Alfalfa stem tissues: Impact of lignification and cell length on ruminal degradation of large particles

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Abstract

A series of experiments were conducted with alfalfa to determine how extensively rumen microorganisms can degrade various tissues within large stem pieces. The seventh internode from the base of the stem was collected from alfalfa clone 718 after 4 weeks of regrowth. Internode length and diameter were measured, and approximately 2 cm stem pieces were excised from the internodes. Stem pieces were incubated with rumen fluid in vitro for 24 h. Bee’s wax was used to coat the stem pieces to prevent microbial access other than at one end of the stem pieces. After exposure to the rumen microorganisms, stem pieces were serially cross-sectioned starting at the exposed surface. Sections were examined by light microscopy to determine which tissues had been degraded and to what depth into the stem piece degradation had occurred. Non-lignified alfalfa stem tissues (chlorenchyma, collenchyma, cambium, and primary xylem parenchyma) were degraded to great depth (3700–8200 μm) in stem pieces, but degradation of lignified tissues (phloem fibres and xylem fibres) was much more limited (150–1360 μm). Depth of degradation was greater in stem pieces derived from long internodes compared to short internodes. Using longitudinal sections and isolated cells of stem tissues, it was found that mean cell length increased by approximately 50% with a doubling of internode length for all tissues examined. Many cell layers of non-lignified tissues were degraded whereas only the exposed cell layer of lignified tissues exposed at the cut end of the internode pieces was susceptible to degradation. Depth of degradation for non-lignified tissues was attributed to a combination of

Abbreviations: cam, cambium; chl, chlorenchyma; coll, collenchyma; epi, epidermis; pf, phloem fibre; pith, pith parenchyma; pxp, primary xylem parenchyma; S.E.M., standard error of the mean; xylem, xylem fibres and vessels

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1. Introduction

Alfalfa (*Medicago sativa* L.) is considered a good quality forage because of its high protein content and digestibility compared to many other forages. While this conclusion is valid for immature alfalfa, as the crop matures digestibility declines substantially. The decline in alfalfa quality partially results from the increasing proportion of the crop accounted for by stems compared to leaves (Nordkvist and Aman, 1986). Stems of alfalfa contain more cell wall material than leaves and cell wall polysaccharides are less digestible than cytoplasmic carbohydrates. Also, alfalfa stems accumulate increasing amounts of cell wall material as the plants mature and the cell walls become more lignified and less digestible with age (Buxton et al., 1987; Nordkvist and Aman, 1986). Although some alfalfa stem tissues (cambium, chlorenchyma, collenchyma, and epidermis) remain non-lignified and completely degradable throughout the maturation process, alfalfa stems accumulate lignified cell wall material during maturation because xylem tissue proliferates through cambial activity, and xylem is highly lignified and almost completely indigestible (Jung and Engels, 2002).

Engels (1989) suggested that in maize (*Zea mays* L.) sclerenchyma the lignified middle lamella/primary wall region may act as a barrier to degradation of forages because rumen microorganisms cannot degrade lignin and this lignified region would prevent access to the potentially degradable cell wall polysaccharides in adjoining plant cells. Wilson and co-workers (Wilson, 1993; Wilson and Mertens, 1995; Wilson and Kennedy, 1996) extended Engels’ accessibility hypothesis to predict that forage particle size will limit digestion in the rumen because larger particles will consist of blocks of cells, many of which are not ruptured. Wilson and Mertens (1995) calculated that almost half of the cells in a 1500 μm × 250 μm grass forage particle are intact. This calculation implies that the majority of the potentially degradable cell wall polysaccharides are not available to rumen degradation because these polysaccharides cannot be accessed by rumen microbes. For forages with high cell wall concentrations such as alfalfa stems, this accessibility barrier could have a profound impact on nutrient utilization.

Because ingested forage particles are relatively large (McLeod and Minson, 1988), the ability of rumen microorganisms to degrade tissues with structurally intact cells is critical to extent of degradation. We investigated the depth to which rumen microbes could degrade individual alfalfa stem tissues utilizing a unique experimental system. By serially sectioning stem internode pieces after exposure to in vitro ruminal degradation, it was possible to determine microscopically how many cell layers could be degraded when cells were not
disrupted. The impact of alfalfa internode length and length of individual plant cells on depth of degradation was examined.

