himmelsbach DS, Gamble GR (1993) Investigation of the ester- and ether-linked phenolic constituents of cell wall types of normal and brown midrib pearl millet using chemical isolation, microspectrophotometry, and ¹³C NMR spectroscopy. *J. Sci. Food Agric.* **63**: 329-337.
- Ohlde GW, Becker K, Akin DE, Rigsby LL, Lyon CE (1992) Differences in rumen bacterial degradation of morphological fractions in eight cereal straws and the effect of digestion on different types of tissues and mechanical properties of straw stalks. *Anim. Feed Sci. Technol.* **36**: 173-186.
- Thorstensson, EMG, Buxton DR, Cherney JH (1992) Apparent inhibition to digestion by lignin in normal and brown midrib stems. *J. Sci. Food Agric.* **59**: 183-188.

Discussion with Reviewers

J.H. Cherney: My primary concern with any anatomical study investigating degradability is the selection of representative and comparable tissues. Millet internodes elongate for about 1 week, but lignify over an extended time period. After elongation, lignification proceeds up a given internode with differing amounts of lignin throughout the internode, until it is fully lignified. Care should be used when selecting a representative section from within an internode in one plant and comparing it to another plant. It is very unlikely that the same internode (counting down from the top) is developing at the same time in a bmr vs. normal plant, and also the internode selected in a bmr vs. normal plant would probably not be at the same stage of lignification. Is there any way to provide a little more confidence in this tissue of representative tissue selection, since it would appear to affect UV absorption results?

Authors: Vegetative plants were chosen for this study to have biomass representative of a stage used for forage and, therefore, before the plant was fully mature. Stem internodes were chosen to try to maximize the lignin present in all plant types. Therefore, center portions of full internodes just above the harvest cut, which were the oldest parts of the plant, were chosen as our best effort to have comparable samples. We feel that evaluation of bulk samples from several plants and also of individual layers in thin sections of other plants provide representative samples.

J.H. Cherney: The authors have concentrated on bmr/N pearl millet. Although the bmr trait has numerous similarities in millet, sorghum, and maize, there are also significant differences between them (for example, digestion rates differ between bmr and N sorghum, but not maize). What are the implications of the use of this technology if bmr/N UV patterns differ by species.

Authors: UV microspectrophotometry is a powerful tool for investigating aromatics in plant cell walls, but interpretations related to biodegradation are not always clear as shown in the present study. Progress toward resolving these situations would be facilitated by knowledge of the type of aromatic within a region of the cell wall and the minimum concentration of an aromatic that limits biodegradation. These factors may differ for various plants. Therefore, considerably more work is needed for other plant species and for specific cell walls and wall layers. As more information is available from this technique on a wide array of plants, it may be possible to predict digestibility based on UV absorption. Currently, the technique allows characterization of aromatics within cell walls and cell layers and appears useful to clarify chemical structure related to biodegradation within specific comparisons.

J.H. Cherney: Leaves mature and lignify quickly, such that any area of leaf sampled will likely be fully lignified. Internodes, however, elongate and then lignify in acropetal succession, taking about 1 week to elongate and many weeks to become fully lignified. How does the degree of lignification in selected representative regions of a given internode affect UV absorption results?

Authors: A UV absorption study was carried out on the midpoints of bermudagrass internodes at three stages of maturity (Akin and Hartley, 1992b). This study showed changes in spectral patterns and absorbance with age for secondary and primary/middle lamella layers related to biodegradation. To our knowledge, no work of this nature has been reported on regions within an internode.

P.J. Weimer: The use of three significant figures in the absorbance data seems unnecessary and inappropriate in light of the very large variations in the data among replicates (Tables 1-3). The authors claim that thin sections provide more uniform samples for spectral characterization. This could be quantitatively verified by indicating the average coefficients of variation in absorbances for the ground (Table 1) versus thin sectioned (Tables 2 and 3) samples. However, it is still rather curious that such large variation is observed among carefully-prepared thin sections of identical thickness. To what might this variation be attributed?

Authors: We prefer to maintain the format for reporting used in previous publications. Part of our meaning of "uniformity" of thin sections refers to the fact that cell walls are of the same type and number of layers, whereas bulk samples may be several layers of walls with perhaps an unavoidable mixture of cells. Even though we try to obtain exactly 4 μm thick sections, there is unavoidable variation in any sectioning. Further, there is obviously variation among the various cell sites that contributes to the standard deviation. However, one problem with presentation of data is that the cell wall itself occludes light, and while there is not absorption maxima due to cellulose and hemicellulose, there is non-specific "background" absorption. This greatly affects the statistical data (Tables), and differences can often be more easily seen from the spectra.

P.J. Weimer: While the authors clearly indicate that the 124 h digestion time was used to maximize differences in digestibility, it should be pointed out that retention times of feed particles in the rumen are normally much shorter. Have the authors done any microspectrophotometric studies on digestion times in the 20-50 h range?

Authors: No and in fact this is the first work where we have evaluated plant material by UV absorption after digestion has occurred. Dr. Weimer's comments

suggest an important avenue that needs further exploration.

P.J. Weimer: Why would extremely thin-walled cells within the rind not produce clear spectral patterns? In the transmission mode, such thin sections would be expected to give strong signals.

Authors: I believe the problem with these walls is related to instrumentation. The size of cell wall was small in comparison with the aperture used to delimit the area for collection, possibly leading to an inadequate concentration of aromatics for interference of the background.

P.J. Weimer: Overall, the spectra correlate reasonably well with known aspects of structure and digestibility of the normal and brown midrib varieties. However, the spectra peaks are very broad and relatively featureless. Can additional information be obtained from transformation of the data (e.g., by using first or second derivatives)?

Authors: We have not tried to transform the data, but we hope to use software that will allow us to sharpen the peaks. I think it is highly likely that more information can be derived from these approaches.

P.J. Weimer: The authors only briefly allude to the potential contribution of the aromatic residues of suberin to the observed spectra. This point merits further discussion. In what tissue types and wall regions is suberin localized? Are there spectral anomalies in these tissues and wall regions that might be explained by significant concentrations of suberin? Are there any contributions of other structural classes (e.g., cutins) to the UV absorbances?

Authors: Dr. Weimer has brought up an important area, but unfortunately little is known about suberins or cutins other than in the cuticle. With the exception of some work on parenchyma bundle sheaths of warm-season grass leaves (Wilson and Hattersley, 1983. *Grass For Sci* 38:219-223), I am not aware of results showing the presence of suberin in cell walls. It is likely that such compounds do exist in other tissues and influence digestion and hopefully research will provide information related to this question. We mention suberin in our Discussion to alert readers to this possibility that aromatics in suberin could contribute to our results, but lack of information precludes more being written at this time.

L.H. Harbers: We have observed differences in fluorescence of parenchymal tissues of sorghum stem internodes. Did you make any observations on stems other than the 6th to 8th internodes from the apex within the same individual plant?

Authors: We did not make observations on internodes other than those listed in the present study. However, we have evaluated bermudagrass internodes at different maturities (Akin and Hartley, 1992a). In this

work, immature parenchyma had no UV maxima in spectra, but as walls matured UV absorption maxima occurred and spectra were indicative of ester-linked aromatics. The middle lamella regions showed greater absorption than other wall regions.