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Cell wall thickness and cell dimensions in plant parts of eight forage species

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SUMMARY

To appreciate more clearly some of the physical characteristics of forages which may be important in relation to digestibility and structural integrity, different parts of eight plant species were examined for the proportion of thick-walled, thin-walled and epidermal cells, the thickness of the cell walls and the diameter, length and volume of the cells. 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 compared with later harvesting in each of two years. The plants were grown in a heated glasshouse in spring–summer.

The plant parts with the lowest proportion of thick-walled cells (3–6 % of cross-sectional area) were the legume leaflets and those with the highest proportion (47–57 %) were the leaf blades and stems of *C. ciliaris*. The plant parts with the highest proportion of thin-walled cells were the legume leaflets and petioles and the *Z. mays* stems and leaf sheaths. The walls of the cells categorized as thick-walled were thinnest (0·9 μm) in *L. perenne* leaf blades and *T. repens* leaflets and thickest (2·0–2·3 μm) in the leaf blade midribs, leaf sheaths and stems of *Z. mays* and in the stems and petioles of *T. repens*. The thinnest outer walls of epidermal cells (0·9 μm) were recorded for the leaf blades of *L. perenne*.

The largest cells within the categories and plant parts examined (1 100 000 μm^3) were thin-walled cells in the stems of *Z. mays*. The longest cells recorded (180 μm) were thin-walled cells in the petioles of *T. repens*. The thick-walled cells were particularly small (1800–2600 μm^3) in *L. perenne* leaf blades and sheaths and in *T. repens* leaflets. The largest thick-walled cells in the study were in the stems and petioles of *T. repens*. The epidermal cells of *D. intortum* leaflets, petioles and stems were particularly small (2000–3000 μm^3).

INTRODUCTION

The composition of plant tissue in terms of size of cells, thickness of cell walls and the proportion of thick-walled cells is likely to have a large, if as yet imperfectly understood, effect on the feeding value of the plant. Thus, for example, tissue with a high proportion of small, very thick-walled cells is likely to be tough and difficult to eat (Vincent 1990), restricting intake, and also to be incompletely digested because there is insufficient time for microbes to degrade the full thickness of potentially degradable cell walls during the time the material stays in the digestive tract (Wilson & Mertens 1995). Conversely, tissue containing mainly large, thin-walled cells is likely to be easier to eat, with a high voluntary intake, and in some legume species there may even be a danger of too rapid release of cell contents, leading to bloat (Lees *et al.* 1981). Thin-walled cells are usually the metabolic cells, containing high levels of cell solubles, with walls which are likely to be completely digested, leading to high tissue digestibility. Too high a proportion of thin-walled cells, however, may leave a plant too structurally weak to be productive and competitive.

As noted by Wilson & Mertens (1995), there is not much published, quantitative information regarding the cellular composition and wall thickness of forage plant tissue. Consequently, to contribute information which can be related to already known forage characteristics, we have examined different plant parts of eight of the twelve species studied by Wilman *et al.* (1996*a*), recording the proportion of different cell types, the thickness of cell walls and the diameter, length and volume of individual cells.

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MATERIALS AND METHODS

The plant material studied was grown in the experiment described by Wilman *et al.* (1996*a*). Eight species with early and later harvesting were studied using a randomized block design 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 in 25-cm pots in a glasshouse; details are given by Wilman & Rezvani Moghaddam (1998). Supplementary heat was supplied when necessary to keep the minimum temperature $> c.$ 13 °C; mean daily minimum temperature was 14·5 °C and mean daily maximum 31 °C (Wilman & Rezvani Moghaddam 1998). There was no supplementary lighting. At each harvest the plants were cut 2 cm above the soil surface and material stored in a freezer. Subsamples were later defrosted and separated into plant parts to provide leaflets, petioles and stems in the case of legumes, and leaf blades, leaf sheaths and stems in the case of the grasses. Dead material and inflorescences were excluded. In *T. repens*, *D. intortum* and *Z. mays*, midribs were examined separately from the rest of the leaflet or leaf blade. Petioles of *M. sativa* and stems of *L. perenne* and *F. arundinacea* were not studied, because they were a very low proportion of total herbage in this experiment (Wilman *et al.* 1996*a*). The growth stage of each species at each harvest is indicated in Table 2 of Wilman & Rezvani Moghaddam (1998) by the proportions of leaf and stem. There was no stem development in *F. arundinacea* and very little in *L. perenne*; in the remaining species, particularly *C. gayana*, the proportion of stem was rather higher at the later than at the early harvest. The diameter of the stem internodes of the different species is given in Table 5 of Wilman *et al.* (1996*a*); the range in mean diameter was from 2·5 mm in *M. sativa* and *C. ciliaris* to 13·6 mm in *Z. mays*. The *Z. mays* plants were grown three to a pot and were not staked.

