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Volume, surface area and cellular composition of chewed particles of plant parts of eight forage species and estimated degradation of cell wall

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SUMMARY

In order to understand more about the nature of forage particles before they enter the rumen and about the degradation of cell walls in the rumen, particles of different plant parts produced by sheep when eating each of eight plant species were described in terms of volume and exposed surface area and in terms of numbers of cells and volume and surface area of cell wall. Estimates were made of the extent to which the walls would be degraded in the rumen. The eight species were: *Trifolium repens* L., *Medicago sativa* L., *Desmodium intortum* (Mill.) Urb., *Lolium perenne* L., *Festuca arundinacea* Schreb., *Chloris gayana* Kunth, *Cenchrus ciliaris* L. and *Zea mays* L. In each case early harvesting was compared with later harvesting in each of two years. The plants were grown in a heated glasshouse.

The plant parts which were chewed to the smallest particle size during eating, and which produced particles with the largest proportion of total cell wall area exposed on the outside of the particle (6–10 %), were the legume leaflets and the grass leaf blades and sheaths. The proportion of total cell wall area exposed on the outside of chewed particles derived from stems was only 3–4 %. The number of thick-walled cells per mm^3 of chewed particle was greatest in particles derived from the leaf blades and stems of *C. ciliaris*, the leaf blades and leaf sheaths of *L. perenne* and the stems of *C. gayana*. The average number of thick-walled cells per particle was 860 000 in particles derived from the stems of *C. gayana* and *C. ciliaris* and 260 000 in particles derived from *L. perenne* leaf blades and sheaths. The volume and surface area of walls of thin-walled cells per mm^3 were greatest in particles derived from legume leaflets. The total surface area of walls of epidermal cells per mm^3 was greatest in particles derived from leaflets and leaf blades and least in those derived from stems. The estimated percentage of wall thickness of thick-walled cells which would be degraded in the rumen was relatively high in the case of leaf blades, sheaths and stems of *C. ciliaris*, leaf blades of *Z. mays*, leaf sheaths of *L. perenne* and *F. arundinacea* and stems of *C. gayana*, relatively low in the case of petioles of *T. repens* and stems of *D. intortum* and *M. sativa* and close to zero in legume leaflets. The estimated percentage of wall thickness which would be degraded did not seem to be related to the concentration of lignin in the walls, either in grasses or in legumes.

INTRODUCTION

The extent to which forage is broken down into particles during eating affects the extent to which cell contents are released immediately and the extent to which rumen microbes have immediate access to cell walls. Some particles are small enough to pass through a 1 mm sieve when forage is eaten by cattle (Kennedy 1985) or sheep (Wilman *et al*. 1997) and are therefore presumed small enough to pass out of the reticulorumen easily (Poppi *et al*. 1985). The majority of particles produced during eating, however, are likely to be larger than this and to require further breakdown, mainly by rumination (Kennedy 1985), before they are small enough to pass out of the reticulorumen.

The composition of chewed particles in terms of numbers of cells, the volume of cell walls and the surface area of those walls has so far received little attention. The extent to which forage is digested,

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however, is likely to be very much affected by the extent to which cells are broken to release cell contents, the extent to which microbes gain access to cell walls, the amount of time they have available for degrading the walls and the thickness of the walls. If the rate of degradation of cell wall by rumen microbes is only *c*. 0·02 μm/h (Wilson & Mertens 1995), if forage particles typically remain in the rumen only *c*. 25–33 h (Minson 1982) and if sclerenchyma cells are typically arranged in tightly packed blocks to which bacteria have very limited access (Wilson & Mertens 1995), it is important, for an understanding of forage digestion, to know how many cells there are in particles, what types of cells are present, how much cell wall is potentially available for degradation and how large a surface area is available for colonization by bacteria. It is also important to know to what extent the lignification of cell walls of different species and plant parts restricts the degradation of those walls. According to Wilson & Mertens (1995), secondary wall material of grasses is degradable, even though lignified, whereas the lignified cell walls of legumes have a very high concentration of lignin and are not degraded.

