# Effect of Phenolic Structures on the Degradability of Cell Walls Isolated from Newly Extended Apical Internode of Tall Fescue (*Festuca arundinacea* Schreb.)

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Apical internodes of tall fescue (*Festuca arundinacea* Schreb. var. Clarine) harvested at flowering were sectioned into 5 or 10 equal parts to study in situ degradability and cell wall composition, respectively. The basal (youngest) section had the greatest primary wall content. Cell walls in the upper (older) sections had the highest xylose/arabinose ratio and lignin content and a lignin rich in syringyl units, all typical of extensive secondary wall development. Almost all of the *p*-coumaric (*p*-CA) and about half of the ferulic acid (FA) were released by 1 M NaOH and presumed to be ester-linked. The total FA content was approximately double that of *p*-CA in all sections other than the youngest with a distribution similar to that of total *p*-CA. However, the ratio of esterified to ether and ether plus ester linked (Et & Et+Es) FA differed with age. Whereas the esterified form remained essentially constant (~4.5 g/kg of cell wall), Et & Et+Es ferulate increased with increasing age of the tissue and was significantly related to lignin deposition ($r = 0.79$, $P < 0.01$). The extent of cell wall degradation after 48 h of incubation in the rumen was inversely related to maturity, falling from 835 g/kg of dry matter in the youngest section to 396 g/kg in the oldest. Both the rate and extent of cell wall degradation were significantly negatively related to the ratio of xylose to arabinose, lignin content, proportion of syringyl units present in lignin, and concentration of Et & Et+Es FA present. A positive relationship between Et & Et+Es FA was also found, with the rate ($P < 0.01$) being better correlated than the extent ($P < 0.05$) of cell wall degradation. Application of the newly extended internode model to fescue produced results consistent with the view that both the lignin content and the extent to which lignin was covalently bound to the other wall polymers crucially influenced the rate and extent of degradation.

**Keywords:** *Tall fescue; cell wall; lignin; phenolic acids; rumen degradability*

## INTRODUCTION

Many studies have demonstrated the overriding effect of phenolics in reducing the extent of cell wall degradation by the rumen microflora. However, there is considerable heterogeneity in the composition and in the manner in which lignin and the structurally simpler phenolic acids are associated with the other components of cell walls in grasses and cereals (Chesson, 1988; Besle et al., 1994). As a result, forages can follow quite different kinetics of degradation depending on variety, age, and agronomy.

The mechanisms that govern the resistance of forage cell walls to microbial attack have been extensively studied, and some general principles have emerged. There is now good evidence that bacterial attack of the cell wall is a superficial process (Chesson et al., 1997a; Gardner et al., 1999) in which lignin presents an essentially inert surface resistant to adhesion by the attacking microflora and to degradation by microbial enzymes. What remains unknown is whether lignins and other phenolics are evenly distributed in the wall and thus have a uniform effect at all stages of cell wall degradation (Chesson, 1993) or whether the effect varies with a gradient of structures within the cell wall, resulting in a changing topochemistry at the cell wall surface (Jung and Deetz, 1993). One approach to this question has been to study the degradation pattern in various tissues or cell types with different compositions and at different stages of development. As expected, the rate and extent of degradation of the thin parenchyma cell walls were greater than those of the thicker, more extensively lignified sclerenchyma from both ryegrass (Chesson et al., 1986) and cocksfoot (Grabber and Jung, 1991). However, rates of degradation were independent of the degree of maturity within the same cell types isolated from maize (Lopez et al., 1993).

