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INFLUENCE OF DELIGNIFYING AGENTS ON TISSUE STRUCTURE IN BERMUDAGRASS STEMS

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Abstract

Permanganate, dioxane, ozone, and hydrogen peroxide, which delignify plant materials, were evaluated for their disruptive action on plant structure, their modification of histological reactions for lignins, and their change in in vitro digestibility by rumen microorganisms of young (second internode from top) and old (fourth and fifth internodes) bermudagrass stems. Epidermis, sclerenchyma ring, and vascular tissue (except phloem) gave positive reactions with acid phloroglucinol (AP) or chlorine-sulfite (CS) in all samples, whereas cortex and parenchyma in older stems gave a positive reaction with CS. Treatment with delignifying agents reduced the reactions for lignin, with permanganate-treated tissues having the least reaction. Gravimetric data indicated that $KMnO_4$ removed about 25% of the dry matter, with the other treatments resulting in no loss with ozone to 34% with hydrogen peroxide. Scanning electron microscopy (SEM) indicated that delignifying agents distorted parenchyma tissues, often resulting in collapsed cell walls. Permanganate especially disrupted parenchyma and the more rigid vascular bundle tissue and caused the most destruction of all the treatments. Ozone was effective in partially breaking down lignified vascular tissue in one sample set but not in a second, more mature set. Delignification resulted in improved in vitro dry matter digestibility by rumen microorganisms for the resultant fiber compared with neutral detergent fiber for all treatments, with permanganate causing the largest increase for both sample sets. SEM showed that chemical treatments improved degradation of the cortex and parenchyma, whereas the epidermis, sclerenchyma ring, and vascular tissue (except phloem) still resisted microbial breakdown.

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Introduction

Electron microscopy has been useful in providing information on plant cell wall degradation by rumen microorganisms from a unique perspective (Akin, 1979; Harbers, 1985). The availabilities of specific tissues in many forages for rumen bacterial fermentation have been elucidated, and anatomical and structural factors which limit microbial degradation have been identified. Scanning electron microscopy (SEM) coupled with light histological techniques have shown that cell walls containing lignin are the least digested tissues in forages. Lignin, a polymer of phenylpropanoid units, is chemically associated with the structural carbohydrates of the plant fiber (Himmelsbach and Barton, 1980) and is recognized as a major limitation to forage utilization (Van Soest, 1973; Waldo et al., 1972).

Lignins vary in type based upon the predominant monomeric unit of the polymer, i.e., p-coumaryl, coniferyl, or syringyl units (Barton et al., 1983). Histochemical tests have also shown differences in types of lignin within cell walls, with the acid phloroglucinol test indicating cinnamaldehyde units and the chlorine-sulfite test indicating syringyl units (Vance et al., 1980).

Stems of grasses decrease in digestibility with increased maturity more than leaf materials (Hanna et al., 1976). Stem anatomy consists of a large proportion of highly lignified and poorly digested tissues, and increased maturity results in even more of the tissues becoming lignified and resisting microbial degradation (Akin et al., 1984; Hanna et al., 1976; Pigden, 1953). The result of increased age is a rigid plant residue in which only a small portion of the parenchyma is digested. It is likely that the rigid stem anatomy in grasses contributes to the lower feed consumption by ruminants generally noted for grasses vs. legumes (Demarquilly and Jarrige, 1974) and for grass stems vs. grass leaves (Laredo and Minson, 1973), possibly by influencing the retention time of undigested fiber in the rumen (Hendricksen et al., 1981). In another study the greatest result of KOH treatment to improve forage utilization was that the parenchyma, which reacts with chlorine-sulfite, was made available for digestion whereas acid phloroglucinol reacting-tissues, although disrupted, were still not available for microbial utilization (Spencer et al., 1984). Similarly, treatment of 100 μm sections of bermudagrass with $KMnO_4$ removed phenolic compounds preferentially from the parenchyma and made this tissue more

available for digestion (Akin et al., 1985). These data indicate that the lignin in parenchyma differs in a significant manner from that in the sclerenchyma ring or vascular tissues, such that the parenchyma cell walls are more susceptible to chemical treatment.

By oxidizing the lignin in cell walls, researchers have been able to make plant fiber more available to microbial fermentation (Barton and Akin, 1977; Ben-Ghedalia and Miron, 1981). However, it is clear that all lignified cell walls are not the same, and information is not available on the effect that oxidizing agents have on specific types of lignified fiber in stem. The influence that delignification has on specific plant structure and on cell wall digestibility by rumen microorganisms is important for developing strategies to improve forage utilization. Therefore, the objective of the present work was to evaluate the response of specific tissues of bermudagrass stems at two maturities to various delignifying agents. The stems were evaluated for the response to histochemical tests, the degree of structural alteration, dry weight loss due to extraction, and the improvement in in vitro degradation by rumen microorganisms.