In the present paper, eight contrasting forage species are considered. In an earlier paper, Rezvani Moghaddam & Wilman (1998) referred to the proportions of thick-walled, thin-walled and epidermal cells present, cell wall thickness and cell dimensions in different plant parts of the eight species. For the present paper we measured the volume and exposed surface area of forage particles which had been produced by sheep during eating, by analysing swallowed boli, and calculated the numbers of cells of different types and the volumes and surface areas of cell wall which the particles contained. The extent to which the walls of the thick-walled cells in these particles are likely to be degraded in the rumen was estimated.

MATERIALS AND METHODS

The plant material studied was obtained from eight of the twelve species grown in the experiment described by Wilman *et al*. (1996).

The experimental treatments comprised all combinations of eight plant species and early *v*. later harvesting. A randomized block design was used, with two blocks. The eight species were: *Trifolium repens* L., *Medicago sativa* L., *Desmodium intortum* (Mill.) Urb., *Lolium perenne* L., *Festuca arundinacea* Schreb., *Chloris gayana* Kunth, *Cenchrus ciliaris* L. and *Zea mays* L. Early harvesting was on 25–26 June 1991 and 22–25 June 1992, except that the three leguminous species were harvested on 23–24 July in 1991 (because of slower development). Later harvesting was on 23–24 July 1991 and 22–25 July 1992, except that the three legumes were harvested on 20 August in 1991. The plants were grown at a density of 3–18 plants per pot according to species (Wilman *et al*. 1996) in 25-cm pots containing potting mix in a heated glasshouse.

At each harvest the plants were cut *c*. 2 cm above the soil surface. Fresh forage of the sown species was offered to two oesophageal-fistulated sheep (one in 1992) in small meals of 100 g fresh weight. At each meal, a sheep was allowed to eat for only one 1-min test period and the amount eaten was recorded (Wilman *et al*. 1996). The plants in each pot provided two meals in each year for this purpose. The forage was chopped to 7-cm lengths before feeding. The proportion of plant parts in the forage fed was as in the harvested crop (Table 6 of Wilman *et al*. 1996), except that the *Z. mays* meals were made up of 80 g of leaf blade and 20 g of stem. The chewed forage was collected from the fistula in a polythene bag, within a linen bag tied round the sheep's neck. A throat plug was used to ensure that all chewed particles passed out through the fistula. The sheep were Clun Forest adult castrate males, 56 kg liveweight.

A subsample from each bolus sample was examined by Mtengeti *et al*. (1996) for numbers and dimensions of particles, numbers of veins per particle and extent of damage to particles. The remaining material from each sample was stored in a freezer until required for the present study. This material was defrosted and a subsample separated into particles derived from different plant parts as follows: those from leaflets, petioles and stems (stolons) of *T. repens*, those from leaflets and stems of *M. sativa* and *D. intortum*, those from leaf blades and leaf sheaths of *L. perenne* and *F. arundinacea*, those from leaf blades, sheaths and stems of *C. gayana* and *C. ciliaris* and those from leaf blades of *Z. mays*. Ten randomly-selected particles from each plant part fraction per subsample were examined. The length, width, thickness, surface area and shape of each particle and the number of tears and the length of each tear were recorded, using a binocular light microscope, eyepiece grid and micrometer. From these records, the volume of each particle and the exposed external surface area (including the exposed surface area of tears) were calculated. From these records and those reported in a previous paper (Rezvani Moghaddam & Wilman 1998) the numbers of cells of different types, and the volumes and surface areas of cell walls per particle and per mm^3 of particle, were also calculated. It was assumed in these calculations that randomly-selected particles from each plant part fraction in the boli would, on average, have approximately the same proportion of thick-walled, thin-walled and epidermal cells as the unchewed forage. The numbers of cells of different types per mm^3 of particle were obtained by allocating a share of the mm^3 to thick-walled, thin-walled and epidermal cells on the basis of their percentage in cross-sectional area (from Table 2 of

