PLANT STRUCTURE IN RELATION TO EASE OF PHYSICAL BREAKDOWN IN THE MOUTH AND RUMEN

by

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Aberystwyth, September 1993

DECLARATION

I hereby declare that the work embodied in this thesis for the degree of *Philosophiae Doctor* of the University of Wales is the result of my own investigation except where indicated in the text.

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CERTIFICATE

I hereby certify that the work embodied in this thesis has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

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DEDICATION

To my family

SUMMARY

Grassland species were grown in the field and glasshouse and research was carried out for two years to investigate plant vascular structure in relation to physical breakdown in the mouth and rumen. Plant part morphology, vascular structural proportion and arrangement, fresh plant diet eating rate by sheep and particle breakdown characteristics were recorded.

Anatomical differences, especially vascular tissue arrangement, appeared to be a major cause of the differences in intake rate between legumes and grasses. Four legumes did not differ significantly in terms of intake rate but differed significantly in terms of the proportion of vascular tissues, neutral detergent fibre content and digestibility. Tropical grasses had a higher proportion of vascular tissues, higher neutral detergent fibre content, lower digestibility, lower intake rate and were chewed into smaller particles than temperate grasses. Legumes were eaten faster and had larger particles after chewing than grasses. Legume and grass particles had a similar number of perforations or ruptures per particle.

Legume leaf petiole and stem particles were longer than leaflet particles and increased the overall mean particle length of the chewed material. Petioles and stems are, therefore, important in legume particle size reduction in the mouth and rumen. Regardless of grass species, the veins of the leaf sheaths were more widely spaced than those in leaf blades, but the two plant parts were chewed to a similar particle size.

Despite having a high proportion of stem and neutral detergent fibre, lucerne and spurrey were eaten quickly, showing the advantage, in respect of intake rate, of having thin pliable and/or fragile stems.

The results of the present project indicate that the physical structure of plants, especially the arrangement of the vascular tissue, and plant morphology have great influence on the physical breakdown of the plants in the mouth and rumen.

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INTRODUCTION

The most important feeds for ruminants are forage grasses and legumes which are grazed or eaten after conservation as hay or silage. Bulk fodder crops such as maize and rape are also important in feeding the animals, especially when forage grasses and legumes are not growing actively or of low quality. In most cases grassland swards contain some unsown dicotyledonous species which may also be palatable and nutritious to the grazing animal. In various parts of the world, crop residues form part of the ruminant diet either at times of year when forages are in short supply or sometimes throughout the year. Mastication of these various feeds during ingestion results in a wide distribution of different sizes of particles entering the reticulo-rumen (Lee & Pearce 1984; Kelly & Sinclair 1989; Wilson *et al*. 1989). Clearance of these particles from the reticulo-rumen has long been recognized as one of the most important factors affecting the intake and nutritive value of these feeds (Ulyatt *et al*. 1986). Clearance of the reticulo-rumen depends largely on the rate and extent of particle size reduction (Moseley 1982). Forage particles must be reduced, mainly by chewing during eating and ruminating (McLeod & Minson 1988), to a critical size of c. 1 mm before they can pass out of the reticulo-rumen (Poppi *et al*. 1980). This critical size of particles is similar for all forage species, for leaf and stem fractions (Poppi *et al.* 1981) and for sheep and cattle (Poppi *et al.* 1985).

The ease of physical breakdown varies with the type of forage or feed material. For example, both perennial ryegrass and white clover are usually herbages of high feed value and similar digestibility, yet it has been found that white clover is broken down more rapidly and cleared faster from the rumen than perennial ryegrass (Moseley 1982). It has, however, been shown that differences between forages in voluntary intake and retention time in the rumen can be reduced substantially by grinding and pelleting the forage before feeding (Minson 1982). Since grinding and pelleting was not accompanied by any significant chemical change, it was concluded that differences in voluntary intake and retention time were caused by differences in physical rather than chemical properties (Laredo & Minson 1975). It could be that the most important plant physical properties causing differences in resistance to breakdown in the mouth and rumen are the arrangement of structural elements and their proportion in plant parts. This view supports the suggestion by Wilson (1985) and McLeod *et al.* (1990) that plant anatomy could be the cause of large differences in resistance to plant physical breakdown in the mouth and rumen, thus leading to differences in intake of different plants or plant parts even at the same level of digestibility or cell wall content. For example, grasses were chewed more and had a lower intake rate than legumes (McLeod & Smith 1989) and the chewed particles of the grasses were longer but thinner (reflecting the parallel venation of their leaf blades) than those of the legumes (which have reticulate venation in their leaflets) (Kelly & Sinclair 1989). During chewing, therefore, the parallel venation seems to be more resistant to breakdown than the reticulate venation. It is for this reason that a study of plant physical structure in relation to ease of particle breakdown in the mouth and rumen could help to find forage plants that require less effort to harvest and chew, as a result increasing the daily voluntary intake of the animal. Little has been done on this subject (Wilson 1991).

The objectives of the present research project were therefore:

(i) to investigate plant vascular structure in terms of the size, arrangement and proportion of vascular tissues in plant parts;

(ii) to relate these plant vascular structures to the ease of plant physical breakdown in the mouth and rumen, measured by eating activity (i.e. eating rate, chewing rate and chews per g dry matter intake) and particle breakdown characteristics (i.e. the size, internal structure and damage characteristics of the chewed particles);

(iii) to investigate the influence of plant part proportion and morphology on eating activity and particle breakdown characteristics;

(iv) to determine forage *in vitro* dry matter digestibility and neutral detergent fibre content and relate them to plant physical structure, eating activity and particle breakdown characteristics.

CHAPTER 2

REVIEW OF THE LITERATURE

Introduction

Some forage species or plant parts have higher intake than others even at the same level of digestibility and cell wall content. Leaves have higher intake than stem (Laredo & Minson 1973; 1975). Legumes have higher intake than grasses (Poppi *et al.* 1981; McLeod & Smith, 1989; McLeod *et al.* 1990). The higher intake of the leaf fraction and legume have been associated with their shorter retention time in the rumen (Thornton & Minson 1973; Laredo & Minson 1975; Poppi *et al.* 1981). These differences in intake and retention time in the rumen are, however, largely eliminated by grinding and pelleting the forage (Minson 1982). It appears that the proportion and arrangement of structural elements in the plant parts of different forage species have a great influence on the resistance of forage to physical breakdown in the mouth and rumen of the ruminant animal. The role of structural proportion and arrangement of plant vascular tissues in relation to resistance to physical breakdown has so far received little attention among ruminant researchers. For example, it seems that due to their reticulate dicotyledonous venation and many angular junctions between short lengths of veins, legume leaves are easier to break down into small rather spherical particles and thus disappear faster from the reticulo-rumen than grass leaves which have a parallel girder system of vascular bundles running their full length (Wilson 1985). Understanding of these concepts could help in breeding forage plants of higher potential intake.

The principal means of physical breakdown of forages are chewing during eating and rumination, microbial attack in the reticulo-rumen and the action of rumen contractions. Of these processes, chewing during eating and rumination account for up to 75% of the breakdown of large forage particles into small particles which can pass out of the reticulo-rumen (McLeod & Minson 1988). The efficiency of chewing during eating is determined by the animal species, the age of the animal, the frequency of chewing (chews/minute), the time spent chewing (chews/g dry matter) and the nature of the diet (Ulyatt *et al.* 1986). Forages with high tensile strength and thick cell walls require a high number of chews and result in a low rate of eating and a high proportion of large particles (Ulyatt *et al.* 1986, McLeod *et al.* 1990).

The ease with which a plant is eaten by an animal can also be affected by its plant part morphology (Stobbs 1973a). Data for plant morphology as related to eating behaviour and plant particle breakdown characteristics is scarce.

In the present review of the literature, therefore, plant morphology and anatomy as related to nutritive quality, the role of chewing during eating and factors affecting particle breakdown characteristics and forage intake rate are emphasised.

Plant morphology in relation to quality

'Quality' in this context is based on the chemical composition, intake and digestibility of the forage plant.

The most important plant morphological factor that has been related with plant quality is the leaf:stem ratio. Leafiness in forage plants is commonly associated with forage quality because there is usually a positive correlation between leaf percentage in a given plant species and its protein content and dry matter digestibility (Norton 1982). Differences between species in leaf:stem ratio are, however, not always an indication of differences in their quality. Norton (1982) noted that, despite higher leaf percentage, *Dactylis glomerata* was of lower digestibility than *Lolium perenne* due mainly to differences in leaf digestibility. Leafiness, however, has a positive influence on dry matter intake (Norton 1982) although Milford & Minson (1966) found a poor relationship between the leafiness and dry matter intake of tropical pastures.

For a given forage species, the voluntary intake of leaves is higher than that of stems (Hendricksen *et al*. 1981; Laredo & Minson 1973; 1975). This is because animals chew each gram of dry matter ingested (both of legume and grass) more in the case of the stem fractions than in the case of leaf (McLeod *et al*. 1990).

Stems have a particularly strong structure and, if unchewed, maintain the essence of their structure after digestion even for as long as three weeks in the rumen (Wilson 1990). Fodder stems like those of maize, sorghum and rape become thick and hard as they mature and can be difficult to prehend, chew and form into a bolus. These stems will be rejected by an animal or eaten very slowly unless chopped and/or crushed to small particles before feeding. Little information has been documented as to how plant dimensions such as stem diameter and leaf length and width influence intake.

It has been suggested that the size, arrangement and distribution of leaves in the sward canopy can influence the ease with which animals can select and ingest the plant material when grazing (Stobbs 1973a). For example, the large leaves of tropical legumes such as siratro, desmodium, pueraria and lablab interfere with the biting behaviour of grazing animals (Stobbs 1973a) and animals will take longer to harvest them as compared to white clover. Crowder & Chheda (1982) commented that a measure of herbage yield without a description of its physical distribution may give no indication of the ease with which the feed can be harvested by animals.

In a number of cases the dry weight of a plant leaf has been related to its dry matter digestibility. Wilson *et al.*(1989) found that grass leaf dry matter digestibility was negatively related to leaf dry weight ($r = -0{\cdot}77$) and specific leaf weight

(weight/leaf area) (r = −0·82). The cell wall content of a grass leaf is positively correlated with specific leaf weight and with leaf bulk density (weight/leaf volume) (Wilson *et al.* 1983). Wilson & Hattersley (1989) found that the proportion of leaf midrib was negatively related to dry matter digestibility and positively related to the cell wall and lignin content of the leaf blade. This characteristic was predominantly shown by C_4 grasses. Wilson *et al.* (1989) found no relationship between stem diameter and dry matter digestibility.

Perennial ryegrass selected for low shear strength (John *et al.* 1989) had leaves which were shorter, narrower and lighter and had less sclerenchyma tissue than perennial ryegrass selected for high shear strength. The low shear strength variety was consumed at a higher rate than the high shear strength variety (Mackinnon *et al.* 1988). This might be due to the faster rate of breakdown during chewing of the low shear strength variety.

Plant anatomy in relation to quality

Introduction

The plant that is readily eaten is likely to increase animal production. Plant structure greatly influences the ease with which the plant can be consumed by an animal. The anatomical structure of the plant is primarily adapted to help it to survive and compete well in its particular ecological niche (Wilson 1990). For their survival, plants have often developed specific features to protect themselves from being overgrazed or from perishing in a harsh environment. Characteristics such as a leaf cuticle covered with wax help to reduce excessive water loss through transpiration (Hanna *et al*. 1977). A large number of vascular strands per unit leaf width help to make the leaf rigid (Sant & Rhodes 1970) and expose more surface area to capture light energy for photosynthesis.

Leaf

Morphologically a leaf may have two main parts, the blade and the sheath or stalk. In legumes the leaf blade may be divided into leaflets. Histologically the leaf is composed of three types of tissue, epidermis, mesophyll and vascular tissue. Of great importance in limiting animal utilization are epidermis and vascular tissue (Akin *et al*. 1973). Mesophyll tissue is readily digested (Akin & Burdick 1975). Epidermal tissue can become a barrier to the degradation of the leaf during mastication (Wilson *et al*. 1989) and digestion by the rumen microbes (Monson *et al*. 1972; Hanna *et al*. 1977). This depends on the epidermal cell wall arrangement (Wilson *et al*. 1989) and outer wall structure of the cells (Hanna *et al*. 1977). Wilson *et al*. (1989) found that the straight sided intercostal cells of perennial ryegrass were easily separated during mastication and digestion allowing the epidermis to split, whereas sinuous walls in green panic were resistant to splitting. They also found that the linking of ryegrass epidermis to the vascular tissue was by thin walled mesophyll cells so that the epidermis was shed early during digestion, whereas in green panic the linkage was via thick walled bundle sheath cells causing the epidermis to remain attached for much longer, which reduced the rate of particle width reduction during digestion.

The outer walls of epidermal cells are normally covered with a cuticle. The cuticle is of varying thickness in different plants (Fahn 1990) and its quantity is generally increased under conditions of high temperature, light and aridity (Wilson 1990) and it is greater on the upper surface of the leaf (Fahn 1990). Normally, cuticle is indigestible and restricts the access of micro-organisms into the leaf (Monson *et al*. 1972). The surface of the cuticle may be smooth, rough, ridged, or furrowed and sometimes covered by wax that may give the 'bloom' of many plant leaves (Fahn 1990). The bloom makes the cuticle waterproof and resistant to penetration by micro-organisms. Hanna *et al*. (1977) reported that leaves of bloomless sorghum lost 31% more dry matter per unit time from penetration of rumen microbes through imperfections in the cuticle than did leaves from its near isogenic bloomed counterpart. In histochemical studies of tropical grass structures and digestion, the *in vitro* dry matter digestion of fresh forage of *Pennisetum typhoides*, *Pennisetum purpureum* and *Cynodon dactylon* was increased by perforating the leaf (Monson *et al*. 1972; Monson & Burton 1972). This indicated that the rate of digestion may be influenced by structural factors which are not shown by chemical analysis of forage.

A strand of vascular tissue in the leaf is called a vein (Fahn 1990). The vein may include the vascular tissue (phloem and xylem) with non-vascular tissue (e.g. sclerenchyma). Phloem tissue cell walls are readily digested (Akin & Burdick 1975) while those of xylem and sclerenchyma are digested very slowly and contain a substantial indigestible component (Akin 1989; Wilson 1990). The extent of indigestibility depends on the degree of lignification (which increases with age) and on the chemical nature of the lignin/phenolic components (Akin 1989). The proportion of these tissues has a great influence on the dry matter digestibility of the leaf. The dry matter digestibility of *Cenchrus ciliaris* leaf was negatively correlated ($r = -0{\cdot}76$) with the proportion of thick walled tissues in the cross section area of the leaf (Wilson *et al*. 1989). John *et al*. (1989) found that the number of leaf sclerenchyma fibres was positively correlated with leaf shear strength ($r = +0{\cdot}82$). Shear strength in this case is a measure of resistance to breakage under a force applied at 90° to the length of the leaf, i.e. compressional force (Mackinnon *et al*. 1988). Vincent (1990) suggested that the number of veins present in leaves could determine the amount of leaf harvested by a grazing animal.

The proportion of thick walled and/or lignified tissues within any plant part cannot fully answer why there is a difference in intake of the plant parts at the same level of digestibility or cell wall content. Therefore, in addition to the proportions of thick walled tissues, the arrangement of these tissues in the plant parts could be important in creating differences in forage intake at the same digestibility and cell wall content. Little has so far been reported on how the arrangement of vascular tissues affects intake.

There are two types of leaf venation (vein arrangement) in angiosperms: reticulate venation which is common among the dicotyledons and parallel venation which is common among monocotyledons. In reticulate venation, the veins are of different sizes (Fahn 1990). The largest vein (primary vein) passes through the median part of the leaf from which smaller veins branch. The smaller veins (secondary veins) branch to even smaller veins (tertiary veins) and so on. As a result of branching and fusing, a network of veins is formed. There are many types of reticulate pattern as classified by Hickey (1979). For example pinnate and simple craspedodromous where all secondary veins and their branches terminate at the margin, e.g. white clover, lucerne and sainfoin and cladodromous where secondary veins are not terminating at the margin but rather freely ramify toward the margin, e.g. birdsfoot-trefoil (Lees *et al*. 1982). In parallel venation the main vein and other veins continue throughout the length of the leaf but approach one another and fuse at the leaf tip or both at the leaf tip and at the base. These parallel veins are interconnected by very thin commissural bundles which are scattered throughout the leaf blade (Fahn 1990). Parallel venation can also be found in certain dicotyledons, e.g. *Plantago spp*. and reticulate can also occur in certain monocotyledons, e.g. genera of Orchidaceae. The parallel veins may be of the same thickness or of different thicknesses. The central vein is usually the thickest (the midrib).

The arrangement of the veins in the leaf may have a great influence on the strength of the leaf during harvesting, chewing and microbial degradation in the rumen of the animal. It may also affect the size and shape of the chewed particles and influence the passage of these particles out of the rumen. To date, this area of research has received little attention among the plant-animal relationship researchers (Akin 1989; Wilson 1991). The measurements of the relative size and shape of digesta particles in the omasum indicated that the digesta from alfalfa hay comprised more short, broad particles approaching a cubical shape, whereas in the digesta of grass hays the prevailing particle shape was longer, thinner and more needle like (Troelsen & Campbell 1968). This could be due to the differences in the arrangement of the veins in the grass and legume leaves. The angular vascular connections in the legume leaflets appear to be points of physical weakness allowing breakage of the minor veins into small chunky pieces while the parallel 'girder' system of veins running the full length of grass leaves are more resistant to breaking into shorter particles (Wilson 1985, 1990).

Leaf sheath and leaf stalk

Leaf sheath and leaf stalk anatomy has been little studied in relation to nutritive quality, yet this part of the plant may be a fairly substantial proportion of herbage yield (Wilson 1990). The vascular tissues in the leaf sheath run parallel to each other. All of them enter the stem, providing an extensive vascular connection (Fahn 1990). Sheath has a higher percentage of sclerenchyma than the leaf blade but a similar percentage of vascular tissue (Wilson 1990). Sclerenchyma and vascular tissue as a proportion of leaf sheath increase with age (Wilson 1990) and probably this is one cause of the decrease in sheath dry matter digestibility with maturity (Terry & Tilley 1964). The arrangement of vascular tissues in leaf petioles may be different at the base, middle and apex (Fahn 1990). Information on the nutritive value of leaf petiole is still scarce.

Stem

Structurally a grass stem has a narrow outer cortical zone, a lignified sclerenchyma ring in which vascular tissue is included, and central pith parenchyma which contains isolated vascular bundles (Wilson 1990). The parenchyma may be absent as in the case of C3 grasses, leaving the centre of the stem hollow.

The epidermis, sclerenchyma ring and vascular xylem occupy c. 30% of the stem cross sectional area of some grasses (Akin 1989). According to Akin (1989) these tissues are totally undigested, giving rise to a great potential for a reduction in dry matter digestibility as lignification proceeds with plant maturity. It has been shown that grass stem dry matter digestibility is inversely related to its number of vascular bundles (Wilson *et al.* 1989) and also to the combined area of vascular bundles as a percentage of the cross section area of the stem(Schank *et al*. 1973). Reduction in the number of vascular bundles by selection improved the forage quality of *Hemarthrias* (Schank *et al*. 1973). Any work done on reduction of the percentage of indigestible tissue or reduction in the rate of lignification could help to improve forage quality. Legume stems can be highly digestible in young plants, but as they mature may decrease considerably in digestibility and contribute largely to the reduced digestibility of the herbage (Terry & Tilley 1964). Data for vascular structure of forage legumes as related to quality is scarce.

The role of chewing during eating

Introduction

Voluntary intake of ruminant animals is thought to depend on the rate at which large particles are reduced to a size small enough to leave the rumen (Poppi *et al*. 1985; McLeod *et al*. 1990). The principal means of physical breakdown of forages are chewing during eating and rumination, microbial fermentation and the action of rumen contraction (detrition). Of these processes McLeod & Minson (1988) found that chewing during eating and rumination play a major role in the physical breakdown of forages. These researchers observed the breakdown of large feed particles by cattle eating perennial ryegrass and lucerne hays and found that:

(a) Chewing during eating was responsible for the breakdown of 25% of the large particles in the feed to particles with 1·18 mm diameter or less, and breakdown was significantly higher in the leaf fractions than in the stem fractions. The values reported by other workers for mature temperate forages range from 23 to 40%, (Lee & Pearce 1984; Chai *et al*. 1984) and tropical forages range from 9 to 39% (Hendricksen *et al*. 1981).

(b) Chewing during rumination was responsible for the breakdown of a further 50% of the large particles. Other reported values for temperate forages range from 50 to 73% (Ulyatt *et al*. 1986) and those for tropical forages range from 54 to 89% (McLeod *et al* 1990).

Most of the eaten forage therefore, is broken down to small particles through chewing during eating and rumination. Microbial digestion is known to weaken eaten forage particle structure so that breakdown during rumination is facilitated (Evans *et al*. 1973). During the churning and tumbling of the rumen contents weakened particles are broken. Wilson *et al*. (1989) found that digestion was able to reduce the width but not the length of particles of *Panicum maximum* var. *trichoglume* cv. Petrie and *Lolium multiflorum* cv. Tetila.

Purposes of chewing activity:

(a) Chewing during eating helps

(i) to reduce long forage pieces to a size that can be incorporated into a bolus and then swallowed;

(ii) to release soluble nutrients especially from fresh forages for fermentation;

(iii) to damage the epidermal surfaces of plant material and expose the inner structure to the invasion of rumen microbes; and

(iv) to stimulate saliva production and thus help to buffer the rumen fluid.

(b) Chewing during rumination helps

(i) to damage regurgitated digesta particles to increase the surface area of the particles and further expose internal structures for rumen microbial attack;

(ii) to reduce the particle size for easy passage of the digesta residues out of the rumen; and

(iii) to stimulate further the secretion of saliva to buffer the rumen fluid.

Factors affecting chewing activity

(a) Factors related to the animal

Chewing during eating is more thorough in small than large ruminants (Owens & Goetsch 1988). It has been found that on rations of hay, sheep spent 6-19 times longer on eating, ruminating and total chewing per kg dry matter intake than dry cows (Thomas & Campling 1977). Furthermore, sheep have a higher frequency of chewing during eating than cattle (125-150 chews/min (Ulyatt *et al*. 1986) versus 72-81 chews/min (Gill *et al*. 1966).

These differences in chewing activity may be due to their teeth and rumen anatomy. Cattle have a larger reticulo-omasal orifice and larger tooth surface area (De Boever *et al*. 1990; Welch & Smith 1970) and therefore need fewer chews/kg dry matter during eating. In their review of literature on the factors affecting chewing activity De Boever *et al*. (1990) noted that the difference between sheep and cattle in chewing activity is greater for bad quality than for good quality forages and also more pronounced for grass silage in the long than in the chopped form.

Other factors related to the individual animal that may affect chewing activity include age, productive potential, physiological stage, ingestive capacity, and body size (Ulyatt *et al*. 1986; Owens & Goetsch 1988; De Boever *et al*. 1990). Younger ruminants need more chews/kg dry matter during eating than older ones (Owens & Goetsch 1988). This could partly explain why young ruminants spend less time ruminating, although a weaker jaw strength and incomplete rumen function are main reasons (Hooper & Welch 1983).

De Boever *et al*. (1990) quoted Coulon *et al*. (1987) who found a strong positive relationship between milk yield and eating rate. They ascribed this relationship to the greater feeling of hunger owing to the larger requirements of nutrients by the higher producing cows. Campling (1966a) found that pregnant animals need more time per kg of hay dry matter to eat and ruminate than non-pregnant cows. This lowered chewing rate persisted for a few days after parturition (Journet & Remond 1976). Animals with a higher intake capacity have been found to need less time to eat and ruminate per unit of ingested feed (Deswysen *et al*. 1987). Time of access to feed and feeding level can affect the chewing activity (Freer *et al*. 1962; Campling 1966b). Gill *et al*. (1966) found that the mean number of jaw movements per bolus was significantly less at the beginning of a meal than at any other time and there was a tendency for the highest values to be at the end of a meal. Restricting eating time of herbage from 24 to 4·5 hours per day with dry cows resulted in an increase of eating rate, which was totally (Freer *et al*. 1962; Campling 1966b), or partly (Campling 1966b) compensated by a longer ruminating time per kg dry matter intake. De Boever *et al*. (1990) concluded that changing from restricted to *ad libitum* feeding results in a longer eating and a shorter ruminating time but total chewing time is not affected.

(b) Factors related to the diet

During eating ruminants masticate only enough to mix the ingesta with saliva to form a bolus for swallowing (Bailey 1961; Owens & Goetsch 1988). For this reason fresh forages and silages are chewed less and swallowed faster than dried forages which have to be held in the mouth until moistened sufficiently to swallow (Gill *et al*. 1966). In this case chewing of dry feeds during eating requires more jaw movement per bolus than wet feeds. The mean number of jaw movements per bolus of feed was significantly less with a diet of mature Italian ryegrass herbage (21/bolus) than with mature timothy hay (26/bolus) (Gill *et al*. 1966).

More fibrous feeds require more chews per unit dry matter ingested than do those which are less fibrous (McLeod & Smith 1989). Leaf fraction and legume diets require fewer chews than stem fraction and grass diets (McLeod & Smith 1989; McLeod *et al*. 1990; John & Reid 1986). The numbers of chews per unit dry matter intake were 5·5 and 4·4/g for leaf and 10·1 and 8·6/g for stem of *Panicum maximum* and *Lablab purpureus*, respectively (McLeod *et al*. 1990). John & Reid (1986) reported that the numbers of chews per gram dry matter of fresh perennial ryegrass, lucerne and red clover diets were 36·4, 10·3 and 13·3, respectively. These low numbers of chews/g dry matter intake of leaf and legume diets were associated with a high intake rate (McLeod *et al*. 1990; John & Reid 1986).

Grinding of feed, thereby destroying its physical structure, reduces chewing per unit dry matter of feed consumed (Shaver *et al*. 1988) and increases intake dramatically (Minson 1982). Therefore, the physical characteristics of the fibre tissues and/or their arrangement within plant parts appears to influence the chewing activity more than does the cell wall content of the diet. Fewer chews per unit dry matter intake of the feed might be required to disrupt the weak reticulate venation of legume leaf compared to the stronger parallel venation of grass leaf. Possibly temperate grass leaves with widely spaced veins (Akin 1989) might require fewer chews per unit dry matter intake than leaves of tropical grasses with closely spaced veins (Akin 1989). Leaf and stem anatomy vary between plant groups, genera, species and lines within species. Therefore a study of the role of the physical characteristics of the fibre tissues, and/or their arrangement within plant parts, in relation to chewing activity, will enhance the prospects for improving intake through plant selection. To date, this area of research has received little attention (Akin 1989; McLeod *et al*. 1990; Wilson 1991).

Ingestive mastication can be influenced by the form in which the forage is presented to the animal. Dougherty *et al*. (1989) studied the ingestive behaviour of beef cattle when grazing lucerne or eating from a swath of freshly cut or wilted lucerne. The swath lucerne had a stem length of c. 40-50 cm. Swathing lucerne forced or allowed cattle to take large bites (1·2, 4·9, 6·7 g/bite for grazing, fresh swath and wilted swath, respectively) that required many jaw movements to form a bolus before swallowing. Therefore, both external physical features and the internal structure of a forage plant may influence the chewing activity of the animal. The influence of the external and internal physical structure of forage plants on the chewing activity of animals requires further investigation.

Factors affecting the particle breakdown characteristics of a chewed forage

Several factors determine the size, shape, edges and surface features of a chewed particle. These include the plant species, plant part, plant maturity, moisture content, chemical composition, feed processing, and animal factors. The size, shape and features of the edges and surfaces of the chewed particles during eating are important for the subsequent particle breakdown during rumination and microbial degradation and eventual passage out of the rumen. Information on factors affecting the particle breakdown characteristics of chewed forages is scarce.

Chewed particles from grasses and legume differ in shape (Troelsen & Campbell 1968; Moseley 1982) and in their edges and surface features (Kelly & Sinclair 1989). Chewed grasses produce long, thin or thread-like particles (Moseley 1982) while legumes produce more blocky and irregular shaped particles (Troelsen & Campbell 1968). This seems to be related to the arrangement of vascular tissues in the leaves which form the major part of the grazed herbage. The reticulate venation of legume leaves appears to provide many weaker points for breaking in all directions during chewing, thus producing shorter, blocky particles (Wilson 1985). Kelly & Sinclair (1989) observed that the length of grass leaf particles in the rumen was 8-18 times the width compared to twice the width for those from legume leaves. Grasses and legumes, however, did not differ in the length/width ratio of the stem particles. Kelly & Sinclair (1989) also observed that fractures were parallel to the bundles in perennial ryegrass leaf blade particles but in legume leaflets and petioles and perennial ryegrass leaf sheaths particle fractures occurred in all directions, i.e. both across bundles and parallel to the bundles. They also found that red clover leaves produced shorter and fatter particles than their leaf petioles, while perennial ryegrass leaves, sheaths and stems produced particles of similar size and shape. These observations show that plant part and their vascular structural arrangement affect the size and shape of the chewed particles.

Some studies have shown that chewed tropical grasses produce shorter particles than temperate grasses (Pond *et al.* 1984; Wilson *et al.*1989). In a study to investigate forage particle breakdown during the eating of tropical and temperate grass by cattle, Wilson *et al.* (1989) found that green panic (*Panicum maximum* var. trichoglume) fresh feed pieces were reduced from their original 50·1 mm length and 8·4 mm width to 5·4 mm length and 1·6 mm width, compared to Italian ryegrass (*Lolium multiflorum*) feed pieces that were reduced from 51·7 mm length and 5·5 mm width to 11·6 mm length and 2·4 mm width. Earlier, Pond *et al.* (1984) found that there was greater reduction in particle size during chewing in coastal bermuda-grass than in Italian ryegrass.

A possible explanation for the small size of chewed particles of tropical grasses is that highly lignified tissues tend to break at all angles whereas tissues with low lignin tend to bend rather than break under pressure (Van Soest 1982). Also Lee & Pearce (1984) found that the greatest reduction in particle size during chewing by cattle of low quality feeds occurred in the feeds with the highest cell wall and lignin content. Lee & Pearce (1984) did not measure the length of the particles, but used a sieving method to estimate particle size.

Ulyatt *et al*. (1986) indicated that chewed fresh herbage produced a larger mean particle size than chewed hay. Gill *et al*. (1966) found a significant decline during the course of a meal in median particle size of swallowed mature timothy hay, but not of swallowed fresh Italian ryegrass. They also observed that median particle size of swallowed fresh forage was greater than that of swallowed hay. This observation fits the concept that ruminants mix the ingesta with saliva to form a bolus for swallowing and that they do less chewing per bolus with fresh grass and silage than with dried grass or hay (Bailey 1961).

The greater particle size reduction achieved by chewing in tropical grass, mature and dried forages might be expected to promote faster subsequent digestion and a faster rate of particle passage out of the rumen and thus lead to higher intake. This has not been the case so far. Pond *et al*. (1987) found that cattle grazing mature coastal bermudagrass had more large and small particles in the rumen than those consuming immature coastal bermudagrass. They also observed a decreased passage rate of particulate matter and a decreased intake by cattle grazing the mature forage. The rate of digestion of chewed fresh particles was lower with green panic than with Italian ryegrass (Wilson *et al*. 1989).

The reasons why greater size reduction achieved during chewing in the case of tropical grasses, mature forages and dried forages does not lead to greater digestibility and intake may be associated with the chemical composition of the plant and the animals' eating behaviour. The higher proportion of cell wall in tropical than in temperate grasses (Wilson *et al*. 1983) is likely to contribute to the lower digestion rate of the former (Wilson *et al*. 1989). Increased lignification with advancing maturity (Cherney *et al*. 1990) may lead to a cell wall structure more resistant to microbial attack. Lee & Pearce (1984) found that the proportion of particles greater than 1 mm varied significantly between steers, but there was a low overall correlation between the level of intake and the extent of particle size reduction. However, they found that when each feed was examined separately there was a high correlation between intake rate and particle size: steers with the highest intake rate had the highest mean particle size in the extrusa. The characteristics of masticated particles during eating can vary, therefore, not only with forage type, but also with the individual animal.

Factors affecting forage intake rate

Introduction

The rate at which a forage can be eaten is an important factor influencing animal preference (Kenney & Black 1984) and voluntary intake (Forbes *et al*. 1972; McLeod & Smith 1989). There are several factors affecting the rate at which a forage can be eaten. These include:

- Physical characteristics of the forage such as its ease of fracture during chewing (Vincent 1990), size of particles in the diet (Kenney & Black 1984; Minson 1990), moisture content (Arnold 1962; Gill *et al*. 1966; Suzuki *et al*. 1969; Kenney *et al*. 1984; John & Ulyatt 1987) and cell wall content (McLeod & Smith 1989).
- Accessibility of the forage or pasture component, which depends upon tiller height and size of the plant parts (Black & Kenney 1984; Stobbs 1973a).
- Acceptability of the forage, which depends upon the taste, odour and touch (Grovum 1984; Arnold *et al*. 1980).
- The individual animal and its degree of satiation and physiological and psychological state (Weston 1982; Grovum 1988).

Physical characteristics of the forage

Stem fractions and grasses are eaten at a slower rate than leaf fractions and legumes (McLeod & Smith 1989; McLeod *et al*. 1990). Tropical grasses are eaten at a slower rate than temperate grasses (McLeod & Smith 1989). The slower rate of eating of stem, grasses and tropical grasses has been associated with their high cell wall content (McLeod & Smith 1989) which leads to high resistance of large particles to breakdown during eating (McLeod *et al*. 1990). The eating rates (g/min) were 55, 63, 99 and 102 for leaf fractions and 36, 49, 48 and 36 for stem fractions of

Panicum maximum, *Lolium multiflorum*, *Lablab purpureus* and *Medicago sativa* (McLeod & Smith 1989). The differences in intake between stem and leaf fractions vary from species to species. For example, with ryegrass (which has relatively thin stems) the difference in intake was only 20% (Laredo & Minson 1975), but it was 87% with *Setaria splendida* (which has thick stems) (Laredo & Minson 1973). Therefore forage intake rate may be most limited by physical characteristics of the forage plants which affect the ease with which they are prehended, chewed and compressed to form a bolus ready for swallowing. Effects of external physical characteristics of the forage plant on its intake rate have been documented (Black & Kenney 1984; Black 1990; Hodgson 1990). Little has, however, been reported on the effects of internal physical characteristics of the forage plant on its intake rate. Variations in the structural strength of plant tissue may be involved in the control of bite dimensions (Poppi *et al*. 1981) and hence rate of intake (Mackinnon *et al*. 1988). This is a potentially fruitful area for agronomist, plant histologist, animal nutritionist and plant breeder to work together to define the relative importance of internal and external characteristics limiting forage plant intake by ruminant animals.