Materials and Methods

Plant samples

Coastal bermudagrass (Cynodon dactylon) was grown in well managed fields near Athens, Georgia. Experiments were conducted on each of two cuttings (experiments 1 and 2) of six-week-old regrowth material harvested in the summer of 1982. Harvested material was stored at -10°C until used. Procedures and treatments were the same for samples in both experiments except that samples in experiment 1 were not treated with H_2O_2 and samples from experiment 2 were not treated with dioxane.

The second internodes from the top of plants (young internodes) and the combined fourth and fifth internodes (old internodes) were hand-separated from leaf blades and sheaths. For gravimetric studies, each sample of stem material was freeze-dried and ground through a 20 mesh (1mm) screen. For light microscopy, free-hand sections were cut from internodes representing five plants for each experiment. For scanning electron microscopy, 3mm sections were cut from 20 stems.

Histological tests for lignin

Free-hand sections were stained for lignin using the acid phloroglucinol (AP) and the chlorine-sulfite (CS) reactions as described by Jensen (1962), except that 2% Na_2SO_3 was used in the CS reaction. Reactions for lignin were scored as + (positive reaction), or O (no reaction).

Chemical delignification

Neutral detergent fiber (NDF) was prepared from ground or intact sections (3mm) of stem material (Van Soest and Wine, 1967, but without sodium sulfite) for subsequent delignification of plant cell walls. $KMnO_4$ delignification was carried out according to procedures of Van Soest and Wine (1967, 1968) as modified by Barton et al. (1976) and Barton and Akin (1977). Ground stems or intact sections of NDF material were extracted with neutral $KMnO_4$ for 3 h and demineralized with buffered oxalic acid in t-butanol for 1.5 h. The stem materials were washed with water and 80% ethanol and acetone 3 times each. The ground material was dried at 105°C overnight and the sections were air-dried at 28 mm Hg.

Dioxane delignification was carried out on 5 g of ground stems or 25 intact sections of NDF. Samples were preextracted with a benzene-ethanol (2.5:1 v/v) mixture at 39°C for 48 h to remove chlorophyll and waxes and freeze dried for 48 h. The samples were then treated with a 1% pepsin - 0.1 N HCl solution for 48 h at 39°C according to the procedure of Routley and Sullivan (1958) to remove protein, followed by centrifugation and freeze drying. The residue was then soxhlet-extracted with 150 ml of a 0.2 N (dry) HCl in dioxane/2, 2-dimethoxy propane solution (6.5:1 v/v) according to the procedure of Bolker and Teraschima (1966) in order to remove lignin. The resultant residue was then washed with fresh dioxane followed by ethylether and air dried.

For ozone delignification, NDF samples (5 g ground or 25 intact sections) were placed in a culture tube fitted with a septum and subjected to an ozone atmosphere generated by a glass micro-ozonizer (Supelco). The procedure was that of Beroza and Bierl (1967) except that the ozonization was performed on dried material. Ozone was introduced into the sample tube until the indicator solution (4 ml of 0.3% tetraphenylcyclopentadieone in CH_2Cl_2 solution) changed color (about 10 min). For experiment 1, the tube was let stand for 24 h and then flushed with nitrogen to remove excess ozone. For experiment 2 the ozone-treated material was not left in ozone for additional time or flushed with nitrogen.

Hydrogen peroxide delignification was carried out according to Gould (1985). One g of ground material or 20 to 40 stem sections were treated with 30 ml of 1% H_2O_2 at room temperature for 6 h. The pH was adjusted initially to 11.5 with 50% NaOH and every 30 min thereafter. After 6 h, samples were washed with distilled water and prepared as with $KMnO_4$ for future use.

In vitro fermentation

The dry weight loss of dried, ground material was determined in duplicate tubes after incubation with rumen microorganisms for 48 h followed by incubation with acidic pepsin for 48 h according to the Tilley and Terry (1963) in vitro dry matter digestibility (IVDMD) procedure. The digestibility of intact tissues in 3mm sections after in vitro incubation with rumen microorganism for 48 h was determined by SEM. For both gravimetric and SEM studies, rumen fluid was collected from a ruminally-cannulated steer fed Coastal bermudagrass hay plus 2 kg per day of grain concentrate (74% oats, 10% corn, 15% soybean meal, 0.5% trace minerals, and 0.5% defluorinated phosphate). The rumen fluid was strained through 4 layers of cheese cloth, then mixed with McDougall's (1948) carbonate buffer (1:2 rumen fluid to buffer), and 30 ml was dispensed into 50ml centrifuge tubes. The mixture was purged with CO_2 and the tubes were then capped with a one-way valve to exclude O_2. Fermentation tubes were incubated at 39°C.