Rezvani Moghaddam & Wilman 1998) and dividing that share by the appropriate volume per cell (from Table 3 of Rezvani Moghaddam & Wilman 1998) (e.g. 1 $mm^3 \times 0{\cdot}2$ proportion of thick-walled cells = $0{\cdot}2$ mm^3; $\div$ 10000 μm^3 volume per cell = 20000 cells). The lumen surface areas of different cell types per mm^3 of particle were calculated (on the basis that the inside of the cell can be regarded for this purpose as the inside of a cylinder) from the internal length and diameter of the cells and the numbers of those cells per mm^3 (e.g. {(50 μm internal length $\times$ 10 μm internal diameter $\times \pi$) + (10 μm internal diameter$^2 \times 0{\cdot}5\ \pi$)} $\times$ 20000 cells per mm^3 of particle = 35 mm^2). In the case of epidermal cells, the area of exposed outer wall was added to the lumen surface area, on the basis that the outer surface would be exposed to rumen microbes. The volumes of cell walls of different types per mm^3 of particle were calculated by multiplying the appropriate numbers of cells per mm^3 of particle by the volume of cell wall per cell. The latter was calculated as the difference between the volume per cell and the lumen volume per cell (e.g. 10000 μm^3 volume per cell minus (50 μm internal length $\times$ 10 μm internal diameter$^2 \times 0{\cdot}25\pi$) = 6073 μm^3).

To estimate the rumen degradation of the walls of thick-walled cells, as a percentage of the initial wall thickness and in μm, the following assumptions were made: (1) *in vitro* dry matter digestibility (DMD) determined by the Tilley & Terry (1963) technique using rumen fluid followed by pepsin with HCl (from Wilman & Rezvani Moghaddam 1998) is a satisfactory estimate of *in vivo* DMD, as found in many laboratories (Van Soest 1994) including our own (e.g. Derrick *et al*. 1993) (although there must be some uncertainty as to whether it will predict accurately the digestibility of all plant parts of all forage species); (2) this *in vitro* estimate of digestibility plus 12·9 percentage units is a satisfactory estimate of true DMD (Van Soest 1967, 1994); (3) neutral detergent fibre (NDF) determined in freeze-dried herbage (from Wilman & Rezvani Moghaddam 1998) is a reasonable estimate of total cell wall (Van Soest 1994); (4) cell contents (100 minus NDF) are *c*. 98 % digestible (Van Soest 1967, 1994) and the remainder of true DMD is accounted for by cell wall degradation; (5) volumes of cell wall per mm^3 of particle provide an acceptable estimate of the distribution of cell wall mass between thick-walled, thin-walled and epidermal cells; (6) the rate of degradation of wall is similar in all types of wall; (7) the walls of cells categorized as thin-walled in the present study (Rezvani Moghaddam & Wilman 1998) are completely degraded, as are the inner walls of epidermal cells except those in the stems (which are thicker); (8) access to cell walls by rumen microbes is not a greater problem with some types of wall than with others. The following is an example of the estimation of the percentage of the wall thickness of thick-walled cells in stems which would be degraded: (750 g/kg true DMD − 210 g/kg contribution of cell content − 20 g/kg contribution of thin walls) $\times$ 95 % of walls of thick-walled cells in (thick + epidermal) wall $\div$ 720 g/kg volume contribution of thick-walled cells to NDF = 69. Clearly, with the above assumptions, the resulting estimates of the wall thickness degraded will not be precise, but it seems worthwhile making the estimates so that they can be compared with those used by Wilson & Mertens (1995) when they considered the extent to which forage digestion may be restricted by cell walls which are too thick to be fully degraded during the time which forage particles spend in the rumen. Assumption (8) in particular may not be true (Wilson & Mertens 1995), in which case the estimates of the average rate of degradation of the walls of the thick-walled cells will be an over-estimate for the less accessible cells and an under-estimate for the more accessible.

The differences between the species were consistent from year to year and the results from the 2 years were analysed together, using the years $\times$ treatments interaction as the error term.

RESULTS

There was very little effect of early *v*. later harvesting and the results are therefore presented as means of the two harvesting treatments and of the 2 years.