Animals prefer and eat faster chopped than long forages (Kenney & Black 1984). Reducing the length of wheaten straw particles from 30 to 10 mm increased intake rate from 5·5 to 12 g/min and resulted in an absolute preference for the short material (Kenney & Black 1984). Kikuyu grass chopped to 10 mm lengths was eaten faster than, and was preferred to, the same grass cut to 40 mm, irrespective of the moisture content.

Kenney *et al*. (1984) found that the rate of intake of fresh forage (i.e. g fresh forage/min) increased as its moisture content increased, but the rate of dry matter intake declined once the dry matter of the forage fell below 40%. This observation supported the earlier observation that fresh feeds are swallowed faster than dried feeds (Gill *et al*. 1966; Suzuki *et al*. 1969). The effect of moisture level on intake rate could be due to its influence on the rate of salivation, as rate of swallowing is correlated with saliva production (Church 1988). Fresh or wet feed does not require lots of saliva to help in chewing and forming a bolus to swallow (Owens & Goetsch 1988) and so can be eaten faster (g fresh feed/min) than dry feeds.

Accessibility of the pasture or forage component

Black & Kenney (1984) found that the rate of intake by sheep grazing artificially constructed pastures (made by placing vegetative grass tillers in holes in pressed hardwood sheets) increased with both the height and density of pasture and was best described by herbage mass per unit area effectively covered by one bite. Intake rate of dry matter ranged from c. 0·5 to 6 g/min and the maximum occurred when pasture availability was a little under 1 t dry matter/ha. Curll & Davidson (1983) have reported feed intakes as low as 0·45 g dry matter/min for sheep grazing sparse pasture. Bush encroachment in rangelands reduces accessibility of the pasture to the grazing animals (Crowder & Chheda 1982) thus could reduce intake rate of the forage dry matter.

Acceptability of the forage

Several studies have shown that the acceptability of a given feed by an animal would be influenced by its taste, odour and texture (Arnold *et al*. 1980; Grovum & Chapman 1988). For example, the intake rate of low quality roughages, found in large quantities in most parts of the world, can be increased by enhancing their acceptability through taste and texture. Grovum (1984) found that the intake rate of coarse-chopped straw by sheep was only 0·91 g/min, but this was increased to 5·17 g/min simply by spraying the straw immediately before feeding to add monosodiumglutamate at a rate of 4% of the air-dry weight of the untreated straw. Grinding and pelleting the untreated straw increased its intake rate from 14·7 g/min on the first day to c. 36·2 g/min from the fourth to the seventh day of feeding. This increase in intake rate may have been due to an improvement in the texture of the feed. An increase in intake rate during the first few days of feeding indicates there is an adjustment period during which an animal adapts itself to the new feed. Arnold *et al*. (1980) found that the voluntary intake of some forages was increased by adding the odour of butyric acid and amyl acetate. They also pointed out that the smell, taste, and touch of the feed may be more important in determining the amount eaten when food is plentiful.

Animal factors

The physiological state of an animal is one of the factors affecting its eating rate. Suzuki *et al*. (1969) found a significant difference between cows in the rate of eating. They also found that dry pregnant cows had a lower rate of eating than lactating cows with both silage and hay. The dry pregnant cows started each meal eating slowly and continued to eat for a longer time than lactating cows. It seems that, in order to compensate for the energy consumed in milk production, lactating cows have to eat faster than dry pregnant cows. Arnold & Birrell (1977) found that thin sheep ate faster than fat sheep and shorn sheep ate faster than unshorn sheep.

It has been reported that animals eat faster at the start than at the end of a meal (Gill *et al*. 1966; Suzuki *et al*. 1969). Gill *et al*. (1966) reported that the rate of production of boli per cow declined from 4·7 and 3·9/min at the start of a meal to 3·5 and 2·3/min at the end of the meal, with fresh herbage and hay respectively. These results were supported by those of Suzuki *et al*. (1969) who found that in all cows, on both silage and hay, the rate of eating was highest during the first or second five minutes, and then declined continuously until the end of eating. A possible explanation for this variation of eating rate during the meal could be fatigue of the jaw muscles. However, according to Suzuki *et al*. (1969), this explanation may not be correct. They took a meal of silage, divided it into two halves, and gave the second half to the cows immediately after they had finished eating the first half. They observed two peaks in the rate of eating, both occurring at the beginning of eating each half of the meal. They suggested that the decline in the rate of eating as the animals continued to eat the offered meal could have been a psychological effect. In this case small, frequent meals could lead to greater daily feed intake than a single large meal given once per day. Black (1990), in his review of literature, noted that the intake rate of chopped forage measured over short periods was three to four times greater than the mean intake rate during eating periods over a whole day.

Agronomy and nutritive value of the grassland plant species and crop residues used in this study

Most swards are not made up of a single grass or a single legume species. A sward can be dominated by one plant species, but some other species may be present in small quantities. Grasses are said to be high in carbohydrates, legumes high in protein and herbs high in minerals. Therefore a sward with several grass, legume and herb species may be closer to meeting the nutritional requirements of a grazing animal than a sward dominated by one plant species. Fodder crops can produce large quantities of dry matter per unit area in a short time. Fodder crops such as maize and rape therefore are very useful for making silage or providing grazing for finishing animals. In many parts of the world, especially where land scarcity is a major problem as in South Asia, crop residues (e.g. rice straw and sweet potato tops) form an important part of ruminant diets.

The eating behaviour of an animal is influenced by the morphology and vascular structure of the plant species and by the way the feed is presented to the animal. Ideally an animal-plant relationship study should include a rather large number of plant species. As many as reasonably possible, therefore, were included in the present study.

White clover (*Trifolium repens* L.)

White clover is the most important creeping perennial pasture legume occurring naturally in many parts of the temperate zone (Carlson *et al*. 1985). It accounts for c. 63% by weight of the herbage legume seed used in the United Kingdom (National Institute of Agricultural Botany 1989). The leaves are borne on long petioles arising from the stolon nodes and flowers are carried on long stalks arising from leaf axils. During cutting or grazing, therefore, leaves, petioles and inflorescences are the only aerial parts harvested leaving the creeping stolons and stolon apices for regrowth. For this reason white clover can survive well as a good pasture legume and also provide herbage which is low in fibre and highly digestible and palatable (Carlson *et al*. 1985). Bloat sometimes occurs in animals grazing lush white clover. Lees *et al*. (1982) related this problem to the low cell wall and low tissue strength in white clover. Bloat can, however, be minimized by maintaining a good proportion of grass in the mixture.

Lucerne (*Medicago sativa* L.)

Lucerne is a herbaceous perennial legume with numerous upright stems and a very strong tap root, penetrating deep into the soil (Barnes & Sheaffer 1985). It can be grown alone or in combination with a grass like cocksfoot, timothy or meadow fescue where it can give a balanced feed for grazing animals or hay and silage making. It is regarded as a key forage for high producing ruminants in many parts of the world because of its richness in protein, palatability and high calcium and vitamin content (Göhl 1981). Terry & Tilley (1964) found that the leaves of lucerne maintained the same cell wall content and dry matter digestibility, while stem became progressively more fibrous and less digestible, with increasing maturity. In order to obtain satisfactory herbage quality, cutting intervals of not > c. 6 weeks are, therefore, recommended (National Institute of Agricultural Botany 1989).

Sainfoin (*Onobrychis viciifolia* Scop.)

Sainfoin is a herbaceous perennial legume with a strong, well developed root system (Hoveland & Townsend 1985), which can grow well on soils with pH ranging from 6·0 to 7·5 (Spedding & Diekmahns 1972). It grows well when sown alone and should be cut for hay or grazed just as the flowers open. Sainfoin has the advantage of not inducing bloat. It is palatable and consumed in greater quantity than ryegrass (Thomson 1982) and lucerne (Osbourn *et al*. 1966).

Desmodium intortum (Mill.) Urb. (Green leaf desmodium)

Desmodium intortum is a large trailing and climbing perennial legume which branches freely and may root at the nodes; it occurs naturally in Central and South America c. 1500-2500 metres above sea level in the equatorial zone and is now familiar in many parts of the tropics (Skerman 1977). It produces a large bulk of palatable herbage and has been used as a companion legume to some grass species, e.g. *Pennisetum purpureum* (Göhl 1981). It can be grazed or cut for zero-grazing or dehydrated to a meal that is an excellent source of protein, riboflavin and vitamin A (Skerman 1977).

Perennial ryegrass (*Lolium perenne* L.)

Perennial ryegrass has good persistence, is widely distributed in temperate regions of the world (Riewe & Mondart 1985) and is by far the most widely sown grass in British Agriculture (National Institute of Agricultural Botany 1989). It is used for grazing, hay and silage making. Its high water soluble carbohydrate content produces a good fermentation without additives during silage making (National Institute of Agricultural Botany 1989). Its intake is greater than that of cocksfoot (Greenhalgh 1966).

Italian ryegrass (*Lolium multiflorum* Lam.)

Italian ryegrass is a tufted, short lived perennial, less winter hardy and less persistent than other common temperate grasses (Spedding & Diekmahns 1972; Riewe & Mondart 1985).

Because of its earliness, together with tall fescue (National Institute of Agricultural Botany 1989), it can provide early spring grazing and can later be conserved as silage (Holmes 1982). Higher intake in Italian ryegrass than in perennial ryegrass has been noted by Jarrige *et al*. (1974).

Tall fescue (*Festuca arundinacea* Schreb.)

Tall fescue is persistent, drought resistant, winter hardy and one of the earliest grasses to grow in spring (National Institute of Agricultural Botany 1989). Tall fescue is rather valuable for hay making because it dries rapidly (Holmes 1982). It is rather a coarse leaved grass and not readily eaten by livestock except in the spring (Holmes 1982).

Chloris gayana Kuth.

Chloris gayana is a perennial grass occurring naturally in most tropical and subtropical areas of Africa (Skerman & Riveros 1990). The popularity of *Chloris gayana* all over the tropical regions has been linked with its good seed production, ease of establishment, tolerance of high soil salinity and ability to combine well with many different legumes (Bogdan 1977; Rotar & Kretschmer 1985). It is used for grazing, hay and sometimes silage making. Stobbs (1973b) found that at 4 weeks of regrowth *Chloris gayana* provided larger bites for grazing cows than *Setaria anceps*. This was because *Chloris gayana* was more leafy and the leaves were smaller and more accessible than those of *Setaria anceps*.

Cenchrus ciliaris L.

Cenchrus ciliaris is a perennial, tufted, rhizomatous grass native to the dry areas of East Africa (Bogdan 1977; Whiteman 1980). Because of its ease of establishment, drought tolerance and high nutritive value it has spread all over the drier regions of the tropics and subtropics where it is used for reseeding denuded or arid pastoral areas for grazing (Whiteman 1980). Voluntary dry matter intakes for *Cenchrus ciliaris* ranging from 52 to 67 $g/kgW^{0.75}$ have been recorded by Minson (1990).

Maize (*Zea mays* L.)

Maize has been extensively used as a grain crop and for fodder production in the countries with more advanced agriculture especially the U.S.A. When used as a forage crop, harvested before full grain ripeness it can be grown in areas with a fairly short growing season. The crop is grown in most parts of the south of the United Kingdom where in addition to silage making it produces a valuable green feed in late summer (Ministry of Agriculture, Fisheries and Food 1985). It is normally harvested for silage as soon as the grains begin to dent (dimple) at the tip, with the ears contributing 50-55% of the total dry matter yield (Ministry of Agriculture, Fisheries and Food 1985). For good quality silage with minimum effluent losses, dry matter content of the plant should exceed 25% (National Institute of Agricultural Botany 1992b).

Rape (*Brassica napus* L.)

Forage rape is a valuable catch crop especially in areas where early mildew is less likely, e.g. in the west and north of the United Kingdom (National Institute of Agricultural Botany 1992a). The main advantage of forage rape is its ability to grow quickly and produce high yields of succulent (dry matter content c. 12%), highly digestible fodder (D -value c. 64%) over a short growing period (Ministry of Agriculture, Fisheries and Food 1983; National Institute of Agricultural Botany 1992a). Cell wall content of forage rape ranging from 21 to 32% have been reported by Kunelius & Sanderson (1989). In the United Kingdom rape has been widely used over the years for fattening lambs and even cattle during the autumn (Ministry of Agriculture, Fisheries and Food 1983). Rape can be grazed *in situ* or zero grazed (National Institute of Agricultural Botany 1992a).

Common or broad-leaved dock (*Rumex obtusifolius* L.)

Broad-leaved dock is a perennial, herbaceous, dicotyledonous species common on dairy farms (Haggar *et al*. 1990). Fairbairn & Thomas (1959) classified dock in the high apparent value weed group. They found that, before flowering, dock had 25% crude protein and low fibre. Wilman & Riley (1993) reported that dock had only 23% neutral detergent fibre suggesting the possibility of high intake.

Dandelion (*Taraxacum officinale* Weber)

Dandelion is a perennial herb common in grassland especially in disturbed areas (e.g. on molehills (Parish & Turkington 1990)).

It is rich in minerals (Marten *et al*. 1987), crude protein and highly digestible (Dutt *et al*. 1982). Dutt *et al*. (1982) found that dandelion had high dry matter digestibility (73·7%) compared to lucerne (61·1%) and grasses (57·1%) (mainly *Bromus inermis* and *Dactylis glomerata*). Therefore its presence in the sward (so long as it is not allowed to suppress grass and clovers) may be advantageous.

Chickweed (*Stellaria media* L.)

Chickweed is an annual herbaceous, dicotyledonous species common in arable land on moist soils, and in pastures and waste places (Clapham *et al*. 1962; Frankton & Mulligan 1987). Its prostrate growth habit restricts the tillering of grasses and the establishment of clover (Haggar *et al*. 1985). Fairbairn & Thomas (1959) classified chickweed as one of the high apparent value weeds that can be used for livestock.

Ribwort plantain or ribgrass (*Plantago lanceolata* L.)

Ribgrass is a common perennial herbaceous, dicotyledonous species widely distributed throughout the British Isles grasslands, particularly on neutral and basic soils (Clapham *et al*. 1962). It has high feeding value, is rich in minerals and is often grazed hard (Stapledon & Davies 1948) and is good for winter and early spring grazing (Fagan & Watkins 1932).

Common spurrey/corn spurrey (*Spergula arvensis* L.)

Common spurrey is an annual herbaceous, dicotyledonous species common in arable land and on road sides and is considered to be an indicator of acid soils in Europe (Frankton & Mulligan 1987). It has been grown as a fodder crop for late autumn/early winter feed in Belgium, France and elsewhere on the continent of Europe, and a crop badly infested with it may be fed off with sheep before the seed ripens (Fream 1900). Very little has been published, however, on the nutritive value of spurrey.

Sweet potato (*Ipomoea batatas* (L.) Lam.)

Sweet potato is a creeping plant with perennial vines and adventitious roots, some of which produce swollen tubers (Göhl 1981). In many parts of the world (in the tropics and warm temperate regions) especially in developing countries, sweet potato ranks fifth on the list of the valuable food crops, and 90% of it is grown in Asia (Woolfe 1992). Both sweet potato roots and tops are valuable as animal feed; roots being more valuable for monogastric animals and tops for ruminants (Göhl 1981). In most cases, however, the roots are used as human food while the tops are used as a crop residue for livestock feed (Woolfe 1992).

Rice (*Oryza sativa* L.)

Rice is grown mainly as food crop for humans. When the grains have been harvested, the straw is fairly palatable although insufficient for animal maintenance (Göhl 1981). Sundstøl (1988) in his review noted that, despite having a high silica content (12-16%), rice straw has a low lignin content (6-7%), and its organic matter digestibility is as high as that of barley and oat straw.

Foxtail millet(*Setaria italica* (L.) Beav.)

Foxtail millet is a fast-growing slender-stemmed and leafy annual cereal that may be cut for hay or for zero grazing within 50 days of sowing or used as pasture or silage for dairy or feed lot (Göhl 1981; Fribourg 1985). In most cases when it is used as food crop, mostly in developing countries, its straw is fed to ruminants.

Wheat (*Triticum vulgare* Vill.)

It is extensively cultivated as a cereal crop and is the leading cereal crop in Europe (FAO, 1992). However its straw is less palatable and digestible than oat straw (Sundstøl 1988), although it has been used especially to maintain ruminants during winter.

CHAPTER 3

EXPERIMENT 1

PLANT PHYSICAL STRUCTURE OF FOUR FORAGES IN RELATION TO EATING RATE BY SHEEP, CHEWING ACTIVITY AND PARTICLE BREAKDOWN CHARACTERISTICS

Introduction

Derrick *et al*. (1993) compared three perennial and two annual herbaceous, dicotyledonous non-legumes with *Lolium perenne* L. (perennial ryegrass) (cv. Melle) in feeding experiments at Aberystwyth and found that the chewing rate was higher on all the dicotyledonous diets than on ryegrass, particularly in the case of spurrey, which had the highest rate of intake because it had a high chewing rate and a low requirement for number of chews/g of dry matter consumed. Earlier, Wilman & Riley (1993) compared 15 grassland species at Aberystwyth using chemical and physical tests and reported that the leaves of all 11 dicotyledonous species were much lower in neutral detergent fibre (NDF) than the leaves of the grasses and most broke down readily when macerated. Moseley (1982), again at Aberystwyth, reported that white clover had a higher intake than perennial ryegrass because, with the former species, particles were broken down more rapidly and cleared faster from the rumen than the particles of the latter species.

The purpose of the present experiment was to develop the subject further by investigating plant physical structure in relation to eating rate by sheep, chewing activity and particle breakdown characteristics. Four plant species with high variation in morphological and anatomical characteristics were used in the present experiment: *Lolium perenne* L. (perennial ryegrass) (cv. Melle) represented grasses and *Trifolium repens* L. (white clover) (cv. Olwen) represented legumes. The two species are very important pasture species in Britain. *Brassica napus* (rape) (cv. Sparta), a broad-leaved non-legume dicotyledonous species, is often grown in Britain as a short term forage crop. *Spergula arvensis* L. (Spurrey), an annual herbaceous non-legume dicotyledonous species, was included in this experiment because it showed the highest potential nutritive value in the previous experiments at Aberystwyth by Wilman & Riley (1993) and Derrick *et al.* (1993).

In order to get further information on how plant physical structure may be associated to its physical breakdown in the mouth and rumen (Wilson 1985), plant part morphology and their vascular structural size, proportion and arrangement, intake rate, jaw activity and particle breakdown characteristics of the four plant species were studied for two years. Among the particle breakdown characteristics investigated were: lengths and widths of particles, number of vascular tissues per particle, edges and surface appearance of the particles. Neutral detergent fibre and *in vitro* dry matter digestibility of the plant species were determined.

Materials and Methods, 1991

Land preparation

The experimental site was in Cae Plas, Penglais Farm, Aberystwyth, a field which had been used mainly for sheep and cattle grazing. On 25 February 1991 the experimental site (53 m x 23 m) was demarcated using wooden pegs and string. The site was then divided into two equal halves by a string. By using a screw auger the top 15 cm of soil of each half was sampled at eight points along the diagonals (Fig. 3.1). The cores from each half of the site were bulked and mixed thoroughly. The soil samples were taken to the Agricultural Sciences Analytical Laboratory at Frongoch. The results of the soil analysis are given in Table 3.1.

Near each soil sampling point the soil was dug using a small spade to observe the soil depth and its visual characteristics. On average the soil depth was 15 to 20 cm. Occasionally, stones were found, starting from 10 cm deep. Earthworms were found as deep as 6 cm from the soil surface. The soil in the eastern half of the site was darker than that in the western half. No place in the experimental site showed signs of waterlogging.

The experimental site was ploughed on 13 March 1991 and fenced off from the rest of the field on 11 to 15 April. The site was lightly harrowed on 17 April and lime and triple superphosphate (45% P_2O_5) were applied at rates of 10 t lime/ha and

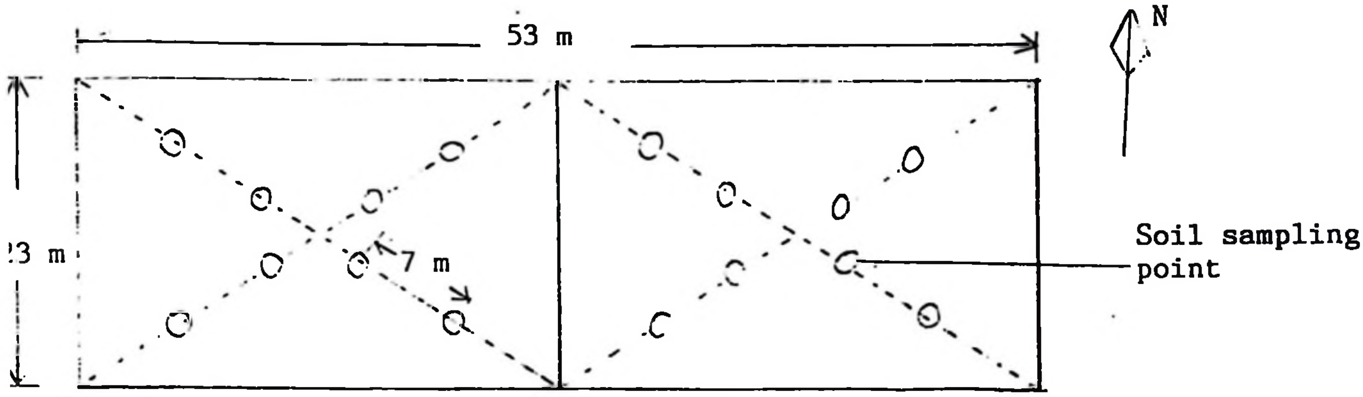

Fig. 3.1. Experimental site: soil sampling points before ploughing

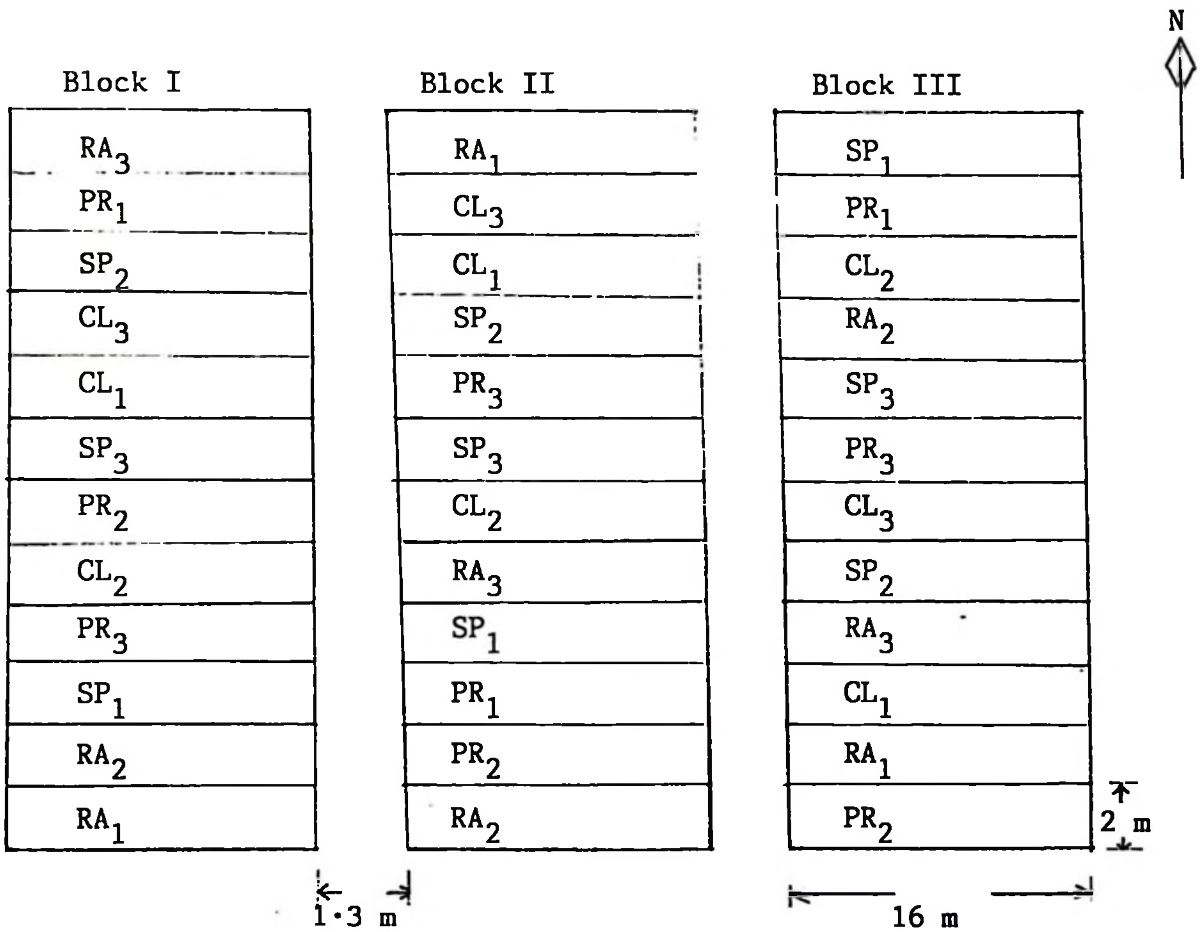

Fig. 3.2. Experimental layout

Key:

1 = First harvest. 2 = Second harvest. 3 = Third harvest.
CL = White clover (*Trifolium repens* cv. Olwen)
RA = Rape (*Brassica napus* cv. Sparta)
SP = Spurrey (*Spergula arvensis*)
PR = Perennial ryegrass (*Lolium perenne* cv. Melle)

Table 3.1. Experimental site soil analysis results

(Sampling date, 25 February 1991)

Portion of the site	pH	Mg/l extractable K	Index K*	Mg/l extractable P	Index P*
Eastern half	5·00	128	2	8·0	0
Western half	5·02	180	2	11·6	1

* MAFF (1986)

100 kg P_2O_5/ha. It was then lightly reharrowed. The site was then left for a month before seed sowing. The aim of resting the site for a month was firstly to allow most weed seeds near the surface to germinate and then to kill the seedlings by harrowing before sowing. Secondly, the delay of sowing was aimed at delaying the time of harvesting so that it would not coincide with the harvesting of Experiment 2.

Crop establishment

By 21 May 1991 weeds had germinated on the experimental site. The site was harrowed in the afternoon using a spike toothed harrow mounted on a tractor. The harrowing was light, just to kill the weed seedlings. Deep harrowing was avoided because it could bring up a lot of weed seeds that had not yet germinated. The experimental site was then demarcated into 36 plots, each 2 m x 16 m. The plots were laid out in 3 completely randomized blocks with 12 plots per block (Fig. 3.2). Each block was sown with four forage species, i.e. Spurrey (*Spergula arvensis* L.), rape (*Brassica napus* cv. Sparta), white clover (*Trifolium repens* L. cv. Olwen) and perennial ryegrass (*Lolium perenne* L. cv. Melle). The forage species were harvested on three different dates (27-30 August, 9-12 September and 23-26 September); in each case this was the first harvest after sowing.

The origin of the forage seeds is shown in Table 4.1, (Expt.2). They were sown on 22-24 May 1991; one day per block from block I to block III. Before sowing, each plot was sub-divided by strings into six sub areas (each 1 m x 5·33 m) in order to broadcast the seeds and fertilizers evenly throughout the plot. Seeds and fertilizers for each sub area were weighed five days before sowing.

The seed rates were:

(i) Perennial ryegrass: 10 g/sub area; equivalent to 18·75 kg/ha.

(ii) Rape: 5·33 g/sub area; equivalent to 10 kg/ha.

(iii) Spurrey: 4·63 g/sub area; equivalent to 8·68 kg/ha.

(iv) White clover: 3·2 g/sub area; equivalent to 6·0 kg/ha.

The fertilizers were:

(i) 'Nitram' (ammonium nitrate 34·5% N): 108 g 'Nitram'/sub area; equivalent to 70 kg N/ha ('Nitram' was not applied to the white clover plots).

(ii) Triple superphosphate (45% P_2O_5): 142 g triple superphosphate/ sub area; equivalent to 120 kg P_2O_5/ha for all plots.

(iii) Sulphate of potash (48% K_2O): 55·6 g sulphate of potash/ sub area; equivalent to 50 K_2O/ha, for all plots.

The seeds were broadcast first, followed by the fertilizers. After sowing, the perennial ryegrass and rape plots were lightly raked, using a wooden rake. The white clover and spurrey plots were not raked.

Seven days after sowing, a few weeds were germinating in the plots. The most important weeds were dock (*Rumex obstusifolius)* and thistle (*Cirsium arvense*). These weeds were removed by hand using a sharp kitchen knife, collected in buckets and taken away from the experimental site.

By 10 June all the sown species were germinating. White clover, however, was very slow to germinate. Rape was the first to germinate. When rape plants started to produce their third leaves, birds started to eat them. Humming lines were set up over the plots and these kept the birds away.

By the first harvest (late August, 1991) the rape had grown strongly. On average rape plants were 50 cm tall, with about 8 to 10 leaves per plant. Spurrey was already mature. Despite regular weeding, only thistle and dock were completely controlled. The plots, especially those of Spurrey and white clover, had lots of chickweed (*Stellaria media* L. Vill) and other dicotyledonous weeds. Rape plots, however, had very few weeds.

Harvesting and sampling

In 1991, each harvest took four consecutive days to complete (Table 3.2). On the first day of each harvest a small amount of herbage (about 1 kg per plot and about 5 plants of rape) was harvested from 12 plots (4 plots per block, one of each forage species). This small amount of herbage was fed to two oesophageal fistulated sheep to study the forage species plant particle breakdown characteristics when eaten. On each of the following three days, a larger amount of herbage (about 2·5 kg/plot and about 15 plants of rape) was harvested from 4 plots in one block (the same plots harvested in that block on the first day) and fed to four sheep to study rate of intake and chewing activity during eating. Normally all forages except rape were harvested at 2 cm above

Table 3.2. Harvesting and feeding programme 1991

Harvest number	Date of harvest	Block (s) harvested	Number of species harvested/ block	Number of sheep fed	Number of meals/ sheep	Oesophageal collection	Jaw recording
1	27 Aug	I,II&III	4	2	12	Yes	No
	28 Aug	I	4	4	8+	No	Yes
	29 Aug	II	4	4	8+	No	Yes
	30 Aug	III	4	4	8+	No	Yes
2	9 Sept	I,II&III	4	2	12	Yes	No
	10 Sept	I	4	4	8+	No	Yes
	11 Sept	II	4	4	8+	No	Yes
	12 Sept	III	4	4	8+	No	Yes
3	23 Sept	I,II&III	4	2	12	Yes	No
	24 Sept	I	4	4	8+	No	Yes
	25 Sept	II	4	4	8+	No	Yes
	26 Sept	III	4	4	8+	No	Yes

+ 4 long and 4 chopped meals

ground level. Rape had very thick stems with a hard base; therefore, it was harvested at 10 cm above ground level. The forages were harvested between 8.00 and 9.00 a.m. and carried in polythene bags to the crop laboratory at Frongoch. All the unsown species were removed by hand from the herbage of each forage species.

On the first day of each harvest, about six tillers of perennial ryegrass, three rape plants and 20-30 g of white clover and spurrey were sampled from the produce of each plot. These samples were placed in labelled polythene grip bags and stored in the freezer. In the case of rape the stem had to be chopped into 10 cm long pieces before it could be properly placed in the polythene bag. The samples were used for the determination of plant morphology (leaf length, stem diameter etc.) and plant part vascular structure. All the remaining forage from each plot sample, after the whole plant sample had been taken, was chopped into 7 cm long pieces and mixed thoroughly. A chopped sample of about 100 g was then taken from the produce of each plot and put in a labelled polythene grip bag and stored in the freezer. This sample was used to determine the plant part proportion and cell wall content of the forage. The remainder of the chopped forage was put into labelled polythene bags, tied by string, ready for taking them at 1.30 p.m. to IGER (AFRC Institute of Grassland and Environmental Research, Gogerddan, Aberystwyth) where they were fed to oesophageal fistulated sheep. Feeding took place around 2.00-3.00 p.m.

On the second, third and fourth day of each harvest, after the unsown species had been removed from the forages, no whole plant samples were taken. Instead each forage sample was divided into two sub-samples, each of about 800 g. One of the subsamples was chopped into 7 cm long pieces and the other was left unchopped. The subsamples were then put in polythene bags, tied by string, taken to IGER at 1.30 p.m. and fed to four intact sheep around 2.00-3.00 p.m.

Feeding activities

1. Oesophageal fistulated sheep

The oesophageal fistulated sheep used in this experiment were two; they were kept indoors at IGER. They were fed ryegrass hay and some sheep nuts and liberally supplied with drinking water. The sheep were Clun wethers, 7 years old, and they had been used for this type of feeding experiment for the previous 4 years. On average the sheep were 56 kg live weight. In order to accustom the sheep to fresh forages, three days before feeding experimental forages, the sheep were given 750 g of mixed fresh experimental forages (c. 12% dry matter) at 9.00 a.m. and 400 g of hay at 4.00 p.m. On the day of feeding experimental forage meals, the sheep were fed 375 g of mixed fresh forages at 9.00 a.m. and 800 g of hay (their normal ration before the experimental period) at 4.00 p.m.

In IGER the chopped forage of each species was poured into a big plastic bowl and mixed thoroughly by hand. About 20-30 g of the chopped forage was then taken and placed in perforated polypropylene bags (W.R.Grace Ltd) and oven-dried at 80°C for 48 hours to determine the dry matter percentage. The dried samples were kept in labelled paper envelopes and taken to the Agricultural Sciences Analytical Laboratory at Frongoch for *in vitro* dry matter digestibility determination. After a sample for dry matter determination had been taken, the rest of the chopped herbage was weighed into 100 g meals and placed in plastic bowls.

After placing the sheep in their metabolism crates the area of the neck around the fistula was washed with warm water and the bung removed from the fistula. A soft clean bung tied with two strings round the sheep's neck was then slotted into the fistula so as to block the lower part of the oesophagus and make sure that all chewed particles passed out through the fistula. A polythene bag for collecting the chewed particles was placed in a linen bag that had four strings. The linen and polythene bag's openings were then placed on the fistulae and the linen bag strings tied round the sheep's neck. With one person holding a stop watch, another one took the two feeding bowls with 100 g chopped forage meals and placed them on the platform fitted on the crates. The sheep were allowed to eat the meals for one minute. At the end of one minute the bowls were removed and the spilled forage pieces on the platform and on the floor collected. The uneaten herbage was weighed and the weight subtracted from 100 g to get the intake rate (g/min) of the forage. After every meal the linen bag was removed from the sheep's neck and the polythene bag containing the chewed particles taken out, tied and placed in a labelled polythene grip bag and stored in the freezer at Frongoch. The soft bung was removed from the oesophagus at the end of the last meal. The fistula bungs were then placed back after the area around the fistula had been washed with warm water. The sheep were then taken out of their crates and put back in their pens and provided with hay and water.