2. Materials and methods

2.1. In vitro ruminal degradation

The seventh internode, counting from the stem base, was harvested from multiple stems of field-grown alfalfa plants (clone 718) after 4 weeks of regrowth during the summers of 1996 (Experiment 1) and 1998 (Experiment 2). The internodes were immediately preserved in ethanol (500 ml L\(^{-1}\)). Prior to the degradation experiments, internodes were immersed in distilled water overnight (4 °C) to exchange the ethanol in the internode pieces with water. Internodes were trimmed to a central 18–25 mm segment. To prevent access of rumen bacteria to stem tissues through the epidermis and stomata, a bee’s wax covering was used. Internode pieces were momentarily dipped in molten bee’s wax (60 °C) to completely coat the internode with wax. To provide an entry point for rumen bacteria the wax coating was removed with a sliding-type microtome. Several 100 μm thick cross-sections were removed from the end of internode pieces until undamaged sections were obtained that contained no wax in the cell lumen. These final sections were mounted in glycerol (500 ml L\(^{-1}\)) on slides and retained as undegraded control sections for each internode piece.

In Experiment 1, a random set of four internode pieces collected in 1996 were compared for depth of degradation by rumen microbes. In addition to the four wax-coated internodes with one exposed end, several additional internode pieces were not covered in wax, completely encased in wax, or with wax only covering the internode piece ends served as controls to test the effectiveness of wax in preventing microbial access. Individual internode pieces were placed in separate, perforated plastic boxes. These boxes were placed in 300 ml glass tubes fitted with rubber stoppers and Bunzen valves that contained ~500 mg of dried and ground maize stover. For Experiment 1 rumen fluid was collected from a rumen-fistulated Holstein steer fed maize stover and a grain concentrate mix, and strained through one layer of cheesecloth. The tubes were inoculated with rumen fluid (10 ml) and McDougall’s buffer (40 ml) (McDougall, 1948).

For Experiment 2, overall internode length was measured prior to trimming stem internodes. Wax-covered internode pieces from five short and six long internodes collected in 1998 were compared for depth of degradation. Perforated boxes with the internode pieces were placed in 50 ml screw-cap centrifuge tubes with ~200 mg ground alfalfa. Rumen fluid was obtained from a rumen-fistulated, lactating Holstein cow fed a total mixed ration of maize silage, alfalfa hay, and a grain supplement. Rumen fluid was strained through four layers of cheesecloth prior to dilution with buffer. Each tube was inoculated with rumen fluid (6 ml) and McDougall’s buffer (24 ml).

In both experiments, rumen fluid was collected 3–5 h post-feeding and internode pieces were incubated at 39 °C for 24 h under anaerobic conditions. At the completion of the incubations, the plastic boxes were removed from the rumen inoculum, gently rinsed by submersion in water, and stem pieces were stored in ethanol. Serial sectioning at 100 μm intervals down the length of the internode pieces provided a profile of degradation with
distance from the original entry point. Light microscopy was used to compare the amount of tissue degradation in sections from internode pieces exposed to rumen microbes with undegraded control sections.

2.2. Cell length measurements

Length of individual cells for specific tissues were determined for internodes collected in both 1996 and 1998. Two random internodes collected in 1996 were longitudinally sectioned (50 µm thick). Individual tissues were identified by appearance and the length of 15 cells from each tissue was measured. Using internodes collected in 1998, four short and four long internodes (similar to the sets used in Experiment 2 for in vitro degradation) and another 11 internodes spanning a range of internode lengths were subjected to a cell separation technique to isolate cells for length measurement. The cell separation procedure utilized hydrogen peroxide oxidation to disrupt the middle lamella region connecting alfalfa cells and is a modification of the method outlined by Ruzin (1999). Internode pieces were trimmed to a 1 cm length. A longitudinal section containing two opposite collenchyma bundles and all tissues in between was made. This section was subsequently cut in half lengthwise. The two half sections were immersed in a 343 g acetic acid and 333 g hydrogen peroxide L$^{-1}$ solution, and refluxed for 90 min. A large volume of water was then added and the half sections were transferred to a microscope slide. After removal of excess water with tissue paper, the softened tissues were covered with glycerol. A cover slip was placed over the sample and samples were gently flattened to separate cells. Cells for each tissue were identified by shape and length of 25 cells from each tissue was measured. Mean cell length was calculated for each tissue type, from each internode piece.