The slide preparation and staining procedures were based on Jensen (1962). The procedures have been described in detail by Rezvani Moghaddam (1996). The de-frozen tissue was fixed in FAA (formalin, acetic acid and alcohol), embedded in paraffin and sectioned at 10–15 μm on a rotary microtome. The sections were stained, some with safranin-fast green and some with haematoxylin-orange G.

Cross-sections of each plant part from each replicate, harvest and year were used to estimate the percentage of the cross-sectional area occupied by thick-walled cells, thin-walled cells and epidermal cells; in each case, one or two representative sections, selected from at least 80, were used; an eyepiece grid fitted to a light microscope, at a magnification of $\times 100$, was used. The cell types recorded as 'thick-walled' and 'thin-walled' in the different plant parts are listed in Table 1. In legume leaflets and grass leaf blades and sheaths, both the abaxial and adaxial epidermis were included in the area occupied by epidermal cells. Measurements were also made of the thickness of the walls of the thick-walled, thin-walled and epidermal cells; in the latter the outer and inner walls were measured separately and the outer wall measurement included any cuticle which was present. Wall thickness was measured for several cells of each category (taking account of the proportions of different cell types within the category) on four or five sections per sample to give a total of 10–20 measurements per sample for each cell category. A micrometer, coupled to a light microscope, at a

Table 1. *Cell types recorded as 'thick-walled' and 'thin-walled' in different plant parts*

Plant part	Thick-walled	Thin-walled
Legume leaflets	Vascular bundles	Mesophyll
Temperate grass leaf blades	Sclerenchyma & xylem	Mesophyll & phloem
Tropical grass leaf blades (including *Zea mays*)	Sclerenchyma, xylem & parenchyma bundle sheath	Mesophyll, parenchyma & phloem
Legume midribs	Phloem fibre & xylem	Parenchyma & phloem
Zea mays midribs	Sclerenchyma, xylem & parenchyma bundle sheath	Parenchyma & phloem
Legume petioles	Collenchyma, phloem fibre & xylem	Parenchyma & phloem
Grass leaf sheaths	Sclerenchyma & xylem	Parenchyma & phloem
Legume stems	Collenchyma, phloem fibre & xylem	Parenchyma & phloem
Grass stems	Sclerenchyma & xylem	Parenchyma & phloem

Table 2. *Percentage in cross-sectional area and wall thickness (μm) of different cell types of plant parts of eight forage species, means of 2 years and of early and later harvesting*

