

## Chapter 3

### BIOMECHANICAL PROPERTIES OF HERBAGE GRASS LEAVES

#### 3.1 Variation in biomechanical properties of leaves among twenty grass species

##### 3.1.1 Introduction

Grasses are, perhaps the most important in plant families for human life such as food production, industry, and sport turf.

The individual organism must be mechanically reliable if it is to survive and reproduce, and thus it is reasonable to assume that the growth and development of each individual must establish a factor of safety against mechanical failure (Niklas *et al.*, 1999).

The objective of this experiment was to determine biomechanical properties of leaves in twenty grass species and to clarify variation in morphological and biomechanical traits.

##### 3.1.2 Materials and methods

Undamaged leaf blades of similar size were selected randomly from 20 grass species of Poaceae family, which were grown at roadside or abandoned field around the Obihiro University of Agriculture and Veterinary Medicine. Five species were weedy annual and the others were perennial (Fig. 3.1.1).

Leaves in polyethylene bag were immediately transported back to the laboratory and stored in a refrigerator until testing.

The specimens were tested on the day of sampling. Basal 10 cm parts of six leaves were sampled in each species. Total of 120 specimens were tested.

Measurements of morphological characteristics and biomechanical properties in grass leaves were carried out using same methods as described in the chapter 2.

### 3.1.3 Results

Values in the morphological characteristics and biomechanical properties of leaf blades were significantly ( $P < 0.001$ ) varied among species (Table 3.1.1 and Table 3.1.2). There were no apparent differences in biomechanical properties between annual and perennial species.

Total length of leaf blade was the greatest in chinese silver grass (*Miscanthus sinensis*) and the shortest in crab grass (*Digitaria adscendens*). Barnyardgrass (*Echinochloa crus-galli*) showed the heaviest fresh weight and redtop grass (*Agrostis alba*) showed the lightest. Barnyardgrass (*Echinochloa crus-galli*) was the largest in cross-sectional area, and Kentucky bluegrass (*Poa pratensis*) the smallest (Fig. 3.1.1).

Shearing strength was the highest in Barnyardgrass (*Echinochloa crus-galli*) and the lowest in crab grass (*Digitaria adscendens*). Orchardgrass (*Dactylis glomerata*) showed the highest tensile strength and crab grass (*Digitaria adscendens*) showed the lowest tensile strength. Bending strength was the highest in chinese silver grass (*Miscanthus sinensis*) and the lowest in redtop grass (*Agrostis alba*) (Fig. 3.1.2, Table. 3.1.2).

Bending strength was highly correlated with the leaf fresh weight. Bending strength was positively related ( $P < 0.001$ ) with many leaf morphological characteristics such as leaf DM weight, width, length, cross-sectional area, but negatively only with leaf DM density, and did not correlated with three tensile parameters such as tensile stress, longitudinal toughness and Young's modulus (Table. 3.1.3 and Fig. 3.1.3).

Safety factor was the highest in chinese silver grass (*Miscanthus sinensis*) and the lowest in crab grass (*Digitaria adscendens*) and which were 15 to 75 times larger than the actual working load in species (Table. 3.1.2).

### 3.1.4 Discussion

Morphological characteristics and biomechanical properties of grass leaves varied broadly among grass species. Biomechanical properties of grass leaves varied widely, depending on species, growth stage, morphological unit, and anatomical

components (sclerenchyma or fiber content) (Wright and Illius, 1995).

In this experiment, a high correlation between bending strength and leaf fresh weight were found. It suggests that heavier grass leaves require higher bending strength. There were extensive variations in within-plant support investments among grass species, and safety from mechanical failure under typical static loads varied from 15 to 75. It is confirmed that aerial stems and leaves must support their own weight bend and twist without breaking during their functional lifetimes (Niklas, 1998).

Table 3.1.1 Length, width, DM weight, cross-sectional area and DM density of leaves of 20 grass species

Species	Leaf length (mm)	Leaf width (mm)	Leaf DM weight (g)	Cross-sectional area (mm <sup>2</sup> )	DM density (mg·DM/mm <sup>3</sup> )
A. Weedy annual					
<i>Digitaria adscendens</i>	120±4	8.2±0.61	0.039±0.0026	1.25±0.088	0.30±0.009
<i>Echinochloa crus-galli</i>	409±8	14.9±1.09	0.195±0.0114	3.23±0.203	0.21±0.011
<i>Setaria faberi</i>	285±7	13.3±0.53	0.114±0.0048	2.45±0.102	0.21±0.008
<i>Setaria glauca</i>	241±6	8.3±0.31	0.049±0.0040	1.43±0.069	0.19±0.006
<i>Setaria viridis</i>	171±3	10.5±0.37	0.066±0.0022	1.34±0.095	0.39±0.027
B. Perennial					
<i>Agropyron repens</i>	236±5	8.9±0.34	0.075±0.0063	1.36±0.053	0.30±0.011
<i>Agrostis alba</i>	124±8	6.6±0.47	0.038±0.0036	1.02±0.107	0.35±0.017
<i>Agrostis scabra</i>	155±9	11.1±0.44	0.067±0.0081	1.44±0.119	0.38±0.008
<i>Anthoxanthum odoratum</i>	344±9	5.3±0.36	0.076±0.0079	1.12±0.106	0.27±0.004
<i>Bromus inermis</i>	254±4	8.8±0.30	0.101±0.0060	1.29±0.085	0.41±0.013
<i>Calamagrostis langsdorffii</i>	258±6	6.6±0.23	0.045±0.0025	0.83±0.041	0.31±0.014
<i>Dactylis glomerata</i>	617±16	10.3±0.44	0.231±0.0175	2.66±0.158	0.23±0.005
<i>Festuca arundinacea</i>	627±35	7.4±0.25	0.166±0.0160	1.89±0.107	0.20±0.003
<i>Festuca elatior</i>	404±10	4.6±0.07	0.054±0.0022	0.94±0.081	0.20±0.016
<i>Lolium perenne</i>	313±11	4.5±0.21	0.043±0.0040	0.88±0.073	0.20±0.031
<i>Miscanthus sinensis</i>	671±12	13.2±0.73	0.289±0.0156	2.72±0.146	0.27±0.028
<i>Phalaris arundinacea</i>	203±7	12.7±0.60	0.102±0.0182	1.69±0.118	0.33±0.012
<i>Phleum pratense</i>	342±16	7.9±0.51	0.080±0.0053	1.29±0.073	0.25±0.008
<i>Poa pratensis</i>	419±17	3.0±0.23	0.057±0.0050	0.63±0.073	0.24±0.020
<i>Stipa pekinense</i>	486±25	10.2±0.57	0.239±0.0242	1.39±0.155	0.47±0.027
sed	65.1	2.38	0.0513	0.536	0.079
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Figures show mean±se.