Preparation for SEM

Stem sections that were not incubated with rumen fluid and those that were incubated with rumen fluid for 48 h were evaluated and included the following treatments: untreated; neutral detergent-treated fiber (NDF); and NDF sections delignified with $KMnO_4$, dioxane (experiment 1 only), ozone, or H_2O_2 (experiment 2 only). Sections were placed into 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4,

TABLE 1: Reactions of bermudagrass stem tissues for lignin with acid phloroglucinol (AP) and chlorine-sulfite (CS).

		Tissues[1]									
		Epidermis		Sclerenchyma Ring		Vascular Bundle		Cortex		Parenchyma	
Internode Age	Exp. No.	AP	CS	AP	CS	AP	CS	AP	CS	AP	CS
Young	1	+*	+**	+	+	+	+	0	0	0	0
Old	1	+	0	+*	+	+	+	0	+	0	+
Young	2	+	+	+	0	+	+	0	+	0	+
Old	2	+	0	+	0	+	+	0	+	+**	+

[1] + (positive reaction), 0 (no reaction) to stains.
*Reaction not present in all cells. **Slight Reaction in only a few sections.

TABLE 2: Reactions of bermudagrass stem tissues for lignin with acid phloroglucinol (AP) and chlorine-sulfite (CS) after chemical treatment (Experiment 1).

		Tissues[1]									
		Epidermis		Sclerenchyma Ring		Vascular Bundle		Cortex		Parenchyma	
Treat-ment	Internode Age	AP	CS	AP	CS	AP	CS	AP	CS	AP	CS
NDF	Young	+	+	+	+	+	+	0	+	0	+*
	Old	+	0	+	+	+	+	0	+	0	+
$KMnO_4$	Young	0	0	0	0	0	0	0	0	0	0
	Old	+	0	+	0	+	0	0	0	0	0
Dioxane	Young	0	+	0	+	0	+	0	0	0	0
	Old	0	+	0	+	0	+	0	+*	0	+*
Ozone	Young	+	+	+*	+*	+	+	0	0	0	0
	Old	+	+	+*	+*	+	+	0	0	0	+*

[1] + (positive reaction), 0 (no reaction) to stains. *Not all cells stained.

at 8°C and fixed for several days. Sections were then postfixed in 1.5% OsO_4 buffered as above for 4 h at 8°C. Sections in experiment 1 were rinsed with 0.1 M cacodylate buffer and air-dried. Because of partial collapse of the parenchyma cells noted in experiment 1, stem sections in experiment 2 were critical point dried in liquid CO_2 after dehydration in a graded ethanol series. Dried sections were adhered to aluminum stubs, coated with Au-Pd alloy, and examined in a Philips 505 T scanning electron microscope at 15 kV.

Results

Histological tests for lignin revealed differences between the young and old internodes in experiment 1 (Table 1). In young stems, the epidermis, sclerenchyma ring, and vascular bundle (except phloem) were positive (+) or slightly positive (±) for both AP and CS, whereas the cortex and parenchyma did not stain for lignin. In old stems, the epidermis, sclerenchyma ring, and vascular tissue (except phloem) were intensely positive for lignin with AP, with a CS+ reaction also occurring in the cortex and the centrifugal cells of the parenchyma. Although internodes from similar positions on the plants were chosen in experiment 2, the second internode samples gave reactions indicative of an older age than young stems of experiment 1, with a positive reaction for CS occurring in the cortex and parenchyma for both ages of stems (Table 1).

Internodes of experiment 1 that had been chemically treated to remove lignin were tested for histological reactions for lignin (Table 2). Reactions for NDF were similar to those in untreated stems, except that a slight or partial reaction with CS occurred in young cortex and parenchyma. All delignifying treatments reduced the reactions in

lignified tissues, but results varied for individual treatments. $KMnO_4$ resulted in the least reaction for lignin, with no reaction in young internodes and only AP+ reactions in epidermis, sclerenchyma ring, and vascular tissues in old stems. Dioxane resulted in CS+ reactions only, with reactions occurring mostly in the epidermis, sclerencyma ring, vascular tissue, and in old cortex and parenchyma tissues. Ozone reduced the lignin reactions in AP+ and CS+ tissues. A reaction with CS in the epidermis after dioxane or ozone treatment compared with the lack of a reaction in control material (Table 2) may indicate that a greater availability or different phenolic entities occurred after treatment, resulting in a stronger histological reaction. While not as rigorously evaluated, H_2O_2 - treated stem sections from experiment 2 showed that this delignifying agent also reduced the AP reaction for lignin compared with NDF samples.