2. Intact sheep

The sheep were Clun wethers, 1½ years old with an average live weight of 56 kg. They were selected from twelve sheep of the same age and size. The sheep had been used once before in similar feeding experiments. The twelve were put together in one big pen and given hay, lamb nuts and drinking water. The four sheep used in this study were then chosen from the twelve on 20 August 1991, put in the metabolism crates and accustomed to fresh forages in the same way as the oesophageal fistulated sheep. During the three days of feeding the experimental diets the sheep were fed 375 g of fresh forages at 9.00 a.m. and 400 g of hay at 4.00 p.m. (800 g of hay at 4.00 p.m. on the third day). From 20 to 26 August, the sheep were tested three times with the experimental feeds to see how well their chews could be recorded by the jaw movement recording equipment.

In order to record the eating activities, each intact sheep was offered 160 g of unchopped (long meal) and then chopped (chopped meal) meal from each forage for 1·5 minutes per meal. Each intact sheep was given 8 meals (i.e. 4 long and 4 chopped) per harvest day (Table 3.2). Two people were involved in feeding the sheep and one person held a stop watch and also monitored the jaw recording equipment. The sheep were fitted with plastic tubes on their jaws to transmit the jaw movement in terms of air pressure to the recording equipment. The tubes were fitted before the first meal and were removed at the end of the last meal. After every meal the uneaten forage was weighed and subtracted from 160 g to work out how much the sheep ate, and then oven-dried at 80°C for 48 hours to find out how much the sheep ate in terms of dry matter. After every two meals (i.e. one long and one chopped meal) of each forage, the length of paper used to record the jaw movements was removed and stored in the file. The numbers of chews per minute for each meal were later worked out as follows:

$$\text{Chewing rate (chews/min)} = \frac{J x P}{L}$$

where J = Number of jaw movements recorded on the paper per meal.

L = The length (cm) of paper with jaw movements recorded per meal.

P = Paper speed in cm/min. In this case it was 10 cm/min.

On the last day of the harvest after feeding the experimental diets, the sheep were taken out of the metabolism crates and kept in pens. Seven days before the next harvest, the sheep were put back in the metabolism crates for adaptation to fresh forages and chewing activity recordings.

On the second day of the first harvest when the four sheep were first used for chewing activity recording, two of them were unfriendly. One of the two was jumpy and the other chewed the plastic tubes transmitting the pressure of the jaw movement to the recording equipment. Therefore the recording did not go well on the first occasion. In the evening of the same day, a solution was put forward to rectify the situation. The solution was to change the two unfriendly sheep for oesophageal fistulated sheep (the same sheep described above). The reason for taking the oesophageal fistulated sheep was that they were friendly and readily ate the offered forage. Therefore it was expected that putting the oesophageal fistulated sheep in between the two intact friendly sheep would improve consumption. In this case the oesophageal fistulated sheep were used without removing the bungs from their fistulae, and thus considered as 'intact sheep'. Starting from the second jaw recording day of the first harvest the solution was put into effect and there were no problems with eating or recording the chews.

Laboratory activities

1. Plant part morphology and proportions

The herbage samples taken before chopping and kept frozen were defrosted. Then different green plant parts were obtained and their morphological dimensions measured. Only fully expanded leaflets or leaf blades were used for measurements. Six leaflets and six leaf petioles per sample of unchopped white clover; one leaf blade, one leaf petiole and one internode from the middle of the stem from each of three plants per rape sample; six leaves and six stem internodes per sample of spurrey; and one leaf blade and one leaf sheath from each of six tillers per sample of perennial ryegrass were measured. The diameters of stem internodes and leaf petioles were measured by a digital vernier caliper. The length and width of leaflets and leaf blades were measured by a transparent plastic ruler. The width of the leaflet was taken at its broadest point and that of leaf blade and leaf sheath at their mid-length. The areas of grass leaf blades were calculated using the formula of Kemp (1960) (leaf area = length x width x 0·905). The areas of the legume leaflets and rape leaf blades were determined by counting the squares they covered on the graph paper.

The leaflets and leaf blades, after their morphological dimensions had been measured, were oven-dried. The oven-dry weight per leaflet or leaf blade was used to calculate oven-dry weight per unit area of leaflet or leaf blade.

In order to determine the proportions of plant parts, about 100 g of each chopped sample was separated into green leaf, dead leaf, green stem, green leaf sheath, green leaf petiole and inflorescence, and oven-dried at 80°C for 48 hours. Inflorescence included the flower stalk. All dead parts of the leaf were cut with a pair of scissors and included in the dead proportions.

In vitro dry matter digestibility of the forages was determined according to the method of Tilley & Terry (1963), while neutral detergent fibre (as an estimate of cell wall content) was determined according to the method of Van Soest & Wine (1967).

2. Plant part vascular structure observations and measurements

Plant parts for vascular structure observations and measurements were obtained from the same unchopped defrosted forage sample used for plant morphology measurements. Only 7 cm of the middle section of the leaf blade and leaf sheath of perennial ryegrass, leaf petiole of white clover and rape and stem internode of spurrey were used for this investigation. The vascular structure of the whole leaflet and leaf blade of white clover and rape was studied. Two specimens of each plant part from each forage sample were used in this experiment. In the case of rape, two thin transverse sections of stem internodes were used because its vascular bundles could not be counted easily by splitting the internode longitudinally as in the case of spurrey, due to much pithy material.

The specimens were put in petri dishes (13·8 cm diameter and 1·7 cm deep) and cleaned in 2·5% NaOH (sodium hydroxide) at 40°C for 40 to 60 hours. Then, the NaOH was poured away and within their petri dishes the specimens were gently washed three times with tap water. Within their petri dishes and submerged in water about 2–3 mm deep, the specimens were observed under a binocular light microscope at 14x magnification. The veins of grass leaf blades and leaf sheaths were counted from one leaf blade or sheath margin to another. The fainter veins of the leaf blade were called 'small veins' and the fewer conspicuous veins were called 'large veins'. Leaf sheaths had veins which were all of approximately the same size. For the white clover leaflets and rape leaf blades, secondary veins were counted on both sides of the primary vein (i.e. the midrib) from the junction of the leaflet or leaf blade with its petiole to the leaf apex (Fig. 3.3). Tertiary veins were also counted. Spurrey leaf blades had no veins, but just a rudimentary midrib. White clover and rape petiole and spurrey stem internode specimens were held at one end by a needle and a scalpel was used to make one longitudinal incision. The specimen was then stretched to form a mat-like structure. The vascular bundles were then counted under the microscope. For the thin rape stem internode transverse sections the vascular bundles were reddish-brown and easily counted.

After the microscopic observations, the water in the petri dishes was poured away and 5-10 drops of 95% ethyl alcohol were added to the specimen, which was left to dry and harden overnight. Stem and leaf petiole specimens, however, took about 2-3 days to dry and become hardened. The reason for adding ethyl alcohol was to dry and harden the cleared soft specimens for easier handling and measuring.

After the specimens had dried, the diameters of leaf blade and leaf sheath veins, leaf blade midrib or primary vein, leaf petiole and stem internode vascular bundles were measured to the

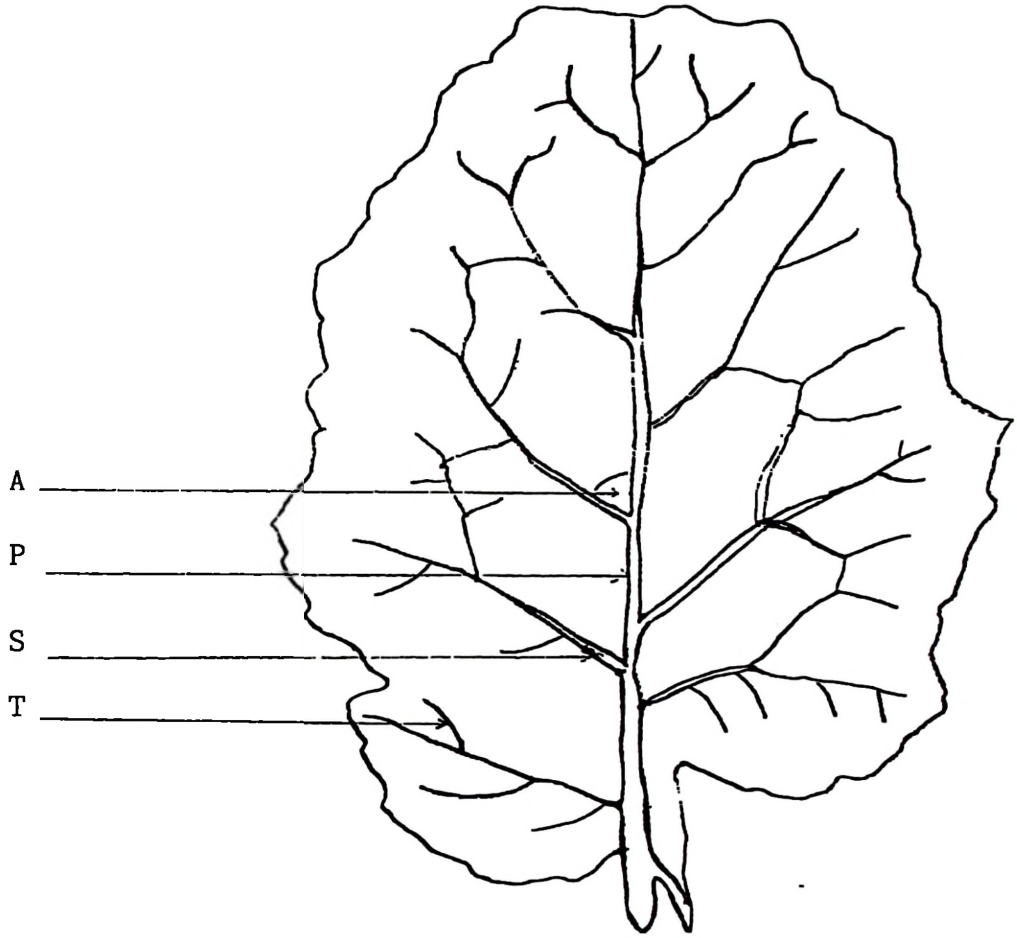

Fig.3.3. A tracing of a rape leaf blade

A = Angle of attachment of secondary vein to the primary vein.

P = Primary vein.

S = Secondary vein.

T = Tertiary vein.

nearest 0·01 mm under a light microscope fitted with a micrometer eyepiece with magnification 10x. The angle of attachment of secondary veins to the primary vein (Fig. 3.3) of white clover leaflets and rape leaf blades was relatively difficult to measure. The leaflet or leaf blade was left stuck on the bottom surface of the petri dish. Then the petri dish was turned upside down. With a 15 cm transparent ruler a line was drawn on the outer surface of the petri dish (using a ball pen) corresponding to the primary vein of the leaflet or leaf blade attached on the inner surface of the petri dish. Then two lines were drawn corresponding to the secondary veins originating from each side of the primary vein roughly at the mid-point of the leaflet or leaf blade length. These lines formed two angles with the line of the primary vein. The two angles were measured by a transparent protractor and their average recorded.

The distances between adjacent secondary veins of white clover leaflets and rape leaf blades and between adjacent leaf blade and leaf sheath veins of perennial ryegrass were calculated as follows:

Distance between adjacent secondary veins (mm)

$$= \frac{\text{Total number of secondary veins per leaflet or leaf blade}}{\text{2x length of leaflet or leaf blade}}$$

Distance between adjacent leaf blade and leaf sheath veins (regardless of size) (μm)

$$= \frac{\text{Leaf blade or leaf sheath width at mid-point} - \text{Leaf blade or leaf sheath width occupied by diameters of veins (and midrib in case of leaf blade)}}{\text{Total number of veins/leaf blade or sheath}}$$

3. Particle breakdown characteristics

In order to analyse particle breakdown characteristics, the frozen chewed particle samples were thawed completely without disturbance so as to avoid particle breakdown due to shattering. After thawing, each chewed particle sample was poured onto a polythene sheet spread on a table and then gently mixed. The chewed particle sample was then quartered repeatedly until two lots of about 1 g wet weight were sampled. The two lots were each placed in a petri dish with a diameter of 13·8 cm and a depth of 1·7 cm. The remainder of each chewed sample was gently replaced in its polythene bag and stored in a freezer. Sufficient tap water was added to the petri dishes containing chewed particles to disperse the particles evenly. The chewed particles from one lot were counted and then placed in a heat-resistant glass petri dish of known weight, dried at 100°C for 24 hours and weighed. The total number of particles per 100 mg of chewed particles was then calculated. The particles counted were $\geqslant$ 0·4 mm in width.

The second lot of chewed particles was taken near the light microscope, thoroughly mixed (but gently) and about 100 particles picked at random and placed in 10 petri dishes (each with 10 particles) containing sufficient water to disperse the particles. The size of the 10 petri dishes was 8·8 cm diameter and 1·2 cm deep. In order to avoid observing the same particle twice, each particle was picked from its respective petri dish and placed in a petri dish cover (with about 2-3 mm of water) and observed individually under a dissecting microscope fitted with a micrometer eyepiece with magnification 10x. Total magnification under which every particle was observed was 14x. After it had been observed, the particle was discarded.

The principal particle breakdown characteristics observed under the microscope were:

(i) The identity of the particle (i.e. if for example it was a leaf or stem particle). The identity of the individual particle was easily determined for large particles ($\geqslant$ 2 mm in width). For small particles ($\leqslant$ 1·5 mm in width) sometimes original plant parts had to be used for comparison with the particle before it could be identified.

(ii) Mean length and width of the particles ($\geqslant$ 0·4 mm in width) were measured under the microscope fitted with the micrometer eye piece as mentioned above. The lengths and widths of the chewed particles were recorded, as the shape and size of the particles may reflect the vascular structure of the forage tissue (Moseley 1982) and because the escape of particles from the reticulo-rumen may be affected by their shape (Troelsen & Campbell 1968) as well as by their size (Poppi *et al.* 1980).

(iii) Total number of veins or vascular bundles per particle were counted. Some particles, like petiole particles of white clover and stem particles of spurrey, were only reduced in length and otherwise remained intact after chewing. The total number of vascular bundles per intact leaf petiole or stem particle was taken to be the same as the average number of vascular bundles of such a plant part obtained during the study of plant part vascular structure.

(iv) The types of damage to the surface and edges of the particle were observed and recorded, as the role of mastication is not just to reduce particle size but also to disrupt barrier tissues such as the cuticles and vascular elements to expose more potentially digestible surfaces to the reticulo-rumen microbes (Ulyatt *et al.* 1986). Under the light microscope the particle surface was recorded as smooth or rough or rippled, perforated or ruptured. A particle surface might have a complete rupture (that allows light to show through clearly) or perforation and still be recorded as having a smooth surface, if its internal structures were not exposed. Rippled surfaces were recorded normally when a particle (especially long particles) surface showed several crushed points or teeth marks made during chewing. Rippled surfaces also indicated the points where the particles could have broken into more particles but, perhaps due to toughness or to the short time of chewing before swallowing, the breakdown was not completed. In order to be recorded, the perforation or rupture should be complete (i.e. could allow light to show through clearly from below the microscope stage). Particle surface damage characteristics were therefore recorded as follows:

(a) Percentage of total observed particles with a rough surface.

(b) Percentage of total observed particles with a rippled surface.

(c) Number of perforations or ruptures per particle.

(d) Percentage of total observed leaflet, leaf petiole, or stem particles which remained intact after chewing.

(e) Percentage of total observed particles with jagged ends or jagged sides.

Some of the leaf particles, especially those of white clover leaflets and rape leaf blades, were not rectangular but rather oblong or even sperical in shape. In the case of oval or spherical particles the ends of the particles were taken as those two ends of the particle which were furthest apart if joined by a straight line.

Materials and Methods, 1992

After the last harvesting date of 1991, on 26 September, all the remaining forages and some regrowths in every plot were cut and removed from the experimental site. In the perennial ryegrass plots there were a few bare patches (especially in the plots harvested last). These bare patches were oversown in mid-November 1991.

The perennial crops (i.e. perennial ryegrass and white clover) survived the 1991/92 winter well. Since the plan was to plant the annual crops (i.e. spurrey and rape) on 29 June, there was a need to manage the perennial crops so that they would not become over mature before the harvesting and feeding programme for 1992 (Table 3.3) began. Therefore on 6 April, 5 May, 2 June and 29 June 1992 the perennial ryegrass and white clover plots were cut, leaving a stubble height of 4-5 cm, raked clear, using strings each plot was divided into six sub areas (as during sowing in 1991) and fertilizers were applied at the following rates:

(i) 'Nitram' (ammonium nitrate, 34·5% N): 54·2 g/sub area, applied only to perennial ryegrass; equivalent to 35 kg N/ha.

(ii) Triple superphosphate (45% P_2O_5): 35·5 g/sub area, applied to all white clover and perennial ryegrass plots; equivalent to 30·0 kg P_2O_5/ha.

(iii) Sulphate of potash (48% K_2O): 55·6 g/sub area, applied to all white clover and perennial ryegrass plots; equivalent to 50 K_2O/ha, for all plots.

On 14-15 April 1992 glyphosate ('Roundup', Monsanto plc) was applied to all the rape and spurrey plots to kill weeds. Because of dry conditions in June 1992, c. 10 mm of water was applied to the rape and spurrey plots on 29 June, using a slurry spreader which had been thoroughly washed. The soil was sampled for pH, which was 5·8. On 30 June the rape and spurrey plots were raked by hand and seed and fertilizer applied at the same rates of application and following the same procedures as in 1991, except that triple superphosphate (45% P_2O_5): 71 g/sub area, equivalent to 60 kg P_2O_5/ha was applied to all rape and spurrey plots.

By July spurrey had germinated well, but the rape plots had very poor germination. By 18 July spurrey plots were establishing well and those of rape had very few plants and these were being eaten by slugs from the adjacent clover and ryegrass plots. Bare patches on the rape plots were reseeded on 18 July at a rate of 24 g/plot, equivalent to 7·5 kg/ha. Then slug pellets (3% w/w metaldehyde) were spread around all the rape plots.

By 27 July spurrey and rape were both established well. Perennial ryegrass and white clover were cut and fertilizers applied at the same rate as spurrey and rape plots in 1992. The harvesting and feeding programme started on 24 August as shown in Table 3.3.

Feeding took place at IGER - Trawsgoed, 15 kg south-east of Aberystwyth, instead of Gogerddan. During the 1991/92 winter, one of the two oesophageal fistulated sheep had died. Therefore, the 1992 feeding programme changed slightly from 1991. The diets were fed to the remaining one sheep for two days (Table 3.3).
In order to give company to the experimental sheep, a second sheep was kept in an adjacent metabolism crate and given a meal of fresh herbage each time the experimental sheep was given a meal.

The differences between the species were consistent from year to year for most of the variables studied. The data for the individual years has been lodged in the Department of Agricultural Sciences, University College of Wales, Aberystwyth.

New intact sheep were used in 1992 for recording chewing activity. Six sheep, one year old, weighing, on average, 42 kg, were trained in the chewing activity recording procedures for two weeks before the first harvest. Then four out of the six sheep were selected for the experiment and fed as shown in Table 3.3.

The procedures followed in the laboratory work were the same as in 1991.

Statistical Analyses

After analyses of the data for each year (1991 and 1992) separately, it was found that harvesting date had a negligible effect on the variables studied. The results of the two years were therefore combined and statistically analysed using the following analysis of variance:

Source of variation	df
Years	1
Species	3
Harvesting date	2
Species x harvesting date	6
Error	11
Total	23

Where one species was very different from others, e.g. in some morphological variables, the results for that species were analysed separately using the following analysis of variance:

Source of variation	df
Years	1
Harvesting date	2
Error	2
Total	5

Table 3.3. Harvesting and feeding programme 1992

Harvest number	Date of harvest	Block (s) harvested	Number of species harvested/ block	Number of sheep fed	Number of meals/ sheep	Oesophageal collection	Jaw recording
1	24 Aug	I,II&III	4	1	12	Yes	No
	25 Aug	I,II&III	4	1	12	Yes	No
	26 Aug	I	4	4	8+	No	Yes
	27 Aug	II	4	4	8+	No	Yes
	28 Aug	III	4	4	8+	No	Yes
2	7 Sept	I,II&III	4	1	12	Yes	No
	8 Sept	I,II&III	4	1	12	Yes	No
	9 Sept	I	4	4	8+	No	Yes
	10 Sept	II	4	4	8+	No	Yes
	11 Sept	III	4	4	8+	No	Yes
3	21 Sept	I,II&III	4	1	12	Yes	No
	22 Sept	I,II&III	4	1	12	Yes	No
	23 Sept	I	4	4	8+	No	Yes
	24 Sept	II	4	4	8+	No	Yes
	25 Sept	III	4	4	8+	No	Yes

+ 4 long and 4 chopped meals

Results

Plant part morphology and vascular structure

White clover leaflets had more secondary veins than rape leaf blades (Table 3.4). White clover and rape were not significantly different in terms of the number of tertiary veins per leaflet or leaf blade. However, white clover leaflets had many more secondary + tertiary veins per unit leaflet area than rape leaf blade. The angle of attachment of secondary veins to the primary vein was wider in rape leaf blades than in white clover leaflets. The diameter of the primary vein of rape leaf blades was $>$ 10x greater than that of white clover leaflets. Secondary veins in rape leaf blades were c. 9x more widely spaced than those in white clover leaflets.

White clover leaf petioles were much narrower and had fewer vascular bundles than those of rape (Table 3.5). On the other hand the vascular bundles of rape leaf petioles were rather thinner and occupied much less leaf petiole cross sectional area than those of white clover.

In perennial ryegrass, leaf blades and leaf sheaths had similar numbers of veins (Table 3.6). However, only one third of the leaf blade veins were as thick as those of the leaf sheath. The distance between adjacent veins regardless of size was almost twice as great in leaf sheaths as in leaf blades. The percentage of the width at the mid-point occupied by the sum of vein diameters was lower in leaf sheath than in leaf blade (in the latter case the sum of vein diameters and midrib diameter).

Table 3.4. White clover leaflet and rape leaf blade vascular structure. Means (± SE's) of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape
Number of secondary veins per leaflet or leaf blade	51·4	32·7 ± 0·93*
Number of tertiary veins per leaflet or leaf blade	165	192 ± 9·5 *
Angle of attachment of secondary veins to the primary vein	47·3	67·0 ± 0·74*
Number of secondary + tertiary veins per cm^2 of leaflet or leaf blade	32·5 ± 3·71	1·00 ± 0·074
Diameter (mm) of primary vein	0·205 ± 0·0024	2·40 ± 0·091
Distance (mm) between adjacent secondary veins	1·35 ± 0·064	12·58 ± 0·307

* S.E. for both species

Table 3.5. Leaf petiole morphology and vascular structure of white clover and rape. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	SE±
Diameter (mm) of leaf petiole	2·25	6·75	0·059
Number of vascular bundles	5·9	23·7	0·63
Diameter (μm) per vascular bundle	224	194	5·4
Percentage of cross sectional area of leaf petiole occupied by vascular bundles	6·09	2·08	0·324

Spurrey stem internodes were much thinner and had far fewer and thinner vascular bundles than those of rape (Table 3.7). The vascular bundles in spurrey stem internodes, occupied a greater percentage of the stem cross sectional area than those of rape.

The ranking of species in terms of leaflet or leaf blade size was: width: perennial ryegrass < white clover < rape; length: white clover < rape < perennial ryegrass; area per leaflet or leaf blade: white clover < perennial ryegrass < rape (Table 3.8). In perennial ryegrass, leaf blades were narrower than leaf sheaths. Spurrey leaf blades were about 1 mm in width and 25 mm in length.

Spurrey had the highest dry matter percentage followed by perennial ryegrass and then white clover and rape (Table 3.9). The ranking of species with respect to dry weight per leaf blade or leaflet was rape > perennial ryegrass > white clover. Perennial ryegrass leaf blade and white clover leaflet did not differ significantly in terms of dry weight per unit area, but were significantly lighter than rape leaf blade.

Table 3.6. Perennial ryegrass leaf blade and leaf sheath vascular structure. Means (± SE's) of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

		Leaf blade	Leaf sheath
Number of veins (excluding the mid rib)	Large	5·73 ± 0·101	15·05 ± 0·303
	Small	10·5 ± 0·47	
Diameter (μm) of veins	Large	113·0 ± 1·96	106 ± 5·0
	Small	71·2 ± 3·18	
Diameter (μm) of mid rib		152 ± 8·0	–
Distance (μm) between adjacent veins regardless of size		162 ± 2·6	315 ± 7·9
Percentage of leaf blade or sheath width (at mid point) occupied by the sum of vein diameters and mid rib diameter		37·4 ± 0·72	25·7 ± 0·60

Table 3.7. Stem internode morphology and vascular structure of rape and spurrey. Means (± SE's) of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	Rape	Spurrey
Diameter (mm) per stem internode	16·9 ± 0·98	1·52 ± 0·048
Numbers of vascular bundles per stem internode	155 ± 5·5	12·11 ± 0·246
Diameter (μm) per vascular bundle	296	111 ± 3·4*
Percentage of cross sectional area of stem internode occupied by vascular bundles	2·6	7·0 ± 0·59*

* SE for both species

Table 3.8. Leaflet, leaf blade and leaf sheath morphology of white clover, rape and perennial ryegrass. Means (± SE's) of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Perennial ryegrass
Leaflet and leaf blade width (mm)	28·2* ± 1·98	146* ± 2·8	4·12+ ± 0·112
Leaflet and leaf blade length (mm)	34·5 ± 1·08	203 ± 4·2	330 ± 6·0
Area (cm^2) per leaflet and leaf blade	6·9 ± 0·73	236 ± 17·9	12·74 ± 0·019
Leaf sheath width (mm)	-	-	6·29 ± 0·111

* At the broadest point

+ At the mid-point

Table 3.9. Dry matter percentage, dry weight per leaflet or leaf blade and weight per unit area of leaflet or leaf blade. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±
Dry matter percentage	11·5	10·6	18·4	15·4	0·92
Dry weight (mg) per leaflet or leaf blade	20·6	1398*	–	40·7	4·60†
Dry weight per unit area of leaflet or leaf blade(mg/cm^2)	3·0	6·1	–	3·3	0·57

* SE ± 55·8 (DF = 2)

† Excluding rape

Proportions of plant parts and intake rate by oesophageal fistulated sheep of fresh chopped forage

Spurrey had the lowest proportion of green leaf (Table 3.10). White clover and rape were not significantly different in terms of the proportion of green leaflet or leaf blade but were significantly lower than that of perennial ryegrass. White clover had a much higher proportion of green leaf petiole than did rape. Perennial ryegrass had more than 60% green leaf blade and more than 10% dead leaf blade. The proportion of green stem in spurrey was almost 50% and was twice as great as in rape. A small proportion of spurrey was dead leaf and c. 40% was inflorescence.

The neutral detergent fibre (NDF) contents of rape and white clover were much lower than those of perennial ryegrass and spurrey (Table 3.11). Spurrey had the lowest *in vitro* dry matter digestibility. The *in vitro* dry matter digestibilities of perennial ryegrass, white clover and rape were not significantly different. The intake rates of fresh white clover, rape and spurrey did not differ significantly, but were significantly higher than that of perennial ryegrass. In terms of dry matter intake rate, however, spurrey had the highest and white clover, rape and perennial ryegrass did not differ significantly. The neutral detergent fibre intake rate was highest in spurrey, lowest in rape and intermediate in white clover and perennial ryegrass. The digestible dry matter intake rate of spurrey was higher than that of perennial ryegrass, but not significantly different to those of white clover and rape.

Table 3.10. Proportion of plant parts (% in dry matter) in the fresh chopped diet. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±
Green leaflet or leaf blade	52·1	50·0	11·42	63·4	±3·38
Green leaf petiole	47·9	26·3	–	–	±1·26
Green leaf sheath	–	–	–	25·1	±0·69
Green stem	–	24·3	48·65	–	±6·14
Dead leaf blade			1·28 ± 0·353	11·65 ± 0·220	
Inflorescence			38·7 ± 2·42		

Table 3.11. Neutral detergent fibre content and *in vitro* dry matter digestibility of the chopped diets and the intake rate of the oesophageal fistulated sheep. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
Neutral detergent fibre content (in % DM)	27·4	24·6	56·8	52·0	2·76	40·7
In vitro dry matter digestibility (%)	80·5	84·3	57·2	78·1	2·52	75·0
Fresh forage intake rate (g/min)	72·0	77·0	68·5	50·6	3·92	67·0
Dry matter intake rate (g/min)	8·5	8·1	12·5	7·7	0·51	9·2
Neutral detergent fibre intake rate (g DM/min)	4·39	1·93	7·10	3·98	0·283	4·35
Digestible dry matter intake rate(g/min)	6·80	6·67	7·15	6·00	0·333	6·66

Eating activity of long and chopped fresh forage by intact sheep

White clover had the highest intake rate of long fresh forage, followed by spurrey and rape and then perennial ryegrass (Table 3.12). On the other hand, the intake rates of fresh chopped white clover and rape did not differ significantly and were higher than that of perennial ryegrass. The intake rate of fresh chopped spurrey was intermediate between those of rape and perennial ryegrass. The consumption rate of fresh chopped rape was higher than that of fresh long rape. The difference was, however, not statistically significant when expressed in terms of dry matter intake rate.

Whether fed in long or chopped form, spurrey had the highest dry matter intake rate. White clover had a significantly higher long dry matter intake rate than rape. Perennial ryegrass, rape and white clover were not significantly different in terms of chopped dry matter intake rate.

The consumption rates of neutral detergent fibre of long or chopped spurrey were much higher than those of the other three species. Long or chopped white clover and rape did not differ significantly in terms of neutral detergent fibre intake rate, but that of rape was lower than that of perennial ryegrass.

Digestible dry matter intake rate of long forage was lowest in rape. White clover, spurrey and perennial ryegrass did not differ significantly in terms of long forage digestible dry matter intake rate. When fed in the chopped form, the species did not differ significantly in terms of digestible dry matter intake rate.

Fresh long spurrey was chewed faster than rape and perennial ryegrass, and the chewing rate of white clover was intermediate between those of spurrey and perennial ryegrass (Table 3.13). The chewing rates of fresh chopped white clover, rape and perennial ryegrass did not differ significantly, but that of the latter species was significantly lower than that of spurrey.

The number of chews per gram of fresh matter ingested of either long or chopped perennial ryegrass was higher than those of white clover and rape but not very different to that of spurrey. Chopped white clover, rape and spurrey did not differ significantly in terms of the number of chews per gram of fresh matter ingested. Long spurrey was, however, chewed more per gram fresh matter ingested than white clover.

White clover, rape and perennial ryegrass did not differ significantly in the number of chews per gram of dry matter ingested, either long or chopped. Rape, however, was chewed more, per gram of dry matter ingested, than spurrey.

Table 3.12. Intake rate of the intact sheep. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
Long fresh forage intake rate(g/min)	64·1	49·6	50·6	40·9	2·48	51·3
Chopped fresh forage intake rate(g/min)	66·4	63·0	55·0	47·1	2·92	57·9
Long forage dry matter intake rate (g/min)	7·51	5·36	9·37	6·22	0·495	7·12
Chopped forage dry matter intake rate (g/min)	7·69	6·70	10·27	7·55	0·529	8·05
Long forage neutral detergent fibre intake rate (g DM/min)	1·90	1·32	5·32	3·23	0·455	2·94
Chopped forage neutral detergent fibre intake rate(g DM/min)	1·98	1·76	6·01	3·93	0·440	3·42
Long forage digestible dry matter intake rate (g/min)	6·05	3·07	5·20	4·86	0·480	4·80
Chopped forage digestible dry matter intake rate (g/min)	6·20	5·62	5·71	5·90	0·421	5·86

Table 3.13. Chewing activity by intact sheep when eating fresh forage. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
Chewing rate(chews/min) of long fresh forage	139·9	129·2	144·0	135·3	2·60	137·1
Chewing rate(chews/min) of chopped fresh forage	132·9	133·9	138·1	129·5	2·02	133·6
Number of chews per gram of long fresh forage ingested	2·32	2·78	3·09	3·61	0·174	2·95
Number of chews per gram of chopped fresh forage ingested	2·15	2·36	2·58	3·11	0·156	2·55
Number of chews per gram of long forage dry matter ingested	20·4	26·3	17·5	23·1	2·23	21·8
Number of chews per gram of chopped forage dry matter ingested	19·5	24·1	15·1	19·0	1·77	19·4

Characteristics of the chewed particles

Perennial ryegrass had more chewed particles per 100 mg dry matter than the other species (Table 3.14). White clover, rape and spurrey did not differ significantly in terms of the number of chewed particles. The ranking of species in terms of the number of veins per leaf blade or leaflet particle was perennial ryegrass > white clover > rape. Leaf sheath particles had more vascular bundles than leaf petiole particles. Rape had fewer vascular bundles per leaf petiole particle than white clover. Spurrey had more vascular bundles per stem particle than rape.

The ranking of species in terms of leaflet or leaf blade particle width was rape and white clover > perennial ryegrass > spurrey (Table 3.15). The widths of the white clover leaf petiole particle and perennial ryegrass leaf sheath particle were not significantly different and were less than that of rape leaf petiole particles. Spurrey had narrower stem particles than rape. The ranking of species in terms of overall particle width (regardless of plant part) was rape > white clover > perennial ryegrass > spurrey.

Perennial ryegrass had the longest leaf blade particle. The other three species were similar to each other in lengths of the leaflet or leaf blade particle. The ranking of species in terms of the length of leaf petiole or leaf sheath particle was white clover > perennial ryegrass > rape. Spurrey had longer stem particles than rape. The overall particle lengths of perennial ryegrass, white clover and spurrey were not significantly different and were greater than that of rape.

Table 3.14. Number of particles (≥ 0·4 mm in width) per 100 mg DM in chewed plant material from the oesophageal fistulas and the number of veins or vascular bundles per particle. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
Number of particles per 100 mg DM	91	82	68	144	9·3	96
Mean number of veins per leaflet or leaf blade particle	6·25	1·72	–	8·27	0·292	5·41
Mean number of vascular bundles per leaf petiole or leaf sheath particle	4·30	2·85	–	6·36	0·314	4·50
Mean number of vascular bundles per stem particle	–	3·54	9·88	–	0·301	6·71

Table 3.15. Widths (mm) and lengths (mm) of particles derived from different plant parts. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
Leaflet or leaf blade						
Width	5·00	5·44	0·78	2·04	0·287	3·32
Length	9·5	9·2	8·8	15·9	0·48	10·9
Leaf petiole or leaf sheath						
Width	1·96	3·46	–	1·92	0·167	2·45
Length	23·0	16·4	–	20·2	0·47	19·9
Stem						
Width	–	3·84	1·15	–	0·158	2·50
Length	–	14·3	22·0	–	1·36	18·2
Overall						
Width	3·50	4·59	1·00	1·99	0·195	2·77
Length	16·2	13·0	15·4	17·5	0·77	15·5

More than 70% of leaflet or leaf blade particles of white clover, rape and perennial ryegrass had jagged ends (Table 3.16). Leaf sheath and leaf petiole particles were not significantly different in terms of the percentage of particles with jagged ends. Spurrey had a lower percentage of stem particles with jagged ends than rape.

