

MORPHOLOGICAL STUDY BY SCANNING ELECTRON MICROSCOPY OF RUMEN DEGRADATION OF WHEAT STRAW TREATED WITH AMMONIA AND SULPHUR DIOXIDE

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Summary

Ammonia and/or sulphur dioxide treated and untreated wheat leaf sheaths were compared for cell wall digestion by incubation with rumen liquor for 24 and 48 hours. Scanning electron microscope (SEM) was used to study the relative rate and extent of cell wall digestion. Treated wheat straw leaf sheaths were distorted, with more distortion observed in ammonia and sulphur dioxide combined treatment than any other treatment. Rumen liquor digestion for 24 hours of untreated leaf sheath showed disrupted phloem, partially ruptured parenchyma and vascular tissues and further partially distorted inner bundle sheaths and vascular bundle after 48 hours incubation. Sulphurated leaf sheaths showed extensive degraded parenchyma and sclerenchyma material in 24 hours incubation, however, all tissues were irregularly shaped in 48 hours incubation. In ammoniation, epidermal cell walls and small vascular bundles began to disintegrate by 24 hours incubation, extensively changed structure and degraded epidermal tissue by 48 hours incubation. Combination treatment of leaf sheaths degraded all cell walls of parenchyma, phloem and vascular bundle by 24 hours incubation, however, structures only of inner bundles sheath with extended lam, sclerenchyma and cutinized epidermal cell walls remained.

(Key Words: Morphological, Scanning Electron Microscopy, Rumen Liquor, Wheat Straw, Ammonia, Sulphur Dioxide)

Introduction

The extent of improvement in rumen degradability of cereal straws treated with ammonia was due to a greater response in the degradability of stem fraction, associated with enhanced microbial degradation of sclerenchyma and parenchyma cells (Goto, 1989). Ammonium hydroxide ruptured the inner cuticle and separated parenchyma cells, but had no effect on vascular bundles, thick-walled sclerenchyma and epidermal silica (Harbes et al., 1982). Ammonia and sulphur dioxide pretreatment of cereal straws has been shown to improve their nutritive value by increasing crude protein content and digestibility (Dryden and Leng, 1988; Song et al., 1991c).

Anatomical studies on ruminal digestion of cereal straws have been reported for ammoniation (Itoh et al., 1981; Harbers, 1982; Goto, 1989; Horn et al., 1989; Song et al., 1993).

The objective of this experiment was the observation by scanning electron microscopy (SEM) of ruminal digestion of wheat straw treated by ammonia and/or sulphur dioxide.

Materials and Methods

Wheat straw (Japonica type, var. Nourin No. 61) collected at the Kyushu University Farm in 1989 was dried for 48 hr at 70°C prior to cutting of the internodes of the midportion of the straw into about 2-3 cm length. The straw was moistened with water (450 g/kg DM) and incubated at 20°C for 4 days in a polyvinyl jar. Sulphur dioxide (40 g/kg DM) was injected into the jar for storing at 70°C for 3 days or anhydrous ammonia (30 g/kg DM) at 20°C for 28 days and their combination treatment of ammoniation following by sulphuration was performed. The treated straw was aired for 3 days to exclude

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Received August 5, 1992

Accepted February 18, 1993

excess chemicals and cut into cross sections of 2 to 5 mm in length. The leaf sheaths of wheat straw were incubated in the rumen liquor (Minson and McLeod, 1972) of three Tokara goats fitted with rumen cannulae and fed on alfalfa hay. Digested leaf sheaths were retrieved for microscopy (WET-SEM) by the modified method of Akin and Amos (1975). The intact and incubated materials were washed with distilled water and then kept in a refrigerator to restore for a few days. Thereafter, leaves sheaths were placed in 4% glutaraldehyde in 0.1 M cacodylate buffer and then postfixed in 1.5% buffered osmium tetroxide at 4°C for 3 days. Specimens were washed in buffer and dehydrated for 5 minutes in 65%, 10 minutes in 75%, 15 minutes each in 85, 95, 99% and four times in 100% ethanol (v/v). Specimens were dried in a desiccator and then observed at 15 kv using a Akishi Beam Tech. (ABT-32).