2.3. Statistical analysis

Data from Experiment 1 were not statistically analyzed for depth of degradation because of limited replication. Depth of degradation data for individual stem tissues in alfalfa internodes were analyzed from Experiment 2 as a completely randomized design with two treatments (long and short internode length groups). Pearson correlation coefficients for length of cells for individual stem tissues types from the 1998 samples with internode length were determined. The PROC GLM and PROC CORR procedures in PC-SAS were used (SAS, 1985). Statistical significance was declared if $P < 0.05$.

3. Results

3.1. Depth of degradation

Coating internodes with bee’s wax was an effective barrier against microbial access to potentially degradable alfalfa stem tissues in Experiment 1. When the wax was not removed from one end of the coated internode pieces prior to exposure to rumen microorganisms, no signs of tissue degradation were observed in the serial sections. A wax cap on both ends of the internodes also prevented microbial access to potentially degradable alfalfa tissues if
Fig. 1. A sequential series of cross-sections removed from an alfalfa internode piece exposed to degradation by rumen microorganisms for 24 h. The control section illustrates the structure of tissues before exposure to rumen fluid (epi, epidermis; coll, collenchyma; chl, chlorenchyma; pf, phloem fibre; cam, cambium and secondary phloem; xylem, xylem fibres and vessels; pxp, primary xylem parenchyma; pith, pith parenchyma). Non-degraded tissues are indicated in the section from 1000 μm below the cut end of the internode piece. Those tissues which were still degraded at 2000 and 3100 μm below the end of the internode piece are indicated in subsequent sections. Scale bar = 20 μm.

The epidermis was not damaged. When the epidermis was damaged, rumen microorganisms were able to degrade non-lignified alfalfa tissues (collenchyma, chlorenchyma, secondary phloem, and cambium) located to the exterior of the xylem ring; however, microbes did not gain access to the potentially degradable tissues inside the xylem ring (data not shown). Internode pieces with no wax coating exhibited degradation of tissues from both ends and wherever the epidermis was damaged.

The xylem ring structure was intact in all sections exposed to ruminal degradation in Experiment 1. Other tissues were initially absent from sections near the exposed end of the internode pieces. Fig. 1 illustrates the degree of alfalfa stem tissue degradation at various depths below the surface exposed to the rumen inoculum. At a depth of 1000 μm complete degradation of most tissues had occurred, with only phloem fibre and xylem remaining intact. At 2000 μm some residual collenchyma structure was visible whereas the cambium and primary xylem parenchyma were completely degraded. At a depth of 3100 μm below the original exposed surface, only the primary xylem parenchyma was still degraded.

Phloem fibre bundles were often difficult to locate in sections near the exposed end of the internode pieces because this tissue collapsed when non-lignified tissues around the phloem fibre bundles were degraded. Visual inspection of internode pieces without any wax
coating indicated the presence of these phloem fibre bundles as fine threads near the ends of the internodes. Although difficult to assess, in some phloem fibre bundles degradation occurred to only a limited extent. From non-degraded control sections it appeared that these phloem fibre cells had virtually no cell lumen space because of extreme thickening of the secondary wall. Intercellular spaces were observed in the chlorenchyma, but not in other tissues.

Based on evaluation of four stem pieces in Experiment 1, the maximal depth of degradation for non-lignified collenchyma (4150 μm) and primary xylem parenchyma (5600 μm) were 10× greater than observed for lignified tissues such as phloem and xylem fibres (800 and 300 μm, respectively). The non-lignified additional secondary wall layer present in many xylem fibre cells was completely degradable at the exposed surface of internode pieces (Fig. 2). However, at a depth of 100 μm below the exposed surface, the additional secondary wall layer remained non-degraded in some xylem fibre cells. This additional wall layer was again present in most xylem fibre cells at a depth of 200 μm below the original exposed surface.

Mean depth of degradation observed for all tissues was almost twice as great in two of the internode pieces examined compared to the other two internode pieces (data not shown). This observation lead to our decision to determine length of internodes in Experiment 2.
Table 1
Maximal average depth of 24-h in vitro ruminal degradation in Experiment 2 for individual alfalfa tissues in 18–20 mm long internode pieces from long (55–65 mm, \(N = 6\)) and short (18–22 mm, \(N = 5\)) internodes harvested after 4 weeks of regrowth in 1998