It is evident that whole forage or even forage plant parts are too complex models with which to study mechanisms of cell wall degradation. The newly extended internode provides a unique model in which the spatial distribution of the temporal events governing secondary wall development allows the internode to be sectioned to supply cell wall material at different developmental stages. Such a model has been applied to cell wall development in reed (Joseleau and Barnoud, 1976), wheat, and phalaris (Lam et al., 1992) and to the

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**Table 1. Cell Wall Content and Cell Wall Composition of the $^1/_{10}$ Sections of the Apical Internode of Tall Fescue[a]**

| section[b] | cell wall (g/kg of DM) | cell wall constituents (g/kg of cell wall) | | | | | | nitrobenzene oxidation products (g/kg of ABL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | glu | xyl | ara | xyl/ara | TP | ABL | V | S | NBO | S/V |
| A | 872 | 330 | 188 | 18.4 | 10.2 | 151 | 137 | 190 | 117 | 335 | 0.48 |
| B | 778 | 393 | 216 | 19.4 | 11.1 | 148 | 134 (136) | 199 (195) | 119 (118) | 334 (335) | 0.50 |
| C | 775 | 427 | 216 | 19.4 | 11.1 | 162 | 147 | 172 | 114 | 300 | 0.55 |
| D | 779 | 407 | 213 | 19.4 | 11.0 | 143 | 128 (137) | 179 (176) | 126 (120) | 321 (311) | 0.59 |
| E | 782 | 414 | 211 | 19.4 | 10.9 | 158 | 143 | 158 | 111 | 283 | 0.59 |
| F | 800 | 424 | 221 | 19.4 | 11.4 | 158 | 142 (143) | 140 (149) | 99 (105) | 255 (269) | 0.59 |
| G | 781 | 426 | 229 | 20.5 | 11.2 | 133 | 117 | 141 | 102 | 262 | 0.61 |
| H | 758 | 418 | 215 | 20.5 | 10.5 | 142 | 126 (121) | 140 (140) | 94 (98) | 257 (260) | 0.68 |
| I | 703 | 405 | 233 | 24.6 | 9.5 | 108 | 92 | 152 | 83 | 265 | 0.46 |
| J | 568 | 331 | 190 | 45.0 | 4.2 | 104 | 94 (93) | 103 (133) | 37 (65) | 158 (224) | 0.30 |
| SE | 8.5 | 32.5 | 22.3 | 3.1 | 0.6 | 7.5 | 7.6 | 8.4 | 5.4 | 14.9 | 0.02 |

[a] glu, glucose; xyl, xylose; ara, arabinose; TP, total phenolics; ABL, acetyl bromide lignin; V, vanillin; S, syringaldehyde from nitrobenzene oxidation; NBO, *p*-hydroxybenzaldehyde + vanillin + syringaldehyde; S/V, syringaldehyde to vanillin molar ratio. Data in parentheses are the calculated figures for the $^1/_5$ internode sections used in subsequent experiments; SE, residual standard error from duplicate/triplicate analysis. [b] Section A represents the uppermost and oldest section and J the basal section, which includes the meristematic tissue.

relationship between wall development and degradability in maize (Scobbie et al., 1993). The present study investigates the pattern of rumen microbial degradation of the different tissues of an extended apical internode of a typical forage grass, tall fescue, in relation to the composition, structure, and fate of the structural components.

## MATERIALS AND METHODS

**Plant Material.** Apical internodes of tall fescue (*Festuca arundinacea* Schreb. var. Clarine) stem were harvested at flowering stage and air-dried at 50 °C for 48 h. Internodes were fractionated into either 10 equal sections, designated A (upper, oldest section) to J (basal, youngest section) for biochemical study or into 5 equal segments, similarly designated AB, CD, EF, GH, and IJ, to measure the in situ kinetics of degradation. Corresponding sections from apical internodes from a number of plants were pooled to give sufficient experimental material. The fractions were dried, weighed, and ground with a blade mill to pass a 1 mm screen.

**Chemical Analysis.** Analyses were performed on cell walls and the residues of cell walls recovered after exposure to rumen microorganisms (CWR) obtained by water extraction at 40 °C followed by sequential refluxing with ethanol 95% and toluene/ethanol (2:1 v/v) in a Soxhlet apparatus (Jarrige, 1961).