TABLE 3: Dry weight loss of NDF due to chemical extraction.

Chemical treatment	Internode age	% dry weight loss Experiment 1	Experiment 2
$KMnO_4$	Young	23.3+0.3	28.7+0.7
	Old	20.2∓0.6	27.7∓0.9
Dioxane	Young	19.8[1]	ND
	Old	16.1	ND
Ozone	Young	0	0
	Old	0	0
H_2O_2	Young	ND	33.4+0.4
	Old	ND	34.9∓0.2

[1]Bulked samples and no standard deviation.
ND = Not determined

The delignifying chemicals removed variable amounts of materials on a dry weight basis from the ground NDF sample (Table 3). $KMnO_4$ removed an average of 25% while H_2O_2 removed an average of 34% and dioxane an average of 18%; ozone resulted in virtually no dry weight loss. In all but the H_2O_2 treatment, old internodes gave up less dry matter during extraction than did young internodes, but differences were small (1 to 4 percentage units).

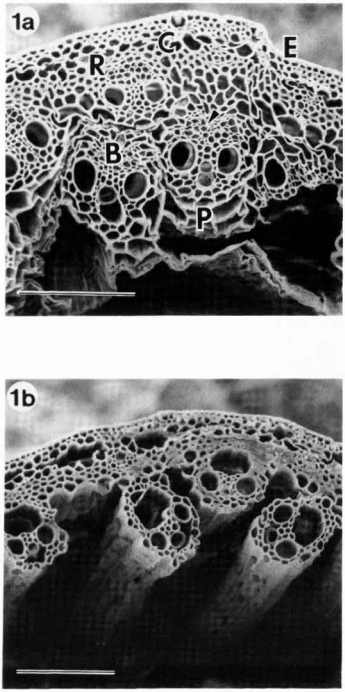
The dry matter digestibilities of untreated and delignified NDF by rumen microorganisms are shown in Table 4. The old stems in both experiments were less digestible than young ones, but material of each age in experiment 2 was less digestible than that for the corresponding position in experiment 1. All chemical treatments improved the IVDMD of the resulting fiber with old stems showing more improvement for all treatments. However, the degree of improvement varied markedly with treatments (Table 4) and further improvement might be possible under optimum conditions. $KMnO_4$ resulted in the greatest improvement in digestibility for both ages of samples and for both experiments.

SEM showed the responses of individual tissues to chemical treatment and to incubation with rumen microorganisms (Figs. 1-10). In experiment 1 (without critical point drying) most of the tissues in the top stems were intact and easily recognized; however, often the parenchyma tissues were collapsed. The NDF stems (Fig. 1a) were similar to untreated sections (not shown). Incubation with rumen fluid for 48 h resulted in loss of parenchyma, phloem, and cortex (Fig. 1b). $KMnO_4$ produced marked distortion and collapse of tissues such that only small parts of the stem could be recognized (Fig. 2a). That cell wall material had been markedly altered was confirmed by the substantial destruction of the stems and removal of all but the most rigid tissue after incubation in rumen fluid (Fig. 2b) and the recovery of mostly small pieces after fermentation. Treatment of top stems with dioxane (Fig. 3a) and ozone (Figs. 4a) produced distortion and collapse in tissues,

TABLE 4: IVDMD of NDF bermudagrass stems treated with delignifying agents.

Chemical treatment	Internode Age	IVDMD Experiment 1 %	Experiment 1 % increase of NDF	Experiment 2 %	Experiment 2 % increase of NDF
NDF	Young	58.7+9.5		49.6+0.8	
	Old	43.0∓0.7		39.6∓0.0	
$KMnO_4$	Young	73.5+0.6	25	66.0+2.6	33
	Old	59.6∓2.6	39	69.2∓1.1	75
Dioxane	Young	61.7+1.6	5	ND	ND
	Old	59.7∓1.3	39	ND	ND
Ozone	Young	63.5+1.4	8	50.3+0.1	1
	Old	ND	ND	50.0∓0.4	26
H_2O_2	Young	ND	ND	56.6+0.4	14
	Old	ND	ND	54.7∓0.0	38