The ranking of species in terms of the percentage of the leaflet or leaf blade particles with jagged sides was rape > white clover > perennial ryegrass (Table 3.16). More than 90% of rape leaf petiole particles had jagged sides. The percentages of perennial ryegrass leaf sheath particles and white clover leaf petiole particles with jagged sides were not significantly different and were c. 30% lower than that of rape leaf petiole particles. The percentage of stem particles with jagged sides was c. 40% lower in spurrey than in rape.

Perennial ryegrass and rape were not significantly different in terms of the percentage of leaf particles with a rough surface (Table 3.17). White clover had a lower percentage of leaflet particles with a rough surface. The ranking of species in terms of the percentage of leaf petiole and sheath particles with a rough surface was rape > white clover > perennial ryegrass. Almost all rape stem particles had a rough surface. However, only c. 50% of spurrey stem particles had a rough surface.

Table 3.16. Percentage of particles with jagged ends or jagged sides in (a) particles derived from leaflets or leaf blades, (b) those derived from leaf petioles or leaf sheaths, and (c) those derived from stems. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
(a) Leaflet or leaf blade						
Ends	71·7	76·8	–	79·6	1·81	76·0
Sides	71·1	80·9	–	43·4	2·15	65·1
(b) Leaf petiole or leaf sheath						
Ends	78·4	83·9	–	81·1	2·17	81·1
Sides	62·0	94·4	–	53·9	2·73	70·1
(c) Stem						
Ends	–	90·8	67·8	–	1·55	79·3
Sides	–	98·0	57·0	–	1·64	77·5

Table 3.17. Percentage of particles with a rough or rippled surface in (a) particles derived from leaflets or leaf blades, (b) those derived from leaf particles or leaf sheaths, and (c) those derived from stems. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
(a) Leaflet or leaf blade						
Rough surface	22·3	34·6	–	31·5	1·46	29·5
Rippled surface	6·8	6·9	–	7·3	1·12	7·0
(b) Leaf petiole or leaf sheath						
Rough surface	50·1	93·4	–	36·8	2·63	60·1
Rippled surface	11·4	–	–	10·9	0·92	11·2
(c) Stem						
Rough surface	–	99·0	51·2	–	2·14	75·1
Rippled surface	–	–	10·5	–	1·72	10·5

White clover, rape and perennial ryegrass had a similar percentage (c. 7%) of leaflet or leaf blade particles with a rippled surface. Perennial ryegrass and white clover did not differ significantly in terms of the percentages of leaf sheath or leaf petiole particle with a rippled surface. About 10% of spurrey stem particles had a rippled surface, while none of the rape stem particles had a rippled surface.

The leaflet particles of white clover had more perforations or ruptures per particle than the leaf blade particles of perennial ryegrass (Table 3.18). The number of perforations or ruptures per rape leaf blade particle was not significantly different to those of white clover and perennial ryegrass. The species did not differ significantly in the number of perforations or ruptures per particle of leaf petiole or leaf sheath.

The percentages of white clover leaf petiole and spurrey stem particles which remained intact (i.e. not split longitudinally after chewing) were 49 ± 2·5 and 65 ± 2·6, respectively.

Table 3.18. Mean number of perforations or ruptures per particle. Means of two years (1991 and 1992) and of three dates of harvest (27 August and 9 and 23 September)

	White clover	Rape	Spurrey	Perennial ryegrass	SE±	Mean
Leaflet or leaf blade	2·01	1·29	–	1·21	0·230	1·50
Leaf petiole or leaf sheath	0·68	0·92	–	0·83	0·308	0·81
Stem	–	1·06	0·52	–	0·181	0·79

Discussion

The four species used in the present experiment showed some notable differences both in their plant part morphology and vascular structure. The small white clover leaflets had more veins per unit area than the large rape leaf blades. The thinner leaf petioles of white clover had fewer vascular bundles that occupied a higher percentage of its cross sectional area than the thicker leaf petioles of rape. The thicker stem internodes of rape had many more vascular bundles than the thinner stem internodes of spurrey. However, a greater cross sectional area of the stem internodes of spurrey was occupied by vascular bundles than in rape. In perennial ryegrass the parallel veins in the leaf blade were c. 2x closer than those in the leaf sheath, and c. 8x closer than secondary veins of white clover leaflets.

Rape had low neutral detergent fibre and high dry matter digestibility, possibly due to its low number of veins per unit area of leaf blade and low proportion of vascular tissue in the cross sectional area of leaf petioles and stem internodes. Rape is noted for its relatively high digestibility (Spurway *et al*. 1974, Kunelius & Sanderson 1989) and low neutral detergent fibre (Kunelius & Sanderson 1989). With its high dry matter yield, therefore, rape gives a good catch crop for grazing animals in autumn when the nutritive value of grasses has gone down, but when some animals require extra energy to finish or prepare for winter.

Spurrey with a high proportion of stem (c. 50%) and inflorescence (c. 40%) had a similar proportion of neutral detergent fibre to perennial ryegrass but lower *in vitro* dry matter digestibility. Low digestibility of spurrey, both *in vitro* and *in vivo*, and high neutral detergent fibre have been reported by Derrick *et al.* (1993). Low *in vivo* dry matter digestibility of spurrey could be partly due to a high rate of passage of digesta (Derrick *et al.* 1993), but *in vitro* dry matter digestibility is also low, suggesting that the high proportions of stem, flower stalk and neutral detergent fibre are major reasons for low digestibility. As noted elsewhere (Minson 1990), white clover and perennial ryegrass had similar digestibility, but the latter species had higher neutral detergent fibre than the former.

Since intake rate is affected by the proportion of fibre in the diet (Hogan *et al.* 1987), the intake rate of spurrey could be low. The dry matter intake rate of spurrey was, however, the highest, both in oesophageal fistulated and in intact sheep. White clover, rape and perennial ryegrass did not differ significantly in terms of dry matter intake rate. Spurrey was palatable to the sheep, as noted by Fream (1900) and reported by Derrick *et al.* (1993). Spurrey's thin stems with fragile nodes and thin fragile (needle like) leaves, and with its downy sticky herbage, was readily drawn into the mouth and masticated quickly to particle sizes consistent with swallowing and therefore it required few chews/g of dry matter consumed.

The form in which forage is offered to the animal can have a marked effect on intake rate (Kenney *et al*. 1984). In the present experiment, however, chopping the forages did not significantly increase the consumption rate of the species. However, when expressed in terms of g fresh matter intake per minute, long rape was consumed at a slower rate than chopped rape. This could be due to the morphological characteristics of rape. When given long rape, the sheep ate only the leaves and left the thick stems. Also the sheep took some time manipulating the long rape in order to get the right presentation to bite the large leaf blade. In the case of chopped rape,the sheep finished the leaves quickly and then struggled with the stems for as long as time allowed. Rape, therefore, required an unexpectedly high number of chews/g dry matter consumed. It seems, therefore, that the type of forage given will determine whether chopping can or cannot improve diet consumption rate. Chopping can be more important in fodder with thick stems and large leaves (such as rape and maize), stemmy grass, and in conserved forages such as hay and silage, as noted by Minson (1990).

The chewed material of perennial ryegrass had a high number of particles per given weight because the grass was leafy and comprised only leaf blade and leaf sheath. On the other hand, the chewed materials of white clover, rape and spurrey had heavy particles of petiole and/or stem. This could mean that the surface area/100 mg dry matter of chewed materials exposed to the reticulo-rumen microbes was higher in perennial ryegrass than in the other species. The mean number of vascular strands per particle was higher in perennial ryegrass than in white clover and rape. This was in agreement with Kelly & Sinclair (1989) and suggests greater resistance to particle size reduction in perennial ryegrass. In the present experiment, the leaf blades of perennial ryegrass and rape were chopped to about the same length (70 mm); yet chewing during eating reduced the length of leaf blade particles 4x in perennial ryegrass and c. 8x in rape. Also chewing during eating reduced the width of chopped leaf blade or leaflet pieces of perennial ryegrass, rape and white clover by 2x, 27x and 5x, respectively. Leaf sheath of perennial ryegrass and leaf petiole of white clover and rape were chewed to almost the same length, which was longer than the leaf blade and leaflet particles. White clover and rape leaf petiole particles were c. 2x longer than their leaflet or leaf blade particles. The proportion of petiole in the forage, therefore, substantially affects the overall mean length of the chewed particles, particularly, it seems, in species with relatively thin petioles.

Leaf particles of spurrey were as long as leaflet or leaf blade particles of white clover and rape. Stem particles of spurrey were as long as leaf sheath or leaf petiole particles of perennial ryegrass and white clover. Generally spurrey particles were thinnest. Leaf particles of spurrey were $\leq$ 1·0 mm in width, and thus presumably able to pass through the reticulo-rumen without further reduction in width (Poppi *et al.* 1980). Further, the overall particle width of spurrey was just 1·0 mm. This can partly explain why spurrey had a higher voluntary intake and higher faeces output by lambs than perennial ryegrass (Derrick *et al*. 1993). In the present experiment, spurrey was eaten avidly and a high proportion (c. 65%) of its stem internode particles passed through the mouth without being split longitudinally. This was further evidence as to how thin and fragile (at the nodes) spurrey stems were.

The particles of rape were generally shorter and wider than those of the other three species. The thick stems and leaf petioles of rape could not pass through the mouth without being chewed properly to reduce their particle size. Rape stem internode contained a ring of vascular bundles towards the periphery and a large inner area filled with pithy material. The pithy particles had no vascular bundles. This contributed to the lower mean number of vascular bundles per rape stem particle, that could lead to easier chewing during eating and rumination.

The role of mastication is not just in particle size reduction but also in the disruption of barrier tissues such as the cuticle and vascular tissues to expose more potentially digestible surfaces to the reticulo-rumen microbes (Ulyatt *et al*. 1986). More than 70% of the particles of white clover, rape and perennial ryegrass had jagged ends, showing that, although it is rather difficult to break across the vascular tissues (Vincent 1990), chewing exposed more surface area of the particles, that could allow effective invasion by the rumen micro-organisms. Almost all particles of rape leaf petiole and stem had a rough surface, giving them a better chance of being weakened by rumen microbes and perhaps of being easier to break by chewing during rumination. The percentage of chewed particles with a rippled surface ranged from c. 7 to 11%, possibly indicating how some pliable plant particles can get trapped in the bolus and thus receive only a few bruises by teeth before swallowing.

Ruptures or perforations on the particle surface increase the invasion of rumen microbes into the internal structures of the particle (Monson *et al*. 1972; Monson & Burton 1972). In the present experiment there were only a few ruptures or perforations per particle even in the broad particles of white clover leaflet and rape leaf blade. However, most of the ruptures were long (not measured) and ran in all directions (even across the veins) in the case of particles of white clover leaflet and rape leaf blade, but ran rather parallel to the vascular tissue in case of particles of leaf stalk and perennial ryegrass leaf blade, suggesting some resistance to breaking across the particle length in the latter cases.

Conclusion

The results of the present experiment indicated that the quality differences between the species studied could not be fully explained by their chemical composition, digestibility and voluntary intake, without information on other aspects such as plant morphology and vascular structure. Rape suffered two main problems, low dry matter percentage and poor presentation of its edible parts to the animal. The large leaves and thick stem of rape were not easily prehended by the animal. Rape was, however, palatable and the animal strove to consume its food for as long a time as it was allowed. The study of the plant morphology and vascular structure of spurrey has illustrated the potential of slender, fragile plants to enhance intake. While the study of plant part particle size has illustrated how leaf petiole chewed particles can substantally affect the overall mean length of the chewed particles, particularly, it seems, in species with relatively thin petioles such as white clover. Particle size reduction (in terms of width and length) during eating was generally lower in perennial ryegrass than in white clover and rape. However, because perennial ryegrass was leafy and comprised only leaf blade and leaf sheath, it had a large number of particles and possibly a large surface area exposed to the reticulo-rumen microbes per given weight of chewed material.

CHAPTER 4

EXPERIMENT 2

PLANT PHYSICAL STRUCTURE AND PARTICLE BREAKDOWN CHARACTERISTICS DURING EATING BY SHEEP OF FRESH TEMPERATE AND TROPICAL FORAGE SPECIES

Introduction

Chewing during eating and ruminating is the most important way in which plant particles are reduced to a size small enough to pass through the rumen (McLeod & Minson 1988). The ease with which plant particles are reduced in size varies between plant parts, legumes and grasses, tropical and temperate grasses and even between cultivars within a species (McLeod & Smith 1989; Wilson 1991). This variation depends substantially on the resistance of the plant species or plant part to breakdown (Weston 1985; McLeod *et al*. 1990). Chewing during eating reduced grass particles more in length than width and more in tropical than in temperate grasses (Wilson *et al*. 1989). McLeod *et al*. (1990) reported greater number of chews per unit dry matter intake and greater particle size reduction in grasses than in legumes. The damage characteristics of the chewed plant particles differ from species to species and even within species and plant parts (Lees *et al*. 1982; Pond *et al*. 1984; Kelly 1988; Kelly & Sinclair 1989). Pond *et al*. (1984) observed the masticates of grazing cattle and found that the cuticle of leaf blade particles of a tropical grass (*Cynodon dactylon*) was more resistant to being separated from the epidermis by chewing and thus more particles of this species had jagged ends and sides than those of a temperate grass (*Lolium multiflorum*). Kelly & Sinclair (1989) found little difference between forages in the number of fractures per 100 particles. Lees *et al.* (1982), however, observed that leaflets of sainfoin and cicer milk-vetch were less fractured by shaking with glass beads than those of white clover and lucerne.

Wilson (1991) noted that much of the variation between plant species or plant parts in the resistance to particle breakdown and the damage characteristics of the chewed particles might be explained by examination of the anatomical characteristics of the plants or plant parts. Little work has so far been done on this topic (Wilson 1991). Four temperate forage species (perennial ryegrass, white clover, rape and spurrey) were used in the first experiment of the present project (Chapter 3) to determine the effect of plant structure on eating activity and particle breakdown characteristics. These four species each represented one of four different forage types as explained in Chapter 3. However, the variation within forage type (e.g. within legumes or grasses) was not investigated. The purpose of the present experiment was to increase understanding of the effect of plant structure on eating activity and particle breakdown by examining a larger number of species including grasses, legumes, maize, rape and spurrey. White clover (*Trifolium repens* cv. Olwen), rape (*Brassica napus* cv. Sparta), spurrey (*Spergula arvensis* L.) and perennial ryegrass (*Lolium perenne* cv. Melle) were described in Chapter 3. Lucerne (*Medicago sativa* cv. Europe) and sainfoin (*Onobrychis viciifolia* cv. Cotswold Common), shown to have higher intake than ryegrass (Jarrige *et al*. 1974; Thomson 1982), and *Desmodium intortum* (cv. Green leaf), a tropical legume which produces a large bulk of palatable herbage (Göhl 1981), were used in the present experiment so as to have a wider view of legume vascular structure and ease of physical breakdown during eating. *Chloris gayana* (cv. Callide), an important tropical and subtropical grass for short term leys for grazing, hay and silage making, and *Cenchrus ciliaris* (cv. Biloela), a tropical grass which is drought resistant and remains fairly palatable to maturity (Skerman & Riveros 1990), were included in the present experiment to represent tropical grasses. The aim was to explore further the well known difference between tropical and temperate grasses in terms of vascular structure (Akin 1989; Minson 1990; Wilson 1990) and relate this difference to physical breakdown during eating. Italian ryegrass (*Lolium multiflorum* cv. RvP), a good grass for silage making, and tall fescue (*Festuca arundinacea* cv. Dovey), a persistent but rather coarse leaved grass, both of which produce early spring growth (National Institute of Agricultural Botany 1989), were used in the present experiment to increase the range of vascular structure and physical breakdown during eating. Maize (*Zea mays* cv. LG 2080), valuable for its high yield of green feed in late summer for zero grazing and/or silage making (National Institute of

Agricultural Botany 1992b), was included to represent a broad leaved, thick stemmed, monocotyledonous species.

The plants were grown in a greenhouse because of the inclusion of the three tropical species. In the present experiment chewing activity was not determined because the amount of forage which could be produced in the pots was insufficient. The experiment was carried out in each of two years.

Materials and Methods, 1991

Establishment

Twelve forage species (3 tropical and 9 temperate) were used in this experiment (Plates 1 & 2). The forages were grown from seed in 48 clay pots each filled with 5·5 kg of composite soil on 25 April 1991. The composite soil comprised 6 parts of sterilized soil, 4 parts of peat and 2 parts of horticultural grade lime-free washed grit (nominally ⩽ 5 mm). To every 40 kg of composite soil, 227 g of John Innes fertilizer and 227 g of ground limestone were added.

The ingredients of the John Innes fertilizer were:

Total nitrogen	5·1%	
Total phosphorus pentoxide	8·2%	(3·5% P)
Phosphorus pentoxide soluble in water ...	7·2%	(3·1% P)
Phosphorus pentoxide insoluble in water .	1·0%	(0·4% P)
Total potassium oxide soluble in water ..	10·0%	(8·5% K)

Plate 1. One pot each of (from left to right) (a) sainfoin, white clover, lucerne and *Desmodium intortum*, (b) rape and spurrey, (c) Italian ryegrass, perennial ryegrass and tall fescue on 20 July 1992

Plate 1

(a)

(b)

(c)

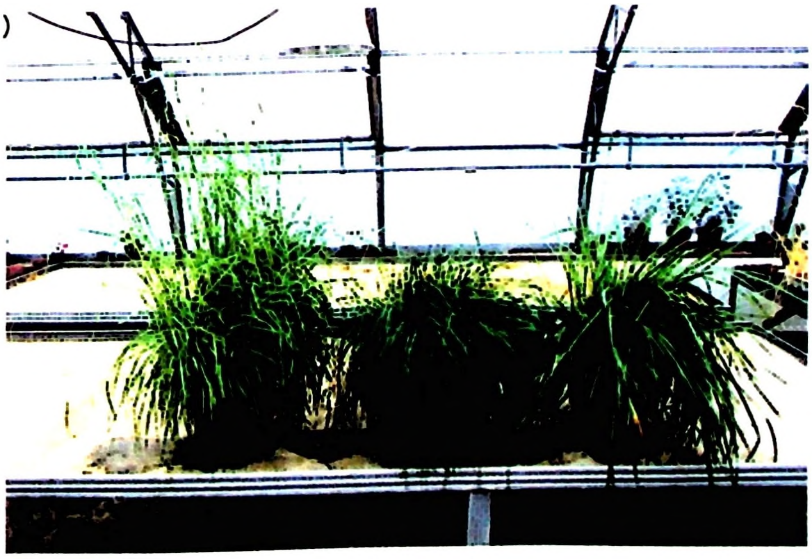

Plate 2. (a) One pot of maize on 1 June 1991.

(b) One pot each of *Cenchrus ciliaris* (on the left) and *Chloris gayana* on 20 July 1992

Plate 2

(a)

(b)

The supplier and weight of each forage seed lot sown is indicated in Table 4.1. The weight of seed sown depended on the germination test done before sowing (Table 4.2), and on the size and weight of the seed.

White clover, spurrey, rape, *Desmodium intortum* and lucerne seeds were scattered evenly on the composite soil followed by a slight disturbance of the soil and slight compaction using the back of the hand. All grass seeds were sown c. 0·5 - 1·0 cm deep in the composite soil followed by slight compaction by hand. Maize seeds were sown c. 2·5 cm deep. The seeds were sown in the pots in the potting house at Frongoch Field Station, Aberystwyth, and thereafter moved to an adjoining glasshouse where they were arranged in two completely randomized blocks (Fig.4.1) in a 4·00 m x 4·33 m compartment (within the glasshouse) with heating facilities and a minimum-maximum thermometer. The compartment had a window which could be opened or closed to regulate the compartment ventilation.

The pots containing *Desmodium intortum* seeds were then inoculated with *Rhizobium* CB 627 culture (from CSIRO Australia). 2 g of Rhizobium culture was mixed with 200 ml of tap water in a watering can and sprayed evenly into all four *Desmodium intortum* pots. The daily minimum and maximum temperatures of the compartment were recorded from sowing in spring 1991 to the end of the experiment in summer 1992 (Table 4.3). Mean daily minimum/maximum temperatures were 15/31°C in the period 26 April to 22 August 1991, 14/31°C in the period 19 April to 25 July 1992 and 14/23°C in the winter period 1 November 1991 to 28 March 1992.

Table 4.1. Experiment 2: forage species used, suppliers of seed and weight of seed sown per pot

Forage species	Supplier	Weight/pot (g)
White clover (*Trifolium repens* cv.Olwen)[1]	IGER U.K.	0·5
Lucerne (*Medicago sativa* cv.Europe)[1]	IGER U.K.	0·4
Sainfoin (*Onobrychis viciifolia* cv.Cotswold common)[1]	British Seed Houses U.K.	2·5
Desmodium intortum (cv.Green leaf) (tropical legume)[2]	CSIRO,Australia	0·5
Rape (*Brassica napus* cv.Sparta)[3]	ICI U.K.	0·5
Spurrey (*Spergula arvensis*)[6]	B & S Weed Seed Suppliers, Nottingham U.K.	1·0
Perennial ryegrass (*Lolium perenne* cv.Melle)[1]	IGER U.K.	0·5
Italian ryegrass (*Lolium multiflorum* RvP)[1]	IGER U.K.	0·5
Tall fescue (*Festuca arundinacea* cv.Dovey)[1]	IGER U.K.	0·5
Chloris gayana (cv.Callide) (tropical grass)[4]	CSIRO,Australia	1·0
Cenchrus ciliaris (cv.Biloela) (tropical grass)[4]	CSIRO,Australia	1·0
Maize (*Zea mays* cv. LG2080)[5]	IGER U.K.	7 seeds

1 = National Institute of Agricultural Botany (1989).

2 = Göhl (1981).

3 = National Institute of Agricultural Botany (1992a).

4 = Skerman & Riveros (1990).

5 = National Institute of Agricultural Botany (1992b).

6 = Derrick (1989)

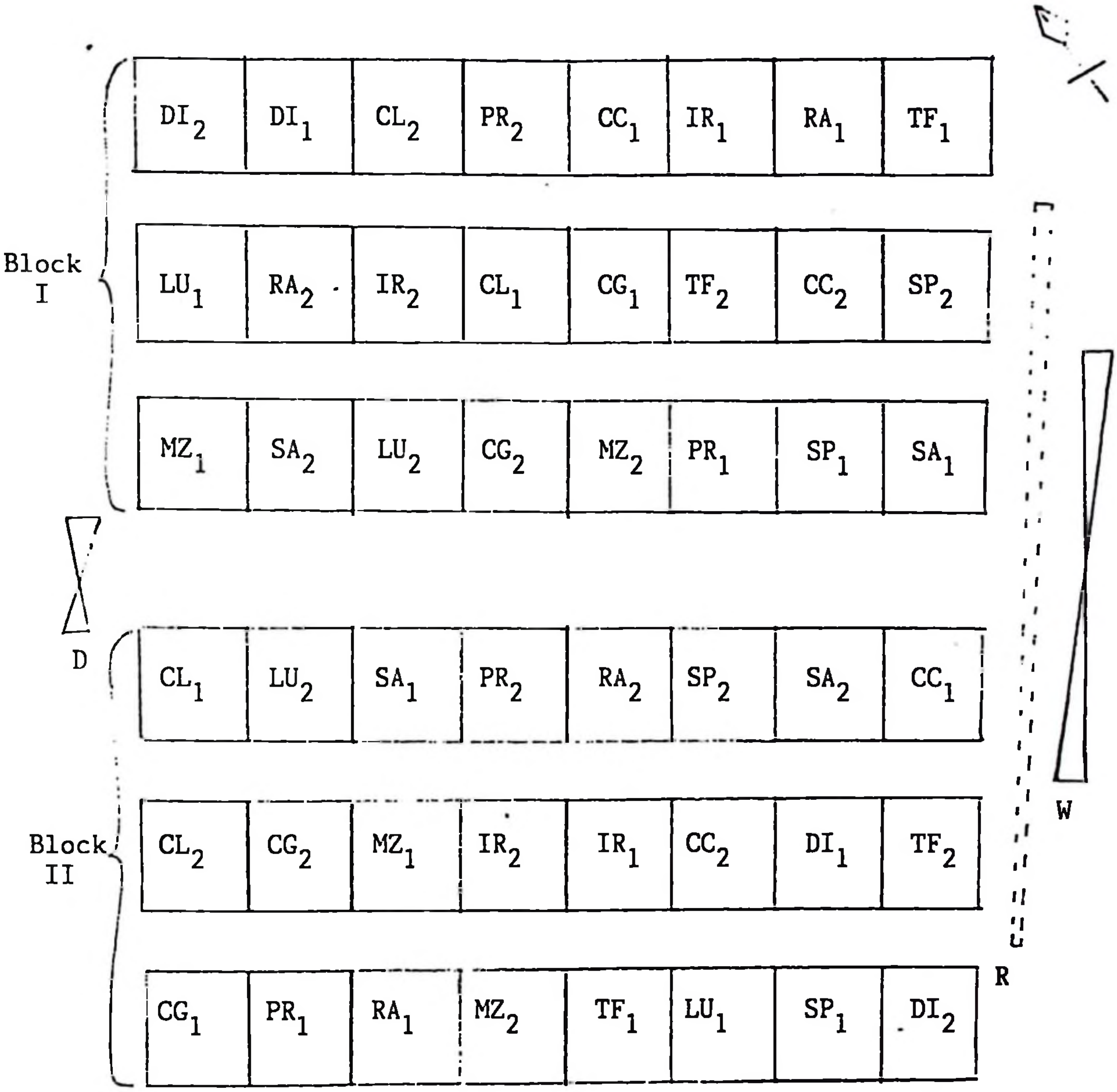

Fig.4.1. Experiment 2 layout

Key

1 = First harvest, 2 = Second harvest, D = Compartment door,

R = radiator (heater), W = compartment window

CL = White clover PR = Perennial ryegrass

Lu = Lucerne IR = Italian ryegrass

SA = Sainfoin TF = Tall fescue

DI = *Desmodium intortum* CG = *Chloris gayana*

RA = Rape CC = *Cenchrus ciliaris*

SP = Spurrey MZ = Maize

Pathway between blocks 1·2 m; space between pots 0·4 m

Table 4.2. Seed germination percentage during a germination test and one week after sowing in the glasshouse

	Germination (%) after 7 days	
Forage species	In petri dishes	In pots
White clover	33	63
Lucerne	90	64
Sainfoin	55	14
Desmodium intortum	100	76
Rape	100	83
Spurrey	70	95
Perennial ryegrass	88	15
Italian ryegrass	90	63
Tall fescue	100	54
Chloris gayana	28	74
Cenchrus ciliaris	15	56
Maize	NT	100

NT = Not tested because there were very few seeds.

Sainfoin had the lowest germination score in the pots (14%) 7 days after sowing (Table 4.2). Seed germination percentage during a test before sowing (done on moist filter paper in petri dishes) was lowest in *Cenchrus ciliaris* followed by *Chloris gayana*. Within the first week after germination there was a damping off of some seedlings in some pots. The condition was most apparent (3–5% by score) among the dicotyledonous species, except sainfoin. Among the grasses, only *Chloris gayana* showed damping off of a few seedlings. The cause of the damping off was assumed to be high humidity and temperature. The door and the window of the compartment were, therefore, opened during the day so as to increase ventilation. The amount of water splashing on the compartment floor during watering was also reduced. The damping off seedlings were removed from the glasshouse. To protect the plants against fungus attack they were sprayed regularly with Benlate (fungicide) (15 g in 4·54 l of water).

The grasses and maize were thinned to 18 and 3 plants per pot respectively on 17 May. On average the grasses already had c. one tiller per plant. While Italian ryegrass plants had 3 tillers per plant, *Cenchrus ciliaris* plants had not yet produced tillers. On the same day, immediately after thinning all pots containing grasses and maize were topped up with 500 g of composite soil of the same composition as used at sowing time. This was because the maize and tropical grasses were growing tall and had started to show the roots. The legumes, spurrey and rape plants were thinned a week later because they had grown more slowly.

Table 4.3. Mean daily minimum and maximum temperatures during 7-day periods from sowing 1991 to the end of the experiment in 1992

Week No.	Dates	Min.°C	Max.°C	Week No.	Dates	Min.°C	Max.°C
1	26. 4- 2. 5.91	15	31	34	15.12-21.12.91	13	19
2	3. 5- 9. 5.91	16	30	35	22.12-28.12.91	13	20
3	10. 5-16. 5.91	16	29	36	29.12- 4. 1.92	15	21
4	17. 5-23. 5.91	17	32	37	5. 1-11. 1.92	16	19
5	24. 5-30. 5.91	16	39	38	12. 1-18. 1.92	12	20
6	31. 5- 6. 6.91	11	27	39	19. 1-25. 1.92	12	20
7	7. 6-13. 6.91	13	26	40	26. 1- 1. 2.92	10	24
8	14. 6-20. 6.91	13	29	41	2. 2- 8. 2.92	15	21
9	21. 6-27. 6.91	15	32	42	9. 2-15. 2.92	16	23
10	28. 6- 4. 7.91	18	34	43	16. 2-22. 2.92	13	24
11	5. 7-11. 7.91	18	32	44	23. 2-29. 2.92	14	27
12	12. 7-18. 7.91	15	31	45	1. 3- 7. 3.92	15	27
13	19. 7-25. 7.91	14	30	46	8. 3-14. 3.92	13	26
14	26. 7- 1. 8.91	17	33	47	15. 3-21. 3.92	15	24
15	2. 8- 8. 8.91	15	32	48	22. 3-28. 3.92	14	24
16	9. 8-15. 8.91	15	29	49	29. 3- 4. 4.92	11	26
17	16. 8-22. 8.91	17	34	50	5. 4-11. 4.92	15	32
18	23. 8-29. 8.91	15	33	51	12. 4-18. 4.92	12	27
19	30. 8- 6. 9.91	16	33	52	19. 4-25. 4.92	15	29
20	7. 9-13. 9.91	14	33	53	26. 4- 2. 5.92	16	30
21	14. 9-20. 9.91	15	33	54	3. 5- 9. 5.92	14	31
22	21. 9-27. 9.91	13	25	55	10. 5-16. 5.92	12	29
23	28. 9- 5.10.91	16	29	56	17. 5-23. 5.92	16	33
24	6.10-12.10.91	15	29	57	24. 5-30. 5.92	15	28
25	13.10-19.10.91	17	32	58	31. 5- 6. 6.92	12	27
26	20.10-26.10.91	14	26	59	7. 6-13. 6.92	14	36
27	27.10- 2.11.91	16	25	60	14. 6-20. 6.92	13	34
28	3.11- 9.11.91	14	23	61	21. 6-27. 6.92	14	33
29	10.11-16.11.91	13	22	62	28. 6- 4. 7.92	16	31
30	17.11-23.11.91	12	19	63	5. 7-11. 7.92	15	30
31	24.11-30.11.91	15	21	64	12. 7-18. 7.92	15	29
32	1.11- 7.12.91	14	23	65	19. 7-25. 7.92	15	30
33	8.12-14.12.91	11	21	66	26. 7- 1. 8.92	14	29

Desmodium intortum and rape were thinned to 10 plants per pot, white clover to 11 plants per pot, sainfoin and lucerne to 17 plants per pot and spurrey to 18 plants per pot. No composite soil was added to the legume, spurrey or rape pots after plant thinning.

On average the number of leaves per plant (per main shoot in the grasses) on 25 May were 8, 5, 5, 3, 3, 3, 3, 4, 3, 3, 3 for maize, *Cenchrus ciliaris*, *Chloris gayana*, Italian ryegrass, perennial ryegrass, tall fescue, rape, *Desmodium intortum*, lucerne, white clover and sainfoin respectively. Spurrey stems had two internodes and each internode had at least one branch. *Cenchrus ciliaris* plants were growing tall very quickly and were starting to lodge. They were, therefore, supported by sticks and strings. The same support was given to *Chloris gayana*. On 31 May, John Innes fertilizer was applied to all pots at a rate of 3·5 g/pot. This was done on the assumption that the fertilizer would replace nutrients lost by leaching and plant uptake.

When the rape plants had about 5 leaves per plant, they were attacked by aphids. An insecticide (14·2 ml of Dimethoate mixed with 4·5 l of water) was sprayed on all rape plants and all other forage species to protect them from the aphids. 'Phostrogen' fertilizer was applied weekly from 17 May to one week before each harvest to the pots containing maize. The 'Phostrogen' (8·75 g) was mixed with water (4·5 l) before applying (c. 1 litre per pot). The composition of 'Phostrogen' was:

Total nitrogen (N)	10%
Phosphorus pentoxide (P_2O_5) soluble in neutral ammonium citrate and water	10% (4·4% P)
Potassium oxide (K_2O) soluble in water ...	27% (22·4% K)
Iron (Fe)	9·4%
Magnesium (Mg)	1·3%
Manganese (Mn)	200 mg/kg

By the beginning of the second week of June 1991 old leaves of the tropical grasses and rape were senescing. At two months old, maize and *Cenchrus ciliaris* plants were flowering and almost all the species were growing vigorously and sometimes during sunny days required watering three times a day. Sainfoin plants were, however, growing slowly. As a result sainfoin pots were supplied weekly from 13 June to one week before each harvest with John Innes fertilizer (3·5 g/pot). Sainfoin plants in the pot for harvest two in block one were growing even more slowly than sainfoin in the other pots. At the beginning of July these sainfoin plants were attacked by fungus. Fungicide (Benlate) was applied and the plants recovered, although they had lost some of their leaves. Sainfoin produced no stem.

During the establishment period the number of tillers per pot of the grass species were counted on three different dates (Table 4.4). Italian ryegrass had the greatest number of tillers per pot, while *Cenchrus ciliaris* had the least on all counting dates.

Table 4.4. Number of grass tillers (excluding main shoots) per pot during the establishment period

Grass species	Counting dates		
	25.5.91	10.6.91	26.6.91
Perennial ryegrass	68	180	585
Italian ryegrass	133	517	546
Tall fescue	25	129	130
Chloris gayana	32	67	124
Cenchrus ciliaris	2	54	57
Mean	52	189	288

Harvesting and sampling

The forage species were left to grow without interruption up to their respective harvesting dates (25-26 June, 23-24 July and 20 August 1991). *Cenchrus ciliaris*, *Chloris gayana*, Italian ryegrass, perennial ryegrass, tall fescue, rape, spurrey and maize in the pots assigned to harvest one in block one were harvested on 25 June and those in block two on 26 June. The same forage species in pots assigned to harvest two in block one were harvested on 23 July and those in block two on 24 July. White clover, lucerne, sainfoin and *Desmodium intortum* in pots for harvests one and two were harvested on 23-24 July and 20 August instead of 25-26 June and 23-24 July because by late June their herbage mass was still small.