Neutral monosaccharides were determined in duplicate as their alditol acetates by GC (Blakeney et al., 1983) after acid hydrolysis (72% w/w $H_2SO_4$ for 1 h at 30 °C followed by 1 M $H_2SO_4$ for 3 h at 100 °C). The total phenolic content was measured in duplicate according to the acetyl bromide method (Iiyama and Wallis, 1990) using ferulic acid as standard. Total phenolic acids were determined by 4 M NaOH microwave digestion (Provan et al., 1994) modified to use smaller samples (Chesson et al., 1997b) with analysis by HPLC. Ester-only linked phenolic acids were defined as those phenolic acids released by treatment with 1 M NaOH at ambient temperature and were analyzed using the same HPLC protocol. Values for ester-linked acids were subtracted from total phenolic acids to provide a measure of the presumed ether and ether plus ester linked (Et & Et+Es) fraction. Lignin monomers were released by alkaline nitrobenzene oxidation (Venverloo, 1971) as described by Bernard Vailhé et al. (1996). The total of the main products obtained [*p*-hydroxybenzaldehyde (H), vanillin (V), syringaldehyde (S)] was designated NBO. The methanol extracts were analyzed by HPLC with a reverse phase column (Lichrosorb R*p*-18, 5 $\mu$m, 250 × 4.6 mm i.d.) and eluted with an $H_2O$/MeOH/$H_3PO_4$ gradient (Mosoni et al., 1994).

**In Situ Degradation.** In situ degradability was determined on the $^1/_5$ sections by using the nylon bag method (Demarquilly and Chenost, 1969) adapted to handle small amounts of dry matter. The bags (pore size = 50 $\mu$m, Ankom) were incubated in duplicate for 2, 6, 12, and 48 h in the rumen of two fistulated sheep. After incubation, they were frozen, thawed, thoroughly washed, dried for 48 h at 60 °C, weighed, and Soxhlet extracted as described above to obtain the CWR. Water-soluble and fine particle losses were determined by placing additional bags in a vessel containing water, vigorously shaking for 4 h at 40 °C, and filtering the extracts on sintered glass (porosity = 2) (Michalet Doreau, 1990). The in situ dry matter (DMD) disappearances were calculated on the basis of dry weight at 60 °C by taking into account the fine particle losses. The in situ cell wall disappearances (CWD) were calculated by subtraction of CWR from the cell wall content of the undegraded material.

**Statistical Analysis.** The kinetics of in situ CWD were fitted [NLIN procedures of SAS (1989)] to the exponential model of Dhanoa (1988) modified to allow a confident calculation of the rate for the conditions of our experiment in which the plateau of degradation was not reached. The derivative of the equation for cell wall degradation is $dY/dt = bc\ \exp^{-c(t-d)}$ ($b$ = slowly degradable material, $c$ = rate, $d$ = lag phase). When $t = d$, $dY/dt = bc = e$, and $b = e/c$. The rate $c$ was determined during the exponential phase with the equation $Y = e/c(1 - \exp^{-c(t-d)})$ (J. Van Milgen, personal communication). Similarly, the rate of dry matter degradation was calculated using $Y = a + e/c(1 - \exp^{-ct})$.

The data on kinetics parameters and 48 h in situ disappearances were subjected to one-way analysis of variance using the GLM procedure of SAS (1989). The data on cell wall content and CWR composition were classified according to the hierarchical agglomerative cluster analysis (tree procedure).

## RESULTS

**Chemical Composition of the Fescue Internode.** The cell wall content increased with age, with the greatest change found within the younger sections J (568 g/kg), I (703 g/kg), and H (758 g/kg) (Table 1). Thereafter, the cell wall content remained roughly constant at ~780 g/kg, although it rose again to a maximum value in the oldest section A (872 g/kg). Polysaccharides, composed mostly of glucose and xylose (Table 1), accounted for a consistent proportion of the cell wall in sections of all ages. Whereas the glucose-to-xylose ratio (1.75) varied little from the base upward, the proportion of arabinose units in section J was almost double that of other sections, and this was reflected in the much lower xylose-to-arabinose ratio (Table 1).