Plant part and species	Percentage in cross-sectional area			Wall thickness			
						Epidermal cells	
	Thick-walled cells	Thin-walled cells	Epidermal cells	Thick-walled cells	Thin-walled cells	Inner wall	Outer wall
Leaflets or leaf blades							
*Trifolium repens**	3·3	78·7	18·0	0·86	0·310	0·35	1·27
Medicago sativa	4·6	78·0	17·4	1·46	0·233	0·29	1·70
*Desmodium intortum**	5·9	78·6	15·5	1·12	0·219	0·28	1·43
Lolium perenne	19·8	57·7	22·5	0·85	0·145	0·22	0·87
Festuca arundinacea	22·5	56·5	21·0	1·21	0·269	0·27	4·02
Chloris gayana	36·8	30·8	32·4	1·28	0·226	0·29	3·41
Cenchrus ciliaris	46·5	13·3	40·2	1·30	0·238	0·45	3·22
*Zea mays**	26·4	41·2	32·4	1·44	0·343	0·98	5·03
S.E. (15 D.F.)	1·26	1·05	1·06	0·043	0·0160	0·021	0·119
Mean	20·7	54·4	24·9	1·19	0·248	0·39	2·62
Leaf midribs							
Trifolium repens	10·0	78·6	11·4	1·58	0·326	0·95	2·61
Desmodium intortum	15·6	76·5	7·9	1·65	0·251	0·69	2·40
Zea mays	20·4	73·8	5·8	2·21	0·527	2·13	6·40
S.E. (5 D.F.)	0·81	1·10	0·76	0·046	0·0270	0·036	0·094
Mean	15·3	76·3	8·4	1·81	0·368	1·26	3·80
Petioles							
Trifolium repens	11·8	81·8	6·4	1·98	0·381	0·87	2·63
Desmodium intortum	16·1	78·9	5·0	1·57	0·325	0·72	2·68
S.E. (3 D.F.)	1·17	1·16	0·09	0·046	0·0077	0·021	0·093
Mean	14·0	80·4	5·7	1·77	0·353	0·79	2·65
Leaf sheaths							
Lolium perenne	21·6	60·9	17·5	1·12	0·251	0·97	3·77
Festuca arundinacea	17·0	71·9	11·1	1·45	0·298	0·66	5·10
Chloris gayana	17·4	70·7	11·9	1·39	0·332	0·88	4·90
Cenchrus ciliaris	24·7	61·9	13·4	1·14	0·215	0·25	2·94
Zea mays	15·4	76·4	8·2	2·33	0·598	2·15	6·92
S.E. (9 D.F.)	0·60	1·35	1·10	0·078	0·0309	0·106	0·392
Mean	19·2	68·4	12·4	1·49	0·339	0·98	4·73
Stems							
Trifolium repens	22·6	73·5	3·9	2·23	0·333	1·01	2·88
Medicago sativa	36·6	59·9	3·5	1·84	0·354	1·05	4·43
Desmodium intortum	28·3	69·0	2·8	1·68	0·313	0·71	3·06
Chloris gayana	42·3	54·8	3·0	1·88	0·424	1·36	5·85
Cenchrus ciliaris	56·5	39·5	4·0	1·83	0·165	1·65	6·20
Zea mays	18·3	80·8	1·0	2·03	0·576	1·72	5·11
S.E. (11 D.F.)	3·28	3·43	0·30	0·117	0·0242	0·104	0·318
Mean	34·1	62·9	3·0	1·92	0·361	1·25	4·59

* Excluding midribs.

magnification of $\times 400$ for the thicker walls and $\times 1000$ for the thinner walls, was used. For the same cell categories, diameters were recorded for several cells in 10–20 cross-sections per sample, and lengths measured for a similar number of cells in longitudinal sections. Volume per cell was calculated from the diameter and length.

The differences between the species were consistent from year to year and the results from the 2 years were analysed together, using the years × 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.

Table 3. *Mean volume per cell ('000 μm³) of different cell types in plant parts of eight forage species, means of 2 years and of early and later harvesting*