Table 3.1.2 Biomechanical properties of 20 grass species

Species	Shearing strength (kg)	Shearing toughness (kg·mm/mm <sup>2</sup> )	Bending strength (kg·mm)	Safety factor (g/g)	Tensile strength (kg)	Tensile toughness (kg·mm/mm <sup>2</sup> )
<i>Digitaria adscendens</i>	0.11±0.017	0.16±0.021	0.01±0.001	14.7±1.03	0.62±0.036	0.30±0.031
<i>Echinochloa crus-galli</i>	0.74±0.041	0.78±0.089	0.33±0.015	43.7±2.29	2.73±0.164	0.52±0.063
<i>Setaria faberi</i>	0.36±0.032	0.32±0.028	0.13±0.013	46.1±2.58	2.54±0.112	0.60±0.071
<i>Setaria glauca</i>	0.38±0.033	0.45±0.026	0.05±0.006	23.5±1.45	1.55±0.087	0.77±0.112
<i>Setaria viridis</i>	0.31±0.025	0.47±0.063	0.04±0.005	52.4±8.41	1.76±0.083	0.91±0.118
<i>Agropyron repens</i>	0.27±0.029	0.44±0.028	0.04±0.004	28.0±3.95	2.98±0.214	1.45±0.194
<i>Agrostis alba</i>	0.11±0.015	0.21±0.032	0.01±0.001	21.9±2.13	1.13±0.077	0.94±0.105
<i>Agrostis scabra</i>	0.23±0.044	0.32±0.041	0.01±0.004	15.5±2.80	1.37±0.150	0.60±0.042
<i>Anthoxanthum odoratum</i>	0.26±0.035	0.55±0.062	0.06±0.010	30.8±2.55	4.28±0.413	3.49±0.417
<i>Bromus inermis</i>	0.38±0.028	0.61±0.047	0.05±0.005	34.5±2.22	3.09±0.192	1.74±0.258
<i>Calamagrostis langsdorffii</i>	0.20±0.027	0.40±0.037	0.02±0.002	22.9±1.88	2.42±0.186	2.06±0.311
<i>Dactylis glomerata</i>	0.55±0.055	0.87±0.121	0.31±0.043	41.3±3.67	6.16±0.147	3.08±0.239
<i>Festuca arundinacea</i>	0.56±0.039	0.65±0.042	0.20±0.020	29.3±1.80	4.22±0.180	2.29±0.079
<i>Festuca elatior</i>	0.14±0.008	0.34±0.015	0.05±0.002	17.3±0.68	1.92±0.111	1.69±0.154
<i>Lolium perenne</i>	0.14±0.009	0.40±0.080	0.03±0.005	19.5±1.03	1.43±0.119	1.22±0.200
<i>Miscanthus sinensis</i>	0.64±0.048	0.67±0.045	0.50±0.032	74.9±2.86	4.58±0.571	1.34±0.366
<i>Phalaris arundinacea</i>	0.24±0.037	0.33±0.012	0.02±0.002	20.7±2.73	2.61±0.209	1.28±0.140
<i>Phleum pratense</i>	0.33±0.063	0.42±0.059	0.09±0.018	41.1±7.15	3.18±0.313	1.74±0.196
<i>Poa pratensis</i>	0.15±0.014	0.31±0.036	0.05±0.007	29.0±3.10	2.31±0.167	3.04±0.204
<i>Stipa pekinense</i>	0.58±0.070	0.96±0.114	0.15±0.020	39.7±3.36	5.76±0.399	4.12±0.324
Sed.	0.18	0.28	0.06	16.8	1.15	1.03
Significance	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001

Table 3.1.3 Correlation coefficient between bending strength and other parameters

Parameters	r	Significance
Leaf length	0.78	$P < 0.001$
Leaf width	0.50	$P < 0.001$
Leaf DM weight	0.88	$P < 0.001$
Cross-sectional area	0.77	$P < 0.001$
DM density	-0.20	$P < 0.029$
Shearing strength	0.76	$P < 0.001$
Shearing toughness	0.55	$P < 0.001$
Tensile strength	0.59	$P < 0.001$
Tensile stress	-0.02	ns
Longitudinal toughness	0.10	ns
Young modulus	-0.05	ns

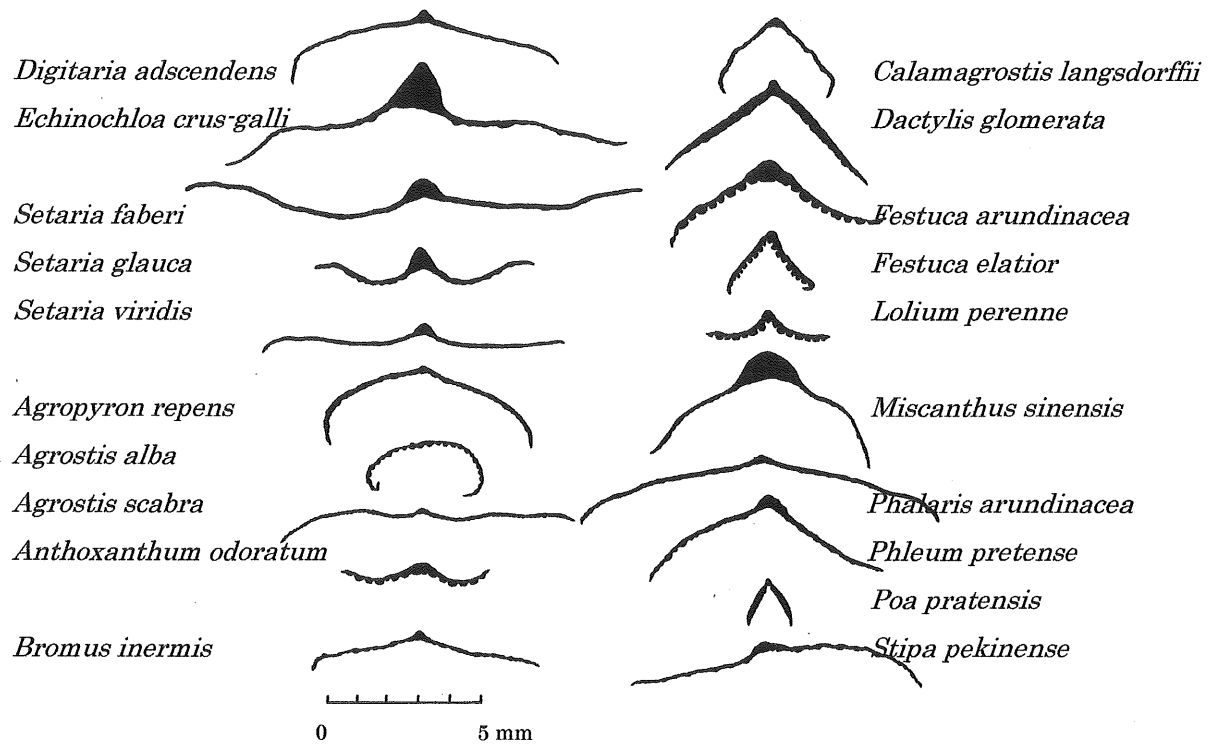


Fig. 3.1.1 Shapes of leaf cross sections of 20 grass species. Upper left 5 species are annual and other species are perennial.