The plants were harvested c. 2 cm above the soil surface at 10.00 a.m. and placed in labelled polythene bags, tied by a string and taken to the crop laboratory at Frongoch. In the laboratory the forages were weighed to record the fresh yield per pot. Then six tillers of each grass species, two or three whole *Desmodium intortum*, lucerne and rape plants and 20-30 g of white clover, sainfoin and spurrey herbage were sampled from the produce of each pot. These samples were placed in labelled polythene grip bags and stored in the freezer for the determination of plant morphology (leaf size, stem diameter etc.) and the vascular structure of different plant parts. Maize plant parts were sampled from two plants per pot. The sample consisted of two leaf blades and their leaf sheaths and stem internodes per plant (one just below an ear position and another below the one holding the flag leaf). Maize leaf blades were separated from their leaf sheaths, the leaf sheaths were left with their stem internodes and both of them were placed in one labelled polythene bag.

The remaining herbage from each pot was chopped into 7 cm-long pieces and mixed well by hand in plastic bowls. Samples of c. 20-30 g were taken from the chopped herbage and placed with labels in perforated polypropylene bags (W.R.Grace Ltd.) and oven-dried at 80°C for 48 hours to determine the dry matter content. The dried samples were put in labelled paper envelopes and taken to the Agricultural Sciences Analytical Laboratory at Frongoch for *in vitro* dry matter digestibility determination (Tilley & Terry 1963). Also from the chopped herbage of all species, samples of c. 70-80 g were placed in labelled polythene grip-bags and stored in the freezer. These samples were used for determining the proportions of plant parts and neutral detergent fibre content (Van Soest & Wine 1967) as an estimate of cell wall content.

Before chopping the herbages, maize leaf blades were separated manually from their stems. The chopped maize leaf blade and 'stem' were kept in separate polythene bags. Maize 'stems' included leaf sheaths, ears and tassels. The cob (i.e. grain and rachis) was discarded before chopping. With its sweet grains maize cob could attract the sheep and this might leave insufficient time to eat the other plant parts. The chopped herbages were then placed in labelled polythene bags tied by string and taken at 1.30 p.m. to IGER Gogerddan, where they were fed to oesophageal fistulated sheep. Feeding took place at c. 2.00 to 3.00 p.m.

Feeding activity

The same oesophageal fistulated sheep used in Experiment 1 were used in the present experiment. The sheep were fed fresh herbage (a mixture of perennial ryegrass and white clover of c. 12% dry matter) beforehand as in Experiment 1. Intact sheep were not used in the present experiment because chewing activity during eating was not recorded.

The procedures for feeding the experimental meals to the oesophageal fistulated sheep, collection of chewed particles from the fistula and recording of diet intake rate (g/min) were as in Experiment 1. The aim was, with each species, to give at least two meals (each of 100 g), one for each sheep, on each day of feeding. In the case of some species, there was only sufficient for one meal, so this was fed to only one sheep (the one referred to as sheep 1 during the whole experiment) and the other sheep (sheep 2) was given a false meal to keep it busy so that it did not disturb sheep 1.

The sheep found it very difficult to chew the pieces of maize stem internode. The sheep, therefore, spat out the 'stem' pieces and only ate leaf blades, ears and tassels. For this reason a maize meal was obtained by mixing 80 g of leaf blade and 20 g of 'stem' pieces so that within one minute the sheep would not waste a lot of time playing with stems, and could find enough palatable material to eat.

Laboratory activities

The procedures followed in the determination of plant part morphology, *in vitro* dry matter digestibility, neutral detergent fibre content, proportions of plant parts, vascular structure arrangement and particle breakdown characteristics were similar to those in Experiment 1. As described in Experiment 1, rape and also maize stem internodes were cut into thin transverse sections for the study of vascular structure.

Maize leaf blade unlike grass leaf blade, had three types of vein, large, small and very small. The veins were arranged in a regular pattern on both sides of the mid-rib. Large veins were wide apart. Between every two large veins there were small veins so arranged that they gave four equal sub distances within which very small veins were closely packed together. These very small veins were not counted. The grass leaf sheath veins, when observed under the light microscope at 14x magnification, appeared to be connected by very fine strands. This character was more conspicuous in tall fescue and maize than in the other grass species.

Materials and Methods, 1992

The perennial species survived the 1991/92 winter well. The glasshouse mean daily temperature during the winter was 14°C minimum and 23°C maximum. The pots were watered regularly throughout the winter. On 3 March 1992, plant regrowths (except those of sainfoin) and dead plant parts were cut and removed from the pots and taken out of the glasshouse. On the following day, 250 g of new composite soil (with similar composition to the one used in 1991) was added to each pot and then 'Phostrogen' fertilizer was applied at the same rate as for maize pots in 1991. By the end of March, the plants had new shoots.

On 23 April, plant regrowths (except those of sainfoin) were cut and removed from the pots. Each pot was then topped up with 200 g of composite soil. Annual species (maize, rape and spurrey) were sown in their respective pots. The same seeding rates as in 1991 were used for all three species. Before sowing, the top layer (c. 2·5 cm) of the old composite soil from each pot was removed and replaced with 1 kg of new composite soil. The top layer of old composite soil was removed for two main reasons: to remove the weeds and to rejuvenate the old composite soil for better establishment of the new crop. All the pots were then watered regularly. The window and door of the compartment were left open during the day and closed at night so as to regulate the compartment ventilation and temperature.

By 7 May, maize, rape and spurrey had germinated and their seedlings had up to 3 leaves. On 19 May, the seedlings of maize, rape and spurrey were thinned to 3, 15 and 20 plants per pot, respectively. Each maize pot was then topped up with 500 g of composite soil and 25 g of lime consecutively.

The plants grew well and there were no serious fungal disease or pest problems. The procedures followed in harvesting and sampling were the same as in 1991. In 1992 the diets were fed at IGER Trawsgoed. Because there was only one oesophageal fistulated sheep (number 2) (number 1 had died during the 1991/92 winter), each harvest period was lengthened from two to four days. The harvesting dates were 22-25 June (first harvest) and 22-25 July (second harvest). All the 12 species were ready for the 22-25 June harvest. On each day of harvest, the sheep ate twelve meals (each of 100 g), one from each plant species from one block. Only half the produce of a pot was harvested per day. Therefore, at each harvest one block was fed to the sheep over two days. Feeding procedures and laboratory activities were as in 1991.

Statistical Analyses

The differences between the species were consistent from year to year for most of the variables studied. The results of the two years (1991 and 1992) were therefore combined and statistically analysed using the following analysis of variance:

Source of variation	df
Years	1
Species	11
Harvesting date	1
Species x Harvesting date	11
Error	23
Total	47

Where species differences were not consistent from year to year, the results were analysed separately for each year using the following analysis of variance:

Source of variation	df
Blocks	1
Species	11
Harvesting date	1
Species x Harvesting date	11
Error	23
Total	47

Results

Plant part morphology and vascular structure

White clover had the greatest number of secondary veins per leaflet, followed by sainfoin (Table 4.5). The numbers of secondary veins per rape leaf blade and per *Desmodium intortum* leaflet were not significantly different, and that of the latter species was lower than that of lucerne. *Desmodium intortum* had by far the greatest total number of tertiary veins per leaflet. Rape leaf blades and white clover leaflets were not significantly different in terms of the number of tertiary veins. The two species had significantly more tertiary veins per leaf blade or leaflet than lucerne and sainfoin. The number of secondary + tertiary veins per unit area in white clover leaflets was not significantly different to that of lucerne leaflets but was lower than those of sainfoin and *Desmodium intortum* leaflets. Rape leaf blade had the lowest number of secondary + tertiary veins per unit area.

The angles of attachment of secondary veins to the primary veins in lucerne and sainfoin leaflets were similar and lower than those in white clover and *Desmodium intortum* leaflets. The primary vein of rape leaf blades was thicker than those in legume leaflets. Among the legumes, *Desmodium intortum* leaflet primary veins were thicker than those of the other three legumes. The distances between adjacent secondary veins in rape leaf blades and *Desmodium intortum* leaflets were not significantly different. The two species had their leaf blade or leaflet secondary veins more widely spaced than those of white clover, lucerne and sainfoin.

Leaf petioles of lucerne were thinnest, while those of rape were the thickest of all the species (Table 4.6). White clover, sainfoin and *Desmodium intortum* did not differ significantly in terms of leaf petiole thickness. The ranking of the species in respect of the number of vascular bundles per leaf petiole was rape $>$ *Desmodium intortum* $>$ sainfoin $>$ white clover $>$ lucerne. There were no significant differences between the species in the thickness of the leaf petiole vascular bundles. *Desmodium intortum* leaf petiole had the highest percentage of its cross sectional area occupied by vascular bundles. There was no significant difference between white clover, lucerne, sainfoin and rape in percentage of cross sectional area of leaf petiole occupied by vascular bundles.

The tropical grasses had more large veins per leaf blade than the temperate grasses (Table 4.7). Among the temperate grasses, perennial ryegrass had the fewest large veins per leaf

Table 4.5. Legume leaflet and rape leaf blade vascular structure. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)§

Species	Number of secondary veins per leaflet or leaf blade	Number of tertiary veins per leaflet or leaf blade	Number of secondary + tertiary veins per cm^2 of leaflet or leaf blade	Angle of attachment of secondary veins to the primary veins	Diameter (μm) of primary vein	Distance (mm) between adjacent secondary veins
White clover	44·3	141	25·4	43·8	186	1·7
Lucerne	26·4	62	49·1	25·4	145	1·9
Sainfoin	30·7	49	64·5	26·6	136	1·8
Desmodium intortum	20·8	1393	62·4	47·7	263	7·4
Rape	24·1	156	1·96	60·8	941	8·3
SE±	1·33	8·3*	8·98†	1·19	20·0	0·59
Mean	29·3	102*	50·4†	40·9	334	4·2

* Excluding *Desmodium intortum* with SE ±41·7

† Excluding rape with SE ±0·512

§ In 1991, the legumes were harvested on 23 July and 20 August blade. Maize had many more large and small veins per leaf blade than any of the forage grasses. The tropical grasses had significantly more small veins per leaf blade than the temperate grasses. The temperate grasses did not differ significantly in the number of small veins per leaf blade.

Among the forage grass species, tall fescue had the thickest large leaf blade veins. The thickness of the small leaf blade veins of tall fescue was not significantly different to those of the maize and perennial ryegrass leaf blades, but was greater than those of Italian ryegrass and the tropical grasses. Maize large leaf blade veins were thicker than those of the forage grass species.

Maize leaf blade mid ribs were much thicker than those of the forage grass leaf blades. Tall fescue leaf blade mid ribs were thicker than those of the other forage grass leaf blades. The leaf blade veins were closer together in the tropical than in the temperate grasses and intermediate in maize. The leaf blade veins were furthest apart in tall fescue. The percentage of leaf blade width at the mid-point occupied by the sum of vein diameters and mid rib diameter was higher in the tropical grasses than in the temperate grasses and maize.

Maize had much wider leaf sheaths than all the forage grasses (Table 4.8). Among the forage grasses, *Chloris gayana* had the widest leaf sheaths. Both tropical grasses had wider leaf sheaths than the temperate grasses. Within the temperate grasses, tall fescue had wider leaf sheaths than perennial ryegrass. The number

Table 4.6. Leaf petiole morphology and vascular structure of legumes and rape. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)*

Species	Diameter(mm) of leaf petiole	Number of vascular bundles per leaf petiole	Diameter (μm) per vascular bundle	Percentage of cross sectional area occupied by vascular bundles
White clover	1·91	6·0	191	6·5
Lucerne	0·87	3·0	156	9·3
Sainfoin	1·77	7·8	181	9·6
Desmodium intortum	1·93	11·7	182	17·8
Rape	4·90	18·3	195	3·1
SE±	0·081	0·54	18·7	2·14
Mean	2·28	9·4	181	9·3

* In 1991 the legumes were harvested on 23 July and 20 August

Table 4.7. Grass and maize leaf blade vascular structure. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)

Species	Number of veins (excluding the mid-rib)per leaf blade		Diameter (μm) of veins		Diameter (μm) of mid rib	Distance(μm) between adjacent veins regardless of size	Percentage of leaf blade width at mid-point occupied by the sum of vein diameters and mid rib diameter
	Large veins	Small veins	Large veins	Small veins			
Perennial ryegrass	2·80	12	112	78	165	176	34·2
Italian ryegrass	5·68	16	103	69	179	178	33·4
Tall fescue	4·45	11	172	105	219	408	26·1
Chloris gayana	8·22	61	127	58	186	62	51·1
Cenchrus ciliaris	7·60	64	120	50	169	53	54·7
Maize	20·48	474	210	88	2210	112	31·5
SE±	0·437	2·9*	11·1	9·3	8·7†	23·9	3·25
Mean	8·21	34*	142	76	184†	165	38·5

† Excluding maize with SE ±210

* Excluding maize with SE ±4·9

of veins per leaf sheath was greatest in maize and greater in the tropical than in the temperate grasses. Maize had the thickest leaf sheath veins. Those of tall fescue were not significantly different to those of Italian ryegrass but were thicker than those of perennial ryegrass and the tropical grasses. The distance between adjacent leaf sheath veins was less in the tropical grasses than in the temperate grasses and maize. The leaf sheath veins were furthest apart in maize. The veins in tall fescue leaf sheaths were more widely spaced than those in Italian ryegrass leaf sheaths. The percentage of the leaf sheath width at the mid-point occupied by the sum of the diameters of the veins was higher in *Chloris gayana* than in *Cenchrus ciliaris* and higher in both the tropical grasses than in the temperate grasses and maize.

Maize had thicker stem internodes than rape and both had thicker stem internodes than all other species (Table 4.9). Maize had the most vascular bundles per stem internode followed by rape and then *Chloris gayana* and *Cenchrus ciliaris*. Perennial and Italian ryegrasses had similar numbers of vascular bundles per stem internode, lower than *Chloris gayana* and *Cenchrus ciliaris* but not significantly different to white clover, lucerne, *Desmodium intortum* and spurrey.

The vascular bundles of spurrey and perennial ryegrass stem internodes were not significantly different in terms of thickness. The vascular bundles in spurrey, perennial ryegrass and Italian ryegrass stem internodes were thinner than in all the other species.

Table 4.8. Grass and maize leaf sheath widths and vascular structure. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)

Species	Width (mm) of leaf sheath at mid-point	Number of veins per leaf sheath	Diameter(μm) of veins	Distance(μm) between adjacent veins	Percentage of leaf sheath width at mid-point occupied by the sum of vein diameters
Perennial ryegrass	5·8	15	108	284	28
Italian ryegrass	6·4	18	128	253	34
Tall fescue	7·6	17	154	345	33
Chloris gayana	12·2	51	117	84	58
Cenchrus ciliaris	9·7	48	113	146	44
Maize	72·6	103	215	456	32
SE±	0·51*	1·5†	8·9	21·1	2·5
Mean	8·3*	30†	139	261	38

* Excluding maize with SE ±2·36

† Excluding maize with SE ±1·4

Chloris gayana and lucerne stem internode vascular bundles were similar in thickness and not significantly different to those of *Cenchrus ciliaris* and *Desmodium intortum*. Maize, rape and white clover were not significantly different in terms of the stem internode vascular bundle thickness. Maize, however, had thicker stem internode vascular bundles than the forage grasses, spurrey, lucerne and *Desmodium intortum*. The percentage of stem internode cross sectional area occupied by vascular bundles was highest in *Cenchrus ciliaris* and intermediate in *Chloris gayana* and lucerne.

The ranking of the legume species in terms of their leaflet length, width and area per leaflet was *Desmodium intortum* > white clover > lucerne and sainfoin (Table 4.10). Rape leaf blades were much longer, broader and bigger than the legume leaflets. The ranking of forage grasses in terms of their leaf blade length was perennial and Italian ryegrasses < *Cenchrus ciliaris* < tall fescue and *Chloris gayana* and in terms of leaf blade width the ranking was perennial and Italian ryegrasses < tall fescue, *Chloris gayana* and *Cenchrus ciliaris*. Perennial ryegrass had the lowest area per leaf blade followed by Italian ryegrass. Both ryegrasses had smaller leaf blades than tall fescue and the tropical grasses. Spurrey leaf blades were slightly longer than lucerne leaflets, but they were narrow and the least of all the species in area.

Perennial and Italian ryegrasses had similar dry matter percentages which were lower than that of *Cenchrus ciliaris* (Table 4.11 Also *Chloris gayana*, *Desmodium intortum* and lucerne had similar dry

Table 4.9. Stem internode morphology and vascular structure of the legumes, rape, spurrey, grasses and maize. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 25 July)[§]

Species	Diameter(mm) per stem internode	Number of vascular bundles per stem internode	Diameter(μm) per vascular bundle	Percentage of stem internode cross sectional area occupied by vascular bundles
White clover	2·61	10·5	204	6·98
Lucerne	2·51	22·3	183	13·18
Desmodium intortum	3·58	21·6	199	6·97
Rape	8·80	80·0	213	4·82
Spurrey	1·12	11·0	95	8·13
Perennial ryegrass†	1·90	19·4	100	5·50
Italian ryegrass†	1·80	19·7	125	8·80
Chloris gayana	3·87	58·5	183	13·76
Cenchrus ciliaris	2·51	56·3	159	21·51
Maize	13·58	134·3	231	5·71
SE±*	0·364	5·72	9·0	1·025
Mean	4·82	49·3	183	10·13

† 1992 only

* Excluding perennial and Italian ryegrasses

§ In 1991 legumes were harvested on 23 July and 20 August

Table 4.10. Lengths (mm), widths (mm) and area (cm^2) per leaflet or leaf blade of legumes, grasses, rape, spurrey and maize. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)†

Species	Leaflet or leaf blade		
	Length	Width*	Area
Legumes:			
White clover	38·9	28·8	7·6
Lucerne	25·2	10·9	2·1
Sainfoin	27·1	8·4	1·4
Desmodium intortum	76·5	48·7	23·9
SE±	2·06	1·43	1·20
Mean	41·9	24·2	8·8
Grasses:			
Perennial ryegrass	290	4·0	10·2
Italian ryegrass	310	5·7	16·2
Tall fescue	480	8·5	36·7
Chloris gayana	550	8·9	44·0
Cenchrus ciliaris	390	9·4	39·9
SE±	24	0·58	1·81
Mean	400	7·3	29·4
Others:			
Rape	146 ± 6·0	90 ± 3·7	92 ± 4·3
Spurrey	31·1 ± 0·24	0·81 ± 0·046	0·22 ± 0·065
Maize	840 ± 25·0	82 ± 2·6	623 ± 17·0

† In 1991 legumes were harvested on 23 July and 20 August.

* Width at leaflet broadest point and leaf blade mid-length point.

Table 4.11. Dry matter percentage, dry matter yield and oven-dry weight per unit area of leaflet or leaf blade. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)*

Species	Dry matter percentage	Dry matter yield (g DM/pot)	Weight per unit area of leaf (mg/cm^2)
White clover	16·1	85	3·02
Lucerne	19·2	100	2·73
Sainfoin	21·5	27	5·24
Desmodium intortum	19·9	181	3·14
Rape	13·0	63	5·60
Spurrey	18·2	29	-
Perennial ryegrass	16·7	42	3·07
Italian ryegrass	16·7	69	2·30
Tall fescue	17·2	40	4·26
Chloris gayana	19·3	141	4·15
Cenchrus ciliaris	23·1	118	3·50
Maize	21·6	327	4·76
SE±	1·64	20·9	0·422
Mean	18·5	102	3·80

* In 1991 the legumes were harvested on 23 July and 20 August.

matter percentages which were significantly higher than that of rape. Maize had the highest dry matter yield of all the species. *Desmodium intortum* and *Chloris gayana* were not significantly different in terms of dry matter yield, but the former species had higher yield than all the other forage grasses, legumes, rape and spurrey. Among the legumes, dry weight per unit area of leaflet was greatest in sainfoin. Rape and maize were not significantly different in terms of weight per unit area of leaf blade. Rape leaf blades had, however, greater weight per unit area than forage grass leaf blades and legume leaflets other than sainfoin.

Proportions of plant parts, neutral detergent fibre content and *in vitro* dry matter digestibility of the fresh chopped diet

The ranking of the legume chopped diets in terms of the proportion of green leaflet was sainfoin > *Desmodium intortum* > lucerne and white clover (Table 4.12). Rape and *Desmodium intortum* were not significantly different in terms of the proportion of green leaf blade or leaflet in their chopped diets. Spurrey had the lowest proportion of green leaf. The ranking of the forage grasses and maize in terms of the proportion of green leaf blade was tall fescue > perennial ryegrass and *Chloris gayana* > Italian ryegrass > *Cenchrus ciliaris* and maize. The proportion of dead leaf blade in the chopped diet was higher in perennial and Italian ryegrasses than in all the other species.

Lucerne had the highest proportion of green stem followed by *Desmodium intortum*, maize, spurrey and *Cenchrus ciliaris* and then white clover, rape and *Chloris gayana*. Perennial and Italian ryegrasses had the lowest proportion of green stem.

The ranking of the legumes and rape in terms of the proportion of green leaf petiole was sainfoin and white clover > rape > *Desmodium intortum* > lucerne. The proportion of green leaf sheath was highest in Italian ryegrass. The tropical grasses had a lower proportion of green leaf sheath than the temperate grasses. Of all the species spurrey had the highest proportion of inflorescence.

Rape had the lowest neutral detergent fibre content (Table 4.13). *Desmodium intortum* and lucerne were similar in neutral detergent fibre content and higher than white clover and sainfoin. The temperate grasses and maize had significantly lower neutral detergent fibre contents than the tropical grasses. Maize was rather similar to tall fescue in neutral detergent fibre content and spurrey was similar to *Desmodium intortum*. The forage grasses and maize were significantly higher in neutral detergent fibre than the legumes and rape.

Desmodium intortum had the lowest and rape the highest *in vitro* dry matter digestibility of the twelve species. Lucerne, sainfoin, *Chloris gayana*, *Cenchrus ciliaris* and spurrey were not significantly different in terms of digestibility. Among the legume species, white clover had the highest digestibility. The tropical grasses were similar in digestibility and lower than the

Table 4.12. Proportions of plant parts (% in dry matter) in the chopped diets. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)§

	Green leaflet or leaf blade	Dead leaflet or leaf blade	Green stem	Green leaf petiole	Green leaf sheath	Inflorescence
White clover	30·1	7·4	18·6	43	–	5·0
Lucerne	34·7	1·7	58·0	4	–	1·5
Sainfoin	51·5	2·9	–	45	–	–
Desmodium intortum	41·9	1·4	44·8	12	–	–
Rape	43·1	6·3	23·6	27	–	–
Spurrey	12·6	–	42·7	–	–	44·7
Perennial ryegrass	56·8	17·2	6·8†	–	23	0·3†
Italian ryegrass	38·3	18·2	8·2†	–	37	1·2†
Tall fescue	70·0	6·5	–	–	23	–
Chloris gayana	54·0	7·7	22·1	–	16	–
Cenchrus ciliaris	29·4	6·2	46·0	–	15	3·0
Maize	29·3	–	42·3	–	18	9·4
SE±	2·42	2·22	2·59*	1·9	1·8	2·24*
Mean	41·0	7·6	36·9*	26	22	15·5*

§ In 1991 legumes were harvested on 23 July and 20 August.

† For 1992 only.

* Excluding perennial and Italian ryegrasses.

– No data and not included in the analysis of variance.

Table 4.13. Neutral detergent fibre content and *in vitro* dry matter digestibility of the chopped diets. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)*

Species	Neutral detergent fibre (% in dry matter)	*In vitro* dry matter digestibility (%)
White clover	34·9	73·3
Lucerne	50·9	58·8
Sainfoin	38·1	60·3
Desmodium intortum	51·7	53·6
Rape	26·0	83·3
Spurrey	52·6	59·4
Perennial ryegrass	57·0	73·2
Italian ryegrass	58·7	70·7
Tall fescue	62·5	67·1
Chloris gayana	73·8	59·7
Cenchrus ciliaris	75·1	59·8
Maize	62·1	70·7
SE±	1·32	1·83
Mean	53·6	65·8

* In 1991 legumes were harvested on 23 July and 20 August.

temperate grasses and maize. Tall fescue digestibility was significantly lower than that of perennial ryegrass.

Intake rate of fresh chopped forage by oesophageal fistulated sheep

The fresh forage intake rate of white clover was not significantly different to those of spurrey and rape but was higher than that of *Desmodium intortum* on both harvesting dates (Table 4.14). Spurrey and rape were eaten at a similar rate and much faster than some of the legumes and grasses including maize. *Desmodium intortum*, lucerne and sainfoin fresh forage intake rates were not significantly different and at the 23 July harvest only the latter species had a significantly greater intake rate than perennial and Italian ryegrasses. At both harvests, *Chloris gayana*, *Cenchrus ciliaris*, tall fescue and maize were not significantly different in fresh forage intake rates. The fresh forage intake rate was generally lower at the second than at the first harvest, particularly in tall fescue and *Cenchrus ciliaris*.

The dry matter intake rates of white clover, sainfoin and *Desmodium intortum* were higher in 1992 than in 1991, while those of spurrey and *Cenchrus ciliaris* were higher in 1991 than in 1992 (Table 4.15). The dry matter intake rate of spurrey was higher at the second than at the first harvest. On average spurrey had the highest intake rate. Its dry matter intake rate was the highest in 1991, but was not significantly different from that of sainfoin in 1992. At the second harvest, spurrey had the highest

Table 4.14. Fresh forage intake rate (g/min) of the oesophageal fistulated sheep

Species	Mean of two years (1991 and 1992)† 25 June harvest	23 July harvest	Mean of two years (1991 and 1992) and of both harvests (25 June and 23 July)†
White clover	78·2	72·9	75·6
Lucerne	62·2	50·2	56·2
Sainfoin	63·7	62·2	63·0
Desmodium intortum	50·0	50·2	54·1
Rape	88·2	80·1	84·2
Spurrey	87·2	82·8	85·0
Perennial ryegrass	53·9	40·7	47·3
Italian ryegrass	53·3	41·0	47·2
Tall fescue	48·6	28·8	38·7
Chloris gayana	34·3	16·7	25·5
Cenchrus ciliaris	35·8	15·2	25·5
Maize	42·2	27·4	34·8
SE±	6·38	6·38	4·51
Mean	58·8	47·4	53·1

† In 1991 legumes were harvested on 23 July and 20 August.

Table 4.15. Dry matter intake rate (g/min) of the oesophageal fistulated sheep

Species	Mean of two dates of harvests (25 June and 23 July)†		Mean of two years (1991 and 1992)		Mean of two years (1991 and 1992) and of both harvests (25 June and 23 July)†
	1991	1992	25 June†	23 July†	
White clover	9·43	11·49	10·71	10·21	10·46
Lucerne	11·57	12·40	11·62	12·35	11·99
Sainfoin	10·19	15·66	12·59	13·26	12·93
Desmodium intortum	9·92	11·41	10·19	11·14	10·67
Rape	11·04	10·49	10·98	10·55	10·77
Spurrey	16·88	14·36	13·22	18·02	15·62
Perennial ryegrass	8·15	7·46	8·35	7·25	7·80
Italian ryegrass	7·72	7·79	7·88	7·63	7·76
Tall fescue	6·85	6·09	7·73	5·21	6·47
Chloris gayana	5·21	3·93	5·58	3·56	4·57
Cenchrus ciliaris	6·03	4·62	6·52	4·13	5·33
Maize	6·89	6·33	7·00	6·21	6·61
SE±	0·501	0·457	1·165	1·165	0·824
Mean	9·11	9·33	9·31	9·13	9·22

† In 1991 the legumes were harvested on 23 July and 20 August.

dry matter intake rate in both years. Among the legumes, sainfoin had the highest dry matter intake rate in 1992. At the individual harvests, mean of 2 years, however, the dry matter intake rates of all the legumes and rape were not significantly different.

The tropical forage grass dry matter intake rates were low at both harvests particularly the second. The dry matter intake rates of the tropical forage grasses were c. 2·2 g/mm lower at the second than at the first harvest. The dry matter intake rate of maize was similar to that of tall fescue. The legumes, rape and spurrey had higher dry matter intake rates than the forage grasses and maize.

Characteristics of the chewed particles

The numbers of particles per 100 mg DM were below average in the legume and rape chewed material (Table 4.16). On average the number of particles was lowest in sainfoin; similar in white clover, lucerne, *Desmodium intortum* and rape and > 50% above average in perennial and Italian ryegrasses.

White clover, lucerne and sainfoin did not differ significantly in terms of the number of veins per leaflet particle (Table 4.17). The number of veins per leaflet or leaf blade particle was higher in sainfoin than in *Desmodium intortum* or rape. Perennial ryegrass had more veins per leaf blade particle than tall fescue. The tropical grasses and maize had more veins per leaf blade particle than the temperate grasses.

Table 4.16. Number of particles (≥ 0·4 mm in width) per 100 mg dry matter in chewed plant material from the oesphageal fistulas

Species	Mean of two dates of harvests (25 June and 23 July)†		Mean of two years (1991 and 1992)		Means of two years (1991 and 1992) and of both harvests (25 June and 23 July)†
	1991	1992	25 June harvest†	23 July harvest†	
White clover	98	109	118	65	92
Lucerne	71	85	105	75	90
Sainfoin	51	45	54	42	48
Desmodium intortum	86	102	115	73	94
Rape	90	97	117	70	94
Spurrey	160	117	159	118	139
Perennial ryegrass	203	164	185	183	184
Italian ryegrass	190	208	216	183	200
Tall fescue	154	140	161	133	147
Chloris gayana	125	106	129	102	116
Cenchrus ciliaris	95	97	109	83	96
Maize	102	117	153	67	110
SE±	19·4	17·5	13·6	13·6	9·6
Mean	119	116	135	100	118

† In 1991 the legumes were harvested on 23 July and 20 August.

* Only maize leaf blade was eaten by the sheep

Table 4.17. Mean number of veins or vascular bundles per particle of the chewed plant material. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)[§]

Species	Leaflet or leaf blade	Leaf petiole and leaf sheath	Stem
White clover	5·8	4·5	6·4[†]
Lucerne	6·2	3·0	12·8
Sainfoin	8·8	7·1	–
Desmodium intortum	4·0	7·6	8·3
Rape	1·8	5·2	3·9
Spurrey	–	–	9·1
Perennial ryegrass	8·8	6·6	8·9[†]
Italian ryegrass	8·1	7·3	8·6[†]
Tall fescue	5·3	5·4	–
Chloris gayana	16·8	10·9	8·5
Cenchrus ciliaris	19·3	10·6	11·0
Maize	20·0	–	–
SE±	1·12	0·60	1·16*
Mean	9·5	6·8	8·9*

§ In 1991 the legumes were harvested on 23 July and 20 August.

† In 1992 only.

* Excluding perennial and Italian ryegrasses and white clover.

Desmodium intortum and sainfoin had similar numbers of vascular bundles per leaf petiole particle and more than lucerne, white clover and rape. The number of veins per leaf sheath particle was greater in the tropical than in the temperate grasses. Lucerne and *Cenchrus ciliaris* were not significantly different in terms of the numbers of vascular bundles per stem particle. However, lucerne had more vascular bundles per stem particle than any of the remaining species.

Rape had broader leaf particles than white clover, lucerne and *Desmodium intortum* (Table 4.18). Spurrey had the narrowest leaf blade particles. The width of the leaf blade particles of maize was greater than those of the forage grasses. The tropical and temperate forage grasses did not differ significantly in terms of the width of the leaf blade particles. The leaf blade particles of the forage grasses were narrower than those of the legumes and rape.

Lucerne had the narrowest leaf petiole particles. The leaf petiole particles of rape were wider than those of the legumes and wider than the leaf sheath particles of the forage grasses. There was no significant difference between the tropical and temperate grasses in leaf sheath particle width.

Spurrey had the narrowest stem particles. Rape and white clover had wider stem particles than all the other species. There was little difference between the forage grasses and lucerne and *Desmodium intortum* in width of stem particles.

Table 4.18. Widths (mm) of particles derived from different plant parts. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)‡

Species	Leaflet or leaf blade	Leaf petiole or leaf sheath	Stem	Overall mean
White clover	4·22	1·69	2·56†	3·10§
Lucerne	3·60	0·74	1·48	2·44
Sainfoin	4·69	1·57	–	3·14
Desmodium intortum	4·04	1·64	1·59	2·92
Rape	5·00	3·07	2·74	4·05
Spurrey	0·80	–	0·98	0·92
Perennial ryegrass	2·02	2·03	1·55†	2·01§
Italian ryegrass	2·02	2·13	1·65†	2·12§
Tall fescue	2·18	2·14	–	2·18
Chloris gayana	1·93	1·95	1·66	1·79
Cenchrus ciliaris	1·87	1·91	1·46	1·78
Maize	2·95	–	–	2·95
SE±	0·218	0·164	0·091*	0·143*
Mean	2·94	1·89	1·65*	2·45*

‡ In 1991 the legumes were harvested on 23 July and 20 August.

† 1992 only.

* Excluding perennial and Italian ryegrasses and white clover.

§ Excluding stem particles.

The overall mean particle width (regardless of the plant part) was lowest in spurrey and highest in rape. Lucerne had the lowest overall mean particle width among the legumes. The overall mean particle widths did not differ significantly between forage grasses and were lower in the grasses than in white clover, sainfoin and *Desmodium intortum*.

The four legume species did not differ significantly in mean leaflet particle length (Table 4.19). Rape and spurrey had similar leaf blade particle length. Leaf blade particles of the forage grasses were longer than the leaflet or leaf blade particles of all the other species. The temperate grasses had longer leaf blade particles than the tropical grasses.

White clover, sainfoin and *Desmodium intortum* were not significantly different in leaf petiole particle length and all were significantly greater than leaf petiole or sheath particle length in lucerne, tall fescue, *Chloris gayana* and *Cenchrus ciliaris*. Leaf sheath particles were longer in the temperate than in the tropical grasses.

Lucerne, *Desmodium intortum*, rape, *Chloris gayana* and *Cenchrus ciliaris* did not differ significantly in terms of stem particle length. There was no significant difference between white clover and spurrey in stem particle length. Perennial and Italian ryegrasses had the longest stem particles.

White clover, sainfoin and spurrey had similar overall particle length which was greater than those of lucerne and

Table 4.19. Lengths (mm) of particles derived from different plant parts. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)‡

Species	Leaflet or leaf blade	Leaf petiole or leaf sheath	Stem	Overall mean
White clover	7·8	22·2	22·0†	14·8§
Lucerne	7·8	10·6	15·2	10·6
Sainfoin	9·0	20·8	–	14·6
Desmodium intortum	7·4	18·7	14·5	11·4
Rape	9·6	16·6	14·6	12·1
Spurrey	9·6	–	20·4	15·0
Perennial ryegrass	17·2	20·2	25·9†	18·2§
Italian ryegrass	15·6	17·8	27·3†	17·4§
Tall fescue	14·4	13·2	–	14·1
Chloris gayana	12·2	12·3	14·8	12·6
Cenchrus ciliaris	11·9	13·9	16·3	13·8
Maize	8·2	–	–	8·2
SE±	0·71	1·19	0·94*	0·76*
Mean	10·9	16·6	16·0*	12·5*

‡ In 1991 the legumes were harvested on 23 July and 20 August.