ND = Not determined



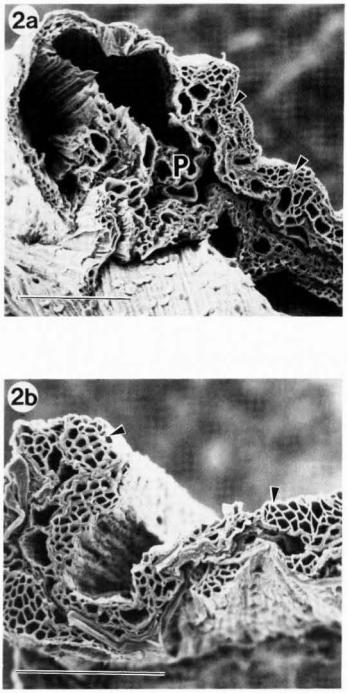
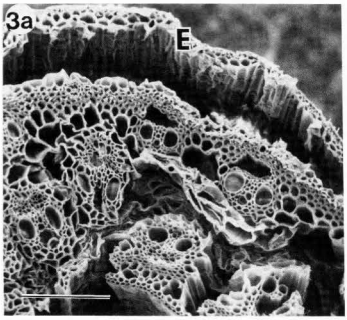


Figure 1. Young internode of NDF. (a). No incubation. All tissues are present but parenchyma is distorted. E = epidermis, C = cortex, R = sclerenchyma ring, B = vascular bundle, P = parenchyma, arrow points to phloem. (b). Incubated with rumen fluid. Parenchyma, phloem, and much of cortex is degraded leaving a residue of epidermis, sclerenchyma ring, and lignified vascular tissue. Bar = 100 μm.

Figure 2. Young internode of NDF treated with $KMnO_4$. (a). No incubation. Tissues are markedly distorted with portions of sclerenchyma ring (arrows) and parenchyma (P) distinguishable. (b). Incubated with rumen fluid. Sclerenchyma ring (arrows) is distinguished but other tissues are disrupted beyond recognition. Bar = 100 μm.

particularly in parenchyma. Microbial fermentation of stems treated with dioxane (Fig. 3b) and ozone (Fig. 4b) showed that only epidermis, sclerenchyma ring, and vascular tissue comprised the residue, with some parenchyma and cortex remaining in some of dioxane-treated stems. Ozone treatment followed by microbial fermentation produced a stem residue in which the extremely resistant xylem cells were partially broken down (Fig. 4b), indicating that this treatment resulted in more breakdown of the most resistant tissues than did dioxane. The H_2O_2 treatment (Figs. 5a,b) resulted in removal of phloem and portions of the parenchyma and appeared less disruptive than other treatments perhaps because of the more rigid nature of the parenchyma in this sample.

In old stems, NDF responded the same as the untreated sections. Microbial fermentation of NDF stems resulted in removal of the phloem and portions of the cortex and parenchyma (Figs. 6a,b). Parenchyma centrifugal to the stem center was less available than that near the center, and the lack of digestion in this tissue is consistent with CS+ reactions for lignin (Table 1). As in young stems, $KMnO_4$ of old stems resulted in marked distortion and collapse of tissues (Fig. 7a) and removal or partial destruction after microbial fermentation of all but the epidermis and sclerenchyma ring; the vascular bundles were especially affected (Fig. 7b).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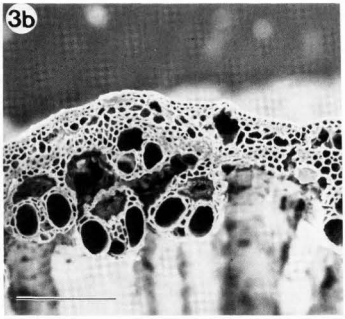
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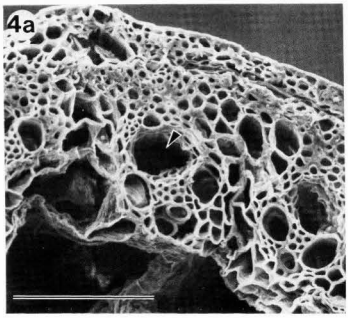
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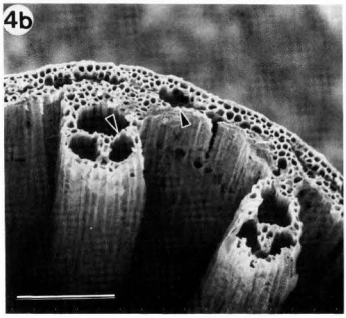
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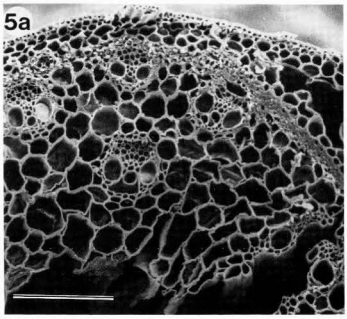
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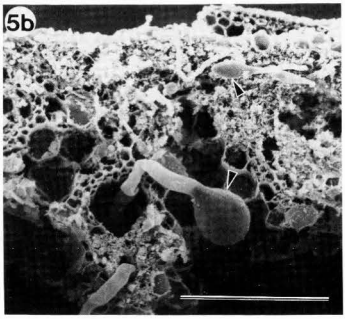
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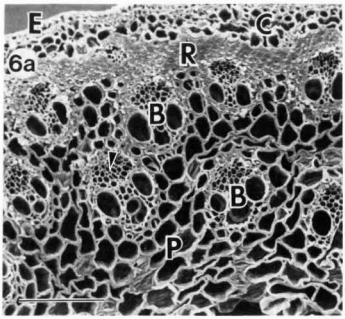