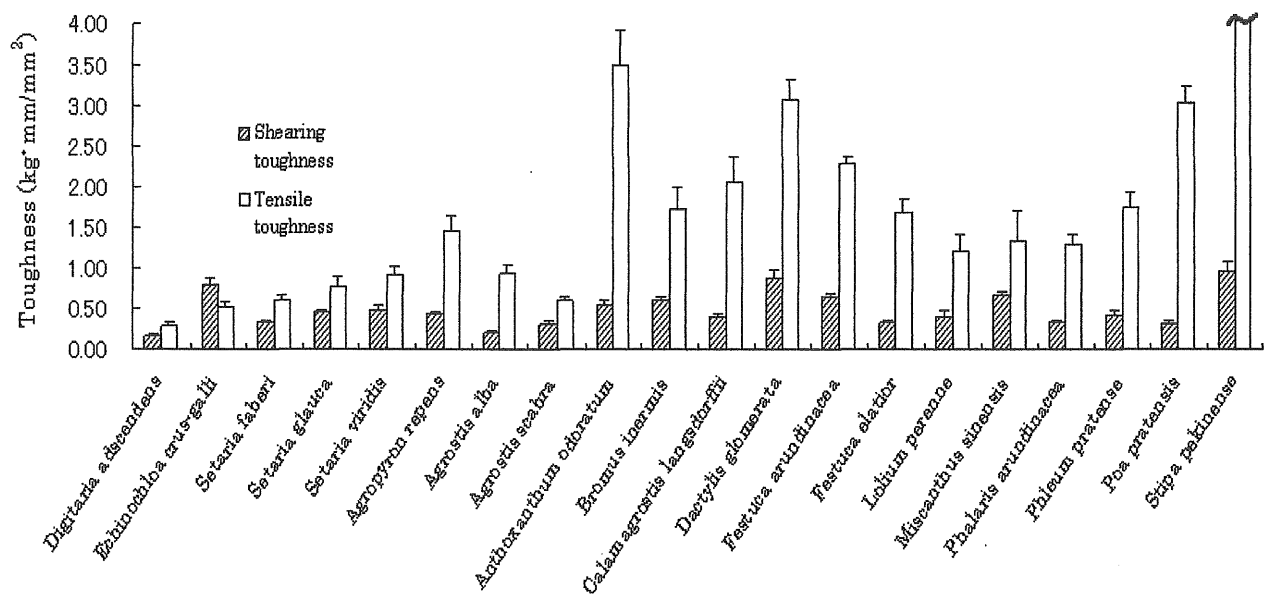


Fig. 3.1.2 Variations in biomechanical properties among 20 grass species



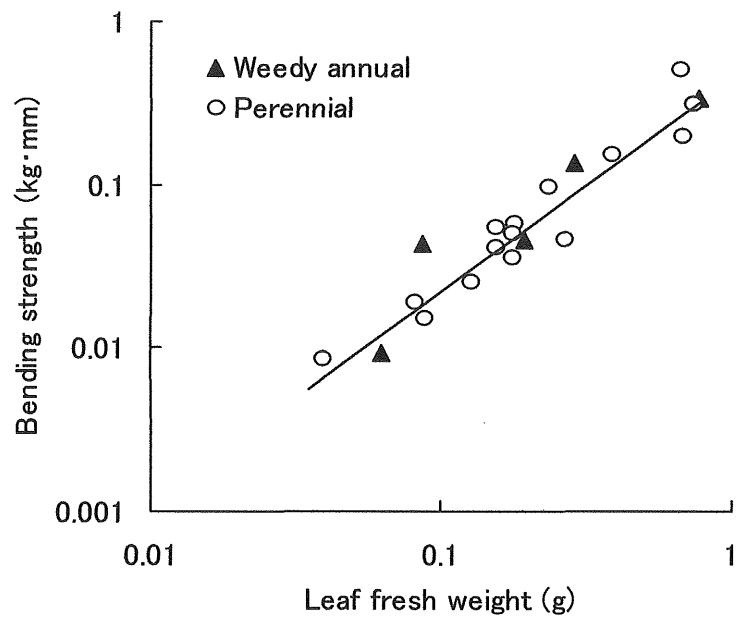


Fig. 3.1.3 Relationship between bending strength and leaf fresh weight in 20 grass species. Correlation equation was as follows:

$$\log_{10} Y = 1.30 \log_{10} X - 0.32 \quad (r = 0.912)$$

## 3.2 Tensile and shearing properties of leaves in festulolium and perennial ryegrass

### 3.2.1 Introduction

Festulolium (*Festulolium loliaceum*) is a hybrid between meadow fescue (*Festuca pratensis*) and Italian ryegrass (*Lolium multiflorum*) or perennial ryegrass (*L. perenne*). It had been bred for a genetic improvement in freezing tolerance, forage yield or persistence (Casler *et al.*, 2001; Casler *et al.*, 2002), or drought resistance (Lesniewska *et al.*, 2001) in regions with severely cold winter. Festulolium plants are also expected to improve feeding values such as palatability, voluntary intake and digestibility (Ghesquiere *et al.*, 1996).

The diploid perennial ryegrass such as Aurora had been selected for higher concentrations of water-soluble carbohydrates, and for more small tillers than the tetraploid. Palatability was higher in Aurora than in other cultivars under rotational sheep grazing (Jones and Roberts, 1991). In addition, total annual sheep production from Aurora swards was higher than that from other cultivars (Munro *et al.*, 1992).

Feeding values and chemical compositions of various elements in festulolium and perennial ryegrass have been well studied. However, few studies were reported with respect to biomechanical properties. In the agronomical field, biomechanical properties affect selective grazing by animals, digestibility of grasses, and resistance of grasses to trampling or mowing, and process of hay-making (Vincent 1982, 1983). This information may be of use to the plant breeder who can select for the important characteristics (Wright and Vincent, 1996). The objective of this experiment was to determine seasonal variation in tensile and shearing properties of leaves in festulolium, compared with those of diploid and tetraploid cultivars of perennial ryegrass.

### 3.2.2 Materials and methods

#### *Grass swards*

The swards of cultivars of perennial ryegrass and festulolium from Wales, UK were established as a pure stand in June 2002 and had been managed by regular

fertilization and harvest for two years: two diploid cultivars (Ba11353 and Aurora) and two tetraploid cultivars (Ba10855 and Prospero) of perennial ryegrass, and two cultivars (Ba11356 and Ba11358) of festulolium. In 2004, the swards at the flowering stage were harvested at 5 cm height on 16 June and applied with a compound fertilizer equivalent to 65-49-65 kg/ha of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O. Then, four successive samples of grass leaves were taken on 7 July, 29 July, 27 August and 22 September. The swards were harvested at 5cm in height immediately after taking samples. An additive compound fertilizer equivalent to 65-49-65 kg/ha of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O was applied after the third sampling time.

### *Measurements of length, width and weight of leaves*

The experiments were conducted in Obihiro, Japan. During the trials, leaves of a similar size were chosen and clipped at a ligule with scissors. Leaves were sprayed with water and stored in a polyethylene bag in a refrigerator. Total length of a leaf was measured. The leaves were cut into 10 cm in length from a ligule side with scissors and had a midpoint marked. The weight was determined using a digital balance ( $\pm 0.0001$  g) after absorbing water on leaf surface with paper towel. The width at midpoint was measured with scaled magnifier ( $\pm 0.01$  mm) under light pressure. Then, leaves were immersed in distilled water for at least 10 min before measurement, so that full turgor within leaves could be achieved (Chan *et al.*, 1999). Twenty leaves were tested in each cultivar.

### *Leaf measurements*

Cross-sectional area and tensile and shearing properties of leaf blades used in this experiment were measured in the same way described in chapter 2.

The stiffness of leaves was estimated by the following equation:

$$E = \text{stress/strain,}$$

where stress is the force per unit area and strain is the relative extension to produce that stress. The stiffness was estimated at the first linear portion of the stress-strain curve (Vincent, 1983).

### *Statistical analysis*

Variables of biomechanical properties were analyzed using a paired t-test and an analysis of variance (Snedecor and Cochran, 1980). The regression analysis was also carried out.

### **3.2.3 Results**

There were no significant differences between two cultivars in each of three species with respect to morphological and biomechanical properties. Therefore, two cultivars in each of three species were included into replication.

#### *Cross-sectional area, DM weight and length of whole leaves*

Cross-sectional area was significantly higher ( $P < 0.001$ ) in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass at all four sampling times (Fig. 3.2.1). Mean values of four samples were  $0.70 \pm 0.016 \text{ mm}^2$ ,  $0.98 \pm 0.019 \text{ mm}^2$  and  $0.97 \pm 0.021 \text{ mm}^2$  in diploid and tetraploid cultivars of perennial ryegrass and festulolium, respectively.

DM weight was significantly higher ( $P < 0.020$ ) in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass from the 1st to 3rd sampling times, but not significantly different ( $P < 0.179$ ) at the 4th sampling times.

Leaf length was not significantly different ( $P < 0.438$ ) between three cultivars, although there were seasonal variations. Leaf width showed constant values at all four sampling times. Tetraploid perennial ryegrass and festulolium had significantly higher ( $P < 0.001$ ) leaf width than diploid perennial ryegrass. Mean values of four samples were  $3.2 \pm 0.04 \text{ mm}$ ,  $3.9 \pm 0.04 \text{ mm}$  and  $3.9 \pm 0.06 \text{ mm}$  in diploid and tetraploid cultivars of perennial ryegrass and festulolium, respectively.

#### *Fracture patterns*

The force-deflection patterns of shearing and tensile fractures are shown in Fig.

3.2.2. Shearing strength reached its peak at the central deflection, coinciding with a shearing point of main vein. Mean values of four samples of tensile strain were  $0.068 \pm 0.0013$ ,  $0.062 \pm 0.0011$  and  $0.069 \pm 0.0010$  in diploid and tetraploid cultivars of perennial ryegrass and festulolium, respectively.

#### *Tensile stiffness and shearing toughness*

Maximum tensile strength was significantly higher ( $P < 0.001$ ) in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass, but tensile stress and stiffness were significantly lower ( $P < 0.001$ ) in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass (Fig. 3.2.3).

Shearing strength and shearing work of fracture were significantly higher ( $P < 0.001$ ) in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass. Shearing toughness showed significant differences between three cultivars ( $P < 0.001$ ) and between four sampling times ( $P < 0.002$ ), but there were seasonally wide variations.