Particle size

The plant parts which were chewed to the smallest particle size during eating by sheep were the legume leaflets and the grass leaf blades and leaf sheaths (Table 1). The particles derived from *F. arundinacea* leaf blades and sheaths were larger than those derived from the leaves of the other grasses. The plant parts which were the largest when swallowed during eating were those derived from *T. repens* stems and petioles and *M. sativa* stems. The particles derived from the stems of *D. intortum*, *C. gayana* and *C. ciliaris* were also larger than the particles derived from the leaflets or leaf blades and sheaths of those species. The exposed surface area of the chewed particles was much larger, per mm^3 of particle volume, in particles derived from leaflets, leaf blades or sheaths than in particles derived from stems or petioles. However, because the particles derived from *T. repens* stems and petioles and *M. sativa* stems were so large, these were the particles with the largest exposed surface area per particle when swallowed (Table 1).

Number of cells

The number of thick-walled cells per mm^3 of chewed particle (Table 1) was greatest in particles derived from the leaf blades and stems of *C. ciliaris*, the leaf blades and leaf sheaths of *L. perenne* and the stems of

Table 1. *Volume and surface area of particles derived from plant parts of eight forage species and the numbers of cells of different types, means of 2 years and of early and later harvesting*

Plant part and species	Volume (mm^3)	Surface area (mm^2)	Surface area/volume (mm^2/mm^3)	Total number of cells per particle ('000) with % thick-walled cells in brackets	Numbers of cells ('000 per mm^3 of particle)		
					Thick-walled cells	Thin-walled cells	Epidermal cells
Leaflets or leaf blades							
Trifolium repens	1·7	37	21·8	398 (8·0)	19	203	12
Medicago sativa	1·4	31	21·6	162 (5·0)	5	92	12
Desmodium intortum	2·1	49	24·4	327 (6·9)	10	90	49
Lolium perenne	3·4	46	13·8	392 (82·0)	96	12	8
Festuca arundinacea	6·8	69	11·3	216 (78·6)	25	5	2
Chloris gayana	0·8	17	21·7	81 (72·3)	70	20	7
Cenchrus ciliaris	1·6	26	16·6	171 (95·2)	101	1	3
Zea mays	1·4	24	17·8	135 (43·8)	43*	49*	4*
S.E. (15 D.F.)	0·70	6·7	1·17	62·1 (2·63)	10·2	6·2	3·0
Mean	2·4	37	18·6	235 (49·0)	47	60	13
Petioles							
Trifolium repens	25·9	122	4·7	186 (33·9)	2	1	4
Leaf sheaths							
Lolium perenne	2·3	33	14·7	230 (83·5)	84	12	5
Festuca arundinacea	6·2	60	12·0	147 (76·7)	17	4	2
Chloris gayana	1·8	23	13·0	115 (78·3)	45	1	11
Cenchrus ciliaris	2·8	40	14·6	149 (89·3)	50	3	3
S.E. (7 D.F.)	1·06	6·5	1·52	32·6 (2·28)	7·1	0·7	1·8
Mean	3·3	39	13·6	160 (81·9)	49	5	5
Stems							
Trifolium repens	39·1	123	3·3	354 (39·2)	4	2	4
Medicago sativa	27·5	100	3·8	392 (83·1)	12	2	1
Desmodium intortum	19·1	81	4·6	375 (38·0)	8	2	12
Chloris gayana	10·6	53	5·0	934 (93·1)	83	1	5
Cenchrus ciliaris	7·9	43	5·8	905 (93·1)	111	1	6
S.E. (9 D.F.)	4·43	11·8	0·27	106·4 (2·82)	13·8	0·2	0·5
Mean	20·8	80	4·5	592 (69·3)	44	2	6

* Midribs excluded from the calculations.

C. gayana. Taking into account the size of the chewed particles derived from the stems of *C. gayana* and *C. ciliaris*, the number of thick-walled cells per particle was very large, on average 920000. Even in *L. perenne* leaf blades and sheaths, which were chewed to small particles, the number of thick-walled cells per particle was large, on average 260000. The smallest numbers of thick-walled cells per mm^3 were in particles derived from the leaflets, petioles and stems of the leguminous species. The number of thick-walled cells per mm^3 was also rather low in particles derived from *F. arundinacea* but, because those particles were moderately large, there were, on average, 140000 thick-walled cells per particle. The number of thin-walled cells per mm^3 was greatest in particles derived from legume leaflets and was very low in particles derived from most other plant parts, particularly stems and petioles. The number of epidermal cells per mm^3 was low in most types of particle; the largest number per mm^3 in particles derived from leaflets or leaf blades was in particles from *D. intortum* leaflets and the largest number per mm^3 in particles derived from stems was in particles from *D. intortum* stems. The latter particles were quite large and had 200000 epidermal cells per particle, on average.