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Internode length group</th>
<th>Long ((\mu m))</th>
<th>Short ((\mu m))</th>
<th>S.E.M.(^a)((\mu m))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collenchyma(^*)</td>
<td>6917</td>
<td>3700</td>
<td>988</td>
<td></td>
</tr>
<tr>
<td>Epidermis(^*)</td>
<td>6917</td>
<td>3700</td>
<td>988</td>
<td></td>
</tr>
<tr>
<td>Chlorenchyma(^*)</td>
<td>6983</td>
<td>3700</td>
<td>988</td>
<td></td>
</tr>
<tr>
<td>Primary phloem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(^*) wall(^†)</td>
<td>1360</td>
<td>500</td>
<td>283</td>
<td></td>
</tr>
<tr>
<td>2(^*) wall</td>
<td>533</td>
<td>267</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>Secondary phloem(^*)</td>
<td>6833</td>
<td>3800</td>
<td>959</td>
<td></td>
</tr>
<tr>
<td>Cambium(^*)</td>
<td>6833</td>
<td>3800</td>
<td>959</td>
<td></td>
</tr>
<tr>
<td>Secondary xylem fibre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(^<em>) and 2(^</em>) wall</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Additional 2(^*) wall</td>
<td>200</td>
<td>150</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Primary xylem vessels</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Primary xylem parenchyma(^*)</td>
<td>8200</td>
<td>5380</td>
<td>667</td>
<td></td>
</tr>
<tr>
<td>Pith parenchyma</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) Standard error of the mean.
\(^*\) \(P < 0.05\).
\(^†\) \(P < 0.10\).

before trimming for in vitro degradation. Internodes used in Experiment 2 were assigned to long and short groups. The long internodes averaged 53 ± 4 mm in length (range 55–65 mm), whereas the short internodes were only 20 ± 1 mm long (range 18–22 mm). Internode diameter was also different (2.32 ± 0.39 and 1.54 ± 0.22 mm for long and short internodes, respectively). While long and short internode groups did not overlap in length, internode diameter did overlap slightly (1.63–2.68 and 1.24–1.78 mm for long and short internodes, respectively).

Maximal depths of degradation for long alfalfa internodes in Experiment 2 were greater \((P < 0.05)\) for non-lignified tissues (chlorenchyma, collenchyma, epidermis, and primary xylem parenchyma) than observed for short internodes (Table 1). Cambium, secondary phloem, and primary wall of phloem fibre tissues also showed a trend \((P < 0.10)\) towards greater depth of degradation in long internodes than short internodes. The heavily lignified xylem tissues and pith parenchyma were completely undegradable. The non-lignified wall layers of phloem fibre (secondary wall) and xylem fibre (additional secondary wall) were degraded in the first few sections below the exposed surface. Although long internodes of these phloem and xylem fibre wall layers were found to be degraded to numerically greater depths than for short internodes, the observed differences were not significant. Variability among stem pieces for depth of degradation was substantial as evidenced by the large standard errors encountered for all tissues. As observed in Experiment 1, non-lignified tissues were degraded to greater depth in the stem pieces than observed for lignified tissues.
Table 2  
Length of individual cells for specific tissue types from alfalfa stem internodes harvested in 1996 and 1998

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1996a</th>
<th>1998b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (μm)</td>
<td>Minimum (μm)</td>
</tr>
<tr>
<td>Epidermis</td>
<td>103</td>
<td>64</td>
</tr>
<tr>
<td>Collenchyma</td>
<td>90</td>
<td>56</td>
</tr>
<tr>
<td>Chlorenchyma</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Phloem fibre</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Xylem fibre</td>
<td>211</td>
<td>120</td>
</tr>
<tr>
<td>Pith parenchyma</td>
<td>196</td>
<td>79</td>
</tr>
</tbody>
</table>

a Cell length determined by longitudinal sectioning. Data are means of 15 cells per tissue from two internodes.
b Cell length determined by cell separation technique. Data are means of 25 cells per tissue from 11 internodes.
c Not determined.

3.2. Length of cells in stem tissues

Cell lengths for major alfalfa stem tissues are shown in Table 2. Collenchyma, xylem fibre, and pith parenchyma tissues were measured using both longitudinal sectioning and cell separation methods. Mean cell lengths determined by both methods were similar for collenchyma and pith parenchyma tissues, but xylem fibre lengths were longer when measured using the separated cell technique. Xylem and phloem fibres had very elongated, narrow shapes that made recognition of cell ends difficult in longitudinal sections, making accurate measurements problematic. Chlorenchyma cells were the shortest and phloem fibre cells were the longest of the tissues examined. Epidermis and collenchyma cells were similar in length, and xylem fibre and pith parenchyma cells were also similar to one another in length. The range in cell lengths was large for all tissues (Table 2).