As expected, the total phenolics and lignin contents were lowest in the basal sections J and I and increased in older sections. However, even the youngest section contained an appreciable phenolic content representing approximately two-thirds of the value in the oldest section. There was, however, evidence of compositional

**Table 2. Concentration of Phenolic Acids (Grams per Kilogram of Dry CWR) in the Cell Walls of Each $^1/_{10}$ Fraction of the Apical Internode of Tall Fescue[a]**

| section | ester-only linked | | | Et & Et+Es linked | | | total | | |
|---|---|---|---|---|---|---|---|---|---|
| | *p*CA | FA | FA/*p*CA | *p*CA | FA | FA/*p*CA | *p*CA | FA | FA/*p*CA |
| A | 4.08 | 4.00 | 0.98 | 0.30 | 5.35 | 17.54 | 4.38 | 9.35 | 2.13 |
| B | 3.79 | 3.94 | 1.04 | 0.44 | 5.69 | 12.93 | 4.23 | 9.63 | 2.28 |
| C | 3.95 | 3.64 | 0.92 | 0.63 | 6.25 | 10.00 | 4.58 | 9.90 | 2.16 |
| D | 4.22 | 3.71 | 0.88 | 0.53 | 6.04 | 11.34 | 4.75 | 9.74 | 2.05 |
| E | 3.93 | 4.03 | 1.02 | 0.70 | 5.52 | 7.89 | 4.63 | 9.55 | 2.06 |
| F | 4.36 | 4.30 | 0.99 | 0.73 | 5.38 | 7.39 | 5.09 | 9.67 | 1.90 |
| G | 4.62 | 4.78 | 1.03 | 0.75 | 5.79 | 7.75 | 5.37 | 10.56 | 1.97 |
| H | 4.82 | 5.41 | 1.12 | 0.37 | 5.06 | 13.86 | 5.19 | 10.47 | 2.02 |
| 1 | 4.70 | 6.17 | 1.31 | 0.37 | 4.41 | 11.78 | 5.08 | 10.58 | 2.08 |
| J | 1.44 | 4.48 | 3.11 | 0.56 | 3.36 | 5.99 | 2.00 | 7.84 | 3.92 |
| SE | 0.08 | 0.07 | 0.02 | | | | 0.29 | 0.65 | 0.13 |

[a] *p*CA, *p*-coumaric acid; FA, ferulic acid; Et & Et+Es linked, ether and ether plus ester linked; SE, residual standard error from duplicate analysis.

differences in the lignin. Data from nitrobenzene oxidation (NBO) suggested that the lignin became progressively syringyl-rich. Overall, the NBO recovery was low in section J [16% of acetyl bromide lignin (ABL)] and increased to double in the upper third of the internode. These results allowed classification of the sections into four distinct clusters, J (the most different) and I sections were greatly different from sections B−H and then section A, the oldest.

The total 4-hydroxycinnamic acid (*p*-coumaric acid, *p*-CA) content tended to parallel the deposition of lignin and was least in the youngest section (Table 2). Esterified *p*-CA accounted for 72% of total *p*-CA in section J and for >85% in the upper internodes with the Et & Et+Es *p*-CA content consistently low (<0.75 g/kg of cell wall). The total 4-hydroxy-3-methoxycinnamic acid (ferulic acid, FA) content was approximately double that of *p*-CA in all sections other than J and followed a distribution similar to that of total *p*-CA. However, the ratio of esterified to Et & Et+Es FA differed greatly, and there was some indication that the two subfractions of FA differed in their patterns of deposition (Table 2). Although the esterified form remained essentially constant at ∼4.5 g/kg of cell wall), Et & Et+Es ferulate increased with increasing age of the tissue and was significantly related to lignin deposition ($r = 0.79$, $P < 0.01$). Hierarchical cluster analysis again showed section J to be clearly different, but sections H and I were in the same cluster, indicating that the main variations occurred in the lower third of the internode. The two other clusters were sections B−G and A.