#### *Density-specific stiffness and density-specific strength*

There was the significant relationship ( $P < 0.007$ ) between density-specific stiffness and density-specific strength in a tensile property (Fig. 3.2.4). The grand mean was  $1.40 \pm 0.023$  MNm/kg in density-specific stiffness and  $0.080 \pm 0.0013$  MNm/kg in density-specific strength.

#### **3.2.4 Discussion**

Grass leaves are probably the simplest of all plant leaves from the mechanical point of view (Vincent, 1982). The leaf itself must mechanically sustain its own weight against the influence of gravity. It must also be sufficiently stiff and strong to resist bending and avoid breaking when subjected to large externally applied mechanical forces (Niklas, 1993). In this study, leaves of all cultivars were vertically kept straight. The grand mean was  $282 \pm 2.2$  mm in total leaf length and  $3.7 \pm 0.03$  mm in

leaf width. Thus, long and narrow leaves may keep straight vertically by increased bending strength, which is maintained by interior angles of leaves in a cross section. This maintenance method seems to be very effective for grass species to minimize metabolic investments in leaf-supporting structures (Chazdon, 1986). The shape of cross section suggests that inherent angles as shown in transverse sections (Fig. 3.1.2), which may be maintained under high turgor (Moullia, 2000), especially in the motor cells.

The major chemical constituent of plants is cellulose, a high molecular weight polysaccharide which is directly responsible for stiffness and strength (Atkins and Mai, 1985). Usually, a behaviour under a tensile load depends only on material properties whereas a shearing load depends on structural properties as well (Vincent, 1990). Brittle materials show more frequent and higher peaks with the downwards side of the curve (Vincent, 1992). Shearing fracture pattern (Fig. 3.2.2) suggests that a multi-ridged outline of leaf cross section at the adaxial side may correspond to each small peak.

Two cultivars of festulolium used in this study were bred by back cross between tetraploid perennial ryegrass and F1 hybrid (meadow fescue x tetraploid perennial ryegrass). Therefore, the morphology and biomechanical behaviour of festulolium leaves were quite similar to those of tetraploid perennial ryegrass.

Longitudinal stiffness was significantly lower in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass ( $192 \pm 5.7$  and  $180 \pm 4.5$  versus  $224 \pm 6.4$  MPa). Reversely, cross-sectional area was significantly higher in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass ( $0.98 \pm 0.019$  and  $0.97 \pm 0.021$  versus  $0.70 \pm 0.016$  mm<sup>2</sup>). Thus, narrower or thinner leaves with lower cross-sectional area tend to have higher longitudinal stiffness in other grass species too. The longitudinal stiffness of grass leaf is directly and linearly proportional to the total cross-sectional area of sclerenchyma in the leaf (Vincent, 1990). Further studies are needed on this subject.

### 3.2.5 Summary

The study was carried out to determine tensile and shearing properties of leaves in cultivars (Ba11356 and Ba11358) of festulolium (*Festulolium loliaceum*), compared

with those of diploid (Ba11353 and Aurora) and tetraploid (Ba10855 and Prospero) cultivars of perennial ryegrass (*Lolium perenne*). Four successive samples of grass leaves were taken from pure stands of third-year swards during July to September, 2004 in Obihiro, Japan. Tensile strength was measured using a 100 N load cell of the breaking test machine. Shear strength was measured about 5mm apart from a broken point in the tensile test using a pair of the scissors.

Cross-sectional area, DM weight and width of leaves were significantly higher in tetraploid perennial and festulolium than in diploid perennial. Shearing strength reached a peak at the central deflection, coinciding with shearing point of main vein. In shearing fracture pattern, a multi-ridged outline of leaf cross section at the adaxial side corresponded to each small peak. Tensile strength, shearing strength and work of fracture were significantly higher in tetraploid perennial and festulolium than in diploid perennial, but tensile stress and stiffness were significantly lower in tetraploid perennial and festulolium than in diploid perennial. Narrower or thinner leaves with lower cross-sectional area tend to have higher longitudinal stiffness. Thus, the morphology and biomechanical behaviour of festulolium leaves were quite similar to those of tetraploid perennial ryegrass.

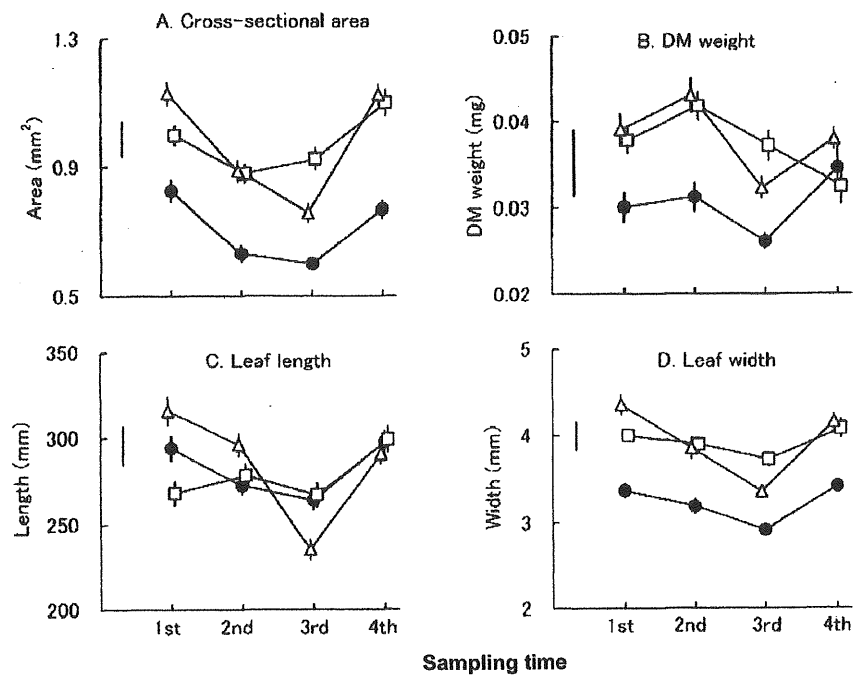


Fig. 3.2.1 Cross-sectional area, DM weight, leaf length and width at four sampling times in diploid (●), and tetraploid (□) cultivars of perennial ryegrass and festulolium (△). Attached lines to symbols show s.e. of mean and vertical lines show s.e.d. of the mean differences.



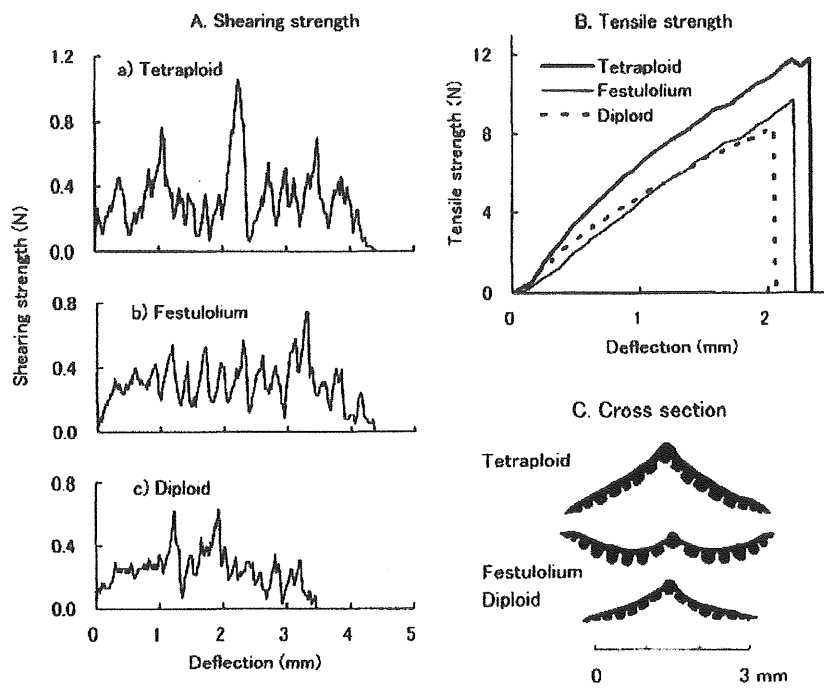


Fig. 3.2.2 Shearing and tensile fracture patterns and cross-sections of diploid (Aurora) and tetraploid (Prospero) cultivars of perennial ryegrass and festulolium. Each measurement was obtained from the same leaf.