Volume of cell wall

The volume of walls of thick-walled cells per mm^3 of particle (Table 2) was greatest in particles derived from the stems of *C. gayana* and *C. ciliaris* and, to a lesser extent, in particles derived from the leaf blades of *C. gayana* and *C. ciliaris* and from the stems of *M. sativa*. In particles derived from stems, the combination of large particles, a high proportion of thick-walled cells and thick walls (Rezvani Moghaddam & Wilman 1998) meant that the total volume of wall of thick-walled cells per particle was high, 2·7 mm^3 on

Table 2. *Volume and lumen surface area of cell walls in particles derived from plant parts of eight forage species, means of 2 years and of early and later harvesting*

Plant part and species	Volume of walls (mm^3/mm^3 of particle)				Surface area of walls (mm^2/mm^3 of particle)			
	Thick-walled cells	Thin-walled cells	Epidermal cells	Total	Thick-walled cells*	Thin-walled cells*	Epidermal cells†	Total
Leaflets or leaf blades								
Trifolium repens‡	0·012	0·083	0·031	0·126	12	259	56	327
Medicago sativa	0·020	0·048	0·037	0·105	12	203	53	268
Desmodium intortum‡	0·019	0·045	0·040	0·104	15	202	71	288
Lolium perenne	0·081	0·013	0·020	0·114	83	91	65	239
Festuca arundinacea	0·074	0·017	0·043	0·134	54	63	39	156
Chloris gayana	0·131	0·015	0·071	0·217	88	66	67	221
Cenchrus ciliaris	0·177	0·004	0·058	0·239	115	16	58	189
Zea mays‡	0·111	0·037	0·102	0·250	64	105	54	223
S.E. (15 D.F.)	0·0087	0·0017	0·0039	0·0080	6·4	4·3	3·1	7·0
Mean	0·078	0·033	0·050	0·161	55	126	58	239
Petioles								
Trifolium repens	0·044	0·020	0·018	0·082	19	51	17	87
Leaf sheaths								
Lolium perenne	0·098	0·023	0·064	0·185	72	92	44	208
Festuca arundinacea	0·068	0·022	0·053	0·143	40	75	24	139
Chloris gayana	0·076	0·017	0·065	0·158	45	51	35	131
Cenchrus ciliaris	0·094	0·012	0·038	0·144	71	60	32	163
S.E. (7 D.F.)	0·0075	0·0019	0·0071	0·0108	3·9	2·8	4·2	7·5
Mean	0·084	0·019	0·055	0·158	57	69	34	160
Stems								
Trifolium repens	0·083	0·019	0·012	0·114	33	56	10	99
Medicago sativa	0·144	0·018	0·008	0·170	67	48	5	120
Desmodium intortum	0·097	0·016	0·010	0·123	52	49	11	112
Chloris gayana	0·239	0·017	0·018	0·274	96	40	9	145
Cenchrus ciliaris	0·295	0·005	0·028	0·328	124	32	11	167
S.E. (9 D.F.)	0·0259	0·0012	0·0020	0·0260	6·5	3·4	0·9	5·4
Mean	0·172	0·015	0·015	0·202	74	45	9	128

* Inner wall of cells only.
† Inner wall of cells plus exposed outer wall.
‡ Midribs excluded from the calculations.

average. The volume of walls of thick-walled cells per mm^3 was low in the particles derived from legume leaflets. The volume of walls of thin-walled cells per mm^3 was greatest in particles derived from legume leaflets, particularly those of *T. repens*. The volume of walls of epidermal cells per mm^3 was greatest in particles derived from *Z. mays* leaf blades; however, these particles were fairly small and the greatest volume of walls of epidermal cells per particle was in particles derived from the petioles and stems of *T. repens*.