Length of cells for all tissues examined was positively correlated with length of the internode from which the cells originated. Phloem fibre cells were longer in internodes from the long treatment group in Experiment 2 than for the short group (1802 μm versus 774 μm, P < 0.05). Cell lengths for other tissues were not determined for the two internode length treatment groups in Experiment 2 because of accidental disposal of samples. For the 11 internodes of varying length from 1998, the cells from collenchyma, phloem and xylem fibres, and pith parenchyma all exhibited significant positive correlations with internode length (Fig. 3). A doubling of alfalfa internode length resulted in approximately a 50% increase in length of individual stem tissue cells.

4. Discussion

The observed great depth of degradation for non-lignified tissues in stem internode pieces indicated that cell walls between adjoining cells must have been removed by microbial action. Many cell layers of non-lignified tissues must have been degraded during exposure of large stem pieces to rumen microbes. Using the overall means for maximal depth of degradation (Table 1) and cell length of individual tissues (Table 2), we calculated that
Fig. 3. Relationships for mean cell lengths of collenchyma, phloem fibre, xylem fibre, and pith parenchyma tissues with overall internode length for alfalfa stem internodes collected in 1998.

66 cell layers were degraded on average for collenchyma and 52 cell layers for epidermis tissues. Chlorenchyma cells were shorter in length (Table 2) but degraded to the same depth, therefore, we calculated that approximately 175 cell layers were degraded in depth for this tissue.

Jung and Engels (2001) calculated rates of cell wall degradation in alfalfa stem tissues by rumen microbes for collenchyma, phloem fibre, primary xylem parenchyma, and pith parenchyma of 0.11, 0.07, 0.04, and 0.11 μm h⁻¹, respectively. Using these previous estimates for rates of cell wall degradation, we calculated that for collenchyma tissue with a mean cell wall thickness of 0.90 μm (Jung and Engels, 2001) approximately 1080 h would be required for rumen microbes to degrade all the collenchyma cell walls to the depth of degradation that was actually observed after only 24 h in the current study (Table 1). In stem cross-sections, chlorenchyma primary walls were degraded very rapidly, within 4 h (Jung and Engels, 2001). Chlorenchyma cell walls were at the limit of light microscope observation and we estimated a thickness of 0.25 μm for two adjacent cells. If we assumed
the rate of cell wall degradation for chlorenchyma was similar to that of collenchyma, then it would take up to 400 h for degradation to reach the depths observed after only 24 h. These calculations suggest that the observed depths of degradation could not have occurred simply by successive degradation through the walls within columns of cells. Of course the time intervals calculated here are over-estimates because only a small opening in a portion of a cell’s wall need be created by degradation before bacteria can begin colonizing the next plant cell.

A partial explanation for the great depths of degradation may involve the numerous large intercellular spaces observed between alfalfa chlorenchyma cells and underneath stomata in the epidermis. This suggests that degradation was possible partly because of diffusion by rumen bacteria through the stem pieces via such intercellular spaces, followed by degradation of non-lignified walls to gain access to cell lumens at greater depth in the stem piece. Rate of diffusion through intercellular spaces should be faster than calculated for the cell lumen of grass sclerenchyma fibre cells by Wilson and Mertens (1995) because the alfalfa intercellular spaces were much larger in diameter (10–30 μm) than the small lumen of sclerenchyma fibres (2.2 μm). Based on diffusion of a molecule through a channel of pores (Nye and Tinker, 1977), the ratio of bacterial diameter to intercellular space size should greatly impact the rate of diffusion. However, even with no retardation of diffusion because of bacterial size relative to intercellular space size, the depth of degradation observed still exceeded that predicted by the equation of Nye and Tinker (1977). The exact mechanisms accounting for the great depth of degradation remain unclear. Perhaps evolution of gas by the bacterial fermentation alters diffusion dynamics through inter- and intra-cellular spaces, plus the number of bacteria in the plant tissue increases through division.

Degradation of non-lignified secondary walls of alfalfa phloem fibre supported the prediction of Wilson and Mertens (1995) that slow bacterial diffusion in long narrow cells limit degradation. In mature alfalfa stems, the secondary wall of phloem fibre remained non-lignified but often appeared undegraded. This result may originate from the fact that in mature internodes the lumen of phloem fibre cells was almost completely occluded by extensive secondary wall deposition. In this case only 1.7 μm in depth of phloem fibre secondary wall, from the exposed surface of the stem piece, might be degraded in 24 h. The mean cell length of phloem fibres was 1598 μm. Therefore, only approximately 0.001 of the potentially degradable secondary wall would be degraded from each cell layer of phloem fibre. In alfalfa there were 16–20 phloem fibre bundles in stems (Engels and Jung, 1998), which represent a considerable amount of cell wall material. Breeding for alfalfa varieties with less extensive phloem fibre secondary wall development, thereby having a significant cell lumen space in mature stems, could increase total cell wall degradability of stems.