**Degradability of Internode Sections.** The 48 h in situ disappearances of the $^1/_5$ internode sections decreased with maturation (Table 3). Both the DMD and CWD after 48 h of incubation were greatest in the youngest section IJ (90 and 84%, respectively) and decreased in a curvilinear fashion in sections GH, EF, and CD, with another marked decrease in the upper section AB. However, the degradation curves failed to reach a plateau after 48 h of incubation (Figure 1). Values for the kinetic parameters (Table 4), obtained with a model taking into account the lack of an experimental plateau, fitted well with the experimental data. For DM disappearance, the high values for *b* implied that the two lower $^1/_5$ sections were potentially fully degradable. The oldest fifth (AB) was the only section predicted to have a consistent undegradable fraction of ∼27%. The rates of CWR degradation for the four upper sections, the sections with the greatest

**Table 3. Disappearance (Percent) of Dry Matter, Cell Wall Dry Matter, and Cell Wall Constituents from $^1/_5$ Sections of the Apical Internode of Tall Fescue after 48 h of Incubation in a Sheep Rumen**

| section | degradability | | | | | ester-only linked | | Et & Et+Es linked | | total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM | CW | ABL | V | S | *p*CA | FA | *p*CA | FA | *p*CA | FA |
| AB | 48.9 | 39.6 | 11.7 | 14.5 | 9.7 | 46.8 | 68.2 | −107.0 | 24.2 | 33.6 | 42.6 |
| CD | 59.2 | 47.6 | 8.1 | 26.7 | 20.7 | 60.9 | 76.7 | −107.8 | 41.8 | 39.9 | 54.9 |
| EF | 57.1 | 46.6 | 14.4 | 23.4 | 17.4 | 57.0 | 80.0 | −93.9 | 29.7 | 34.8 | 51.4 |
| GH | 65.7 | 56.0 | 8.1 | 31.2 | 28.2 | 65.4 | 83.3 | −58.2 | 49.1 | 52.4 | 65.6 |
| IJ | 89.7 | 83.5 | 56.8 | 77.9 | 57.0 | 82.6 | 93.6 | −2.3 | 70.6 | 71.4 | 83.9 |
| SE | 0.6 | 0.8 | 1.6 | 1.1 | 1.4 | 0.6 | 0.3 | 3.7 | 1.1 | 1.0 | 0.7 |

[a] DM, dry matter; CW, cell wall; ABL, acetyl bromide lignin, V, vanillin; S, syringaldehyde from nitrobenzene oxidation; Et & Et+Es linked, ether and ether plus ester linked; *p*CA, *p*-coumaric acid; FA, ferulic acid; SE, residual standard error.

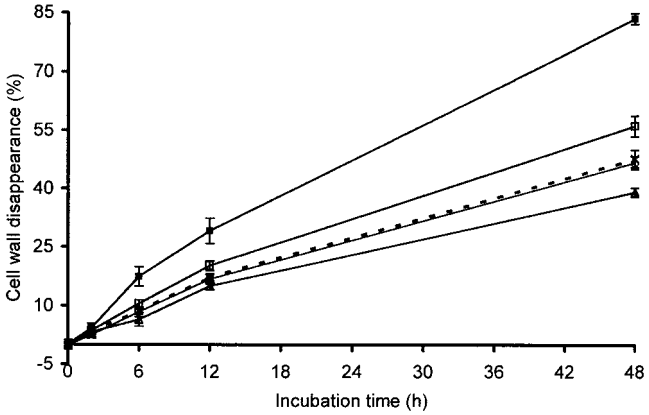


**Figure 1.** Kinetics of cell wall disappearance (CWD) from $^1/_5$ sections of the apical internode of fescue incubated for 48 h in a sheep rumen: (▲) AB, the oldest section; (×) CD; (○) EF; (□) GH; (■) IJ, the youngest section.

**Table 4. Kinetics of Dry Matter and Cell Wall Dry Matter Disappearance from Each $^1/_5$ Section of the Apical Internode of Tall Fescue Calculated from Data Obtained over 48 h of Incubation in a Sheep Rumen**

| section | dry matter disappearance | | | cell wall disappearance | | |
|---|---|---|---|---|---|---|
| | *a* | *b* | *c* | *b* | *c* | *d* |
| AB | 14.5 | 58.4 | 1.84 | 61.8 | 2.28 | 0.23 |
| CD | 13.1 | 78.9 | 1.83 | 75.6 | 2.18 | 0.07 |
| EF | 12.5 | 75.8 | 1.86 | 67.8 | 2.45 | 0.55 |
| GH | 14.0 | 90.1 | 1.78 | 82.9 | 2.36 | 0.22 |
| IJ | 28.6 | 79.2 | 3.08 | 104.9 | 3.36 | 0.90 |
| SE | 0.5 | 2.6 | 0.12 | 4.8 | 0.2 | 0.45 |