Surface area of cell wall

The total lumen surface area of walls of thick-walled cells per mm^3 was greatest in particles derived from the stems and leaf blades of *C. ciliaris* and *C. gayana*, and in those from the leaf blades of *L. perenne*, and least in particles derived from legume leaflets (Table 2). The total surface area of walls of thin-walled cells per mm^3 was greatest in particles derived from legume leaflets and, to a lesser extent, in particles from *Z. mays* leaf blades and *L. perenne* leaf blades and sheaths, and least in particles derived from *C. ciliaris* leaf blades. The total surface area of walls of epidermal cells per mm^3 was greatest in particles derived from leaflets and leaf blades and least in those derived from stems.

The proportion of total particle cell wall area which was exposed on the outside of a particle was greater in particles derived from leaf sheaths and leaflets or leaf blades than in particles derived from stems or petioles (Table 3); the proportion exposed was greater in leaf blades of *C. gayana* and *C. ciliaris* than in those of *L. perenne*.

Estimated degradation of cell walls

The estimate of the percentage of wall thickness of

Table 3. *Estimates of the percentage of cell wall surface area of a chewed particle exposed on the outer surface of the particle and of the wall thickness of the thick-walled cells which would be degraded in the rumen in plant parts of eight forage species*

Plant part and species	Percentage of cell wall area exposed	Percentage of wall thickness degraded	Wall thickness degraded (μm)
Leaflets or leaf blades			
Trifolium repens	6·7	*	*
Medicago sativa	8·1	*	*
Desmodium intortum	8·5	*	*
Lolium perenne	5·8	60	0·50
Festuca arundinacea	7·2	50	0·61
Chloris gayana	9·9	58	0·75
Cenchrus ciliaris	8·8	69	0·90
Zea mays†	8·0	65	0·93
S.E. (15 D.F.)	0·59	2·3‡	0·040‡
Mean	7·9	60	0·74
Petioles			
Trifolium repens	5·5	38	0·70
Leaf sheaths			
Lolium perenne	7·0	78	0·88
Festuca arundinacea	8·7	66	0·96
Chloris gayana	10·1	50	0·68
Cenchrus ciliaris	9·0	66	0·76
S.E. (7 D.F.)	0·97	2·7	0·076
Mean	8·7	65	0·82
Stems			
Trifolium repens	3·3	61	1·35
Medicago sativa	3·1	44	0·82
Desmodium intortum	4·1	40	0·68
Chloris gayana	3·5	68	1·28
Cenchrus ciliaris	3·5	67	1·22
S.E. (9 D.F.)	0·22	3·2	0·108
Mean	3·5	56	1·07

* Close to zero.
† Midribs excluded from the calculation.
‡ 9 D.F.

thick-walled cells which would be degraded in the rumen was relatively high in the leaf blades, sheaths and stems of *C. ciliaris*, leaf blades of *Z. mays*, leaf sheaths of *L. perenne* and *F. arundinacea* and stems of *C. gayana*, relatively low in the petioles of *T. repens* and stems of *D. intortum* and *M. sativa* and close to zero in legume leaflets (Table 3). The estimate of the wall thickness (in μm) of thick-walled cells which would be degraded was relatively high in the stems of *T. repens*, *C. gayana* and *C. ciliaris* and relatively low in the leaf blades of *L. perenne*.

DISCUSSION

The rate of intake of the tropical grasses, *Chloris gayana* and *Cenchrus ciliaris*, in 1-min meals was low (Wilman *et al.* 1996), but the chewing was evidently effective in breaking the leaf blades and leaf sheaths into particles which were generally somewhat smaller than the equivalent particles derived from temperate grasses (Table 1 and Mtengeti *et al.* 1996). The particles derived from grasses in the present experiment were larger, on average, than some derived from grasses fed to sheep (Kelly & Sinclair 1989) and some derived from grasses fed to cattle (Wilson *et al.* 1989; Wilson & Kennedy 1996), judging from the length and width of the particles (Mtengeti *et al.* 1996). The particles derived from the leaf blades and sheaths of *C. gayana* and *C. ciliaris* contained very large numbers of thick-walled cells per mm^3, a large volume of thick wall per mm^3 and a high proportion of thick-walled cells. This might imply low digestibility, but the *in vitro* digestibility was not especially low (Wilman & Rezvani Moghaddam 1998) and it seemed from the estimate in Table 3 that at least half the thickness of these cell walls would probably be