Degradation of secondary wall of lignified phloem fibres and the additional secondary wall of lignified xylem fibres was apparently limited to a single cell layer because average cell length of these tissues (Table 2) was longer than the observed depth of degradation (Table 1). Only the non-lignified wall layers of lignified cells that had been ruptured by sectioning and directly exposed to microbial access were degraded. As hypothesized by Engels (1989) and others (Wilson and Mertens, 1995; Wilson and Kennedy, 1996), lignified walls acted as absolute barriers to rumen microorganisms gaining access to potentially degradable cell wall layers if the alfalfa cells had not been mechanically ruptured.
Fig. 4 illustrates how lignified walls limit depth of degradation in alfalfa stems, and how the pattern of gradual appearance of non-degraded tissue in serial cross-sections can occur due to variation in cell length and staggering of cell columns in stems. Removal of material from the top of a stem piece exposes the lumen of all cells to microbial degradation of non-lignified wall layers. When the internode is subsequently sectioned 100 μm below the original exposed surface, examination of the newly exposed top surface of the internode shows no degradation in the short cell column and the center cell column because there are lignified cross walls above this level in the cell columns of the first section. After removing the second section, only cells in the column on the right edge of the drawing still show degradation of walls at the newly exposed surface because lignified barrier cross walls are present above this position in the other columns of cells. Lack of cell wall degradation in the column of long cells on the right edge would only be observed at the surface of the fifth section because the lignified barrier cross wall in this cell column is located in the fourth section.

The difference in depth of degradation between long and short internodes may result from the more compact structure of the short internodes due to shorter cell lengths and possibly smaller intercellular spaces. For the particular alfalfa clone studied, it is clear that length of cells in stem tissues increased as internode length increased. Because cell
length only increased approximately 50% with a doubling of internode length, we conclude that longer internodes must contain both longer cells and also more cells. Whether this relationship between cell length and internode length is specific for this particular alfalfa clone or a general trait of alfalfa, or also true of other forage species, remains to be determined.

If cell and internode lengths are generally positively correlated, then several important hypotheses concerning stem degradability can be suggested. Because non-lignified alfalfa tissues are completely degradable at all stages of maturation (Jung and Engels, 2002), degradation of this cell wall material should be maximized when internodes are short. The shorter cell lengths of these non-lignified tissues in short internodes should reduce the time necessary for rumen bacteria to diffuse down the lumen inside of cells and through intercellular spaces to reach the next degradable cell wall surface. In contrast, maximizing degradation of potentially degradable cell wall layers in lignified tissues would occur when internodes and cells are long. This should be true because only cells mechanically ruptured are available for degradation and long cells increase the amount of potentially degradable wall in each cell. Also, longer cells will increase the probability that mastication or feed grinding will rupture a given cell within a specific size particle. This advantage of longer cells to be mechanically disrupted will also occur for non-lignified tissues. Whether long or short internodes would be most beneficial to the total feeding value of alfalfa stems will depend on the total amount of potentially degradable cell wall available from non-lignified versus lignified tissues in stems, and the amount of other nutrients (protein, soluble sugars, etc.) trapped in these tissues. Research to address these hypotheses is currently in progress.

5. Conclusions

Degradation of non-lignified alfalfa stem tissues in large particles occurred by microbial degradation through the walls between adjacent cells and by movement of microorganisms through intercellular spaces in the chlorenchyma tissue. Many cell layers could be degraded within 24 h in non-lignified tissues. In contrast, degradation of non-lignified cell wall layers in phloem and xylem fibre cells was limited to those cells which were mechanically ruptured to allow microbial access to the cell lumen. The lignified primary wall was an absolute barrier to microbial degradation from one lignified cell through the intervening walls to an adjacent cell. In alfalfa stems, individual cells became longer as internode length increased for both lignified and non-lignified tissues. This increase in cell length resulted in greater depth of degradation through alfalfa stem pieces. The relationship between cell length and lignification of the cell wall was postulated to have differential impacts on extent of degradation for various tissues.

References