[a] Rapidly degradable material. [b] Potentially degradable part of the insoluble material ($b = e/c$, $e$ = rate when $t = d$). [c] Degradation rate (%·h$^{-1}$). [d] Lag time (h).

secondary wall content, were roughly similar (∼2.3%·h$^{-1}$). In contrast, the rate of degradation of the IJ section, rich in primary walls and parenchyma, was 1.5-fold higher than that of the other sections. The lag phase was consistently shorter in the four upper sections than in the youngest (IJ) section (0.9 h).

Over half (57%) of the lignin was released from the cell walls of the youngest section (IJ) after 48 h in the rumen. This was in marked contrast to the other sections in which only 8−14% was lost (Table 3). The proportion of guaiacyl units in the lignin remaining with the undigested residue after 48 h of degradation appeared to be lower than in the initial sample, especially in section IJ (Table 5). The syringyl content was less

**Table 5. Lignin Content and Lignin Composition of Residues of Each 1/5 Section of the Apical Internode of Tall Fescue after 48 h of Incubation in a Sheep Rumen[a]**

| section | ABL (g/kg of CWR) | nitrobenzene oxidation products (g/kg of ABL) | | | |
|---|---|---|---|---|---|
| | | V | S | NBO | S/V |
| AB | 198 | 189 | 121 | 326 | 0.54 |
| CD | 241 | 141 | 96 | 263 | 0.57 |
| EF | 229 | 133 | 101 | 253 | 0.63 |
| GH | 253 | 105 | 76 | 199 | 0.61 |
| IJ | 243 | 68 | 65 | 156 | 0.79 |
| SE | 6.9 | 12.7 | 8.4 | 22.8 | 0.02 |

[a] ABL, acetyl bromide lignin; CWR cell wall residue; V, vanillin; S, syringaldehyde from nitrobenzene oxidation; NBO, *p*-hydroxybenzaldehyde + vanillin + syringaldehyde; S/V, syringaldehyde to vanillin molar ratio; SE, residual standard error from analysis in triplicate.

affected, with the result that the syringaldehyde to vanillin (S/V) ratio was only slightly greater than in the initial substrate in the upper four sections. However, some of the vanillin detected after NBO could be attributed to degradation of FA (Billa et al., 1996). When the value for vanillin was corrected using the difference between total FA and FA measured by NBO, the percentage losses of guaiacyl and syringyl units were approximately equal and the S/V ratio was unchanged in the four upper sections. The difference in proportional loss of guaiacyl and syringyl units from the youngest section IJ (77% for vanillin and 57% for syringaldehyde) remained. The proportion of total phenolics increased in all CWR, but this increase was most marked in the youngest section (from 93 to 243 g/kg of cell wall and CWR, respectively).

The extent of release of the saponifiable FA and *p*-CA (released by 1 M NaOH) closely mirrored the extent of cell wall breakdown and, consequently, was greatest from cells walls of the youngest section (IJ). The Et & Et+Es fraction of FA showed a similar pattern of release also related to the extent of cell wall breakdown, although the proportion lost from the more lignified fractions was considerably less than the esterified counterpart (Table 3). Only the small amount of Et & Et+Es *p*-CA present appeared to behave in a different fashion and was apparently preferentially retained during degradation in all sections. However, these values have to be treated with some caution as they derive from small difference values and were not directly determined.

**Relationships between Degradability and Cell Wall Components.** Both the rate and extent of cell wall degradation after 48 h were significantly negatively related to the cell wall content, the ratio of xylose to arabinose, the lignin content, and the proportion of syringyl units present in lignin (Table 6). A positive relationship with the concentration of Et & Et+Es FA was also found, with the rate ($P < 0.01$) being better correlated than the extent ($P < 0.05$) of cell wall degradation.