Results and Discussion

1. Micrographs of cross section of untreated and treated wheat straw before incubation

Cross section of untreated leaf sheath showed that all cell walls of tissues are intact and

maintained structural integrity. Sulphur dioxide (SO_2) treatment of leaf sheaths distorted parenchyma (arrow), and degraded part of phloem (figure 4). Ammonia (NH_3) treatment of leaf sheaths disrupted parenchyma, vascular tissues appeared to be distorted, and a part of the phloem degraded (figure 7). Potassium hydroxide treatment separated parenchyma and inner bundle sheath and lignified sclerenchyma and inner sheath tissues (Akin, et al., 1977). However, bermuda grass treated with potassium hydroxide did not separate sclerenchyma cells (Spencer and Akin, 1980). NH_3 and SO_2 treatment of leaf sheaths had little effect on the epidermis and sclerenchyma, however parenchyma (arrow), vascular tissues and phloem were degraded or more distorted than other treatment (figure 8). Rice straw showed similar response to ammonia and sulphur dioxide treatment (Song et al., 1993).

2. Micrographs of cross section of untreated and treated wheat straw incubated for 24 hr

Untreated wheat leaf sheath after 24 hr incubation showed that phloem was disrupted, and parenchyma and vascular tissues were partially ruptured, however, lignified tissues were not degraded (figure 2). The phloem tissue in fescue

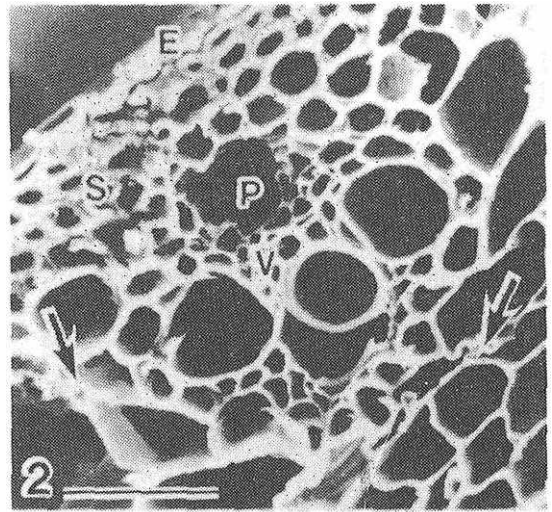
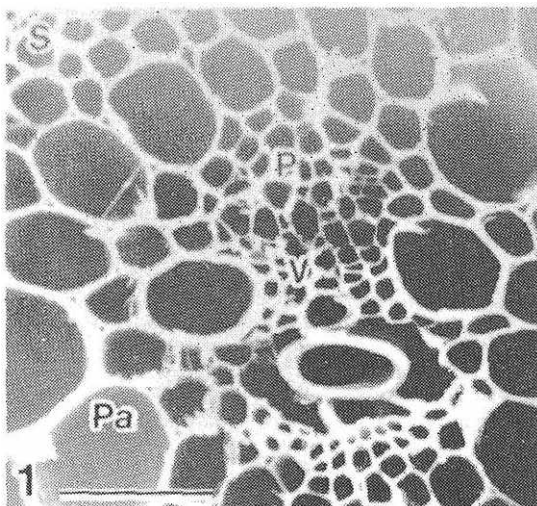


Figure 1. Cross section of untreated wheat straw. The parenchyma (Pa), phloem (P) and vascular tissue (V) maintained structural integrity.

Figure 2. Cross section of untreated wheat straw incubated with rumen liquor for 24 hr. The parenchyma tissue (arrow) and phloem were greatly collapsed. The sclerenchyma and epidermal cell walls were extended.

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appeared to be in the initial stages of digestion (Akin et al., 1973).

Sulphur dioxide-treated leaf sheaths maintained internal structure after 24 hr digestion, however degradation of parenchyma (arrow) and scleren-

chyma material was extensive (figure 5). The epidermal cell walls and small vascular bundles began to be removed, phloem tissue was completely removed and vascular tissues lacked structural integrity, although removal was not com-