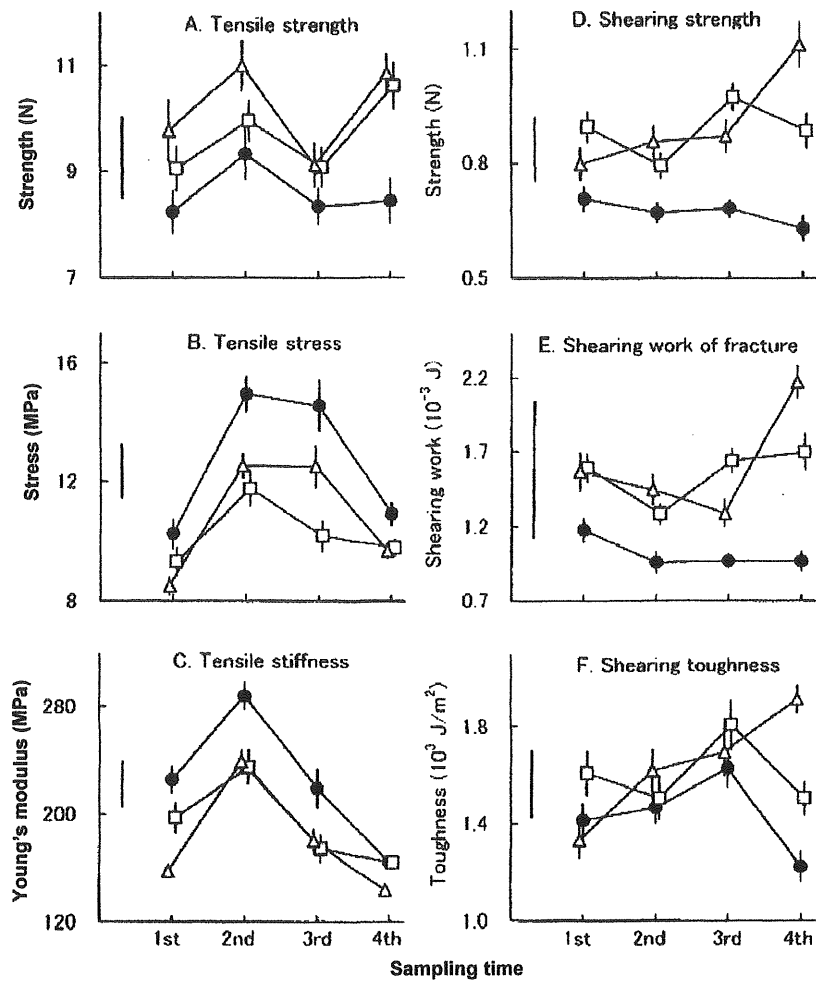


Fig. 3.2.3 Tensile and shearing properties at four sampling times in diploid (●) and tetraploid (□) cultivars of perennial ryegrass and festulolium (△). Attached lines to symbols show s.e. of mean and vertical lines show s.e.d. of the mean differences.

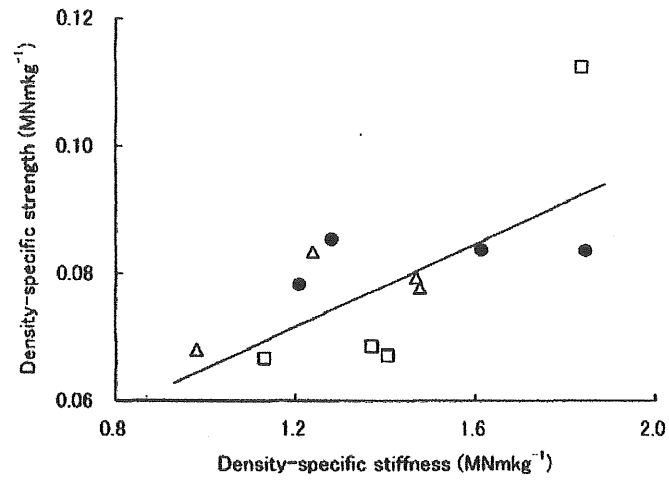


Fig. 3.2.4 Relationship between density-specific stiffness and density-specific strength at four sampling times in diploid (●) and tetraploid (□) cultivars of perennial ryegrass and festulolium (△).

$$Y = 0.032x + 0.035 \quad (r = 0.666, P < 0.007)$$

### 3.3 Relationships between biomechanical properties and morphological characteristics of herbage grass leaves

#### 3.3.1 Introduction

The farm intensification depends mainly on grass-fed systems. Better knowledge of biomechanical properties of herbage grasses should facilitate better management and animal production.

Biomechanical properties of grass leaves vary widely, depending on species, growth stage, morphological unit, anatomical components (sclerenchyma or fiber content) (Wright and Illius, 1995), water content, and its inner and outer structures.

In order to analyze the plant mechanically, the contributions of the different components should ideally be quantified. The relationship between mechanical and anatomical properties of plant tissues has been the subject of considerable speculation because it is evident that, aside from their physiological functions, every tissue type contributes in some way to the mechanical behaviour of organs (Schwendener, 1874; Carlquist, 1961, 1969, 1975; Wainwright *et al.*, 1976; Niklas, 1992; Speck, 1994; Spatz *et al.*, 1995).

The tensile and shearing properties of the leaves, stems of agriculturally important grasses that influence the choice of grass species by grazing animals, the resistance of plants to lodging and trampling, and the behavior of materials during harvesting and processing has been studied (Silk, Wang, and Cleland, 1982; Vincent, 1982, 1983; Ennos, 1989, 1991; Paolillo and Niklas, 1996). However, the complex investigations of the biomechanical property of grass leaf organs (blade, ligule and sheath), parts (midrib and blades) at different sites are scarce.

The objective of this experiment was to determine the relationship between biomechanical properties and morphological characteristics of grass leaves, and to clarify the mechanism of contribution of plant parts and organs to the plant biomechanical properties. Specimens from different organs of a shoot (leaf blade, ligule and sheath), different sections along the length of a leaf blade, and longitudinally dissected parts of a leaf blade (midrib and wings of a leaf blade) in two grass species were investigated in comparison. Four experiments were conducted. The first experiment tested biomechanical properties of leaf blade versus ligule versus sheath, the second experiment did those of different sections from tip

to base of a leaf blade, the third experiment did those of midrib versus wings of a leaf blade, and the fourth experiment examined the importance of an interior angle of a leaf blade in the leaf biomechanical property.

### 3.3.2 Materials and Methods

Vegetative shoots of orchardgrass (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*) were used in the experiments. For the experiment 1, vegetative shoots of a similar size were chosen and cut. With 8 cm length of the sheath, ligule and blade were sampled from six shoots in each species (Fig. 3.3.1). This experiment aims to determine and compare biomechanical properties of the leaf organs (sheath, ligule and blade) and clarify their role in plant mechanical support.

In the experiment 2, undamaged leaves of similar size were selected and clipped at the ligule. For the examination, each leaf blade was marked into four pieces with equal length, and initial parts of 7 cm of each marked piece were cut and sampled (Fig. 3.3.2) so that biomechanical properties at the four positions along the length of a leaf blade are tested in comparison. Six replications from each species, in total of 48 specimens, were tested.

The objective of the experiment 3 was to investigate contribution of midrib and the rest halves of leaf blade (wing) to the biomechanical properties of a leaf blade at different sites along the length of a leaf blade. Similar-sized leaf blades were chosen and clipped at the ligule. In the same way as we did in the experiment 3, each leaf was marked into four pieces of equal length, and initial parts of 7 cm of each marked piece were cut. Afterwards, the prepared four pieces were dissected longitudinally with a razor blade under a binocular microscope for partitions into three pieces; midrib and two remained wings of the leaf blade (Fig. 3.3.3). Six leaves from each species, a total of 144 specimens were tested.