## DISCUSSION

The extent of fescue cell wall degradation decreased with maturation as previously reported for internodes of maize (Scobbie et al., 1993) and wheat (Lam et al., 1993). The youngest section (IJ), which contained the highest proportion of primary tissues as indicated by the low X/A ratio (Morrison, 1980) and phenolic content, was extensively degraded after 48 h. The extent of degradation was substantially reduced in the remaining sections with the oldest (AB) showing less than half of the cell wall loss shown by the youngest section over the same time period. Despite differences after 48 h, degradation kinetics indicated that only the uppermost section had a truly undegradable fraction. Rates of dry matter and cell wall loss were similar in the upper four sections and were significantly different from the considerably greater extent and rate of degradation seen in the youngest section. Similar differences in extents of degradability in parenchyma or sclerenchyma walls isolated from the lowest and the uppermost sections of maize internodes were observed by Lopez et al. (1993). However, in contrast to this study, but consistent with the observations of Grabber and Jung (1991) made with different tissues of cocksfoot, the rate of degradation of IJ was greater than in upper sections. As this significant decrease in degradability between sections IJ and GH was accompanied by a relatively small increase in lignin content, other factors relating to the structure and organization of the wall evidently contributed to the determination of degradability.

**Table 6. Correlation between the Rate (Percent Loss per Hour) or Extent of Degradability Measured after 48 h of Incubation in a Sheep Rumen and the Composition of the 1/5 Sections of the Apical Internode of Tall Fescue[a]**

| constituent | units | DM disappearance | | CW disappearance | |
|---|---|---|---|---|---|
| | | extent | rate | extent | rate |
| cell wall content | g/kg of DM | −0.98** | −0.97** | −0.98** | −0.92* |
| X/A | g/kg of CW | −0.91* | −0.99** | −0.93* | −0.96** |
| ABL | g/kg of CW | −0.94* | −0.90* | −0.96** | −0.88* |
| V | g/kg of ABL | −0.77 | −0.53 | −0.76 | −0.67 |
| S | g/kg of ABL | −0.95* | −0.89* | −0.96* | −0.96** |
| NBO | g/kg of ABL | −0.88* | −0.70 | −0.87* | −0.82 |
| S/V | molar ratio | −0.66 | −0.88* | −0.70 | −0.80 |
| ester-only *p*CA | g/kg of CW | −0.47 | −0.78 | −0.50 | −0.67 |
| ester-only FA | g/kg of CW | 0.86 | 0.71 | 0.87 | 0.82 |
| ester-only FA/*p*CA | g/kg of CW | 0.92* | 0.96** | 0.94* | 0.98** |
| Et & Et+Es *p*CA | g/kg of CW | −0.15 | −0.37 | −0.20 | −0.24 |
| Et & Et+Es FA | g/kg of CW | −0.85 | −0.92* | −0.88* | −0.98** |
| Et & Et+Es FA/*p*CA | g/kg of CW | −0.51 | −0.28 | −0.47 | −0.41 |
| total *p*CA | g/kg of CW | −0.44 | −0.76 | −0.47 | −0.63 |
| total FA | g/kg of CW | 0.01 | −0.39 | −0.02 | −0.30 |
| total FA/*p*CA | g/kg of CW | 0.65 | 0.87 | 0.69 | 0.75 |

[a] CW, cell wall; X/A, xylose-to-arabinose ratio; ABL, acetyl bromide lignin, V, vanillin; S, syringaldehyde from nitrobenzene oxidation; NBO, *p*-hydroxybenzaldehyde + vanillin + syringaldehyde; S/V, syringaldehyde to vanillin molar ratio; Et & Et+Es, ether and ether plus ester linked; *, **, significant at $P < 0.05$ and $P < 0.01$, respectively.