Plant part and species	Thick-walled cells	Thin-walled cells	Epidermal cells
Leaflets or leaf blades			
*Trifolium repens**	1·8	4	15
Medicago sativa	8·6	9	15
*Desmodium intortum**	6·2	10	3
Lolium perenne	2·1	51	28
Festuca arundinacea	9·2	131	94
Chloris gayana	5·4	18	46
Cenchrus ciliaris	5·0	100	125
*Zea mays**	6·7	9	77
S.E. (15 D.F.)	0·72	7·5	6·8
Mean	5·6	42	51
Leaflet or leaf blade midribs			
Trifolium repens	19·6	30	20†
Desmodium intortum	18·5	30	9†
Zea mays	22·3	875	43†
S.E. (5 D.F.)	1·94	22·1	2·3
Mean	20·1	312	24†
Petioles			
Trifolium repens	49·5	872	16†
Desmodium intortum	36·3	319	3†
S.E. (3 D.F.)	2·55	96·2	2·0
Mean	42·9	596	10†
Leaf sheaths			
Lolium perenne	2·6	54	41
Festuca arundinacea	9·9	195	86
Chloris gayana	4·3	557	13
Cenchrus ciliaris	5·2	209	58
Zea mays	16·8	506	99†
S.E. (9 D.F.)	0·84	56·7	9·8
Mean	7·8	304	59
Stems			
Trifolium repens	63·9	418	11
Medicago sativa	30·6	368	55
Desmodium intortum	35·9	470	2
Chloris gayana	5·5	556	7
Cenchrus ciliaris	6·1	399	7
Zea mays	10·9	1076	13†
S.E. (11 D.F.)	2·33	64·7	1·9
Mean	25·5	548	16

* Excluding midribs.
† Excluding cuticle.

Proportions of thick-walled, thin-walled and epidermal cells

The plant parts with the lowest proportion of thick-walled cells were the legume leaflets (3–6% of cross-sectional area) and, to a lesser extent, the petioles of *T. repens* (Table 2). The plant parts with the highest proportion of thick-walled cells were the leaf blades and stems of *C. ciliaris* (47–57%) and, to a lesser extent, the stems and the leaf blades of *C. gayana* and the stems of *M. sativa*. Except in *L. perenne*, grass leaf sheaths had a lower proportion of thick-walled cells than the equivalent leaf blades. *Z. mays* had a much lower proportion of thick-walled cells than *C. ciliaris* and *C. gayana* in its stems and leaf blades and a rather lower proportion than those two grasses in its leaf sheaths.

The highest proportion of thin-walled cells were in the legume leaflets and petioles and the *Z. mays* stems and leaf sheaths (Table 2). The plant parts with the lowest proportion of thin-walled cells were the leaf blades of *C. ciliaris*.

The proportion of epidermal cells was lowest for the stems, particularly those of *Z. mays*. The

Table 4. *Mean diameter and length (μm) of cells of different types in plant parts of eight forage species, means of 2 years and of early and later harvesting*

Plant part and species	Diameter			Length		
	Thick-walled cells	Thin-walled cells	Epidermal cells	Thick-walled cells	Thin-walled cells	Epidermal cells
Leaflets or leaf blades						
*Trifolium repens**	9·8	15·8	25·4	24·2	19·9	28·7
Medicago sativa	12·5	23·0	26·7	70·7	20·9	25·4
*Desmodium intortum**	15·3	24·6	14·5	33·2	19·7	19·3
Lolium perenne	8·0	31·3	17·6	43·0	66·1	119·4
Festuca arundinacea	15·1	45·9	28·9	50·8	78·7	158·2
Chloris gayana	18·4	28·6	27·5	20·2	26·7	81·6
Cenchrus ciliaris	15·8	56·6	41·0	25·1	39·1	98·4
*Zea mays**	14·0	22·3	31·9	43·2	22·2	100·5
S.E. (15 D.F.)	0·94	1·63	1·14	2·39	2·31	5·66
Mean	13·6	31·0	26·7	38·8	36·7	78·9
Leaf midribs						
Trifolium repens	14·7	28·0	17·6	113·8	47·8	101·0
Desmodium intortum	16·2	19·9	19·3	89·2	95·9	36·3
Zea mays	14·7	97·3	23·4	132·1	117·8	149·2
S.E. (5 D.F.)	0·51	1·56	0·95	4·50	2·93	2·65
Mean	15·2	48·4	20·1	111·7	87·2	95·5
Petioles						
Trifolium repens	20·2	77·4	17·5	155·0	183·8	83·2
Desmodium intortum	22·9	77·7	12·5	89·5	66·3	33·3
S.E. (3 D.F.)	1·39	5·08	0·75	5·41	2·42	3·64
Mean	21·6	77·6	15·0	122·3	125·1	58·3
Leaf sheaths						
Lolium perenne	9·5	32·8	19·8	37·1	63·6	144·9
Festuca arundinacea	14·3	44·6	23·7	61·7	125·0	158·5
Chloris gayana	13·2	69·1	15·3	30·3	145·9	81·9
Cenchrus ciliaris	12·3	54·3	24·8	43·9	88·8	110·0
Zea mays	17·1	86·2	41·6	72·5	86·1	92·9
S.E. (9 D.F.)	0·82	3·25	1·16	2·75	6·44	6·33
Mean	13·3	57·4	25·0	49·1	101·9	117·6
Stems						
Trifolium repens	23·4	66·1	17·0	149·8	120·2	56·7
Medicago sativa	18·0	60·6	36·4	120·4	127·1	58·1
Desmodium intortum	18·5	83·5	12·6	133·3	84·9	26·3
Chloris gayana	12·3	64·4	14·2	45·7	168·3	73·9
Cenchrus ciliaris	13·4	58·4	12·9	40·3	149·4	97·2
Zea mays	12·3	94·6	14·4	92·1	152·2	129·6
S.E. (11 D.F.)	1·09	3·84	0·68	5·23	5·71	2·90
Mean	16·3	71·3	17·9	96·9	133·7	73·6