In the experiment 4, in order to clarify the contribution of an interior angle of leaf blade to mechanical support of the leaf, the data on whole leaf blade was compared to the data on the compound of separated midrib and leaf wings (Fig. 3.3.4). The data on the compounds were obtained from measuring biomechanical properties of midrib and two halves of a leaf blade as one. Therefore, an investigation of the compound is intended to eliminate the supporting role of interior angles.

The above samples were all tested in bending, shearing and tension for determination of their biomechanical properties as described in the chapter 2.

### 3.3.3 Results

The values in the morphological characteristics such as DM weight, fresh weight and cross sectional area showed significant variations ( $P < 0.05$ ) between the three organs of a shoot in both species (Table 3.3.1 and Fig. 3.3.5). Cross sectional area (Fig. 3.3.6) in OG is significantly decreasing from sheath ( $3.03 \pm 0.123 \text{ mm}^2$ ) to ligule ( $2.28 \pm 0.256 \text{ mm}^2$ ) and to leaf blade ( $2.17 \pm 0.092 \text{ mm}^2$ ), whereas it is significantly increasing in TF ( $2.79 \pm 0.192 \text{ mm}^2$ ;  $3.20 \pm 0.121 \text{ mm}^2$  and  $3.35 \pm 0.112 \text{ mm}^2$  respectively). Three organs of a grass shoot; sheath, ligule and leaf blade were similar in DM density. However, shearing toughness at ligule ( $0.51 \pm 0.040 \text{ kg} \cdot \text{mm}/\text{mm}^2$  in OG;  $0.78 \pm 0.207 \text{ kg} \cdot \text{mm}/\text{mm}^2$  in TF) and tensile stress at leaf blade ( $5.56 \pm 0.238 \text{ kg}$  in OG;  $6.60 \pm 0.255 \text{ kg}$  in TF) were significantly higher than other organs (Fig. 3.3.5). Yet, there is no variation in bending strength between organs in both species.

In the experiment 2, values in the morphological characteristics of leaf blade such as width, fresh weight and cross sectional area linearly decreased from the base to the apex of a leaf blade (Table 3.3.2). But density (DM weight per unit volume) did not vary across four positions with almost similar values at all the positions (Fig. 3.3.7). Cross sectional areas of leaf blade were 2.0 and 2.8 times, leaf fresh weight 5.3 and 5.8 times, and leaf width 1.8 and 1.5 times higher at the basal site than those at the apical site in orchardgrass and tall fescue respectively.

Bending moment was much higher at basal sites in both species. In orchardgrass, it was 33.8 times higher at the basal site ( $0.20 \pm 0.023 \text{ kg} \cdot \text{mm}$ ) compared to the apical site ( $0.006 \pm 0.0013 \text{ kg} \cdot \text{mm}$ ), and 14 times higher in tall fescue ( $0.12 \pm 0.007 \text{ kg} \cdot \text{mm}$ ;  $0.008 \pm 0.0016 \text{ kg} \cdot \text{mm}$  respectively). Similarly, other biomechanical parameters of leaf blade significantly decreased from basal to apical positions, although there was no difference in Young's modulus between four positions in both species. Tensile and shearing strength were 5.3 and 4.0 times in orchardgrass, and 6.6 and 5.7 times higher in tall fescue, respectively, at the basal site than those at the apical site.

In the experiment 3, cross-sectional areas of both midrib and wings linearly

decreased from basal to apical positions in both species. Cross-sectional area of OG midrib was smaller than that of its wing, whereas TF has a bigger midrib cross-section than its wing (Fig. 3.3.8) at all positions along the length of a leaf blade. DM density showed almost similar values at all positions in both sections in the two species.

Bending strength and shearing toughness were significantly higher in the midrib part than in the wing part. But the difference decreased from the base to the apex of leaf. There was no significant difference in tensile parameters between midrib and wing of both species (Fig. 3.3.8).

In the results from the experiment 4, the only significant difference between the compound and whole leaf that can be considered to be due to interior angle was in OG bending strength (Fig. 3.3.9 and Fig. 3.3.10). Bending moment of whole leaf ( $0.14 \pm 0.020 \text{ kg} \cdot \text{mm}$ ) of orchardgrass was significantly ( $P < 0.05$ ) higher than that of the compound ( $0.09 \pm 0.012 \text{ kg} \cdot \text{mm}$ ). This difference causes a significant greater tensile energy in a whole leaf of orchardgrass even though the significance is marginal (Fig. 3.3.9).

### 3.3.4 Discussion

Even though DM densities were similar, there were significant variations in biomechanical properties between the three organs of a grass shoot. Ligule is the greatest in shearing toughness in both species perhaps due to adaxial epidermis of the ligule which possesses a thick cuticle (Chaffey, 2000). This suggests ligule of a grass shoot may become a barrier layer for grazing depth by sheep. Leaf blade was the greatest in tensile stress. Leaf longitudinal strength is a function of the content and distribution of fiber cells. The amount of sclerenchyma associated with the fibres is directly related to the tensile strength of grass leaves (Vincent, 1982). Well developed cellulose fiber of grass leaf blade showed highest tensile strength than other organs. Therefore, ligule of grass shoot may require more energy compared to the other two parts of a grass shoot when harvesting and chewing.

Mechanical property of grass leaves depended upon the position along a leaf blade (Greenberg *et al.*, 1989). All morphological and biomechanical parameters significantly decreased from the base to the apex of a leaf blade in both species. In

other words, leaf blades are getting weaker from the base to the tip.

The midrib was higher in physical strength than the leaf wing at all positions in orchardgrass and tall fescue. The results suggest that the midrib provides structural support for the leaf. Chan *et al.* (1999) reported bending strength of midribs of grass leaves disregarding the contribution from the rest of the leaf. The bending strength of the midrib was five to ten times larger than that required to support its mass, which are probably required to withstand the much higher forces of wind and rain. Givnish (1978) reported that optimization of water supply and biomechanical support for a given biomass investment in the midrib suggests that the optimal leaf form requires a larger midrib mass fraction combined with greater physiological activity of the rest of the lamina. However, leaf wings were higher in tensile strength than the midrib at all positions in orchardgrass. The midrib contains about 20% of the total volume fraction of fibres (Vincent, 1982). It suggests that fibres of remainder fraction which is orientated across to the width of leaf wings might cause higher tensile strength.

The only significant difference between the compound and whole leaf that can be considered to be due to interior angle was bending strength in OG. This suggests that maintenance of interior angle in whole leaf of orchardgrass is very important for a creation of bending strength (Fig. 3.3.10). In the case of TF leaf blade, thick midrib might be useful for being upright.

In all experiments, the investment of DM material (leaf DM density) was similar between treatments despite the contrasting biomechanical properties. It confirmed the importance of structural organization and shape of plant organs and parts to the plant biomechanical property.



Table 3.3.1 Morphological and biomechanical properties of sheath, ligule and leaf blade of orchardgrass and tall fescue shoots

		Sheath	Ligule	Leaf blade	<i>P</i>
OG	Cross-sectional area (mm <sup>2</sup> )	3.03±0.123	2.28±0.256	2.17±0.092	0.003
	DM density (mg/mm <sup>3</sup> )	0.19±0.010	0.23±0.033	0.22±0.006	0.379
	Leaf fresh weight (g)	0.84±0.038	0.72±0.014	0.64±0.021	0.000
	Bending strength (kg)	16.23±2.666	18.33±2.309	18.21±2.214	0.847
	Bending moment (kg·mm)	0.16±0.027	0.18±0.023	0.18±0.022	0.847
	Shearing strength (kg)	0.35±0.034	0.41±0.044	0.23±0.024	0.002
	Shearing toughness (kg·mm/mm <sup>2</sup> )	0.36±0.033	0.51±0.040	0.24±0.018	0.000
	Tensile strength (kg)	4.42±0.101	2.45±0.184	5.56±0.238	0.000
	Tensile stress (kg/mm <sup>2</sup> )	1.47±0.066	1.20±0.206	2.59±0.109	0.000
	Young modulus (kg/mm <sup>2</sup> )	25.57±1.139	20.09±5.445	40.27±5.542	0.000
TF	Cross-sectional area (mm <sup>2</sup> )	2.80±0.192	3.21±0.121	3.35±0.112	0.048
	DM density (mg/mm <sup>3</sup> )	0.20±0.011	0.22±0.011	0.22±0.005	0.457
	Leaf fresh weight (g)	1.02±0.112	1.31±0.096	0.97±0.077	0.071
	Bending strength (kg)	21.41±2.300	30.69±5.495	31.16±2.130	0.129
	Bending moment (kg·mm)	0.21±0.023	0.31±0.055	0.31±0.021	0.129
	Shearing strength (kg)	0.64±0.268	0.86±0.564	0.53±0.134	0.044
	Shearing toughness (kg·mm/mm <sup>2</sup> )	0.62±0.077	0.78±0.207	0.43±0.032	0.073
	Tensile strength (kg)	3.68±0.138	3.48±0.340	6.60±0.255	0.000
	Tensile stress (kg/mm <sup>2</sup> )	1.34±0.066	1.10±0.124	1.98±0.058	0.000
	Young modulus (kg/mm <sup>2</sup> )	20.78±1.524	8.71±1.555	26.81±0.709	0.000

The figures show mean±s.e.