† 1992 only.

* Excluding perennial and Italian ryegrass and white clover.

§ Excluding stem particles.

Desmodium intortum. The overall mean particle lengths were greatest in perennial and Italian ryegrasses. The tropical grasses and tall fescue did not differ significantly in terms of overall mean particle length.

White clover had a lower percentage of leaflet particles with jagged ends than lucerne, *Desmodium intortum* and rape (Table 4.20). The forage grasses and maize were not significantly different in terms of the percentage of leaf blade particles with jagged ends. Over 90% of rape and *Desmodium intortum* leaf blade and leaflet particles had jagged sides. Among the forage grasses only tall fescue had >60% of leaf blade particles with jagged sides. Over 80% of maize leaf blade particles had jagged sides.

At least 80% of the leaf petiole particles of *Desmodium intortum* and rape and of the leaf sheath particles of the forage grasses had jagged ends. The percentage of leaf petiole particles with jagged sides in *Desmodium intortum* was greater than in the other three legumes and greater than in the leaf sheath particles of the forage grasses. Among the grasses, perennial ryegrass and *Cenchrus ciliaris* had < 50% of leaf sheath particles with jagged sides and the other forage grasses had ≤60%.

The species did not differ significantly in terms of the percentage of stem particles with jagged ends. Only spurrey had <80% stem particles with jagged ends. *Desmodium intortum*, rape, *Chloris gayana* and *Cenchrus ciliaris* were not significantly different in terms of the percentage of stem particles with jagged

Table 4.20. Percentage of particles with jagged ends or jagged sides in (a) particles derived from leaflets or leaf blades, (b) those derived from leaf petioles or leaf sheaths, and (c) those derived from stems. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)‡

	(a) Leaflet or leaf blade		(b) Leaf petiole or leaf sheath		(c) Stem	
Species	Ends	Sides	Ends	Sides	Ends	Sides
White clover	63	77	68	57	81†	61†
Lucerne	80	76	77	6	87	63
Sainfoin	69	63	72	66	-	-
Desmodium intortum	84	95	86	85	88	87
Rape	83	91	84	83	88	94
Spurrey	-	-	-	-	73	55
Perennial ryegrass	80	38	83	47	85†	64†
Italian ryegrass	79	52	80	55	85†	63†
Tall fescue	79	67	80	60	-	-
Chloris gayana	75	57	82	56	89	86
Cenchrus ciliaris	80	54	81	47	84	85
Maize	78	82	-	-	-	-
SE±	4·5	6·2	4·4	6·5	5·2*	5·6*
Mean	77	68	79	56	85*	78*

‡ In 1991 the legumes were harvested on 23 July and 20 August.

† For 1992 only.

* Excluding perennial and Italian ryegrasses and white clover.

sides and all had a higher proportion of stem particles with jagged sides than the remaining species.

Only *Desmodium intortum* and maize had > 50% of leaflet and leaf blade particles with a rough surface (Table 4.21). Sainfoin had the lowest percentage of leaf particles with a rippled surface. Among the grasses, the percentages of leaf blade particles with a rippled surface were below average in tall fescue and *Chloris gayana*.

The percentages of leaf petiole or sheath particles with a rough surface were < 50% in all species except *Desmodium intortum* and rape, which had ≥ 80% with a rough surface. The ranking of species in terms of the percentage of leaf petiole or sheath particles with a rippled surface was Italian ryegrass and *Desmodium intortum* > perennial ryegrass > *Cenchrus ciliaris* and white clover > sainfoin > *Chloris gayana* > tall fescue.

The percentage of stem particles with a rough surface was higher in *Desmodium intortum*, rape and the tropical grasses than in white clover, lucerne, spurrey and perennial and Italian ryegrasses. The ranking of the legumes with respect to the percentage of stem particles with a rippled surface was white clover > *Desmodium intortum* > lucerne.

The ranking of legumes in terms of the number of perforations or ruptures per leaflet particle was white clover and *Desmodium intortum* > lucerne > sainfoin (Table 4.22). Sainfoin, rape, tall fescue and maize were not significantly different in terms of the

Table 4.21. Percentage of particles with a rough or rippled surface in (a) particles derived from leaflets or leaf blades, (b) those derived from leaf petioles or leaf sheaths, and (c) those derived from stems. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July) ǂ

	(a) Leaflet or leaf blade		(b) Leaf petiole or leaf sheath		(c) Stem	
Species	Rough surface	Rippled surface	Rough surface	Rippled surface	Rough surface	Rippled surface
White clover	32	9·7	46	12·2	61·0†	17·30†
Lucerne	41	7·6	23	–	65·9	7·73
Sainfoin	47	2·3	40	10·4	–	–
Desmodium intortum	62	10·0	80	14·5	89·0	12·55
Rape	46	10·3	90	–	94·1	–
Spurrey	–	–	–	–	43·0	10·68
Perennial ryegrass	29	17·2	38	13·4	67·0†	15·30†
Italian ryegrass	38	19·6	48	14·8	49·1†	18·10†
Tall fescue	47	7·9	43	3·5	–	–
Chloris gayana	28	8·8	41	7·1	80·4	16·12
Cenchrus ciliaris	40	15·3	33	12·4	86·8	9·00
Maize	56	11·0	–	–	–	–
SE±	3·5	1·71	3·8	0·17	3·14*	1·008*
Mean	42	10·9	48	11·0	76·5*	11·22*

ǂ In 1991 the legumes were harvested on 23 July and 20 August.

† For 1992 only.

* Excluding perennial and Italian ryegrasses and white clover.

Table 4.22. Number of perforations or ruptures per particle. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July) ǂ

Species	Leaflet or leaf blade	Leaf petiole or leaf sheath	Stem
White clover	2·75	0·57	0·29†
Lucerne	1·50	–	0·61
Sainfoin	0·71	0·44	–
Desmodium intortum	2·53	1·25	1·14
Rape	1·17	0·41	0·63
Spurrey	–	–	0·57†
Perennial ryegrass	1·91	1·14	1·47†
Italian ryegrass	2·37	1·56	1·03†
Tall fescue	1·42	1·38	–
Chloris gayana	1·85	1·43	1·11
Cenchrus ciliaris	2·15	1·44	1·20
Maize	0·85	–	–
SE±	0·242	0·175	0·128*
Mean	1·75	1·07	0·94*

ǂ In 1991 the legumes were harvested on 23 July and 20 August.

† For 1992 only.

* Excluding perennial and Italian ryegrasses, spurrey and white clover.

number of perforations or ruptures per leaflet or leaf blade particle. Perennial and Italian ryegrass and the tropical grasses did not differ significantly in the number of perforations or ruptures per leaf blade particle.

Rape, sainfoin and white clover differed very little in the number of perforations or ruptures per leaf petiole particle. The three species had, however, significantly fewer perforations or ruptures per petiole than *Desmodium intortum* and fewer than in the forage grass leaf sheath particles. The grasses did not differ significantly in terms of the number of ruptures per leaf sheath particle. The numbers of ruptures per stem particle did not differ significantly between *Chloris gayana*, *Cenchrus ciliaris* and *Desmodium intortum* and were higher in these species than in rape and lucerne.

Some leaflets of lucerne and sainfoin remained intact (i.e. not broken into pieces) after chewing (Plate 3). The ranking of legumes in terms of the percentage of leaf petiole particles which remained intact after chewing was lucerne > sainfoin > white clover > *Desmodium intortum* (Table 4.23).

Relationships between variables

The neutral detergent fibre content of the species was negatively related to the intake rate, overall mean particle width and particle size (length x width) (Table 4.24). Intake rate (excluding spurrey) was positively related to the overall mean particle width and particle size. The relationship between particle

Table 4.23. Percentage of particles which remained intact after chewing in (a) particles derived from leaflet or leaf blade, (b) those derived from leaf petioles or leaf sheaths, and (c) those derived from stems. Means of two years (1991 and 1992) and of two dates of harvest (25 June and 23 July)

Species	(a) Leaflet or leaf blade	(b) Leaf petiole or leaf sheath	(c) Stem
White clover	–	42·5	51·7†
Lucerne	6·9	95·4	41·9
Sainfoin	20·5	62·8	–
Desmodium intortum	–	23·9	–
Rape	–	23·1	–
Spurrey	–	–	63·0
Perennial ryegrass	–	–	28·8†
Italian ryegrass	–	–	33·8†
Chloris gayana	–	–	7·0†
SE±	2·28	3·83*	5·09‡
Mean	13·7	56·2*	52·5‡

In 1991 the legumes were harvested on 23 June and 20 August.

† For 1992 only.

* Excluding rape.

‡ Excluding *Chloris gayana*, Italian ryegrass, perennial ryegrass and white clover.

Table 4.24. Correlation coefficients (r) between neutral detergent fibre, intake rate and the size of chewed particles.

	Neutral detergent fibre content (% DM)	Intake rate g DM/min
(a) All species		
Intake rate (g DM/min)	−0·688* n = 12	
Overall mean width(mm) of chewed particles	−0·700* n = 12	+0·680* n = 11
Mean length x width (mm^2) of chewed particles	−0·748** n = 12	+0·662* n = 11
(b) Forage grasses only		
Overall mean width (mm) of chewed particles	−0·845 n = 5	+0·759 n = 5
Overall mean length (mm) of chewed particles	−0·872 n = 5	+0·952* n = 5
Mean length x width (mm^2) of chewed particles	−0·987** n = 5	+0·982** n = 5

* $P < 0{\cdot}05$

** $P < 0{\cdot}01$

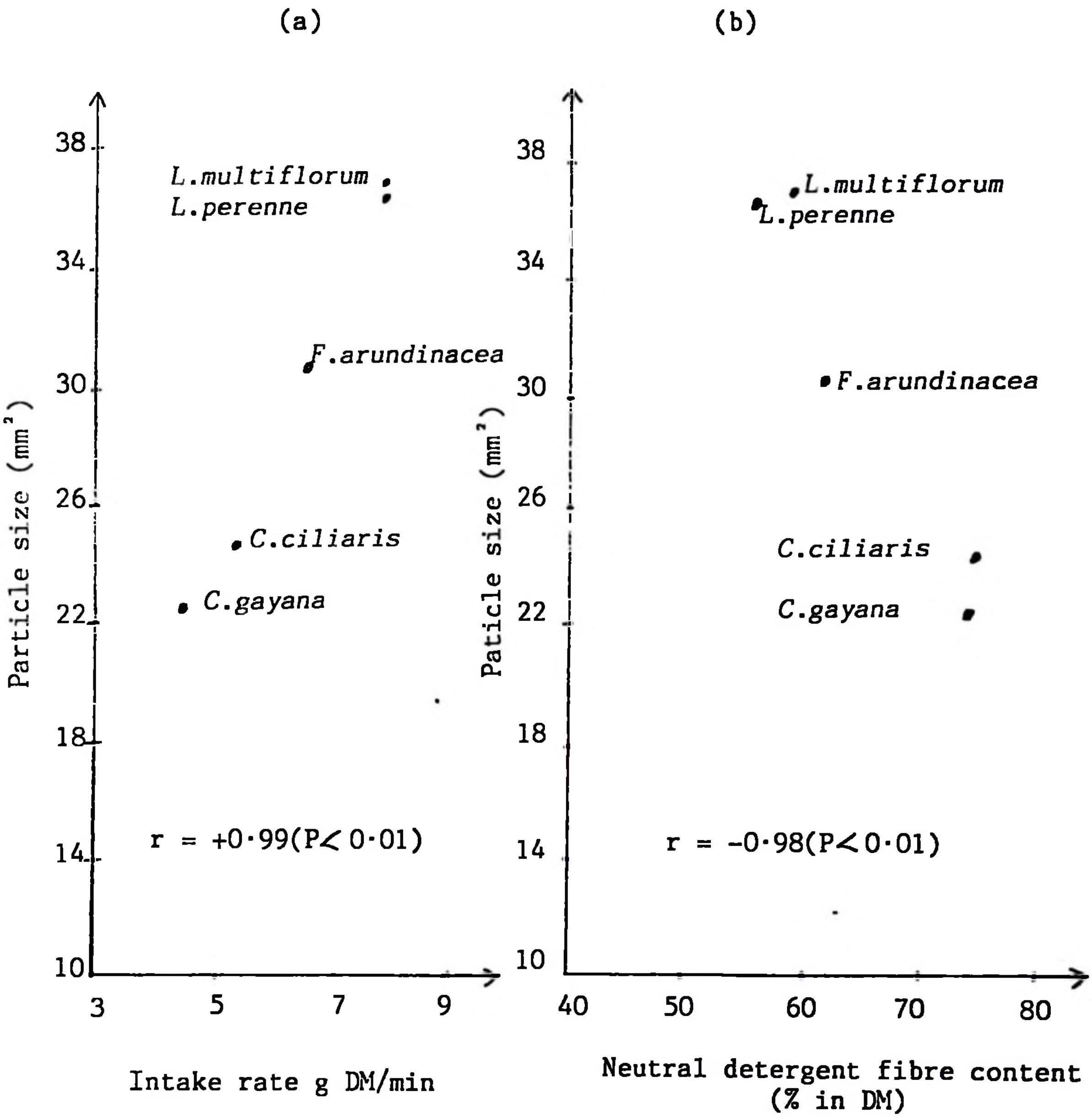

Fig.4.2. Relationships between particle size (length x width) and (a) intake rate, (b) neutral detergent fibre content of forage grasses.

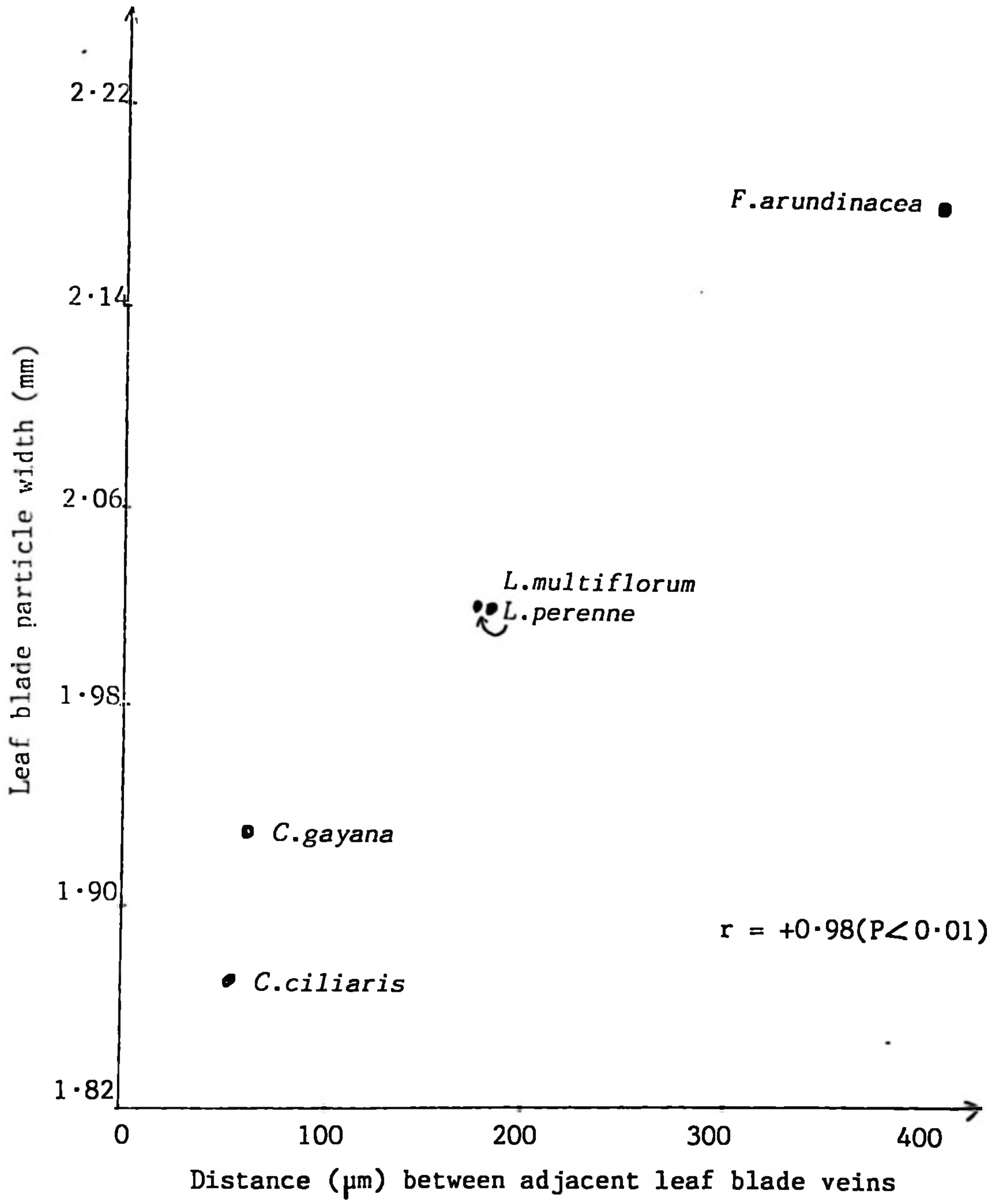

Fig.4.3. Relationship between leaf blade particle width (mm) and distance (μm) between adjacent leaf blade veins

size and both intake rate and neutral detergent fibre in the case of the forage grasses is illustrated in Fig.4.2: the tropical grasses had higher fibre, lower intake rate and were chewed to smaller particles than the ryegrasses, with tall fescue intermediate. The distance between adjacent leaf blade veins was positively related to the leaf blade particle width (Fig.4.3).

Discussion

White clover, lucerne and sainfoin had smaller leaflets with much more closely spaced secondary veins than *Desmodium intortum*. The legumes differed a little in the number of secondary veins per leaflet, but differed greatly in the number of tertiary veins per leaflet. *Desmodium intortum* had >10x more tertiary veins per leaflet than the three temperate legumes. As in Experiment 1, rape leaf blades had thicker primary veins, more widely spaced secondary veins and fewer veins per unit area than legume leaflets.

Desmodium intortum leaf petioles were about the same size as those of white clover and sainfoin, but had more vascular bundles which occupied a higher proportion of the cross sectional area than in the case of the three temperate legumes. This could have led to more chewing of the *Desmodium intortum* petioles, reflected in the high proportion of particles with jagged sides and the low proportion of particles which remained intact after chewing. As in Experiment 1, the thick leaf petioles and stem internodes of rape had only a low proportion of their cross sectional area occupied by vascular bundles. The thick stem internodes of maize had more vascular bundles, which occupied a lower proportion of the cross sectional area, than the stems of the tropical forage grasses. When compared to the tropical grasses, perhaps the thickness of maize stems contributed more to their rejection by the sheep than their toughness.

Temperate grass leaf blades and sheaths had fewer veins which were more widely spaced ($\geqslant 4$x in the leaf blade and $\geqslant 2$x in the sheath) and occupied a lower proportion of the leaf width than those of the tropical grasses. This was in agreement with Wilson *et al.* (1989). The highly vascularized nature of tropical grass herbage has been noted as the cause of its high cell wall content, low digestibility and low intake (Akin 1989; Minson 1990). This is in agreement with the results of the present experiment. In both forage grasses and maize the leaf blade veins were less widely spaced than the leaf sheath veins, possibly because the leaf blade requires more internal support in order to remain sufficiently open and upright to intercept the light for photosynthesis, while the leaf sheath is partly supported by other sheaths and by the shape of the sheath tube and is generally shorter than the blade.

In the present experiment the tropical grasses had the highest neutral detergent fibre content, followed by the temperate grasses and then the legumes. The trend of digestibility was not so smooth. The tropical grasses were significantly less digestible than the temperate grasses. However, among the legumes only white clover was significantly more digestible than the tropical grasses. Lucerne and sainfoin had similar digestibility to the tropical grasses; *Desmodium intortum* was slightly less digestible than the tropical grasses. The lower digestibility of *Desmodium intortum* could be due to its high tannin content (Rotar 1965). The presence of tannin in some legumes has also been blamed for their low palatability (Donnelly & Anthony 1969). However, in the present experiment, *Desmodium intortum* and sainfoin, which contain tannin, were consumed at almost the same rate as the other legumes. Higher intake of sainfoin than ryegrass has been noted by Thomson (1982). Also, Wilman & Asiedu (1983) reported that sainfoin leaves were readily eaten by sheep. Göhl (1981) noted that *Desmodium intortum* produces a large bulk of palatable herbage. The low digestibility of lucerne could be due to its high proportion of stem. Lucerne stem has been noted for its relatively high neutral detergent fibre content and low digestibility and voluntary intake (McLeod & Minson 1988). In the present experiment as in Experiment 1, rape had the lowest neutral detergent fibre content and the highest digestibility and was eaten faster than the forage grasses and maize.

The legumes did not differ in dry matter intake rate and were eaten faster than the forage grasses and maize. This was in agreement with McLeod & Smith (1989) and McLeod *et al.* (1990). The tropical grasses were eaten at a slower rate than the temperate grasses. This difference could be due to the higher vascularization and higher neutral detergent fibre content of the tropical grasses (Minson 1990). A lower intake rate of tropical grass as compared to temperate grass was reported by McLeod & Smith (1989). Due to its high dry matter intake rate, spurrey had high neutral detergent fibre and digestible dry matter intake rates. This illustrates that effort put into improving forage intake is important in increasing energy intake and thus increasing animal production.

The number of particles/g after chewing was high in perennial and Italian ryegrasses. In the present experiment perennial and Italian ryegrasses had stems in the second year of the experiment, and c. 30% of the stem particles remained intact after chewing, which would tend to reduce the number of particles/g. However, the ryegrass stems, unlike those of the tropical grasses, were hollow and low in proportion in the herbage. Therefore, despite having large particles (Fig.4.2) there were more particles per unit weight in the ryegrasses than in the tropical grasses.

Sainfoin had the fewest particles/g after chewing. This might be due largely to the high proportion of sainfoin leaflets and leaf petiole particles which remained intact after chewing. Sainfoin was eaten quickly, sometimes as quickly as spurrey, and 21% and 63% of its leaflets and leaf petioles, respectively, remained intact after chewing. Some of the sainfoin leaflets were just halved (Plate 3) and had no perforations or ruptures. The resistance of sainfoin leaflets to breakdown has also been reported by Lees *et al.* (1982). They reported that sainfoin leaflets were less disrupted than lucerne and white clover leaflets

Plate 3. Chewed particles of six species on 25 June 1992: (a) white clover, (b) rape, (c) lucerne, (d) spurrey, (e) sainfoin, (f) *Desmodium intortum*.

Scale 1:2·1

Plate 3

(a)

(b)

(c)

(d)

(e)

(f)

by shaking with glass beads and that digestion of sainfoin whole leaflets by rumen micro-organisms was less extensive than digestion of lucerne and white clover leaflets. They found that sainfoin leaflets like those of other bloat-safe legumes, were more resistant to breakdown because they had slightly thicker-walled epidermal and mesophyll cells and a thicker cuticle than the leaflets of bloat-causing legumes (e.g. white clover and lucerne). The numbers of veins per sainfoin leaflet particle were high, and the numbers of bundles of its leaf petiole particles were similar to the numbers before chewing. Further work on sainfoin particle breakdown during rumination is necessary as a greater proportion of its particles remained intact after chewing during eating and there were fewer perforations and ruptures per particle than in the case of the other legumes and therefore possibly there would be less weakening by rumen micro-organisms before chewing during rumination.

In the present experiment, the leaflets of *Desmodium intortum*, a species also considered to be bloat-safe (Bryan 1969), were large, with many branching tertiary veins, and had a soft epidermis, which seemed to be weak; chewing disrupted the leaflets and even removed some of the veins and produced holes; as a result the particles had many perforations or ruptures, comparable in number to those of white clover. Rape had large chewed particles, with few vascular bundles per stem particle and per leaf blade particle, but a moderate number per leaf petiole particle. As in Experiment 1, this was a reflection of the low proportion of vascular tissue in the rape plant parts. White clover and lucerne were similar in the number of veins per leaflet particle, but the latter species had fewer perforations or ruptures per leaflet particle and c. 7% of its leaflet particles remained intact after chewing. Further, lucerne stem particles had twice as many vascular bundles as those of white clover, following the same trend as in the unchewed stems. The two species also had some stem particles which remained intact after chewing (52% for white clover and 63% for lucerne) indicating possibly the degree of their pliability and ease of incorporation into a bolus to swallow. For example, despite having the highest proportion of stem, lucerne had a high dry matter intake rate, possibly due to the thin and/or pliable nature of its stems.

Tropical and temperate grasses were not significantly different in the number of vascular bundles per stem particle. However, the number of veins per leaf blade or leaf sheath particle was more than twice as great in the tropical as in the temperate grasses. This suggests that a greater effort might have been required to chew the tropical compared with the temperate grasses, which might also be the case during rumination. This, together with the associated high neutral detergent fibre content, meant that the tropical grasses were eaten slowly and were broken into smaller particles than the temperate grasses, which were eaten relatively quickly (Fig.4.2). The shorter particles of the tropical than the temperate leaf blades after chewing during eating (Plate 4) is in agreement with the results of Wilson *et al*. (1989).

Plate 4. Chewed particles of six species on 25 June 1992: (a) *Chloris gayana*, (b) perennial ryegrass, (c) *Cenchrus ciliaris*, (d) Italian ryegrass, (e) maize, (f) tall fescue.

Scale 1:2·1

Plate 4

(a)

(b)

(c)

(d)

(e)

(f)

Particles derived from leaf blades were similar in mean length and width to particles derived from leaf sheaths in the case of the forage grasses; however, legume leaflet particles were c. 2·5 times the width and c. 0·4 times the length of the leaf petiole particles. The mean particle length was c. 2·0x the mean width in the legume leaflet particles, c. 10·4x in the leaf petiole particles and c. 8·2x in the stem particles, showing that leaf petiole and stem particles can contribute substantially to the mean particle length of chewed legume material. Therefore both leaf petiole and stem require attention when research on increasing legume intake is conducted. In the case of the forage grasses the mean particle length was c. 7·1x the mean width in the case of leaf blade; 7·6x in the case of leaf sheath and 13·3x in stem, showing that a high proportion of stems in forage grasses can substantially increase the overall mean particle length and thus delay the passage of the chewed material out of the rumen. From the point of view of high intake, therefore, it is a good idea to graze grasses while they have young stems or when they are still leafy.

Maize leaf blade is brittle; it may be 'notch-sensitive' (Vincent 1990), allowing the cracks to propagate in all directions, even across the parallel thicker veins. It may not be surprising, therefore, that maize leaf blade was chewed into shorter but wider particles and that a higher proportion (>75%) of these particles had jagged ends and sides and had fewer perforations as compared with those of the forage grasses.

The proportion of particles with jagged sides was higher in legume leaflet and rape and maize leaf blade particles than in forage grass leaf blade particles. This was in agreement with Vincent (1990) who noted that most grass leaf blades are almost completely 'notch-insensitive', not allowing cracks to develop across their parallel veins and allowing cracks to develop easily parallel to the veins, resulting in a high proportion of leaf blade particles with jagged ends but a lower proportion with jagged sides. Grazing animals, as Vincent (1990) suggested, have to use brute force to break grass leaf blades. There was a little difference between legumes, rape and forage grasses in terms of the proportions of leaf petiole or leaf sheath and stem particles with jagged ends. These results suggested that leaf sheath, leaf petiole and stem in most species did not differ much in their mode of physical breakdown. This was in agreement with Kelly & Sinclair (1989) who observed the regurgitated particles (from the sheep rumen) of fresh perennial ryegrass leaf sheath and red clover and lucerne leaflets and leaf petiole; they reported that the fractures on the surface of these particles occurred in all directions. A high proportion of particles with jagged sides in the case of leaf petiole and stem could also mean that extra chewing might have been necessary to break them down before forming a bolus to swallow. This might have been the case with the thick leaf petiole and stem of rape and the harder stems of the tropical grasses and *Desmodium intortum*.

The absence of particles with a rippled surface in rape leaf petiole and stem, lucerne leaf petiole and spurrey leaf may have been due to morphological characteristics: the thick leaf petiole and stem of rape fractured easily upon thorough chewing before swallowing and the thin lucerne leaf petiole and spurrey leaf were easily incorporated into a bolus for swallowing and remained intact after chewing.

After chewing during eating, the forage grass had an equal number or more perforations or ruptures per particle than legumes, rape, spurrey and maize leaf blade (maize stem was not consumed). This would give the rumen micro-organisms equal or greater access in the case of grass particles and an equal or greater opportunity to weaken the particles before chewing during rumination. However, it has been shown that legume particles are reduced into small particles faster during rumination (Moseley 1982) and thus retained in the rumen for a shorter time than are grass particles (Minson 1990). Since the results of the present experiment showed that the particles of grasses and legumes allowed almost equal access to rumen microbes, differences in toughness during rumination chewing could be due to anatomical differences such as cell wall thickness (Wilson 1991) and vascular tissue proportion and arrangement in the particles (Wilson 1985; Kelly & Sinclair 1989). Research to discover rumen microbes or enzymes that can more efficiently destroy or weaken the cell wall (especially the middle lamella) has been suggested by Wilson (1991). Further the results of the present experiment suggest that there is a need for further research on plant physical factors affecting particle breakdown during rumination chewing and on how the characteristics of the chewed particles during eating are related to the further particle breakdown during rumination chewing.

Conclusions

1. In the present experiment, greater variation in dry matter intake rate was found within grasses than within legumes. As in earlier studies legumes were eaten faster and chewed to shorter but broader particles than grasses. Temperate grasses were less vascularized and were eaten faster and chewed to longer particles than the tropical grasses. Tall fescue appeared to be intermediate in neutral detergent fibre content, dry matter intake rate and particle size.

2. The results of the present experiment, as those of Experiment 1, confirmed that the veins of leaf sheaths were more widely spaced than those in leaf blades, but both plant parts were chewed to a similar particle size.

3. The difference between the tropical and temperate grass stem physical breakdown during eating was shown by the results of the present experiment. About 30% of the ryegrass stem particles remained intact after chewing in contrast to the tropical grass stem particles which were almost all crushed before swallowing. This indicated that the solid stems of the tropical grasses (especially when mature) limit forage intake more than the hollow stems of the temperate grasses.

4. An advantage of the thin and/or pliable nature of some species' leaf petioles and stems in relation to intake was clearly indicated in the present experiment by white clover, sainfoin, lucerne and spurrey. All four species had a high

intake rate despite having a high proportion of leaf petiole (white clover and sainfoin) or stem (lucerne and spurrey). A high proportion of these plant part particles, however, remained intact after chewing. Follow up of these intact particles during rumination is necessary so as to understand more about animal response to plant structure.

5. As in Experiment 1 legume leaf petiole and stem contributed substantially to the overall mean particle size of the chewed material and therefore the two plant parts require attention during studies which relate plant structure to forage intake.

6. The results of the present experiment showed that plant physical properties like the arrangement of the vascular tissue could have more effect on eating rate than the proportion of vascular tissue. This was illustrated by *Desmodium intortum*, which had a very large number of tertiary veins per leaflet, a high proportion of neutral detergent fibre and low digestibility, but was eaten at the same rate as white clover and chewed to almost the same particle size.

7. The results of the present experiment support the suggestion of research to discover rumen microbes or enzymes which can more efficiently destroy or weaken the cell wall (especially the middle lamella). This was confirmed after it was observed that chewed particles of grasses and legumes would have allowed almost equal access to rumen microbes and thus an equal opportunity to be weakened if attacked by the right type of microbes or enzymes before chewing during rumination.

CHAPTER 5

EXPERIMENT 3

A STUDY OF PLANT MORPHOLOGY AND VASCULAR STRUCTURE OF SOME GRASSLAND SPECIES IN RELATION TO CHEWING ACTIVITY BY SHEEP

Introduction

The value of grassland herb species in leys has long been recognized (Fagan & Watkins 1932; Fairbairn & Thomas 1959). Moore (1966) noted that hay made from meadows including a large number of grassland herbs was relished by stock and especially by sick animals, which may refuse otherwise good quality hay. He therefore suggested that some grassland herbs may be included in a ley mixture provided they are not allowed to become dominant.

The real value of grassland herb species for livestock has partially been investigated in short studies of palatability, chemical composition and dry matter digestion (Fairbairn & Thomas 1959; Dutt *et al*. 1982; Marten *et al*. 1987). In most cases animals were not included in these investigations and the only intake studies appear to be those of Derrick *et al*. (1993).

If organic farming becomes more common there could be more use of herb species in leys, especially species which survive better in poor soils and have high nutritive value, e.g. ribwort (*Plantago lanceolata* L.) (Fagan & Watkins 1932; Clapham *et al*. 1962) and dandelion (*Taraxacum officinale* Weber) (Long, 1938;

Dutt *et al.* 1982). Wilman & Riley (1993) studied a range of grassland species with respect to chemical composition and physical breakdown when macerated and selected five herbaceous, dicotyledonous species as being of sufficient promise and interest to be compared with perennial ryegrass (*Lolium perenne*) in feeding experiments. These species were: dock (*Rumex obstusifolius* L.), dandelion (*Taraxacum officinale* Weber), chickweed (*Stellaria media* L.) ribwort (*Plantago lanceolata* L.) and spurrey (*Spergula arvensis* L.). The feeding experiments were described by Derrick (1989) and Derrick *et al.* (1993). Samples of the five dicotyledonous species and perennial ryegrass harvested from Derrick's (1987) experiment (referred to by Derrick *et al.* (1993) as Experiment 3) were used in the present project so as to expand further the knowledge of these species with respect to plant structure in relation to eating activities and particle breakdown. The five dicotyledonous species were described briefly by Wilman & Riley (1993).

Materials and Methods

Morphology and vascular structure of different plant parts of the six grassland species (i.e. five dicotyledonous species (see above) and perennial ryegrass) were studied. Samples of these species were harvested on 8-11 September 1987 from R.W.Derrick's experiment at Frongoch Field Station, Aberystwyth, and kept frozen until they were used in the present project in February/March 1991. The field plots were laid out in a randomized block design, with two blocks and six plots per block. Block 1 was sampled on 8 and 10 September and block 2 on 9 and 11 September. On each date one sample was taken from each species. For the present project therefore there were four samples of each of the six species.

On 23 February 1991 samples of perennial ryegrass, ribwort and chickweed were taken out of the freezer to prepare their plant parts for morphological measurements and vascular structure observations and measurements. Samples of spurrey, dock and dandelion were taken out of the freezer on 28 February for the same operation. Perennial ryegrass samples had only leaves. Ribwort had leaves and inflorescence. Chickweed and spurrey had leaves, stem and inflorescence. Dock had mainly leaves with a very small amount of stem and inflorescence on one date (9 September). Two specimens of each green plant part in each sample of each species were cleared in NaOH and their vascular structural arrangement and the number and size of vascular strands were recorded under the light microscope as described in Chapter 3.