* Excluding midribs.

proportion of epidermal cells was > 30 % in the leaf blades of *C. ciliaris*, *C. gayana* and *Z. mays* (in the latter case, excluding the midrib).

Cell wall thickness

The cell walls of the thick-walled tissues were thinnest (0·9 μm) in *L. perenne* leaf blades and *T. repens* leaflets (in the latter case, excluding the midrib) and thickest (2·0–2·3 μm) in the leaf blade midribs, leaf sheaths and stems of *Z. mays*, and in the stems and petioles of *T. repens* (Table 2). In *C. gayana* and *C. ciliaris*, the walls of the thick-walled cells were thinner in the leaf blades and leaf sheaths than in the stems.

The cell walls of the thin-walled tissues were thinnest in *L. perenne* leaf blades and *C. ciliaris* stems and thickest in the leaf blade midribs, leaf sheaths and stems of *Z. mays* (Table 2). The inner walls of the epidermal cells were particularly thin in *L. perenne* leaf blades and *C. ciliaris* leaf sheaths and thickest in *Z. mays* midribs and leaf sheaths and, to a lesser extent, in the stems of *Z. mays*, *C. ciliaris* and *C.*

gayana. The outer walls of the epidermal cells (which included cuticle) were consistently much thicker than the inner walls. The plant parts with the thinnest outer walls of epidermal cells (0·9 μm) were the leaf blades of *L. perenne*. The thickest outer walls of epidermal cells were in the midribs and leaf sheaths of *Z. mays* and the stems of *C. ciliaris* and *C. gayana*. The petioles of *T. repens* and *D. intortum* and the stems of *M. sativa* had thicker outer walls of epidermal cells than the leaflets.

Cell size

The thick-walled cells were particularly small (1800–2600 μm^3) in *L. perenne* leaf blades and leaf sheaths and in *T. repens* leaflets (Table 3); these cells were the narrowest recorded (8–10 μm) (Table 4). In *L. perenne* and *F. arundinacea*, there was not much difference between the leaf blade and the leaf sheath in the size and shape of the various cell types. In *C. gayana* and *C. ciliaris*, there was not much difference between the leaf blade and the leaf sheath in the size of thick-walled cells, but the thin-walled cells of the sheaths were larger than the thin-walled cells of the blades and the reverse was the case with the epidermal cells. The largest thick-walled cells in the study were in the stems and petioles of *T. repens*; these cells were much longer (150–155 μm) than the average for thick-walled cells. The thick-walled cells in the stems of *M. sativa* and *D. intortum* were also large and long. On the other hand, the thick-walled cells in the stems of *C. gayana* and *C. ciliaris* were small and rather short.