degraded in the rumen. The particles of stem of these two grasses were much larger than the particles of leaf blade and sheath and also contained a greater volume of thick wall per mm^3, suggesting that *in vivo* digestibility might be low unless the particles remain in the rumen for a long time. A further factor restricting digestion of these particles would be the close packing of sclerenchyma cells, denying or delaying access by rumen bacteria, as noted by Wilson & Mertens (1995). It appeared, however, from the estimates in Table 3 that at least two thirds of the thickness of the thick cell walls of the stems can be degraded during the time the walls are likely to remain in the rumen; this is in accord with the results of Wilson *et al*. (1991), who incubated 5-μm sections of the stems of three tropical grass species in a rumen fluid/buffer mixture for 48 h and assessed the thinning of the walls. Despite the higher concentration of lignin in NDF in the stems than in the leaf blades or sheaths in the present study (Wilman & Rezvani Moghaddam 1998), it seemed that the thick walls in the stems were at least as digestible as those in the leaves. If two thirds is a satisfactory estimate of the average proportion of thick wall degraded in *C. gayana* and *C. ciliaris* stems and if *in vivo* rumen bacteria do not have access to all the cells, the implication is that those thick-walled cells to which bacteria have immediate access may be almost completely degraded.

The rate of intake of the legume species was higher than that of the grasses (Wilman *et al*. 1996) and the petioles and stems were not reduced to such small particles, although the leaflets, which have a lower NDF content than the petioles and stems (Wilman & Rezvani Moghaddam 1998), were broken into small particles. The legume leaflets had many more thin-walled than thick-walled cells and a large surface area of thin wall per mm^3. This might suggest that a high proportion of the cell wall in the leaflets should be accessible and digestible. However, it seemed from the estimates in Table 3 that the contribution of cell wall to leaflet digestibility was close to zero, as the cell contents alone appeared approximately sufficient to account for the digestibility recorded. The high concentration of lignin in NDF (Wilman & Rezvani Moghaddam 1998) may be one reason for low cell wall digestibility in this case; however, the lignin may be confined to xylem and tracheary cells (Wilson & Mertens 1995), in which case the remaining cell walls of the leaflets would be expected to be digestible. There may be other chemical inhibitors of digestion in legume leaflets, as noted by Wilman & Rezvani Moghaddam (1998), which may mean that the cell contents are incompletely digested. The particles derived from legume petioles and stems had only a small number of thick-walled cells per mm^3, but, because of the large size both of these cells (Rezvani Moghaddam & Wilman 1998) and of the particles, there was an appreciable total volume of thick wall per particle and it seemed from the estimates in Table 3 that this wall contributes appreciably to the digestibility of those plant parts, despite having concentrations of lignin in NDF as high as or higher than those in the leaflets.

Wilson & Mertens (1995) observed that lignin in legumes is confined to xylem and tracheary cells and that the lignin concentration per unit cell wall is very high in these cells and appears to completely prevent the digestion of secondary walls. In the present study there may have been very little contribution to digestibility from the cell wall fraction of the legume leaflets, but there appeared to be an appreciable contribution from the cell wall of the legume petioles and stems, e.g. in lucerne stems the proportion of cell content was only sufficient to account for *c*. 58% of true DMD, leaving cell wall to contribute the remaining *c*. 42%. The contribution of cell wall to the digestibility of legume petioles and stems may have been principally from walls which were thickened but not lignified (collenchyma, phloem fibre and epidermal cells) as well as from the thin-walled cells.