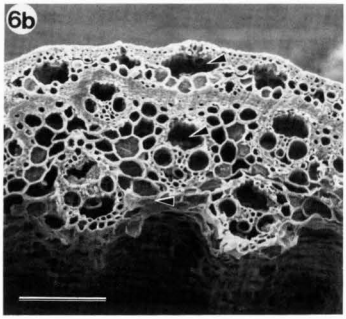

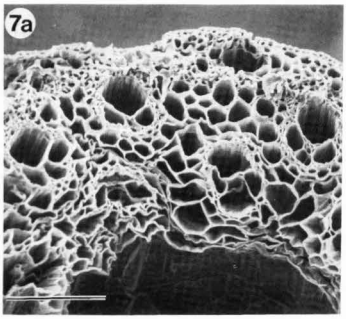

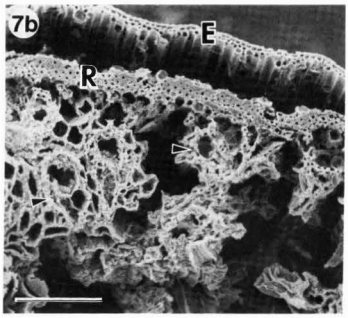

Figure 3. Young internode of NDF treated with dioxane. (a). No incubation. Tissues are mostly present, but parenchyma is collapsed. The epidermis (E) is separated from the remainder of the stem in places, and the whole section is cracked possibly due to air-drying and physical damage during handling. (b). Incubated with rumen fluid. Only epidermis, sclerenchyma ring, and lignified vascular tissue remain, and parenchyma, phloem, and cortex are removed. Bar = 100 μm.

Figure 4. Young internode of NDF treated with ozone. (a). No incubation. Tissues are distorted and phloem (arrow) and parenchyma are removed in some areas. (b). Incubated with rumen fluid. The residue consists of epidermis, sclerenchyma ring, and lignified vascular tissue; the latter two tissues show some evidence of attack (arrows). Bar = 100 μm.

Figure 5. Young internode of NDF treated with H_2O_2. (a). No incubation. All tissues are intact. (b). Incubated with rumen fluid. Degradation of phloem and parenchyma occurred. Rumen fungi are prevalent as shown by the sporangia (arrows). Bar = 100 μm.

Figure 6. Old internode of NDF. (a). No incubation. All tissues are intact, but the parenchyma is distorted. E = epidermis, C = cortex, R = sclerenchyma ring, B = vascular bundle, P = parenchyma, arrow points to phloem. (b). Incubation with rumen fluid. Degradation occurred in the phloem, parts of the cortex, and the centripetal regions of the parenchyma (arrows). Bar = 100 μm.

Figure 7. Old internode of NDF treated with $KMnO_4$. (a). No incubation. Tissues are distorted but intact, except for the loss of phloem. (b). Incubation with rumen fluid. Epidermis (E) and the sclerenchyma ring (R) are intact, but the lignified vascular tissue (arrows) is disrupted. The cortex and a large portion of the parenchyma is removed. Bar = 100 μm.

Marked destruction of tissues subjected to $KMnO_4$ and microbial fermentation occurred in both experiments. Treatment with dioxane (Fig. 8), ozone (Fig. 9), or H_2O_2 (Fig. 10) resulted in removal of phloem and partial degradation of cortex and parenchyma, leaving a residue of epidermis, sclerenchyma ring, vascular tissue, and parts of the parenchyma. Ozone treatment in experiment 1 (Fig. 9) resulted in marked destruction of parenchyma and a greater breakdown of the vascular bundle (Fig. 9b) than with all other treatments except $KMnO_4$. However, stems in experiment 2 did not show this marked destruction of vascular tissue but resembled more the tissue after dioxane or H_2O_2 treatment. The shorter time of treatment and more mature sample in experiment 2 could explain this variation in results.