The largest cells within the categories examined were thin-walled cells in the stems of *Z. mays*, which were large in volume (1 100 000 μm^3), diameter and length (Tables 3 and 4). The thin-walled cells in the leaf blade midribs of *Z. mays* were also large, particularly in diameter, but the thin-walled cells in the remaining part of the leaf blade were very much smaller. The thin-walled cells in the petioles of *T. repens* were large; these were the longest cells recorded (180 μm). Thin-walled cells were larger than thick-walled or epidermal cells in stems, leaf sheaths, petioles and leaf blade midribs, but in the leaflets or leaf blades epidermal cells were commonly at least as large as the thin-walled cells. The thin-walled cells of the leaflets or leaf blades were much smaller than thin-walled cells in stems, leaf sheaths and petioles in almost all the cases examined. The length of thin-walled cells in the leaflets and leaf blades was generally similar to the diameter, except in *L. perenne* and *F. arundinacea* in which length was about twice the diameter. In the petioles and stems, the ratio of length to diameter was greater than in leaflets and leaf blades in all categories of cells.

Among the epidermal cells, those of *D. intortum* leaflets, petioles and stems were particularly small (2000–3000 μm^3) (Table 3). The epidermal cells in the stems of *C. gayana* and *C. ciliaris* were also small, being similar in diameter to those of *D. intortum*, but greater in length (Table 4). The largest epidermal cells were those in the leaf blades of *C. ciliaris* and the leaf sheaths of *Z. mays*, which were wide and long, and those in the leaf blades and leaf sheaths of *F. arundinacea*, which were particularly long (160 μm).

DISCUSSION

A notable feature in the present study was the relatively thin cell walls of *Lolium perenne*, particularly in the leaf blades, compared with the other species examined; the cells classed as thick-walled, those classed as thin-walled and the epidermal cells all had relatively thin walls. If the view of Wilson & Mertens (1995), that the incomplete digestion of very thick cell walls can be a major reason for low digestibility, is correct, vegetative tillers of *L. perenne* should have highly digestible leaf blades and sheaths, and this accords with general experience of this species, as illustrated in a previous paper (Wilman & Rezvani Moghaddam 1998). Despite the thin cell walls, *L. perenne* vegetative tillers are known to be strong and tough enough to withstand the treading of animals relatively well (Edmond 1966) and to compete successfully with other plant species in a suitable environment (Spedding & Diekmahns 1972). The strength and toughness seem to come at least partly from the small size of the thick-walled cells in both the blades and the sheaths and the moderately high proportion of blade and sheath tissue occupied by the thick-walled cells. Some strength will also come from the overlapping layers of leaf sheath (Niklas 1990). There may be some scope for plant breeders to select within *L. perenne* for a lower proportion of thick-walled cells and perhaps for even thinner cell walls, with a view to increasing digestibility and intake, but care will be needed to retain the ability to withstand treading and to be competitive and high yielding.

The leaf blades and leaf sheaths of *Festuca arundinacea* appear to be made up of much larger cells than those of *L. perenne*, with thicker walls; the greater thickness of walls may be a reason for the generally lower digestibility of *F. arundinacea* (Wilman *et al.* 1996*a*, *b*; Wilman & Rezvani Moghaddam 1998) if a greater thickness of the thicker walls is left undegraded when the particles containing the walls pass out of the reticulorumen. The thicker cell walls of *F. arundinacea* suggest scope for the production of varieties with thinner walls, which could be more digestible and more palatable and have a higher intake, but care is needed to retain sufficient strength to display the leaf blades in an optimum position in the canopy and to retain the ability of *F. arundinacea* to withstand drought and extremes of temperature. On the basis of the evidence from different grass species presented by Vincent (1991), a reduction in the

proportion of sclerenchyma in the cross-sectional area could appreciably weaken leaves.