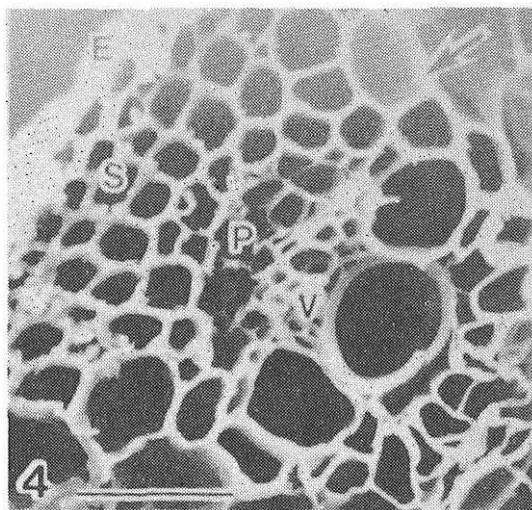
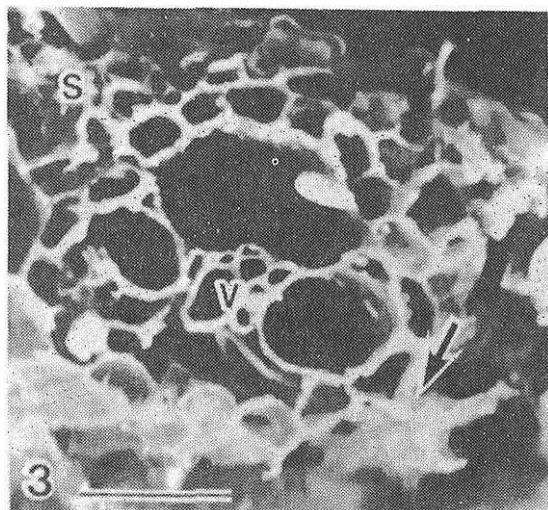


Figure 3. Cross section of untreated wheat straw incubated with rumen liquor for 48 hr. The parenchyma tissue (arrow) and phloem were greatly collapsed. The sclerenchyma and epidermal cell walls were extended.

Figure 4. Cross section of 4% SO₂ treated wheat straw. The parenchyma (arrow), and phloem were slightly shrunk.

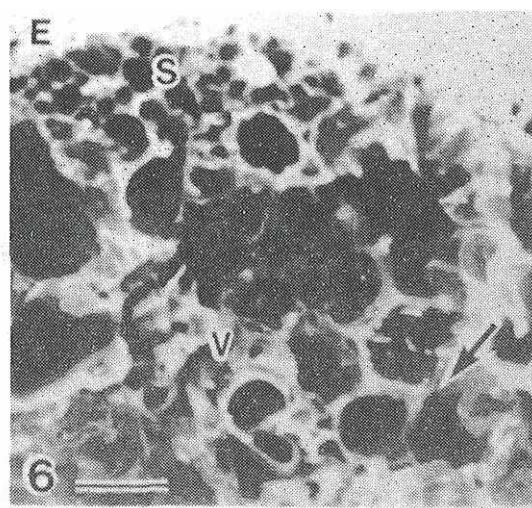
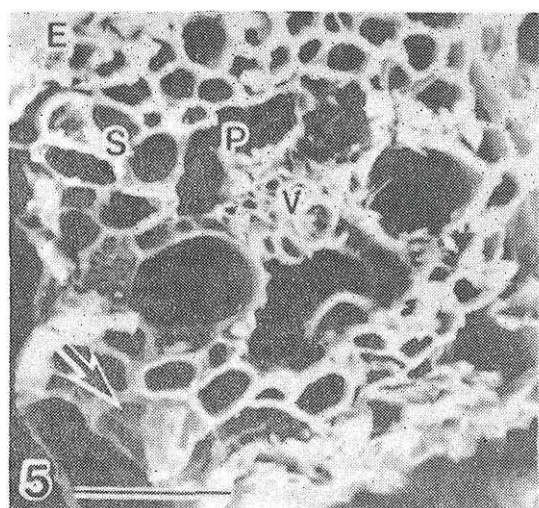


Figure 5. Cross section of 4% SO₂ treated wheat straw incubated with rumen liquor for 24 hr. Extensively degraded parenchyma (arrow), and sclerenchyma, vascular bundle and phloem were slightly collapsed.

Figure 6. Cross section of 4% SO₂ treated wheat straw incubation with rumen liquor for 48 hr. It shows that all tissues (especially parenchyma: arrow) were more degraded than 24 hr incubation.

plete after 24 hr incubation of ammonia-treated leaf sheaths (figure 8). NH_3 and SO_2 treatment of leaf sheaths showed degradation of all cell tissues of parenchyma (arrow), phloem and vas-

cular bundles removed by 24 hr incubation (figure 9). However, rigid residue remained even after 48 hr (epidermis, sclerenchyma ring) in wheat stems (Horn et al., 1989).