Table 3.3.2 Morphological and biomechanical properties at four positions along a leaf blade of orchardgrass and tall fescue

		Base	B-Middle	A-Middle	Apical	<i>P</i>
OG	Cross-sectional area (mm <sup>2</sup> )	2.20±0.172	1.82±0.118	1.26±0.171	0.78±0.071	0.000
	DM density (mg/mm <sup>3</sup> )	0.21±0.009	0.21±0.009	0.21±0.018	0.22±0.012	0.954
	Bending moment (kg·mm)	0.20±0.023	0.09±0.010	0.03±0.002	0.01±0.001	0.000
	Leaf fresh weight (g)	0.71±0.055	0.42±0.036	0.21±0.020	0.13±0.014	0.000
	Safety factor	28.06±2.915	22.12±0.671	13.55±0.904	4.15±0.553	0.000
	Shearing strength (kg)	0.31±0.023	0.28±0.025	0.13±0.016	0.05±0.011	0.000
	Width (mm)	8.89±0.457	8.79±0.442	7.28±0.171	4.93±0.476	0.000
	Shearing toughness (kg·mm/mm <sup>2</sup> )	0.36±0.041	0.44±0.027	0.28±0.022	0.10±0.027	0.000
	Tensile strength (kg)	6.79±0.599	5.54±0.249	2.73±0.285	1.29±0.093	0.000
	Tensile stress (kg/mm <sup>2</sup> )	3.17±0.366	3.14±0.315	2.33±0.327	1.78±0.310	0.037
	Tensile toughness (kg·mm/mm <sup>2</sup> )	4.65±0.637	3.64±0.493	1.94±0.406	1.21±0.137	0.000
	Young modulus (kg/mm <sup>2</sup> )	38.23±3.687	46.02±3.124	50.77±6.022	51.68±9.593	0.652
TF	Cross-sectional area (mm <sup>2</sup> )	1.84±0.072	1.81±0.050	1.57±0.074	0.92±0.081	0.000
	DM density (mg/mm <sup>3</sup> )	0.22±0.007	0.22±0.007	0.21±0.009	0.19±0.016	0.252
	Bending moment (kg·mm)	0.12±0.007	0.11±0.007	0.05±0.007	0.01±0.002	0.000
	Leaf fresh weight (g)	0.55±0.024	0.35±0.018	0.17±0.011	0.10±0.009	0.000
	Safety factor	21.07±1.263	30.07±1.723	25.99±2.629	8.36±1.319	0.000
	Shearing strength (kg)	0.45±0.26	0.43±0.037	0.22±0.033	0.08±0.014	0.000
	Width (mm)	6.65±0.392	7.44±0.407	7.34±0.263	4.48±0.352	0.000
	Shearing toughness (kg·mm/mm <sup>2</sup> )	0.50±0.044	0.42±0.045	0.23±0.043	0.10±0.020	0.000
	Tensile strength (kg)	3.93±0.282	4.01±0.382	2.63±0.216	0.97±0.107	0.000
	Tensile stress (kg/mm <sup>2</sup> )	2.12±0.088	2.20±0.164	1.67±0.098	1.09±0.141	0.000
	Tensile toughness (kg·mm/mm <sup>2</sup> )	2.11±0.232	1.97±0.303	1.29±0.107	0.83±0.109	0.002
	Young modulus (kg/mm <sup>2</sup> )	37.17±2.674	44.25±5.038	34.52±3.033	26.43±5.192	0.082

The figures show mean±s.e.

Table 3.3.3 Morphological and biomechanical properties of midrib versus wing of a leaf blade of orchardgrass and tall fescue

			Base	B-Middle	A-Middle	Apical	<i>P</i>
OG	Cross-sectional area (mm <sup>2</sup> )	Midrib	0.59±0.040	0.40±0.050	0.22±0.016	0.15±0.042	0.000
		Wing	0.87±0.046	0.73±0.025	0.46±0.037	0.25±0.041	
	Bending moment (kg·mm)	Midrib	0.06±0.010	0.03±0.009	0.01±0.001	0.00±0.006	0.002
		Wing	0.03±0.003	0.01±0.003	0.01±0.001	0.00±0.002	
	Shearing toughness (kg·mm/mm <sup>2</sup> )	Midrib	0.71±0.073	0.49±0.064	0.27±0.026	0.07±0.059	0.000
		Wing	0.29±0.036	0.26±0.022	0.19±0.019	0.04±0.020	
Tensile stress (kg/mm <sup>2</sup> )	Midrib	3.19±0.369	3.45±0.303	3.41±0.402	2.14±0.207	1.000	
	Wing	3.51±0.317	3.57±0.299	3.09±0.261	2.02±0.167		
TF	Cross-sectional area (mm <sup>2</sup> )	Midrib	1.18±0.238	0.88±0.129	0.56±0.110	0.29±0.105	0.006
		Wing	0.64±0.063	0.67±0.057	0.48±0.045	0.29±0.036	
	Bending moment (kg·mm)	Midrib	0.11±0.004	0.07±0.007	0.01±0.003	0.00±0.010	0.000
		Wing	0.02±0.005	0.02±0.002	0.01±0.001	0.00±0.002	
	Shearing toughness (kg·mm/mm <sup>2</sup> )	Midrib	0.76±0.097	0.60±0.056	0.20±0.046	0.05±0.071	0.000
		Wing	0.28±0.026	0.21±0.019	0.12±0.014	0.05±0.017	
Tensile stress (kg/mm <sup>2</sup> )	Midrib	2.64±0.230	2.61±0.168	1.72±0.175	1.66±0.139	0.798	
	Wing	2.34±0.136	2.48±0.151	2.10±0.117	1.58±0.092		

The figures show mean±s.e.

Table 3.3.4 Morphological and biomechanical properties of whole leaf versus the compound of midrib and wings of a leaf blade of orchardgrass and tall fescue

		Whole leaf	Midrib+Wing	Midrib	Wing	<i>P</i>
OG	Cross-sectional area (mm <sup>2</sup> )	2.01±0.121	2.09±0.108	0.50±0.044	1.60±0.077	0.629
	Bending moment (kg·mm)	0.14±0.020	0.09±0.012	0.05±0.008	0.05±0.008	0.056
	Shearing energy (kg·mm)	0.82±0.088	0.75±0.085	0.30±0.046	0.45±0.053	0.620
	Tensile energy (kg·mm)	8.32±1.135	5.52±0.641	0.95±0.096	4.57±0.585	0.057
TF	Cross-sectional area (mm <sup>2</sup> )	1.82±0.044	2.34±0.244	1.03±0.143	1.31±0.115	0.063
	Bending moment (kg·mm)	0.11±0.005	0.12±0.010	0.09±0.007	0.04±0.007	0.279
	Shearing energy (kg·mm)	0.84±0.064	0.85±0.072	0.57±0.060	0.28±0.028	0.895
	Tensile energy (kg·mm)	3.77±0.403	4.44±0.514	2.03±0.312	2.41±0.254	0.345

The figures show mean±s.e.