Chloris gayana and *Cenchrus ciliaris*, which are adapted to hotter, more tropical conditions than *F. arundinacea*, did not in general have thicker cell walls or larger cells than *F. arundinacea* under these glasshouse conditions, but they did have a larger proportion of thick-walled cells in their leaf blades and, in *C. ciliaris*, in the leaf sheaths also, than *F. arundinacea*. Wilman *et al.* (1996*a*) noted that the veins were much closer together and occupied a greater proportion of the width of blades and sheaths in *C. gayana* and *C. ciliaris* than in *F. arundinacea* or *L. perenne*. Similarly, Wilson & Hattersley (1989) noted higher proportions of bundle sheath and vascular tissue and lower proportions of mesophyll in leaf cross-sectional area in *C. gayana* and *C. ciliaris* than in *L. perenne*. Higher proportions of bundle sheath and vascular tissue and higher frequency of vascular bundles across the leaf may be characteristic of grasses with C_4 photosynthesis compared with C_3, both between and within genera (Wilson & Hattersley 1989; Wilson 1993). The high proportion of thick-walled cells in *C. gayana* and *C. ciliaris*, and the associated closeness of the veins, presumably help to support the leaf blades when growing in hot, dry conditions, but are a considerable disadvantage if forage of high digestibility and high intake potential is required. Presumably there is scope for selecting and breeding within these species for a lower proportion of thick-walled cells and for thinner cell walls in leaves and stems. When considering selection for higher digestibility in *C. ciliaris*, Wilson *et al.* (1989) suggested a low specific leaf weight and a small number of vascular bundles in the stem as selection criteria which are easily and quickly measured, with a heritability comparable to that for digestibility.

Although *Zea mays* plants grow much larger than those of the other species in the present study, the cell walls of *Z. mays* were not in general outstandingly thick in comparison with those of the other species, nor were the cells in general outstandingly large. Thus, for example, the wall thickness of the thick-walled cells of the leaf blades and stems was not very much greater in *Z. mays* than in *C. gayana* and *C. ciliaris*, and in the leaf blades not very much greater than in *F. arundinacea*. The thick-walled cells in the stems of *Z. mays* in the present study had rather thinner walls than the sclerenchyma cells in the *Z. mays* stems examined by Willemse & Den Outer (1988). The proportion of thick-walled cells was moderately low in *Z. mays*, much lower than in *C. gayana* and *C. ciliaris*, and the proportion of thin-walled cells was high, particularly in the stems and leaf sheaths. The moderately low proportion of thick-walled cells in cross-sectional area need not imply a weak structure: the contribution of the thick-walled cells to the dry weight of the plant organ would be much higher, as was shown in mature *Z. mays* stems by Engels & Schuurmans (1992). The combination of cell walls which are not especially thick in relation to plant size, the moderately low proportion of thick-walled cells and the high proportion of thin-walled cells help to explain the rather high *in vitro* digestibility of the leaf blades, leaf sheaths and stems of *Z. mays* in the present study (Wilman & Rezvani Moghaddam 1998). In farm practice, crops of *Z. mays* harvested for silage often contain a high proportion of grain, which is highly digestible, so that the digestibility of the total crop may be high, even when the stems are fairly mature and when the proportion of green leaf blade is not high (Cabon & Riviere 1989).

A feature of *Trifolium repens* is its low fibre content, particularly in green leaflets, illustrated in the present project by neutral detergent fibre (NDF) (Wilman & Rezvani Moghaddam 1998). A major reason for the low fibre content is evidently a low proportion of thick-walled cells, together with a high proportion of thin-walled cells. In the leaflets the proportion of thick-walled cells was particularly low and the walls of these cells were as thin as the walls of the corresponding cells in *L. perenne* leaf blades; it is not surprising, therefore, that green leaflets have a high proportion of cell content and are highly digestible (Wilman & Altimimi 1984; Wilman & Rezvani Moghaddam 1998). Lees (1984) related thin epidermal and mesophyll cell walls in *T. repens* and *M. sativa* leaflets to low resistance to leaf and cell rupture and hence to greater risk of causing bloat than some other legumes; the walls recorded in the present study were a little thicker than those reported by Lees (1984). The higher proportion of thick-walled cells and the thicker walls in the petioles than in the leaflets are presumably needed to provide sufficient strength to support the leaflets in as favourable a position in the canopy as is feasible for this species. The small size of the thick-walled and thin-walled cells in *T. repens* leaflets presumably helps, with the midrib and main laterals, to keep the leaflets structurally intact.