In order to record the proportions of plant parts in the herbage, whole samples of all species were taken out of the freezer on 11 and 12 March 1991 and completely separated into their plant parts. About 100 g were taken in the case of samples exceeding 100 g. The plant parts were oven-dried at 80°C for 48 hours and their oven-dry weights used to determine their proportion in the sample. Inflorescence included the inflorescence stalk. Yellowish and dead portions of the plant parts were included in the dead category. Chickweed leaf included the petioles (which were very small and negligible in weight) and perennial ryegrass leaves included leaf sheaths which were relatively negligible in size and amount. Many perennial ryegrass leaf blades were without leaf sheath or with a very short (c. 2 cm) leaf sheath.

In the present experiment each species was statistically analysed separately in the case of all the variables studied. For the proportions of plant parts there were four values per species (3 D.F. for S.E.) and for the other variables eight values per species (7 D.F.).

Results

Spurrey leaf blades' vascular structure, as in Experiments 1 & 2 of this project, was very rudimentary. After they had been cleared, only rudimentary leaf primary veins were observed and there were no other veins. The ranking of the dicotyledonous species in respect of the number of secondary veins per leaf blade was dock > dandelion > chickweed > ribwort and in terms of the number of tertiary veins per leaf blade the ranking was dock > dandelion > chickweed, while in terms of veins (secondary + tertiary veins) per unit leaf blade area the ranking was chickweed > dandelion and dock > ribwort (Table 5.1). The arrangement of ribwort leaf blade veins was like that of a monocotyledon. However, ribwort had very few leaf blade veins, just two on either side of the primary vein (the midrib) and these secondary veins were parallel to the primary vein. The ribwort leaf blade veins were more widely spaced than those of perennial ryegrass (Tables 5.1 and 5.2). In contrast to the perennial ryegrass, ribwort leaf blade secondary veins had rudimentary tertiary veins (not as conspicuous as those of the other dicotyledonous species) ramifying within the space between them.

The ranking of the dicotyledonous species with respect of leaf blade primary vein thickness was dandelion > dock > ribwort > chickweed (Table 5.1). When observed under a light microscope (14x magnification), both dandelion and dock leaf blade primary veins were seen to be comprised of three to five veins closely packed together. The distance between adjacent secondary veins of the dicotyledonous leaf blades was greatest in dandelion followed by dock, then ribwort and then chickweed. Leaf blade veins in perennial ryegrass were closer together than the secondary veins of any of the dicotyledonous species leaf blades (Tables 5.1 and 5.2). The thickness of the leaf blade primary vein or midrib of perennial ryegrass was similar to that of chickweed.

Dock leaf petiole had the greatest number of vascular bundles, followed by ribwort and dandelion (Table 5.1). The three species did not differ significantly in terms of the diameter of leaf petiole vascular bundles.

Spurrey had thinner stem internodes than chickweed (Table 5.3). Spurrey stem internodes had more, but thinner, vascular bundles than those of chickweed. Only one sample of

Table 5.1. Leaf blade and leaf petiole vascular structure of dock, dandelion, chickweed and ribwort. Means ± standard error (7 D.F.)

	Dock	Dandelion	Chickweed	Ribwort
Number of secondary veins per leaf blade	38·3 ±2·53	23·4 ±0·87	12·4 ±0·53	4·0 ±0·00
Number of tertiary veins per leaf blade	166·8 ±9·36	60·4 ±0·93	25·3 ±1·91	-
Angle of attachment of secondary veins to the primary vein of leaf blade	53·0 ±1·91	56·2 ±2·11	43·9 ±3·86	-
Number of secondary + tertiary veins per cm^2 of leaf blade	2·79 ±0·286	2·94 ±0·243	15·09 ±2·221	0·19 ±0·009
Diameter (mm) of primary vein of leaf blade	1·111 ±0·0508	1·690 ±0·0911	0·173 ±0·0126	0·315 ±0·0112
Distance (mm) between adjacent secondary veins of leaf blade	6·73 ±0·121	10·16 ±0·425	3·22 ±0·229	4·91 ±0·080
Number of vascular bundles per leaf petiole	12·75 ±0·412	5·00 ±0·000	-	6·00 ±0·000
Diameter (μm) per vascular bundle of leaf petiole	225 ±10·4	218 ±7·3	-	251 ±21·6

Table 5.2. Perennial ryegrass leaf blade vascular structure. Means ± standard error (7 D.F.)

Number of veins (excluding the midrib)	Large	4·63 ± 0·108
	Small	10·15 ± 0·213
Diameter (μm) of veins	Large	110·0 ± 0·75
	Small	80·0 ± 0·58
Diameter (μm) of midrib		164 ± 12·5
Distance (μm) between adjacent veins regardless of size		101 ± 5·3
Percentage of leaf blade width occupied by sum of vein diameters and midrib diameter		48·5 ± 0·78

Table 5.3. Stem internode morphology and vascular structure of dock, chickweed and spurrey. Means ± standard errors (7 D.F.)

	Dock*	Chickweed	Spurrey
Diameter (mm) per stem internode	2·75	1·96 ±0·057	1·32 ±0·037
Number of vascular bundles per stem internode	19·6	5·5 ±0·27	10·6 ±0·65
Diameter (μm) per vascular bundle	240	243 ±19·0	131 ±12·1
Percentage of cross sectional area of stem internode occupied by vascular bundles	8·9	8·5 ±0·45	10·4 ±0·85

* Standard error was not determined because stem was present in only one sample.

dock had stem. Dock stem appeared to be thicker and to have more vascular bundles than the stems of chickweed and spurrey.

Dock, dandelion and perennial ryegrass had the same proportion of green leaf blade, a proportion greater than those of the other three species (Table 5.4). Dock had more leaf petiole than dandelion and ribwort. Spurrey had the lowest proportion of green leaf blade. Chickweed and spurrey had similar proportions of green stem. The ranking of species in terms of the proportion of inflorescence was ribwort > spurrey > dandelion and chickweed. Only one sample of dock had stem and inflorescence.

The lengths of leaf blades of dock and dandelion were similar; the former had wider and hence larger leaves than the other species (Table 5.5). The needle-like leaf blades of spurrey were much thinner and smaller than those of the other species. The ranking of species in terms of area per leaf blade was dock > dandelion > ribwort > perennial ryegrass > chickweed > spurrey.

Table 5.4. Proportion of plant parts (% in dry matter) of dock, dandelion, chickweed, ribwort, spurrey and perennial ryegrass harvested on 8-11 September 1987. Means ± standard error (3 D.F.)

Plant part	Dock	Dandelion	Chickweed	Ribwort	Spurrey	Perennial ryegrass
Green leaf blade	72·6 ±1·36	72·2 ±4·17	36·9 ±2·58	25·8 ±4·90	14·6 ±1·95	72·3 ±7·09
Dead leaf blade	–	–	–	–	–	27·7 ±3·69
Green leaf petiole	23·7 ±3·52	13·6 ±1·99	–	7·8 ±2·03	–	–
Green stem	1·9*	–	57·7 ±2·20	–	52·4 ±2·54	–
Inflorescence	1·9*	14·3 ±6·15	5·4 ±1·01	66·4 ±6·88	33·0 ±4·20	–

* Standard error was not determined because stem and inflorescence were present in only one sample.

Table 5.5. Leaf blade dimensions of dock, dandelion, chickweed, ribwort, spurrey and perennial ryegrass. Means ± standard error (7 D.F.)

Leaf blade dimensions	Dock	Dandelion	Chickweed	Ribwort	Spurrey	Perennial ryegrass
Leaf blade length(cm)	12·8 ±7·71	12·3 ±0·97	1·98 ±0·152	17·9 ±0·37	3·00 ±0·021	20·6 ±0·74
Leaf blade width (cm)†	6·8 ±2·16	3·3 ±0·18	1·38 ±0·075	2·4 ±0·68	0·08 ±0·005	0·27 ±0·164
Area per leaf blade(cm^2)*	80·8 ±11·11	31·0 ±3·23	2·78 ±0·336	21·8 ±1·05	0·24 ±0·008	5·2 ±0·44

† Width at the broadest point for the first four species and at the mid point for the last two species.

* For the first four species area per leaf blade was the area covered by the leaf blade on graph paper and for the last two species area per leaf blade was calculated by using Kemp's (1960) formula (leaf area = length x width x 0·905).

Discussion

Wilman & Riley (1993) reported that chickweed and spurrey had a similar particle breakdown index. Also Derrick (1989) found that chickweed resembled spurrey in physical breakdown. In Experiments 1 and 2 of the present project, spurrey broke down more readily and had a higher rate of intake than the other species and this was associated with low resistance to chewing due to its thin fragile leaves and stems (with fragile nodes). In the present experiment, chickweed had thin stems which were fragile at the nodes like those of spurrey. Chickweed stems had fewer, thicker vascular bundles than spurrey stems. However, the vascular bundles occupied slightly less of the stem cross sectional area in chickweed than in spurrey. Chickweed had small soft leaf blades which had the highest number of veins (secondary plus tertiary) per unit area. It might be due to all these morphological and plant structure features that chickweed had the same physical breakdown and eating activity characteristics as spurrey. From this point of view, then, plant morphology and physical structure seem to be confirmed by the present experiment as being important features affecting plant physical breakdown and intake. Plants with thin fragile leaves and thin fragile stems (breaking easily at the nodes), despite having a greater proportion of stem than leaves in their feed material, seem to promote high intake, with the result that their presence in grassland may be of some benefit.

Wilman & Riley (1993) and Derrick (1989) found that the particle breakdown of ribwort seemed to be closer to that of perennial ryegrass than to that of dicotyledonous species such as chickweed and dock. The parallel arrangement of veins (similar to that of monocotyledonous leaf blades) probably makes the leaf blade more resistant to breakdown in the mouth and rumen than a reticulate arrangement (typical of dicotyledonous leaf blades or leaflets) (Wilson 1985). Ribwort leaf blade veins were, however, much fewer in number and much more widely spaced than those of perennial ryegrass leaf blades and this could be associated with the lower fibre content in ribwort than in perennial ryegrass reported by Wilman & Riley (1993). Another feature which could increase ribwort's resistance to physical breakdown and the sheep's reluctance to eat it (noted by Derrick (1989)) could be its high proportion of tough inflorescence stalks. Like dandelion (Marten *et al*, 1987) and many other species, it seems that reproductive development reduces the palatability and intake potential of ribwort; grazing or cutting before flowering would be an advantage from an intake point of view.

Dandelion had more widely spaced leaf blade secondary veins than any other species and dandelion had also the second highest number of tertiary veins per leaf blade area. These vascular structural features of dandelion might have contributed to its high intake rate, comparable to that of chickweed and spurrey (Derrick *et al*. 1993). However, Wilman & Riley (1993) noted that dandelion leaf blades had a lower particle breakdown index than those of chickweed and Derrick (1989) reported a high percentage (53%) of total dandelion oesophageal extrusa sample dry matter retained above the 1 mm sieve, which was comparable to that in extrusa samples of perennial ryegrass and ribwort and greater than those of chickweed, spurrey and dock. This physical breakdown characteristic of dandelion could be due to its thick leaf blade primary vein and/or perhaps due to its being less manipulated as compared to dock (Derrick *et al.* 1993) and therefore swallowed faster without time for thorough chewing. In Experiments 1 and 2 of the present project most of the quickly eaten species had large particle size in the oesophageal extrusa samples.

The vascular structure of dock's leaf blade helps to explain its high particle breakdown index reported by Wilman & Riley (1993). However, the vascular structure of dock's stem and leaf petiole seems to be more resistant to breakdown. Derrick (1989) reported a high particle breakdown index for both extrusa samples and macerated samples of leafy dock. Derrick (1989) was surprised by the low intake rate of fresh dock (particularly when chopped). He assumed that it could be due to the taste and/or the smell of the dock, especially when chopped. This reinforces the need, as far as possible, to include animals when evaluating the potential nutritive value of grassland species.

Conclusion

The results from the present experiment have clearly indicated the influence of plant morphology on feed material particle breakdown and intake. Despite containing more stem than leaves, chickweed and spurrey were eaten faster and broken down to smaller particles than the other grassland species because the two species had thin fragile stems (breaking easily at the nodes) and the leaves were also fragile, especially those of spurrey.

Vascular structural arrangement is an important factor affecting plant part breakdown and therefore intake. This was confirmed in the present experiment by ribwort. Ribwort although a dicotyledonous species has leaf blade veins with a parallel arrangement resembling that of perennial ryegrass leaf blades. As a result, ribwort particle breakdown is closer to that of perennial ryegrass than to those of other dicotyledonous species.

The experiment suggests that a full investigation of the potential nutritive value of grassland species should include study of morphology and physical structure and of their influence on the ease of particle breakdown in the mouth and rumen and therefore on intake by the animal.

CHAPTER 6

EXPERIMENT 4

EFFECT OF PLANT MATURITY ON THE PLANT PHYSICAL STRUCTURE AND NUTRITIVE VALUE OF FOUR FORAGE SPECIES

Introduction

Plant morphology, histology and chemical composition change with maturity. As the plant matures, the proportions of stem and cell wall content increase and the proportions of leaf and cell content decline (Sullivan 1973; Minson 1990). With increasing maturity, therefore, the quality of forage plants declines. The quality of the stems, however, declines much faster than that of the leaves (Terry & Tilley 1964; Deinum 1974; Wilman & Altimimi 1982) because the cell wall content increases at a faster rate in the stems as compared to the leaf (Reid *et al*. 1973). Also the proportion of vascular tissue is higher in stem than in leaf blade and sheath, giving rise to much greater potential for reduction in digestibility as lignification proceeds (Wilson 1990).

The proportion of vascular tissue varies little with the age of an individual leaf (Akin & Burdick 1975; Wilson 1976) and its position on the stem (Wilson 1990). However, marked changes may occur in their cell wall thickness and/or lignification to cause a decrease in digestibility with age. The proportion and degree of cell wall thickening of vascular bundles can vary considerably between plant genotypes (Schank *et al.* 1973; Wilson *et al.* 1989). Schank *et al.* (1973) reported that a *Hemarthria* selection with the smallest stem vascular bundle cross-sectional area, showed hardly an increase in the percentage of lignin and no decrease in digestibility with age. Wilson *et al.* (1989) reported that in genotypes of *Cenchrus ciliaris* the digestibilities of leaf and stem were inversely related to the proportion of vascular tissue in the leaf and stem cross-sectional areas respectively.

The aim of the present experiment was to develop further the subject of the present project by investigating the effect of plant maturity on the number, arrangement, thickness and proportion of vascular tissues of different plant parts of lucerne, sainfoin, white clover and Italian ryegrass grown in the same field experiment and by relating these vascular tissue characteristics to the nutritive value of the respective plant parts. Such information may help to increase understanding as to how different plant parts contribute to the nutritive value of a given herbage. In the present experiment the intervals between harvests were longer than those of Experiments 1 and 2, allowing the plants more time to mature. The species used in the present experiment have been described in Chapters 3 and 4.

Materials and Methods

The morphology, vascular structure and nutritive value of different plant parts of lucerne (*Medicago sativa* L. (cv. Europe)), Giant sainfoin (*Onobrychis viciifolia* Scop.), white clover (*Trifolium repens* L. (cv. Grasslands Huia)) and Italian ryegrass (*Lolium multiflorum* cv. RvP) were studied. The samples (c. 1 kg fresh weight) had been harvested in 1985 from an experiment established in early May 1984 at Frongoch Field Station Aberystwyth. The experimental treatments were the 12 combinations of the four herbage species and three dates of harvest. The dates of harvest were 31 May to 4 June for the first harvest, 1 to 4 July for the second and 14 to 16 August for the third. The mean monthly rainfall, radiation and soil temperature during May to August 1985 at Frongoch Field Station are shown in Table 6.1. Each plot was 2 x 8 m. A randomized-block design was used, with three blocks. The samples were stored frozen in polythene bags until they were taken out for the present experiment in December 1990 to January 1991.

Samples of blocks I, II & III were taken out of the freezer on 17, 18 and 19 December, respectively, for study of plant morphology and vascular structure. Two (fully expanded) green grass leaf blades, two green legume leaflets, two green legume leaf petioles, two green grass leaf sheaths and two green pieces of stem internodes were obtained from each of the 36 samples. The procedures followed in studying the morphology and vascular structure of these plant parts were the same as those described in Chapter 3.

Table 6.1. Mean monthly rainfall, radiation and soil temperature during May to August 1985 (recorded at Frongoch Field Station Aberystwyth)

	May	June	July	August
Rainfall (mm/month)	65	74	88	93
Total monthly hours of bright sunshine	174	177	185	112
Mean monthly soil temperature (°C) at 10 cm	10·4	12·9	15·3	13·7

After cleaning with NaOH, the strength of the stem and leaf petiole vascular bundles was tested by the blunt side of the dissecting scalpel. With gentle disturbance, using a scalpel, the stem vascular bundles separated eaily longitudinally. The blunt side of the dissecting scalpel was used to try to break the stem and leaf petiole vascular bundles transversely by pressing them hard on the bottom surface of the petri dish. Except for the white clover vascular bundles, which broke easily, the bundles were not easily broken. Sainfoin and lucerne stem vascular bundles needed more force to break than those of Italian ryegrass. The force required to break the vascular bundles was greater for late cut stems than for the early cut stems. Only sainfoin leaf petioles had hard vascular bundles; those of white clover and lucerne required little force to break transversely.

The herbage samples were again taken out of the freezer on 27, 28 and 31 December (each block on a different day) to determine the oven-dry weight per leaf blade or leaflet of the species. From each of the 36 samples six leaf blades or leaflets were obtained. The leaf blades and leaflets were thoroughly examined so as to make sure that they were fully expanded, green and free from holes or breaks. The leaf blades and leaflets were then oven-dried at 80°C for 24 hours.

In order to determine the proportions of plant parts, c. 100 g of each sample was separated into green leaf blade or leaflet, dead leaf blade or leaflet, green stem, dead stem, green leaf sheath, dead leaf sheath, leaf petiole and inflorescence, and the parts were oven-dried at 80°C for 48 hours. Leaf petioles included the stipules and inflorescence included the inflorescence stalk. All dead parts of the leaf or stem were cut away and included in the dead proportions. A part of a leaf blade or leaflet which was yellow or brown was classed as dead. After oven drying and weighing, the green leaf blade or leaflet, green stem, green leaf sheath and leaf petiole samples were taken to the Agricultural Sciences Analytical Laboratory at Frongoch for *in vitro* dry matter digestibility determination using the Tilley & Terry (1963) method (after milling through a 1 mm screen). The amounts of lucerne leaf petiole and white clover stolon were very small and these were discarded after oven drying and weighing. The determination of the proportions of plant parts was conducted on 2 to 10 January 1991. On 15 to 18 January, c. 2·5 to 3·0 g each of green leaf blades or leaflets, green stems, green leaf sheaths and green leaf petioles (except lucerne leaf petioles and white clover stolons which were negligible in the sample) were obtained from each sample and freeze dried for neutral detergent fibre determination (as an estimate of the proportion of cell wall) using the Van Soest & Wine (1967) method.

The results were analysed using the following analysis of variance:

Source of variation	d.f.
Blocks	2
Species	3
Harvesting date	2
Species x harvesting date	6
Error	22
Total	35

The morphology and vascular structure results of Italian ryegrass leaves were analysed independently from those of the legumes by using the following analysis of variance:

Source of variation	d.f.
Blocks	2
Harvesting date	2
Error	4
Total	8

Results

White clover had the most secondary veins per leaflet (Table 6.2). The ranking of species in terms of the number of tertiary veins per leaflet was white clover > lucerne > sainfoin and in terms of the number of veins (secondary + tertiary veins) per unit area the ranking was sainfoin > lucerne > white clover. The distance between adjacent secondary veins was similar in lucerne and white clover leaflets and less in sainfoin leaflets. White clover leaflets had the thickest primary veins followed by lucerne and then sainfoin. The angle of attachment of the secondary veins to the primary vein was similar in lucerne and sainfoin leaflets and wider in white clover leaflets.

Lucerne had the thinnest leaf petioles with fewest vascular bundles (Table 6.3). White clover had thicker leaf petioles than sainfoin. However the two species did not differ significantly in terms of the number of vascular bundles per leaf petiole nor in the diameter per leaf petiole vascular bundle. The proportion of leaf petiole cross sectional area occupied by vascular bundles did not change significantly with date of harvest in white clover and increased with delay in date of harvest in sainfoin and lucerne (Table 6.4). In May the three species did not differ significantly in terms of the proportion of leaf petiole cross sectional area occupied by vascular bundles, but by August the proportion was highest in sainfoin, intermediate in lucerne and least in white clover.

The ranking of species in terms of area per leaf blade or leaflet was Italian ryegrass > white clover > lucerne and sainfoin and in terms of oven dry weight per leaf blade or leaflet the ranking was Italian ryegrass > white clover > lucerne > sainfoin (Tables 6.5 and 6.6). The ranking of the species in terms of oven dry weight per unit leaf blade or leaflet area was, however, lucerne > sainfoin > white clover > Italian ryegrass.

Table 6.2. Leaflet vascular structure of lucerne, sainfoin and white clover. Mean of three harvesting dates (31 May, 1 July and 14 August 1985)

Leaflet vascular structure	Species			SE±	Mean
	Lucerne	Sainfoin	White clover		
Number of secondary veins per leaflet	33·0	35·1	39·8	1·39	36·0
Number of tertiary veins per leaflet	54·3	31·0	65·8	1·98	50·4
Total number of veins (secondary + tertiary) per cm^2 leaflet area	51·6	81·3	21·9	4·74	51·6
Distance (mm) between adjacent secondary veins	1·671	1·192	1·630	0·0461	1·498
Diameter (μm) of leaflet primary veins	178·2	164·1	228·4	4·22	190·2
Angle of attachment of secondary veins to the primary vein	27·9	28·1	52·2	0·93	36·1

Table 6.3. Leaf petiole diameter (mm), number of vascular bundles and mean diameter (μm) of vascular bundles of lucerne, sainfoin and white clover. Mean of three harvesting dates (31 May, 1 July and 14 August 1985)

Leaf petiole	Species			SE±	Mean
	Lucerne	Sainfoin	White clover		
Diameter (mm) of leaf petiole	1·25	1·68	2·33	0·141	1·75
Number of vascular bundles per petiole	3·0	7·8	6·5	0·47	5·8
Diameter (μm) per petiole vascular bundle	243	268	278	10·9	265

Table 6.4. Proportion (as a percentage) of leaf petiole cross sectional area occupied by vascular bundles in lucerne, sainfoin and white clover

	Date of harvest (1985)			
Species	31 May	1 July	14 August	SE±
Lucerne	7·6	10·4	18·1	
Sainfoin	10·6	20·2	29·6	2·92
White clover	6·2	12·6	9·1	

Table 6.5. Morphology and vascular structure of Italian ryegrass leaf blade and leaf sheath. Mean of three harvesting dates (31 May, 1 July and 14 August, 1985)

	Leaf blade	Leaf sheath
Dimensions		
Length (cm)	27·4 ± 1·57	-
Width (cm)	0·56 ± 0·046	0·75 ± 0·123
Area (cm^2)	13·89 ± 0·412	-
Oven dry weight (mg)	21·5 ± 0·70	-
Oven dry weight/leaf blade area (mg/cm^2)	1·55 ± 0·159	-
Vascular structure		
Number of small veins	12·80 ± 0·301	-
Number of large veins	8·00 ± 0·000	19·8 ± 0·83
Diameter (μm) per small vein	73 ± 13·6	-
Diameter (μm) per large vein	117 ± 16·3	135 ± 10·6
Diameter (μm) of midrib	171 ± 7·3	-
Distance (μm) between adjacent veins regardless of size	171 ± 12·2	244 ± 14·1
Percentage of leaf blade and leaf sheath width at the mid point occupied by vein diameters (and mid rib diameter in leaf blade)	36·5 ± 1·21	35·6 ± 1·22

Table 6.6. Area (cm^2), oven dry weight (mg) and oven dry weight per unit area (mg/cm^2) of leaflets of lucerne, sainfoin, white clover. Mean of three harvesting dates (31 May, 1 July and 14 August 1985)

	Lucerne	Sainfoin	White clover	SE±	Mean
Area (cm^2) per leaflet	2·10	0·91	5·70	0·412	2·90
Oven dry weight (mg) per leaflet	7·6	2·5	13·0	0·70	7·7
Oven dry weight per unit leaflet area (mg/cm^2)	3·66	2·97	2·30	0·159	2·97

In Italian ryegrass the veins were more widely spaced in the leaf sheath than in the leaf blade (Table 6.5). Sainfoin had the thickest stems with the most and the thickest vascular bundles (Table 6.7). White clover stolons were thinner than the stems of lucerne. Italian ryegrass and lucerne did not differ significantly in terms of stem thickness. However, the lucerne stems had more and thicker vascular bundles than the Italian ryegrass stems. The ranking of species in terms of the number of stem vascular bundles was sainfoin > lucerne > Italian ryegrass > white clover. The vascular bundles in Italian ryegrass stems were thinner and generally occupied a lower proportion of the stem internode cross sectional area than those in legume stems (Tables 6.7 and 6.8). The proportion of stem internode cross sectional area occupied by vascular bundles was increased by delaying the date of harvest (Table 6.8).

The proportions of both green leaf blade and green leaf sheath in Italian ryegrass herbage decreased with delay of the date of harvest (Table 6.9). The proportion of dead leaf blade increased with delay of the date of harvest while that of dead leaf sheath was lowest in May and did not change significantly between the July and August harvests. The proportion of green stem was highest in July and similar at the May and August harvests. At the May harvest there was no dead stem, in July there was a small proportion and in August the highest proportion. The proportion of inflorescence was higher at the August harvest than in May.

Table 6.7. Stem internode cross sectional area (mm^2), number of vascular bundles and mean diameter (μm) of vascular bundles of lucerne, sainfoin, white clover and Italian ryegrass. Mean of three harvesting dates (31 May, 1 July and 14 August 1985)

	Species					
Stem internode	Lucerne	Sainfoin	White clover	Italian ryegrass	SE±	Mean
Cross sectional area (mm^2)	9·2	12·7	5·9	7·4	0·74	8·8
Number of vascular bundles	27·8	33·3	9·7	20·1	0·50	22·7
Diameter (μm) per vascular bundle	294	363	280	171	8·6	277

Table 6.8. Proportion (as a percentage) of stem internode cross sectional area occupied by vascular bundles in lucerne, sainfoin, white clover and Italian ryegrass

Species	Date of harvest (1985)			SE±
	31 May	1 July	14 August	
Lucerne	12.8	22·4	34·5	2·37
Sainfoin	19·0	30·3	33·5	
White clover	3·7	11·9	18·2	
Italian ryegrass	3·8	7·0	9·5	

Table 6.9. Proportion (as a percentage of total herbage dry matter) of plant parts of Italian ryegrass

Plant part	Date of harvest (1985)			SE±	Mean
	31 May	1 July	14 August		
Green leaf blade	27·4	9·1	6·0	0·68	14·2
Dead leaf blade	2·6	7·7	15·7	1·17	8·7
Green stem	37·3	46·4	37·4	1·10	40·4
Dead stem	–	1·3	10·4	0·29	3·9
Green leaf sheath	25·3	12·3	5·3	0·34	14·3
Dead leaf sheath	0·5	10·9	8·6	1·18	6·7
Inflorescence	6·9	12·3	16·9	2·27	12·0

Of the three legumes, white clover had the highest proportion of green leaflets, green leaf petiole and inflorescence (Table 6.10). Lucerne and sainfoin were not significantly different in the proportions of green leaflets and inflorescence. The ranking of species in terms of the proportion of green stem was lucerne > sainfoin > white clover and the ranking in terms of green leaf petiole was white clover > sainfoin > lucerne. The proportion of green leaflets was generally high at the May and low at the August harvest and vice versa for the proportion of green stem.

The green leaflets of sainfoin were less digestible than the leaflets or leaf blades of the other species on all three dates of harvest (Table 6.11). The Italian ryegrass leaf blades and sainfoin green leaflets were more digestible at the May than at the July and August harvests. The digestibilities of the green leaflets of lucerne and white clover were not significantly affected by the date of harvest.

Neutral detergent fibre contents were higher at the August than at the May harvest in Italian ryegrass green leaf blades and sainfoin green leaflets, and little affected by date of harvest in lucerne and white clover green leaflets (Table 6.11). At all three harvests, Italian ryegrass green leaf blade had the highest neutral detergent fibre content.

On all dates of harvest, the digestibility of green leaf petioles was higher in white clover than in sainfoin (Table 6.12).

Table 6.10. Proportion (as a percentage of whole herbage dry matter) of plant parts of lucerne, sainfoin and white clover. Mean of three harvesting dates (31 May, 1 July and 14 August 1985)

Plant part	Species			SE±	Mean
	Lucerne	Sainfoin	White clover		
Green leaflets	23·5	25·4	41·2	1·68	30·0
Green stem	72·0	60·2	7·7	1·70	46·6
Green leaf petiole	2·1	13·5	45·0	0·65	20·2
Inflorescence	3·6	1·5	9·3	1·00	4·8

Table 6.11. *In vitro* dry matter digestibility (%) and neutral detergent fibre (cell wall content, % in dry matter) of green leaflets of lucerne, sainfoin and white clover and green leaf blade of Italian ryegrass

Species	Date of harvest (1985)			SE±
	31 May	1 July	14 August	
	In vitro dry matter digestibility			
Lucerne	78·9	75·8	72·6	1·99
Sainfoin	68·2	59·0	56·1	
White clover	79·8	77·2	76·6	
Italian ryegrass	83·4	73·2	69·8	
	Neutral detergent fibre			
Lucerne	19·4	22·8	22·9	2·01
Sainfoin	26·4	28·6	38·2	
White clover	17·7	21·4	21·3	
Italian ryegrass	46·6	50·8	58·8	

The digestibility of white clover green leaf petiole did not change with advancing date of harvest. However the digestibilities of sainfoin green leaf petiole and Italian ryegrass green leaf sheath decreased significantly with advancing date of harvest.

On all dates of harvest green leaf sheath had higher neutral detergent fibre than green leaf petioles (Table 6.12). The difference between the sainfoin and white clover green leaf petiole neutral detergent fibre contents was small at the May and July harvests. At the August harvest, however, the petioles of sainfoin were significantly higher in neutral detergent fibre than those of white clover.

The digestibilities of the green stems of lucerne, sainfoin and Italian ryegrass decreased significantly with advancing date of harvest (Table 6.13). Italian ryegrass green stems were more digestible than those of sainfoin and lucerne.

The neutral detergent fibre content of the green stems increased significantly with advancing date of harvest, particularly in lucerne and sainfoin (Table 6.13). At the August harvest lucerne green stem had the highest neutral detergent fibre content.

Table 6.12. *In vitro* dry matter digestibility (%) and neutral detergent fibre (cell wall content; % dry matter) of green leaf petioles of sainfoin and white clover and green leaf sheaths of Italian ryegrass

Species	Date of harvest (1985)			SE±
	31 May	1 July	14 August	
	In vitro dry matter digestibility			
Sainfoin	70·6	64·2	50·4	1·34
White clover	79·3	77·4	77·1	
Italian ryegrass	72·4	63·6	58·7	
	Neutral detergent fibre			
Sainfoin	40·4	45·2	59·0	2·31
White clover	39·4	42·3	39·5	
Italian ryegrass	62·2	63·4	68·9	

Table 6.13. *In vitro* dry matter digestibility (%) and neutral detergent fibre (cell wall content; % in dry matter) of green stem of lucerne, sainfoin and Italian ryegrass

Species	Date of harvest (1985)			SE±
	31 May	1 July	14 August	
	In vitro dry matter digestibility			
Lucerne	57·0	46·6	41·2	
Sainfoin	66·0	52·9	43·0	2·28
Italian ryegrass	70·9	64·8	55·3	
	Neutral detergent fibre			
Lucerne	51·0	58·8	72·2	
Sainfoin	46·6	55·3	64·4	1·55
Italian ryegrass	53·4	55·1	61·9	

Discussion

White clover had the most secondary and tertiary veins per leaflet, followed by lucerne and sainfoin. The same order was followed in terms of area and weight per leaflet. Sainfoin had closely spaced secondary veins and the most veins (secondary + tertiary) per unit area of leaflet. In Experiment 2 sainfoin leaves were less broken down in the mouth than those of white clover and lucerne. Thus the large number of veins per unit leaflet area in sainfoin may not indicate high physical breakdown (Wilson 1985), but presumably contributed to the higher neutral detergent fibre content and lower digestibility of sainfoin noted in Experiments 2 and 4.

As was observed in Experiment 2 Italian ryegrass leaf blade veins were on average $>$ 7X closer than the secondary veins in the leaflets of the legumes. The distance between veins became even less (227 µm to 120 µm; SE± 12·2) following the decrease of leaf blade width (6·5 mm to 4·3 mm; SE± 0·46) and unchanged number of veins (20·0 to 20·5; SE± 0·15) with delay of the date of harvest from May to August. This could mean that with advancing plant maturity and partly due to higher temperature (Table 6.1),Italian ryegrass leaf blades become harder to break in the mouth and rumen not simply because of increased fibre content but also because of the closeness of the veins.

The digestibilities of the green leaf blades or green leaflets of Italian ryegrass, lucerne and white clover were similar, and higher than those of sainfoin. A lower digestibility of sainfoin green leaflets as compared to those of lucerne and red clover and the green leaf blades of hybrid ryegrass has been reported by Wilman & Asiedu (1983). The digestibilities of the green leaflets of sainfoin and the green leaf blades of Italian ryegrass decreased, while those of the green leaflets of lucerne and white clover remained almost constant, with delay of harvest date. This trend reflected the increase in neutral detergent fibre content with delay of harvesting date in the green leaf blades of Italian ryegrass (also reported by Wilman & Altimimi (1982)) and green leaflets of sainfoin, as compared to the neutral detergent fibre content in the green leaflets of white clover and lucerne which was relatively unaffected by date of harvest. This could mean that Italian ryegrass and sainfoin require relatively frequent defoliation if they are to provide leaves with low cell wall content and high digestibility.

Leaf petiole thickness and the number of vascular bundles per leaf petiole did not change significantly with delay of harvesting date. Information on the relationship between the vascular structure and the nutritive value of leaf petioles is rather scarce (Wilson 1991). In the present experiment, the percentage of the cross sectional area of leaf petioles occupied by vascular tissue was negatively related to the digestibility of the petioles ($r = -0{\cdot}96$; $P < 0{\cdot}01$; $n = 6$ (for white clover and sainfoin on three harvesting dates)). The anatomical structure of leaf petioles may therefore have a substantial influence on the nutritive value of forage especially in legumes with a high proportion of leaf petiole.