Discussion

While delignifying agents have been used extensively to improve forage digestibility, the effects on plant structure have not been studied to a large extent. Our data showed that $KmnO_4$ resulted in the greatest reduction in histological staining for phenolic compounds, the greatest loss of dry matter, the greatest disruption of lignified tissues, and the greatest improvement in digestibility of resultant fiber from bermudagrass stems. Other workers (Darcy and Belyea, 1980) reported that treatment with permanganate resulted in an increased rate and extent of cellulose degradation in forage grass. $KMnO_4$ has been shown to preferentially extract phenolics from the CS+ tissues in bermudagrass stems, making them totally digestible (Akin et al., 1985). We have shown in this study that $KMnO_4$ disrupted the AP+ tissues even though some of them still resisted microbial utilization. All of the other three delignifying agents have been shown to improve fiber utilization (Han et al., 1975; Bunting et al., 1984; Kerley et al., 1985). SEM indicated that ozone disrupted parts of the AP+ tissues (i.e., vascular bundles), resulting in more apparent destruction of these lignified cell walls than dioxane or H_2O_2. It has been reported that ozone can oxidize lignin and thereby disrupt the lignocellulose complex (Bunting et al., 1984). Our data suggested that the more resistant AP+ tissues in bermudagrass stems, however, still were not made available to microbial digestion with our method of ozone treatment. Nonetheless, the destruction of vascular tissue with ozone in experiment 1 shows potential for this treatment and it should be explored further. Recently, alkaline peroxide delignification has been used to disrupt the lignocellulose complex (Gould, 1985) and the treatment increased the fermentability of wheat straw (Kerley et al., 1985). SEM of H_2O_2 - treated wheat straw indicates that the plant structure was extensively disrupted, and that bacterial colonization of fiber was improved (Kerley et al., 1985). However, as with dioxane and ozone for the most part, AP+ tissues were often resistant to microbial utilization in H_2O_2 - treated bermuda stems.

Conclusions

Bermuda stems increase in lignification with age, and an increase in the CS+ reaction in the cortex and parenchyma is the most notable change in histological reactions. Various chemical treatments to delignify fiber have resulted in marked improvement in degradation of the cortex and parenchyma, with total degradation in some treatments. The AP+ tissues (i.e., epidermis, sclerenchyma ring, and vascular tissue) are the most resistant tissues, and none of the delignifying agents resulted in total microbial breakdown of these tissues. Observations by SEM on plant cell wall destruction related closely to gravimetric losses for the delignifying agents.

Research to reduce the amount of AP+ tissues in stems or to delay CS+ lignification in parenchyma with maturity would appear to be profitable avenues to explore for improving forage quality. Research to optimize the ozone method might prove especially useful, since this treatment appeared to partially degrade the most resistant, AP+ tissues. Treatments that degrade lignin may produce soluble phenolic compounds that are toxic to rumen microorganisms, and this aspect should not be overlooked in studies to enhance forage utilization.

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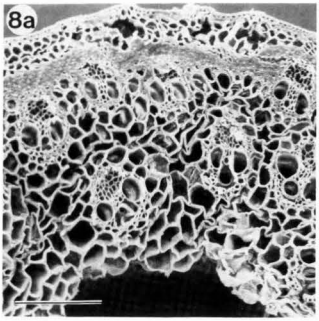
Figure 8. Old internode of NDF treated with dioxane. (a). No incubation. Tissues are intact, but parenchyma is distorted. (b). Incubation with rumen fluid. Only phloem and parts of the cortex and parenchyma are degraded. Bar = 100 μm.

Figure 9. Old internode of NDF treated with ozone. (a). No incubation. Tissues are intact, except for the loss of phloem. Starch granules are prevalent on the section surface. (b). Incubated with rumen fluid. Phloem Cortex, and parenchyma are virtually all degraded. The lignified cells of the vascular bundles also show partial degradation (arrows). Bar = 100 μm.