The leaflets of *Medicago sativa* were similar to those of *T. repens* in the proportions of thick-walled, thin-walled and epidermal cells and are similar to those of *T. repens* in NDF content and digestibility (Wilman & Altimimi 1984; Wilman & Rezvani Moghaddam 1998). The thick-walled cells in the *M. sativa* leaflets were larger than those in the *T. repens* leaflets, but had thicker walls, which would help to keep the leaflets structurally intact. Because the stems of *M. sativa* are erect, while those of *T. repens* creep along the ground, more strengthening tissue is likely to be required in the former, and this seems to be supplied by a higher proportion of thick-walled cells and smaller thick-walled cells, rather than by thicker cell walls (except that the outer wall of the epidermal cells was thicker). Greater lignification of some of the

cell walls (Wilman & Rezvani Moghaddam 1998) may provide some additional strength for the *M. sativa* stems. The much lower digestibility and much higher NDF content of *M. sativa* stems compared with those of *T. repens* (Wilman & Rezvani Moghaddam 1998) similarly seems to be associated with a higher proportion of thick-walled cells, and perhaps with greater lignification of some of the walls, rather than with thicker cell walls (apart from the outer wall of the epidermal cells).

The proportions of thick-walled, thin-walled and epidermal cells and the thickness of the walls in the leaflets of *Desmodium intortum* suggest that these leaflets should be nearly as digestible as those of *T. repens* and *M. sativa*. However, it appears that the leaflets of *D. intortum* are much less digestible than those of *T. repens* and *M. sativa* and that they have higher concentrations in dry matter of NDF and lignin (Wilman & Rezvani Moghaddam 1998). It seems that the lower digestibility cannot be fully explained in terms of the proportion of cell types and the thickness of cell walls and that a chemical explanation, probably involving tannins (Rotar 1965) and lignin, is required. The position seems to be similar in respect of petioles and stems; the much lower digestibility of *D. intortum* petioles and stems (Wilman & Rezvani Moghaddam 1998) than those of *T. repens* seems not to be fully explainable in terms of the proportions of cell types and the thickness of cell walls and again a chemical explanation, probably involving tannins and lignin, is required. The petioles of *D. intortum* support much larger leaflets than the petioles of *T. repens*, and the stems of *D. intortum* are more upright than those of *T. repens*; the additional strength required by the *D. intortum* petioles and stems seems not to be derived from thicker cell walls and only to a moderate extent from a higher proportion of thick-walled cells, but one contributor to additional strength may have been the smaller size of the thick-walled and epidermal cells. The very small size of the epidermal cells in the *D. intortum* leaflets, petioles and stems, together with the very large number of tertiary veins per leaflet (Wilman *et al.* 1996*a*), may contribute to the structural integrity of those organs and may provide a little extra protection against pests and diseases.

NDF and *in vitro* dry matter digestibility were determined by Wilman & Rezvani Moghaddam (1998) in 22 of the 24 plant parts examined in the present study, providing the opportunity to look for overall relationships between the cell wall data and NDF and digestibility. The percentage of thick-walled cells in cross-sectional area and mean cell wall thickness (mean of thick-walled, thin-walled and epidermal cells, weighted for their percentage in cross-sectional area) were both positively related to the NDF content of the plant parts ($r = +0{\cdot}76$ and $+0{\cdot}69$ respectively; $n = 22$; $P < 0{\cdot}001$), as might be expected. If it had been possible to record the dry weight or cell wall volume of different tissues types, as is more feasible in the larger forage species such as sorghum (Wilson 1993), stronger relationships with NDF might have been obtained. Relationships between the present cell wall data and *in vitro* digestibility were not strong, although there was a statistically significant correlation ($r = -0{\cdot}52$; $n = 19$; $P < 0{\cdot}05$) between the percentage of thick-walled cells in cross-sectional area and the *in vitro* dry matter digestibility of milled forage when the *D. intortum* plant parts were excluded from the calculation.

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