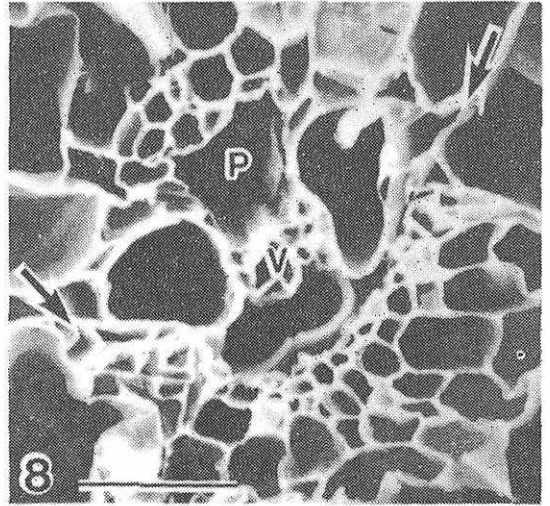
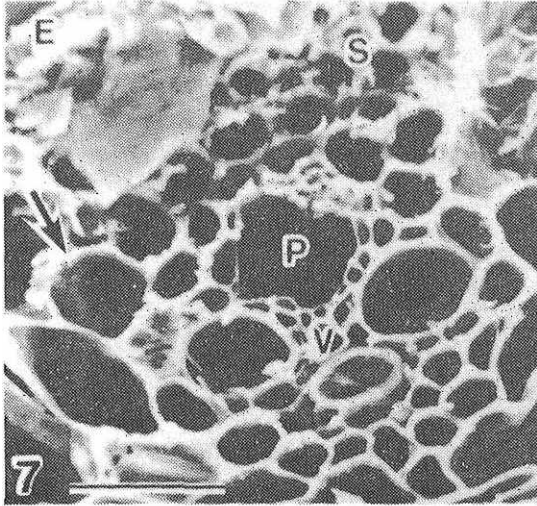


Figure 7. Cross section of 3% NH_3 treated wheat straw. The tissues of parenchyma (arrow), phloem, sclerenchyma were extended than SO_2 treated wheat straw leaf sheath.
 Figure 8. Cross section of 3% NH_3 treated wheat straw with rumen liquor for 24 hr. The parenchyma tissues (arrow), vascular bundle and phloem were extended than wheat leaf sheath not incubated.

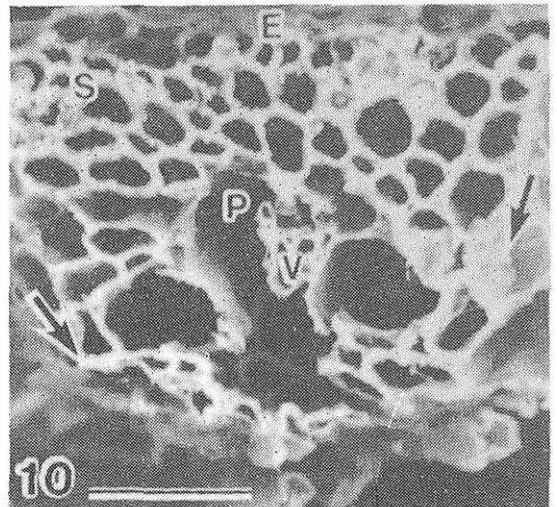
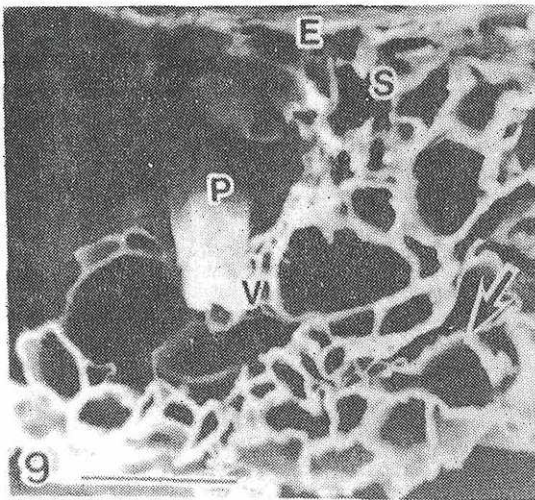


Figure 9. Cross section of 3% NH_3 treated wheat straw incubation with rumen liquor for 48 hr. Additional structural changes (especially parenchyma; arrow) were observed.
 Figure 10. Cross section of 4% SO_2 + 3% NH_3 treated wheat straw. It shows more distortion of tissues (especially parenchyma; arrow) than any other treatment.