The green stems of Italian ryegrass were more digestible than those of lucerne and sainfoin at the July and August harvests. This was in agreement with Demarquilly & Jarrige (1974) who reported that, during the first cycle of growth, the digestibility of lucerne stems decreased faster with plant maturity than that of Italian ryegrass stems. Deinum (1974) noted from the literature that, the higher the proportion of stem vascular bundles, the lower the digestibility and the greater the depression of digestibility with age due to increased lignification. A negative relationship between the proportion of vascular bundles in the stem and the digestibility of the stem of forage species has been reported by Shenk & Elliot (1971) and Wilson *et al*. (1989). Such findings are in agreement with the results obtained in the present experiment. The digestibility of the green stems of the species in the present experiment was negatively related ($r = -0{\cdot}83$; $P < 0{\cdot}01$; $n = 9$ (for lucerne, sainfoin and Italian ryegrass on three harvesting dates)) to the proportion of stem cross-sectional area occupied by vascular bundles. Sainfoin had more and thicker vascular bundles, which occupied a greater stem internode cross sectional area, than lucerne or Italian ryegrass. Also, sainfoin and lucerne had a much higher proportion of stem than Italian ryegrass. Therefore, as suggested earlier by Terry & Tilley (1964) and Wilman & Asiedu (1983), the results of the present experiment confirm that lucerne and sainfoin require rather frequent defoliation if they are to provide stem which is digestible and readily eaten.

The drawback of this is that they are likely to be less persistent and lower yielding with frequent defoliation (e.g. Wilman 1977). Other suggestions given by Shenk & Elliot (1971) and Wilson (1991) are to breed varieties with a reduced proportion of lignified vascular tissue and/or alter the arrangement of the stem vascular tissue, but with care not to destroy the adaptation of the plant to its environment.

Conclusion

In addition to the results of Experiments 1 and 2, the results of the present experiment have shown that in some species the differences between plant parts in terms of nutritive value may not be fully identified through analysis of chemical composition and digestibility without observing the vascular structure. This was the case with white clover, whose leaflets and leaf petioles showed very little difference in terms of digestibility and neutral detergent fibre content, but, due to structural differences, leaf petiole may affect the reduction of herbage particle size during eating as shown in Experiments 1 and 2 of this project.

From the results of the present experiment it seems that there is a need to investigate further how lucerne and sainfoin nutritive value could be improved by breeding of varieties with reduced lignified vascular tissues. Also, as was suggested earlier, lucerne and sainfoin require rather frequent defoliation if they are to provide young stems with high digestibility and low neutral detergent fibre content.

In addition to the results of Experiment 2, the results of the present experiment show that Italian ryegrass should be harvested when leafy, to avoid the stems and narrow leaves produced in a more mature crop, which seem to be tough to break down due to their veins being close together and due to a relatively high neutral detergent fibre content.

Also from the results of the present experiment, it seems that with plant maturity plant part nutritive value may change not mainly due to the number of vascular strands, but also possibly due to the changes in secondary cell wall thickening and size of plant part (which influence arrangement of the vascular strands) that may influence physical breakdown of the plant in the mouth and rumen of the animal.

CHAPTER 7

EXPERIMENT 5

PHYSICAL STRUCTURE AND PARTICLE BREAKDOWN CHARACTERISTICS DURING EATING BY CATTLE OF LUCERNE HAY AND SOME CROP RESIDUES FROM CHINA

Introduction

In many parts of the world crop residues may form a major part of the ruminant diet especially during the dry or winter seasons. Some crop residues are normally green and fresh (e.g. sugar cane tops and sweet potato vines) and some are dry and highly lignified (e.g. cereal straws and stovers). The nutritive value of crop residues is highly variable and depends on the species and variety of crop, farming methods, harvesting date and environmental conditions prevailing in the area (Staniforth 1979; Sundstøl 1988). Among the various crop residues, cereal straw and stovers have generally low intake due to their low rate of particle reduction during eating and rumination (Lee & Pearce 1984; Ribeiro 1989). There is, however, some evidence that straws of some cereal species are eaten more quickly than others (Lee & Pearce 1984; Chesson & Murison 1989). In addition to chemical composition (Chesson & Murison 1989), morphology (Sundstøl 1988) and anatomy (Staniforth 1979) of the different crop residues, the proportions of plant parts may influence intake. Sundstøl (1988) noted that barley and oats straw are eaten more than wheat and rye straw because the former species' straws are softer than those of the latter. The reason why barley and oats straws are softer than wheat and rye straws could be differences in stem internode anatomy noted by Staniforth (1979). He noted that barley and oat straw stem internodes had thinner walls and larger lumen than those of wheat and rye straw. Such an example shows that selection of suitable crop residues for feeding animals can be based partly on the morphological and anatomical structure of the plant parts.

The purpose of the present experiment was to extend the project by examining the morphology and vascular structure of plant parts and particle breakdown during eating by cattle of various crop residues. Lucerne (*Medicago sativa* L.) hay was also included. Lucerne is important in many parts of the world as a protein rich supplementary forage. As it matures the lower half becomes more stemmy than the upper half (Wilman & Asiedu 1983). In the present experiment, therefore, the lucerne hay was divided into two parts, the upper and the lower half. Millet (*Setaria italica* (L.) Beav.) an annual crop that produces a slender stem and leafy straw (Fribourg 1985) was also included. Wheat (*Triticum vulgare* vill.) and rice (*Oryza sativa* L.) are two of the three major cereal crops (another one is maize (*Zea mays*)) of the world (FAO 1992) and their straws were included. Approximately 90% of sweet potato (*Ipomoea batatas* (L.) Lam.) in the world is grown in Asia, and China is the world's largest producer of this crop (Woolfe 1992). The leaves of sweet potatoes are relished by livestock (Göhl 1981). Sweet potato tops were included in the experiment.

Materials and Methods

In September 1991, samples of chewed and unchewed lucerne hay upper and lower half, millet straw leaf, millet straw stem, millet straw (leaf and stem) and winter wheat straw were received from Professor Yi-lun Ji, Shanxi Agricultural University, China. Again, in October 1992, samples of chewed and unchewed samples of sweet potato tops and rice straw were received from the same source.

The lucerne was harvested at 50% bloom leaving a 10 cm stubble and dried for hay. The lucerne was divided into two halves (upper and lower). Sweet potato tops were also dried before feeding. All the crop residues and the lucerne hay were harvested by hand and chopped into 2-3 cm pieces before being fed to rumen fistulated cattle. The chewed samples were obtained as the cattle were eating, by placing a hand through the fistula into the reticulo-rumen at the point where the food leaves the oesophagus. The chewed samples were then rinsed slightly with tap water and oven-dried at 60°C for 48 hours before they were packed and sent over to Aberystwyth.

Samples which arrived here in September 1991 were studied in April 1992 and those which arrived in October 1992 were studied in April 1993. About 20 pieces of each plant part of the unchewed samples of lucerne hay and crop residues were soaked in 2·5% NaOH for 24-48 hours so as to study their vascular structure under the light microscope as in the previous experiments of this project.

Each chewed sample was poured onto a polythene sheet placed on a table. It was then mixed gently by hand and *c*. 1 g of the sample was taken and placed in a petri dish (14 cm diameter). Tap water was then added to the samples in the petri dishes and soaked for a night so as to study the particle breakdown characteristics under a light microscope as in Experiments 1 and 2. In the present experiment, however, the particles were not separated into plant parts and only 50 particles per sample were studied. As the feeds were from dry crop residues and lucerne hay, it was not easy to identify the plant part from which a particle had come.

Results

Lucerne hay upper and lower parts were not different in terms of the number of secondary and tertiary veins per leaflet and the distance between adjacent secondary veins of the leaflet (Table 7.1). However the leaflets of the lower part had thicker primary veins than those of the upper part. Leaf petioles of the upper and lower parts of lucerne hay were not different in terms of thickness and vascular structure. Leaf petioles of sweet potato were thicker and had more vascular bundles than those of lucerne. However, the species did not differ significantly in terms of the leaf petiole vascular bundle thickness or in the percentage of the leaf petiole cross sectional area occupied by vascular bundles. Sweet potato stems were thicker than lucerne stem internodes. The lower and upper parts of lucerne hay did not differ significantly in terms of stem internode thickness or vascular structure.

Table 7.1. Morphology and vascular structure of three types of feed from lucerne hay and sweet potato tops

Plant part	Type of feed: Lucerne hay upper part	Lucerne hay lower part	Sweet potato+	SE±*	Mean
Leaflet or leaf blade:					
Secondary veins per leaflet or leaf blade	24·0	24·1	-	1·21	24·1
Tertiary veins per leaflet or leaf blade	31·0	28·5	-	1·93	29·8
Distance(mm)between adjacent secondary veins	1·88	1·95	-	0·115	1·92
Diameter(μm)of leaflet or leaf blade primary vein	137	185	-	10·9	161
Leaf petiole:					
Diameter(mm)of leaf petiole	1·07	0·92	2·53	0·131	1·51
Number of vascular bundles per leaf petiole	3·00	3·00	9·75	0·405	5·25
Diameter(μm)per vascular bundle	103	103	112	7·8	106
Percentage of leaf petiole cross sectional area occupied by vascular bundles	2·85	3·90	3·62	0·426	3·46
Stem:					
Diameter(mm)of stem internode	3·67	3·86	5·10	0·215	4·21
Number of vascular bundles per stem internode	26·0	27·1	-	0·93	26·6
Diameter(μm)per vascular bundle	174	203	-	12·4	189
Percentage of stem internode cross sectional area occupied by vascular bundles	6·3	7·9	-	1·07	7·1

+ There were no whole leaf blades in the sweet potato sample and its stem internode vascular structure was not recorded because NaOH could not clear it properly and it could not be observed clearly under the microscope.

* 14 D.F. for leaflet, 21 D.F. for leaf petiole and 18 D.F. for stem (except the diameter per stem internode with 27 D.F.).

Wheat leaf blades had fewer veins, thinner midribs and thicker small veins than those of millet (Table 7.2). The veins in wheat leaf blades were more widely spaced than those in rice and millet leaf blades. The percentage of leaf blade width occupied by the sum of vein diameters and midrib diameter was higher in millet than in wheat. Small veins in rice leaf blades were thicker than those in millet leaf blades.

Wheat leaf sheaths had fewer veins that were thinner and more widely spaced than those in millet (Table 7.2). Rice and wheat were not significantly different in terms of leaf sheath vein thickness and the distance between adjacent leaf sheath veins. Rice and wheat were also not different in stem internode thickness and had thinner stem internodes than millet. Wheat and millet had more vascular bundles per stem internode than rice. The species did not differ significantly in terms of the percentage of stem internode cross sectional area occupied by vascular bundles.

The range in particle width was from 2·07 mm for lucerne hay upper half to 4·01 mm for sweet potato tops, and the range in particle length was from 12·0 mm for lucerne hay lower half to 20·9 mm for sweet potato tops (Table 7.3). Sweet potato tops particles had relatively high numbers of vascular bundles and perforations or ruptures. Whole millet straw particles had the second highest number of vascular bundles per particle.

The number of intact particles of the lower part of lucerne hay was half that of the upper part but not very different to that of sweet potato tops (Table 7.4). In all species, the percentage

Table 7.2. Morphology and vascular structure of five feeds from cereal straws

Plant part	Millet straw (leaf)	Millet straw (stem)	Whole millet straw	Wheat straw	Rice* straw	SE±†	Mean
	Type of feed						
Leaf blade:							
Number of large veins per leaf blade	11·8	-	11·5	7·6	-	0·76+	10·3
Number of small veins per leaf blade	94·7	-	106·6	27·1	-	4·29+	76·1
Diameter(μm)of leaf blade midrib	541	-	660	182	-	43·5+	461
Diameter(μm)per large vein	129	-	151	133	125	5·4	135
Diameter(μm)per small vein	51·2	-	59·6	74·0	77·0	3·47	65·5
Distance(μm) between adjacent veins	110	-	72	212	120	8·21	129
Percentage of leaf blade width occupied by the sum of vein diameters and midrib diameter	39·2	-	52·0	31·5	-	2·29+	40·9
Leaf sheath:							
Number of veins per leaf sheath	-	-	41·8	35·0	-	1·96‡	38·4
Diameter(μm)of leaf sheath vein	-	-	148	120	127	8·9	132
Distance(μm)between adjacent veins	-	-	241	310	322	21·7	291
Percentage of leaf sheath width occupied by the sum of vein diameters	-	-	39·3	28·3	-	2·82‡	33·8
Stem:							
Diameter(mm)per stem internode	-	4·71	4·41	3·35	3·29	0·28	3·94
Number of vascular bundles per stem internode	-	37·8	39·7	39·8	25·7	2·52	35·8
Diameter(μm)per vascular bundle	-	172	166	135	175	6·6	162
Percentage of stem internode cross sectional area occupied by vascular bundles	-	5·2	5·9	7·6	7·4	0·86	6·5

* Only parts of leaf blade and leaf sheath width (not entire leaf blade or sheath) were present in the rice straw sample.

† 36 D.F. for leaf blade and stem and 27 D.F. for leaf sheath

+ 27 D.F.

‡ 18 D.F.

Table 7.3. Particle width and length, number of vascular bundles per particle, and number of perforations or ruptures per particle of different types of feed after chewing by rumen fistulated cattle during eating (50 particles from each feed were observed)

Type of feed	Particle width (mm)	Particle length (mm)	Number of veins or vascular bundles per particle	Number of perforations/ruptures per particle
Lucerne hay upper part	2·07 ±0·118	13·5 ±0·86	9·6 ±1·36	1·43 ±0·312
Lucerne hay lower part	2·10 ±0·128	12·0 ±0·86	7·2 ±0·90	1·52 ±0·226
Sweet potato	4·01 ±0·279	20·9 ±1·56	22·6* ±2·85	2·27 ±0·239
Millet straw leaf	3·65 ±0·474	14·8 ±0·90	11·4 ±1·33	1·97 ±0·174
Millet straw stem	2·37 ±0·144	13·6 ±0·56	6·2 ±0·41	1·40 ±0·183
Whole millet straw	3·46 ±0·446	15·1 ±0·88	15·4 ±2·45	1·93 ±0·183
Wheat straw	2·57 ±0·247	12·6 ±0·85	8·6 ±0·93	1·96 ±0·259
Rice straw	2·35 ±0·140	18·8 ±1·19	7·9 ±0·80	1·93 ±0·184

* 45 D.F.: Because intact particles of stem were not included.

of particles with jagged ends was lower than that of those with jagged sides. The particles with jagged ends and sides in millet straw stem were < 50% while in the other species the ranges were from 54 to 76% for jagged ends and 74 to 86% for jagged sides. The percentage of particles with a rough surface ranged from 58% in rice straw to 92% in millet stem. In all species the percentage of particles with a rippled surface was $\leq$ 10%.

Discussion

Wilman & Asiedu (1983) reported that sheep tended to be more selective when grazing the lower half than the upper half of lucerne. Also, Wilman & Altimimi (1984) reported that the lower half of lucerne had a higher proportion of stem, higher cell wall content and lower digestibility than the upper half. The results obtained from Shanxi Agricultural University showed that the upper half of the lucerne hay used in the present experiment had a lower neutral detergent fibre content (39% v. 68%), higher digestibility (63% v. 29%) and a higher rate of intake (6·8 g/min v. 3·9 g/min) than the lower half. In the present experiment the upper and lower half of lucerne hay did not differ significantly in terms of plant part morphology (dimensions of leaflets, leaf petioles and stem internodes) or vascular structure or particle breakdown characteristics. This could mean that in some forage crops (as in the case of lucerne) analysis of chemical composition and digestibility could be nearly enough to predict their nutritive value and thus saving costs of long feeding experiments.

Table 7.4. Percentages of total observed particles in different categories (50 particles from each feed were observed)

Type of feed	Intact*	Jagged ends	Jagged sides	Rough surface	Rippled surface
Lucerne hay upper part	30	64	70	72	4
Lucerne hay lower part	14	76	85	82	6
Sweet potato vines	18	60	80	66	10
Millet straw leaf	0	72	86	74	6
Millet straw stem	0	34	46	92	4
Whole millet straw	0	54	82	88	2
Wheat straw	0	56	80	86	0
Rice straw	0	66	74	58	8

* Lucerne had only intact particles of stem while sweet potato had intact particles of both stem and leaf petiole

The particles of chewed lucerne hays (upper and lower), millet straw stem and wheat straw seemed not to differ significantly in terms of the size and number of vascular strands per particle. However, the particles of the latter two diets had slightly more perforations or ruptures per particle and no intact particles. The absence of intact particles in the cereal straws could possibly indicate that the straws required more thorough chewing before swallowing (i.e. had a low rate of particle breakdown) leading to a lower intake rate than that of lucerne hay or sweet potato tops. The results obtained from China showed that whole millet straw had a lower dry matter intake rate (2·6 g/min) than lucerne hay of either lower or upper parts. Further, the presence of intact particles in chewed lucerne hays (which was also observed in fresh lucerne in Experiment 2 of this project) showed that even dry and/or mature lucerne can have a fast rate of intake. A higher intake rate of lucerne hay than of oat or barley straw was also noted by Lee & Pearce (1984). This confirms the value of lucerne as a supplementary forage especially when the animals are fed low quality roughage.

In Experiments 1 and 2 of this project it was noted that species with a high intake rate had in most cases large particles after chewing. This could be the case with sweet potato tops in the present experiment because it had the largest particles of all diets (no data is available for intake rate). Sweet potato tops digestibility ranging from 60 to 80% and crude fibre ranging from 13 to 27% have been reported by Woolfe (1992) and Göhl (1981).

Sweet potato tops, therefore, seem to be a valuable crop residue. Woolfe (1992), however, noted that little has so far been done to improve the use of sweet potato tops as a ruminant feed.

Of the cereal straw diets, rice straw had relatively long particles and the highest proportion of particles with a rippled surface, indicating that rice straw may be softer than, for example, wheat straw, which had no particles with a rippled surface. This could be true because the stem internodes of rice had fewer vascular bundles than those of millet or wheat. This was also noted by Staniforth (1979). Further, in his review of literature, Sundstøl (1988) noted that rice straw had a lower lignin content and as high a digestibility as barley and oat straw. He, however, noted that the high ash content of rice straw reduces its overall feeding value.

Conclusion

The results from the present experiment have shown that some crop residues (e.g. sweet potato tops) are more valuable than others and therefore require an emphasis to improve their use as animal feed.

Evaluating the nutritive value of cereal straws by observing the eating behaviour of animals and the degradations in the rumen seems to be very important. However, in some forage crops (e.g. lucerne) analysis of chemical composition and/or percentage of leaf could be nearly enough to predict their nutritive value, rather than subjecting them to long feeding experiments.

CHAPTER 8

GENERAL DISCUSSION

The dicotyledonous species

The leaflets or leaf blades of the dicotyledonous species studied in the present project tended to differ more in the number of tertiary veins per leaflet or leaf blade than in the number of secondary veins. The smaller leaflets like those of sainfoin and lucerne had, therefore, more closely spaced secondary veins than the larger leaflets or leaf blades like those of *Desmodium intortum*, rape and dock. Among the legumes, *Desmodium intortum* leaflets had the most widely spaced (7·4 mm) secondary veins. *Desmodium intortum* had the most and sainfoin had the least tertiary veins per leaflet. However, the two species did not differ significantly in terms of the number of veins (secondary plus tertiary veins) per unit leaflet area and had more than white clover and lucerne leaflets.

The leaflets of the four legume species were chewed to almost the same particle size, meaning that chewing reduced the larger leaflets more than the smaller leaflets. Sainfoin leaflets were more resistant to breakdown than those of the other three legumes. This was shown by a high proportion of sainfoin leaflet particles that remained intact or were just halved by chewing and had very few or no perforations or ruptures. This was in agreement with Lees *et al.* (1982), showing one of the characteristics of a bloat safe legume.

The larger leaf blades of rape, dock and dandelion had very few veins per unit leaf blade area and thick primary veins. These leaf blades seemed to require more time for prehension during eating. However, once in the sheeps' mouths it seems the sheep concentrated their chewing more on the thick leaf petioles and/or stems instead of the soft leaf blades and then swallowed quickly to compensate for the time lost in prehending the large leaf blades. For this reason the large leaved dicotyledonous species did not differ significantly from the small leaved dicotyledonous species in intake rate and the former species had slightly larger chewed leaf blade particles than the latter ones.

Two of the dicotyledonous species differed markedly from others in leaf blade morphology and leaf blade vascular structure. Spurrey had thin fragile needlelike leaf blades with just a rudimentary primary vein, and ribwort had a lanceolate leaf blade with four secondary veins running parallel, two on either side of the primary vein. The vein arrangement in leaf blades of ribwort therefore resembled those of monocotyledonous species and it was not surprising that in Derrick's (1989) results the ribwort particle size did not differ from that of perennial ryegrass. These results support the idea that plant vascular tissue arrangement has a profound effect in the ease of particle breakdown (Wilson 1985).

Little has been reported on the effect of leaf petiole vascular structure on forage intake (Wilson 1990). In the present project white clover and sainfoin had a high proportion of leaf petiole and these leaf petioles were thin, long and pliable and a high proportion of the particles derived from these leaf petioles remained intact (43% and 63%, respectively) after chewing. Since these leaf petiole particles were c. 2·5x longer than the leaflet particles, they contributed substantially to the mean length of the chewed particles and this could have an effect on the rate of clearing particles out of the rumen. Despite having about the same thickness as leaf petioles of white clover and sainfoin, *Desmodium intortum* leaf petioles had a higher proportion of vascular bundles and seemed to be rather difficult to incorporate quickly into a bolus to swallow. This led presumably to more chewing of *Desmodium intortum* leaf petioles, reflected in a high proportion of particles with jagged sides and a low proportion of particles that remained intact after chewing.

In Experiment 4 of the present project, it was observed that with advancing plant maturity the neutral detergent fibre content increased and digestibility decreased in leaf petioles of sainfoin and remained constant in those of white clover. Such results suggested that with advancing plant maturity sainfoin leaf petiole particles might be retained longer in the rumen than those of white clover. The thicker leaf petioles of rape were chewed to shorter but wider particles than those of the legumes indicating a positive effect of a low proportion of vascular tissue on intake despite the thickness of the plant part. Lucerne had a very low proportion of leaf petiole and the petioles were very thin and short and most of their particles therefore remained intact after chewing. As was suggested by Wilson (1990; 1991) the present results have shown that leaf petiole proportion, morphology and vascular structure need to be considered in studies of the plant factors affecting intake.

Lucerne had the highest proportion of stem, which presumably contributed to its high neutral detergent fibre content (Terry & Tilley 1964). Its stem internodes had twice as many vascular bundles as those of white clover. The two species were, however, eaten at the same rate and a high proportion of their stem particles remained intact after chewing. This indicated that the high proportion of vascular tissues in lucerne stems could possibly not affect intake rate so long as the stems are green, thin, pliable and readily incorporated into a bolus and swallowed. In Experiment 5 of the present project the cattle ate more slowly and it seems chewed more thoroughly the lower part of lucerne hay and thus a lower proportion of stem particles remained intact after chewing as compared to the upper part of lucerne hay. In Experiment 2 *Desmodium intortum* was eaten at the same rate as lucerne and white clover but the former species had no stem particles that remained intact after chewing possibly due to the rather thicker and more rigid stems that had to be chewed thoroughly before swallowing. In Experiment 4 of the present project, sainfoin had the thickest stem internodes with the highest proportion of vascular tissues. In Experiment 2 of the present project, however, sainfoin did not produce stem and thus its stem particle breakdown characteristics remain to be studied in the future.

Among the dicotyledonous species studied in the present project, rape had the thickest stems with a low proportion of vascular tissues. Possibly it was due to this low proportion of vascular tissues in the rape plant parts that it had the lowest neutral detergent fibre content and was eaten as fast as the legumes. Spurrey had thin fragile (at the node) stems and was eaten fastest and most of the stem particles (c. 65%) remained intact after chewing. Both chickweed and spurrey which were observed in the present project to have similar stem morphology and a higher proportion of stem than leaf in the diet, were also found by Derrick (1989) to have a higher intake rate and to be broken easily into smaller particles than other species.

The study of stem (especially of the dicotyledonous species) morphology, vascular structure and physical breakdown in the present project has therefore suggested that slender, pliable or fragile (at the nodes) stems can be eaten quickly and that the presence in the sward of a reasonable proportion of non-legume dicotyledonous species with plants having such stems may not adversely affect the intake of the grazing animal. These findings should encourage agronomists, ruminant nutritionists and managers in different localities to consider more the potential nutritive value of non-conventional grassland species present in their swards and possibly avoid the expense of removing some of these species.

The monocotyledonous species

Perennial ryegrass had the smallest leaf blades which had slightly fewer veins than those of Italian ryegrass but a similar number to those of the larger tall fescue leaf blades. The veins in tall fescue leaf blades were therefore the most widely spaced and this is possibly associated with its rapid drying during hay making (Holmes 1982). The two tropical grasses (*Chloris gayana* and *Cenchrus ciliaris*) had more veins per leaf blade and leaf sheath that were more closely spaced than the three temperate grasses and they had coarse and solid stems. As a result, these tropical grasses had a higher neutral detergent fibre content, lower digestibility, a lower intake rate and were chewed to rather shorter particles than the temperate grasses. These results were in agreement with Wilson *et al.* (1989) and Minson (1990). Regardless of the grass species, leaf sheath veins were more widely spaced than leaf blade veins, but the two plant parts were chewed to particles of almost the same size.

Whether in tropical or in temperate grass chewed materials, stem particles were rather longer than both leaf blade and leaf sheath particles. This was in agreement with Wilson (1990) who noted that stems have a particularly strong structure and are normally eaten more slowly and chewed for a longer time than leaves (McLeod & Smith 1989) to achieve particle size reduction. The negligible number or absence of intact stem particles remaining after chewing in tropical grasses in contrast to temperate grasses reflected the disadvantage of the solid hard stems that require more chewing and thus delay swallowing. Therefore grasses should be cut or grazed when stems are still young and leafy to avoid reduction of intake due to mature stems.

The coarser temperate grass, tall fescue, was intermediate between the tropical and the other two temperate grasses in almost all nutritional variables recorded in the present project. Tall fescue resembled maize in neutral detergent fibre content and intake rate. However, maize leaf blade (the only maize plant part eaten) was chewed into slightly broader but shorter particles with more jagged sides, fewer perforations or ruptures and a much higher number of veins per particle than all the forage grasses. Such features of maize leaf blade particles after chewing indicated that their internal structures may not be easily accessible to the rumen micro-organisms (Monson & Burton 1972) and that they could spend a relatively long time before being cleared out of the rumen. The thick stems of maize were rejected by the sheep. This possibly indicated that thick stems of monocotyledonous species, due to their hard shiny surface structure,may impair intake more than do the thick stems of dicotyledonous species which are normally covered by thick, soft epidermis, e.g. rape stems.

Comparison of the dicotyledonous and monocotyledonous species

The legumes differed significantly in terms of neutral detergent fibre content and digestibility but did not differ significantly in terms of intake rate. On the other hand, the forage grasses differed significantly in terms of neutral detergent fibre content, digestibility and intake rate. Generally the legumes and rape were eaten faster than the forage grasses and maize. These results were in agreement with McLeod & Smith (1989) and McLeod *et al*. (1990) who reported that grasses were chewed for a longer time and had a slower rate of eating than legumes. Further they were in accord with Wilson (1990) who noted that even if legume species are low in digestibility (55-65%) and have a high fibre content (as was the case with *Desmodium intortum* and lucerne in the present project) their leaf anatomy still gives them a fast rate of breakdown and thus a high intake compared to grasses. For high intake of the grazing animals, therefore, grass/legume mixed swards should be encouraged.

The chewed particles of the legumes and rape were shorter, broader and usually had fewer vascular bundles than those of the forage grasses and maize. This reflected their differences in vascular structural arrangement especially in the leaves as described in Chapter 2. Troelsen & Campbell (1968), Kelly (1988) and Kelly & Sinclair (1989) reported similar differences of particle shape in grass and legume particles obtained from the rumen. A high proportion of short and broad (almost spherical shape) particles have a higher chance of escaping out of the rumen than long rectangular particles (Moseley 1982), making legumes and other dicotyledonous species likely to have a higher intake than grasses. The chewed particles of spurrey (a non-legume dicotyledonous species) were as long but thinner ($\leq$1·0 mm) than those of grasses. If spurrey particles were to meet the rumen opening at an end position they could easily pass out of the rumen because their diameter was below the critical size (c. 1 mm (Poppi *et al.* 1980)) which will pass out of the rumen. It was, therefore, not surprising that in the Derrick *et al.* (1993) experiments, spurrey had a higher voluntary intake and higher faeces output per lamb than perennial ryegrass.

Legumes and rape had larger particles after chewing than forage grasses and maize. Generally there was a positive relationship between particle size and intake rate. It seems that the animals do not need to chew long when eating feeds that are fragile and especially with high moisture like rape. In forage grasses alone there was a negative relationship between particle size and neutral detergent fibre content (Fig. 4.2). This could mean that neutral detergent fibre is more limiting to intake in grasses than in legumes. Again this view agrees with Wilson (1990).

The legumes and rape normally had fewer particles per unit dry weight of chewed material than grasses. This meant that in a given weight of chewed material, grass particles normally had more sites exposed to invasion by rumen microbes than legume particles. However, this may not mean that the grass particles could be weakened by microbes as quickly as legume particles for easy chewing during rumination and quick clearance out of the rumen (Moseley 1982). A high proportion of vascular tissues, a high proportion of cell wall and thick cell walls, as suggested by Wilson (1991), might contribute to the reason why grass particles, despite being given as much opportunity as legume particles to be weakened by rumen microbes, are retained longer in the rumen. The results of this project therefore support the idea of research to find microbial enzymes which can more quickly weaken cell walls especially the middle lamella (Wilson 1991) and therefore increase the intake of forages with a high fibre content.

Crop residues

Pearce *et al.* (1979) found considerable differences in the quality of cereal straws due to variation between species, and between cultivars within species. Also Staniforth (1979) noted that the major slender stemmed cereal crops (i.e. wheat, rice, barley, oats and rye) vary in their stem vascular structure. These reports were in agreement with the results recorded in the present project. For example, rice straw chewed material had the highest proportion of particles with a rippled surface, indicating that rice straw may be softer and eaten more than wheat or millet straw. This could be due to the lower number of vascular bundles per stem internode in the rice straw than in the wheat and millet straws. These results show that screening of cereal straws in terms of their anatomical structure could also help to find ones which are softer and eaten faster than others.

CHAPTER 9

GENERAL CONCLUSIONS AND RECOMMENDATIONS

In the present project grasses differed significantly in terms of the proportion of vascular tissues, neutral detergent fibre content, digestibility and intake rate. The tropical grasses had a higher proportion of vascular tissues, higher neutral detergent fibre content and lower digestibility and lower intake rate and were chewed into smaller particles than temperate grasses. The relatively coarse temperate grass, tall fescue, was intermediate between the tropical and temperate grasses in terms of neutral detergent fibre content, intake rate and chewed particle size. Tall fescue resembled maize in its digestibility, neutral detergent fibre content and intake rate. The legumes on the other hand, did not differ significantly in terms of intake rate despite having differences in the proportion of vascular tissues, neutral detergent fibre content and digestibility. Generally the legumes were eaten faster and had larger particles after chewing than the grasses. These results, therefore, support the suggestion that it is anatomical differences, especially vascular tissue arrangement in the plant parts (e.g. the leaves), which give the legumes and other dicotyledonous species an advantage of higher intake rate than grasses. This was illustrated by *Desmodium intortum* and ribwort. *Desmodium intortum* had a very large number of tertiary veins per leaflet, a high proportion of neutral detergent fibre and low digestibility but was eaten at the same rate as white clover and chewed to almost the same particle size. On the other hand, ribwort, although a dicotyledonous species, has a leaf blade with a parallel arrangement of veins resembling that of a monocotyledonous species leaf blade. As a result, despite having very few veins per leaf blade and a lower fibre content, ribwort particle breakdown was closer to that of perennial ryegrass than to that of other dicotyledonous species such as chickweed and dock. Further, therefore, these results should encourage agronomists and ruminant nutritionists to observe plant vascular arrangement in addition to the proportion of vascular tissues as an anatomical factor affecting forage intake.

In addition to the high proportion of vascular tissues and neutral detergent fibre, the results of the present project showed that, the tropical grasses had an extra disadvantage of hard solid stems that had to be chewed thoroughly before swallowing as compared to the hollow and pliable stems of temperate grasses that presumably required less chewing; about 30% of ryegrass stem particles remained intact after chewing. From the point of view of high intake, therefore, it is a good idea to graze or cut the grasses while they have young stems or when they are still leafy.

The legume leaf petiole and stem particles were longer than leaflet particles and contributed substantially to the overall mean particle length of the chewed material. The proportion and vascular structure of the two legume plant parts require attention during studies which relate plant structure to forage intake.

The results of the present project showed that, regardless of the type of forage grass and even maize, the veins of the leaf sheaths were more widely spaced than those in leaf blades. The two plant parts were, however, chewed to a similar particle size showing that they may be equally important in the passage rate out of the rumen of the chewed grass materials.

In the present project it was observed that legumes had fewer chewed particles per unit dry weight than grasses. This meant that chewed particles of grasses and legumes would have allowed almost equal access to rumen microbes and thus an equal opportunity to be weakened if attacked by the right type of microbes or microbial enzymes before chewing during rumination. However, grass particles are retained longer in the rumen than those of legumes, and the present project results support the suggestion of research to find rumen microbes or enzymes which can more efficiently destroy or weaken the cell wall to facilitate particle breakdown and a high rate of passage out of the rumen.

The results of the present project clearly illustrated the influence of plant morphology on particle breakdown and intake. Despite having a higher proportion of stem than leaves, chickweed and spurrey were eaten faster and broken down to smaller particles than the other grassland species because the two species had thin fragile stems breaking easily at the nodes and the leaves were also fragile, especially those of spurrey. Further, an advantage of thin and/or pliable plant parts such as leaf petioles and stems in relation to intake was clearly indicated in the present project by white clover, sainfoin and lucerne. The former two species had a high proportion of leaf petiole and lucerne had a high proportion of stem but all three species had a high intake rate. However, the advantage of high intake rate shown by the grassland species having thin and/or pliable or fragile plant parts such as stems and leaf petioles was followed by a high proportion of intact particles in their chewed materials. Follow up of these intact particles during rumination is necessary so as to understand more about animal intake as related to plant structure. The morphological disadvantage of bulky crops in relation to intake was also illustrated by the results of the present project. The large leaves and thick stems of rape and maize were not easily prehended by the animal. The sheep rejected the maize stem but strove to eat the palatable rape stem for as long a time as was allowed. Chopping, which had almost no effect on the rate of intake of most of the grassland species, seemed to be more effective on the bulky crop.

The small amount of work done on the crop residues in the present project suggested that some crop residues (e.g. sweet potato tops) are more valuable than others and therefore require some emphasis to improve their use as animal feed. Further, evaluating the nutritive value of cereal straws by observing their vascular structure in addition to the eating behaviour of animals and degradation in the rumen seems to be very important.

In general, therefore, the results of the present project indicate that the quality differences between the species studied cannot be fully identified by their chemical composition, digestibility and voluntary intake, and that information is needed on other aspects such as plant morphology and vascular structure. This should, therefore, encourage further studies of the potential nutritive value of forage species in terms of their physical structure in relation to ease of physical breakdown in the mouth and rumen.

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