Figure 10. Old internode of NDF treated with H_2O_2. (a). No incubation. All tissues are intact. (b). Incubated with rumen fluid. Degradation occurred in the cortex and the centripetal parts of the parenchyma. An obvious microbial mat covers the stem surface. Bar = 100 μm.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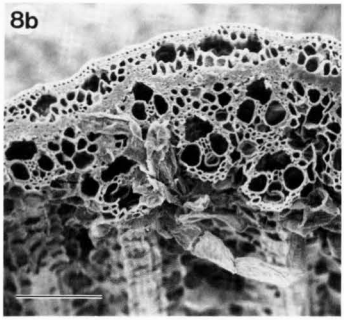
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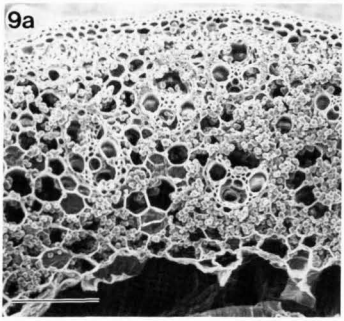
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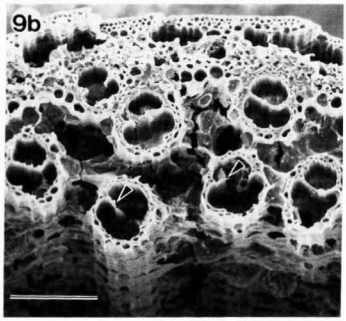
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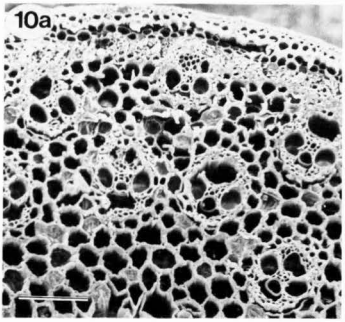
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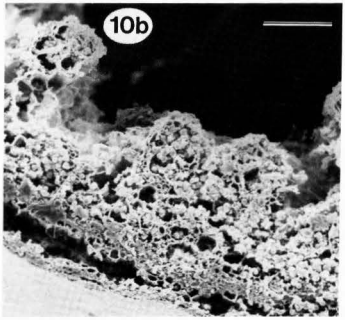
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Discussion with Reviewers

M. J. Gould: Kerley et al. (1985) noted that delignification of wheat straw by alkaline hydrogen peroxide treatment resulted in a dramatic increase in the attachment of rumen microorganisms to the straw surface. The data in this paper seem to show a similar effect with peroxide treated bermudagrass (e.g., Fig. 10). From the micrographs presented, it appears that the other delignifying treatments tested do not cause a similar increase in bacterial attachment. To what factors do the authors attribute this difference, and is it likely to be of significance in vivo, where attachment of bacteria to cellulosic particles is thought to be important for efficient cell wall degradation?

Authors: In our study there was a difference between experiment 1 and 2 in the microbial mass that covered stem surfaces, probably due to the difference in inocula for these two studies. Sections delignified with other treatments and incubated with inocula from experiment 2 also were covered with microorganisms. In our study, the microbial mass on H_2O_2 treated bermudagrass does not appear to be significant for this treatment, and the IVDMD data (Table 4) appear to confirm this. It is our opinion that one should use care in interpreting a microbial cover as attached, active fiber-degrading microorganisms, because at times it may just indicate debris from the inoculum. Methods other than SEM should be used to verify true attachment of cells.

W. W. Hanna: Except for SEM pictures, how does permanganate stem digestion in this study differ from the Akin et al. (1985) paper? To me, both papers provide the same information.

Authors: $KMnO_4$ treatment did cause similar results in other studies. We felt that in order to have a true comparison of the methods to delignify stems, plant material from a common source should be used as we did for this study. We also felt that, since $KMnO_4$ delignification is widely used, information presented along with other treatments would permit a side-by-side comparison by the readers.

L. H. Harbers: The difference in histological reactions between samples for experiments 1 and 2 are interesting. Would environmental factors such as

temperature and rainfall influence rate of lignification?

Authors: Increased growth temperatures have been shown in several studies to result in lower forage digestibility, and often a concomitant increase in lignification occurs. Results of studies on moisture are less consistent. Extreme moisture stress has resulted in increased lignification and depressed digestibility, whereas moderate stress resulted in improved digestibility. With all aspects, it should be remembered that different plants may respond in different ways to environmental stress. It is likely that our bermudagrass samples from experiments 1 and 2 were exposed to different environmental stresses because internodes from experiment 2, even though from similar positions of 6 week old plants, appeared more mature and were 4 to 9 units lower in IVDMD.

L. H. Harbers: It is apparent from your studies that syringyl-type lignin is produced in mature stem parenchyma. Could this lignification be controlled genetically or will we have to rely on early harvest and/or chemical treatment of those types of grasses?

Authors: Some research has been done that suggests that plant cultivars can be developed with reduced lignin in the parenchyma and greater digestibility. It is possible that research which focuses on delayed maturity and reduced lignification in whole plants would result in hybrids of bioengineered plants in which the parenchyma is more digestible and that consumption by ruminants would be improved. Alternately, cultivars of some species have been developed with reduced numbers of vascular bundles in the stem with a resultant increase in stem digestibility. Therefore, it appears that lignin in stems can be manipulated genetically, and efforts to reduce lignin or lignified tissues should be pursued.