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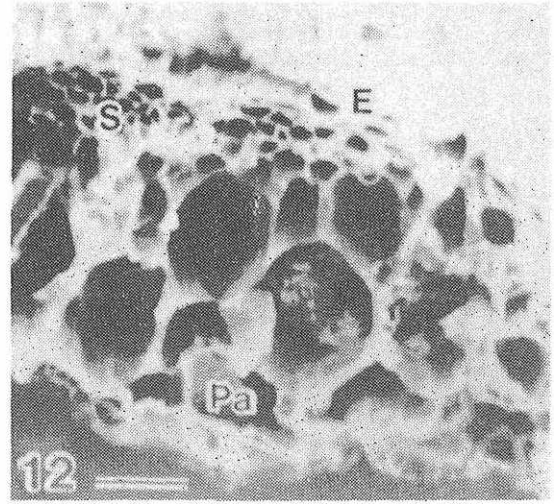
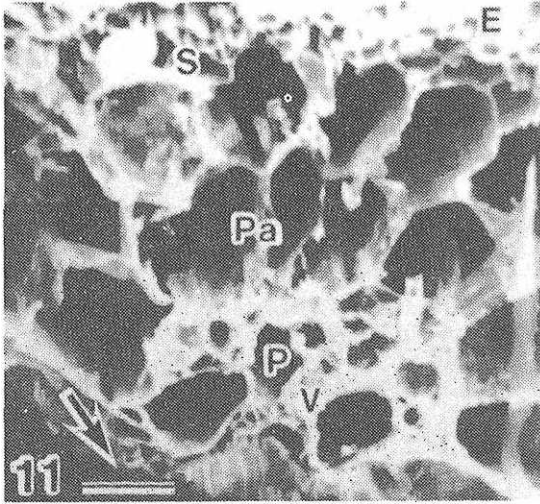


Figure 11. Cross section of 4% SO₂ + 3% NH₃ treated wheat straw incubated with rumen liquor for 24 hr. All tissues of parenchyma (arrow), phloem and vascular bundle were degraded. The sclerenchyma was extended and the inner layer was removed from the outer layer.

Figure 12. Cross section of 4% SO₂ + 3% NH₃ treated wheat straw incubated with rumen liquor for 48 hr. The tissue of epidermis, sclerenchyma and rigid portion of vascular bundle were left.

3. Micrographs of cross section of untreated and treated wheat straw incubated for 48 hrs.

Untreated leaf sheath showed sclerenchyma ruptured, however inner bundle sheath (arrow) and vascular bundles were partially distorted (figure 3). Sulphur dioxide-treated leaf sheaths (figure 6) showed all tissues were largely colonized by the anaerobic rumen fungi after 24 and 48 hrs, but they were no longer present after 72 hrs (Grenet and Barry, 1988). Extensively degraded epidermal tissues were observed in ammoniated leaf sheaths resulted in degradation of internal tissue (figure 9). Ammonia and sulphur dioxide treated leaf incubated for 48 hr showed more degradation than any other treatment: the only remaining structures were inner bundle sheaths with extended band, sclerenchyma and the cutinized epidermal cell walls (figure 12). These results suggest that the cutinized epidermis and lignified tissue usually resist microbial degradation.

Spencer and Akin (1980) found that potassium hydroxide treatment of bermuda grass disrupted tissues and separated lignified thick-walled cells (bundle sheath and sclerenchyma). Different mode of microbial attack on the highly lignified tissues because of their separation suggested that loss

of intercellular material (possibly pectins) may enhance digestion, as reported by Spencer and Akin (1980). Silica and cuticle may be a barrier to microbial penetration of leaf tissue. Lignin prevents digestion of vascular tissue and slows down the hydrolysis of sclerenchyma and epidermal tissues (Harbers et al., 1981). Increases in surface area and decreases in lignin content and crystallinity index by a wide variety of physical and chemical pretreatments of wheat straw enhance the rate of enzymatic hydrolysis of wheat straw.

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