When forage is eaten, the cell walls which are immediately available for colonization by rumen micro-organisms are those on the exposed outer surface of the particles produced during the chewing which precedes swallowing and passage to the rumen, plus any walls which are immediately accessible via gaps between cells in loosely-arranged tissue such as mesophyll. The present study suggests that only a modest proportion (3–10%) of the total cell wall area may be immediately available for colonization, emphasising the importance of further breakdown of the particles, which is likely to be mainly by rumination (Kennedy 1985). Of the material included in the present study, further breakdown may be particularly needed in the case of particles from the stems of *C. ciliaris* and *C. gayana* which are moderately large and contain a high proportion of thick-walled cells which may be tightly packed together, restricting access. Delay in the colonization of walls implies delay in beginning degradation of those walls and increases the likelihood of walls being incompletely degraded or even not degraded at all before passing out of the reticulorumen.

The estimate of wall thickness of thick-walled cells which would be degraded in the rumen in the present study, excluding legume leaflets, was 0·87 μm on average. If forage particles on average spend *c*. 29 h in the rumen (Minson 1982), average degradation rate would be 0·03 μm/h, compared with the 0·02 μm/h cited by Wilson & Mertens (1995). The latter rate was based on mesophyll cells isolated from *Lolium perenne* leaves and incubated for 8 h in nylon bags in a sheep's rumen (Chesson *et al*. 1986). The estimates from the present study suggest that a rate $> 0{\cdot}02$ μm/h is likely to be achieved in practice with grass leaf blades,

sheaths and stems and with legume petioles and stems. This conclusion is in accord with mean degradation rates of 0·03 μm/h *in vitro* of walls of sclerenchyma cells in sections of stems of tropical grasses recorded by Wilson *et al*. (1991) and Akin & Hartley (1992).

The estimated percentages of the wall thickness of the thick-walled cells which would be degraded did not appear to be closely related to the concentrations of lignin in NDF (from Wilman & Rezvani Moghaddam 1998). Thus, in *C. gayana* and *C. ciliaris* stems, with a mean concentration of lignin in NDF of 9·9%, the estimate of wall thickness degraded was 68%, whereas in the leaf blades, with a mean concentration of lignin in NDF of only 4·1%, the estimate of cell wall thickness degraded was 64%. The apparent lack of effect of lignification supports the view of Wilson & Mertens (1995) that digestion of most of the secondary wall of grasses is not prevented by lignification, but it appears to conflict with some commonly held views (e.g. Holmes 1989; McDonald *et al*. 1995). The position may be somewhat similar in legumes. Thus in *M. sativa* stems, with 17·7% lignin in NDF, the estimate of wall thickness degraded was 44%, whereas in the leaflets, with a lower concentration of lignin in NDF, 8·2%, the estimate of wall thickness degraded was close to zero.

Clearly the digestion of forage by ruminants is a complex issue which is by no means fully resolved. Part of the complexity lies in the wide range of plant material which is eaten, from legume leaflets (which are quickly eaten, are likely to pass through the rumen relatively quickly (e.g. McLeod *et al*. 1990) and may contain chemical inhibitors of digestion) to coarse, stemmy material such as that from tropical grasses (which is eaten more slowly, yields larger particles and is likely to stay in the rumen a relatively long time). The approach developed by Wilson (1993) and Wilson & Mertens (1995), considering cell wall accessibility and thickness and the likely rate of degradation of accessible wall, should lead to greater understanding. The approach requires data such as those in the present paper, indicating the size of plant particles as they enter the rumen, their exposed surface area, and their composition in terms of numbers of cells of different types and the volume and surface area of cell walls. This provides a picture of the 'challenge' facing the digestive system and illustrates the extent to which the challenge differs according to the type of plant material eaten. A further development in the present paper is the attempt to predict the wall thickness of thick-walled cells which would be degraded *in vivo*, from the *in vitro* digestibility and NDF content of the plant part and the distribution of cell wall volume between thick-walled, thin-walled and epidermal cells. The estimates of wall thickness degraded prompt consideration of reasons for the large differences recorded. A major reason for the differences between, for example, tropical grass stems and temperate grass leaf blades, may be that particles derived from the stems stay in the rumen longer, allowing more time for degradation. In contrast to the large contribution of the walls of thick-walled cells to digestibility in tropical grass stems, the equivalent contribution in legume leaflets appeared close to zero, partly presumably because of a short residence time in the rumen, partly because such walls are only a minor proportion of leaflet dry matter and partly, it seems, because of chemical inhibitors of digestion.

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