Other important factors could include the extent and pattern of deposition of phenolic acids and the nature of the lignin polymer itself. There is evidence of an increasing incorporation of syringyl units into lignin from the base upward in the internode, and a significant correlation was found between degradability and the syringyl content of lignin (Table 6). This is unlikely to relate to a direct effect of syringyl units or syringyl-rich lignin on the attacking microflora but more probably reflects the nature of the lignin incorporated into the secondary wall; thus, a high syringyl content acts as a marker of wall thickening (Grabber et al., 1997).

Probably of greater importance is the extent and manner of the incorporation of the phenolic acids, particularly FA. Within the Poaceae, FA is found esterified to structural polysaccharides and, in part, provides cross-linking to other polysaccharide chains (Ralph et al., 1992; Grabber et al., 1995; Hatfield et al., 1999). It has been elegantly demonstrated in a maize cell culture model that the extensive cross-linking between polysaccharides by FA dimers will reduce the rate of hydrolysis (Grabber et al., 1998). However, this effect can be largely overcome by polysaccharidase (but not feruloyl esterase) action, suggesting that this form of cross-linking is not a major factor in the determination of the extent of degradation. Esterified FA is also thought to act as a nucleation site for lignin biosynthesis and in so doing forms a link between lignin and the structural polysaccharides of the cell wall (Ralph et al., 1995). The pattern of deposition of ester-linked FA in the fescue internode suggested that proportionally more ferulate was associated with the secondary wall than was present in primary tissue (see Table 2, change from section J to I). This initial rise in ester-linked ferulate concentration was followed by a decrease with maturity, consistent with increased incorporation into lignin. This decrease was not accompanied by a corresponding rise in Et & Et+Es bonded ferulate. However, coupling of ester-linked FA via a radical mechanism favors C−C bond formation, which is not cleaved by 4 M NaOH under the conditions employed. This undefined loss to analysis of part of the FA involved in cross-linking to lignin (Grabber et al., 1995) may explain the variation in the strength of the relationship that has been found between wall degradability and the various fractions of FA in the lignified wall (Goto et al., 1994; Jung et al., 1998). Nonetheless, a strong significant negative relationship between Et & Et+Es linked ferulate and the rate and extent of cell wall breakdown could be demonstrated within the fescue internode.

The nonmethoxylated hydroxycinnamic acid, although occurring to a limited extent ester linked to polysaccharide, is less able to participate in cross-linking reactions than its methoxylated counterparts. ESR studies have demonstrated that this is because of a restricted ability to reduce peroxidase and allow completion of the catalytic cycle (Russell et al., 1999). Most *p*-CA is now thought to be ester-linked to syringyl units in lignin, a view consistent with the commonly made observation that *p*-CA is deposited in parallel with lignin (Ralph et al., 1994; Scobbie et al., 1993).

Estimates of Et & Et+Es *p*-CA are made by a difference method and are prone to error. However, Et & Et+Es *p*-CA was found in greater concentrations in fescue than previously reported for other members of the Poaceae (Provan et al., 1994). The fact that Et & Et+Es *p*-CA was entirely preserved during degradation and accumulated in digested residues suggests that this fraction was not an artifact. Alternatively, it is possible that the susceptibility of Et & Et+Es *p*-CA to alkaline treatment was increased after digestion. However, the nature of the linkage(s) to this fraction of *p*-CA, its association with cell wall polymers, and any implications for wall degradability are unknown.

The extended internode can provide a useful model for the study of the relationships between cell wall development and degradability. Its application to fescue demonstrated that two factors crucially influenced the rate and extent of degradation. These were the lignin content and the extent to which lignin was covalently bound to the other wall polymers. Lignin concentration would be paramount in the determination of the amount of lignin exposed at a cell wall surface and the extent of cross-linking determining its retention at the wall surface. Limited cross-linking to wall polysaccharide would allow the release of lignin by polysaccharidase action degrading the polysaccharide to which the lignin molecule is attached as demonstrated for diferulate cross-links (Grabber et al., 1998). This would become progressively more difficult as the overall amount of lignin and the number of bonds to other polymers increased. However, this does not exclude the possibility that degradation is progressively slowed by a preexisting lignin concentration gradient in the wall as suggested by Jung and Deetz (1993).

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