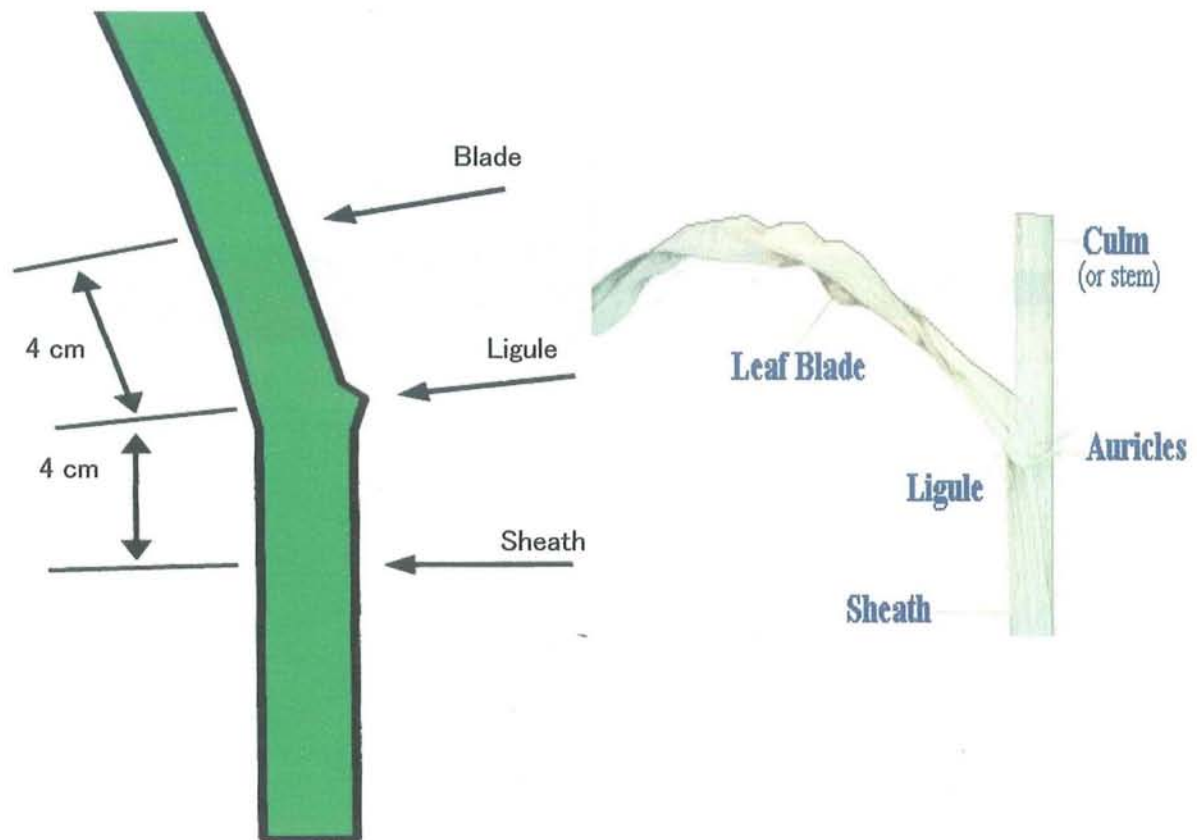


Fig. 3.3.1 Three sites along a grass shoot for measurements of biomechanical properties.

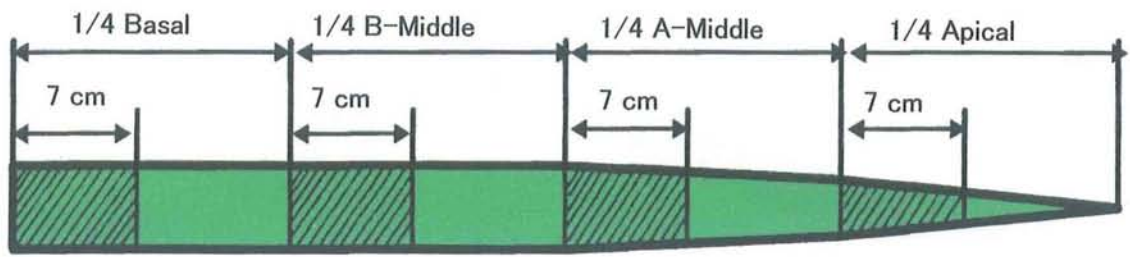


Fig. 3.3.2 Four sampling positions along a leaf blade.

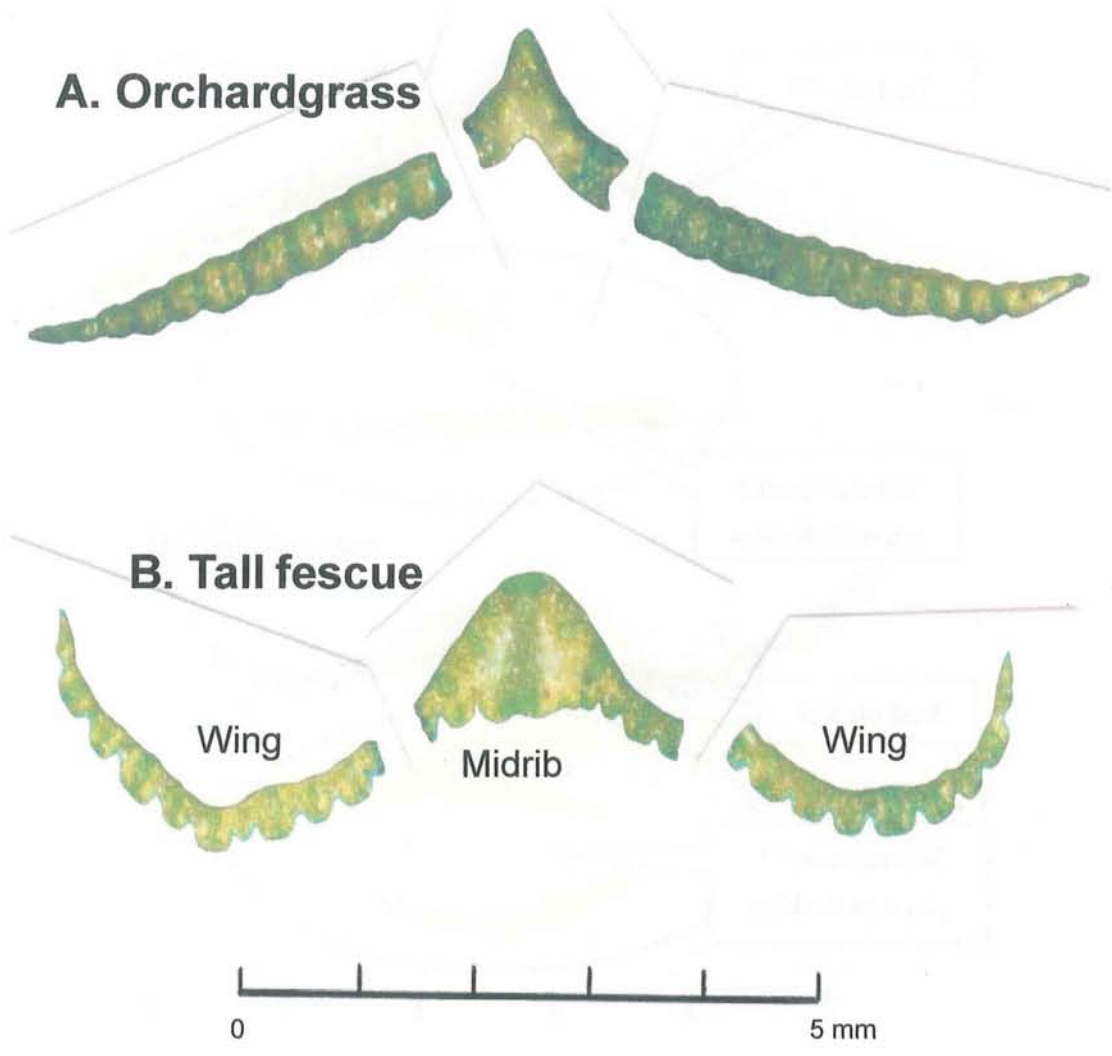


Fig. 3.3.3 Cross-sectional picture of cutting treatments in a leaf blade for partition into three pieces (midrib and two wings of a leaf blade).

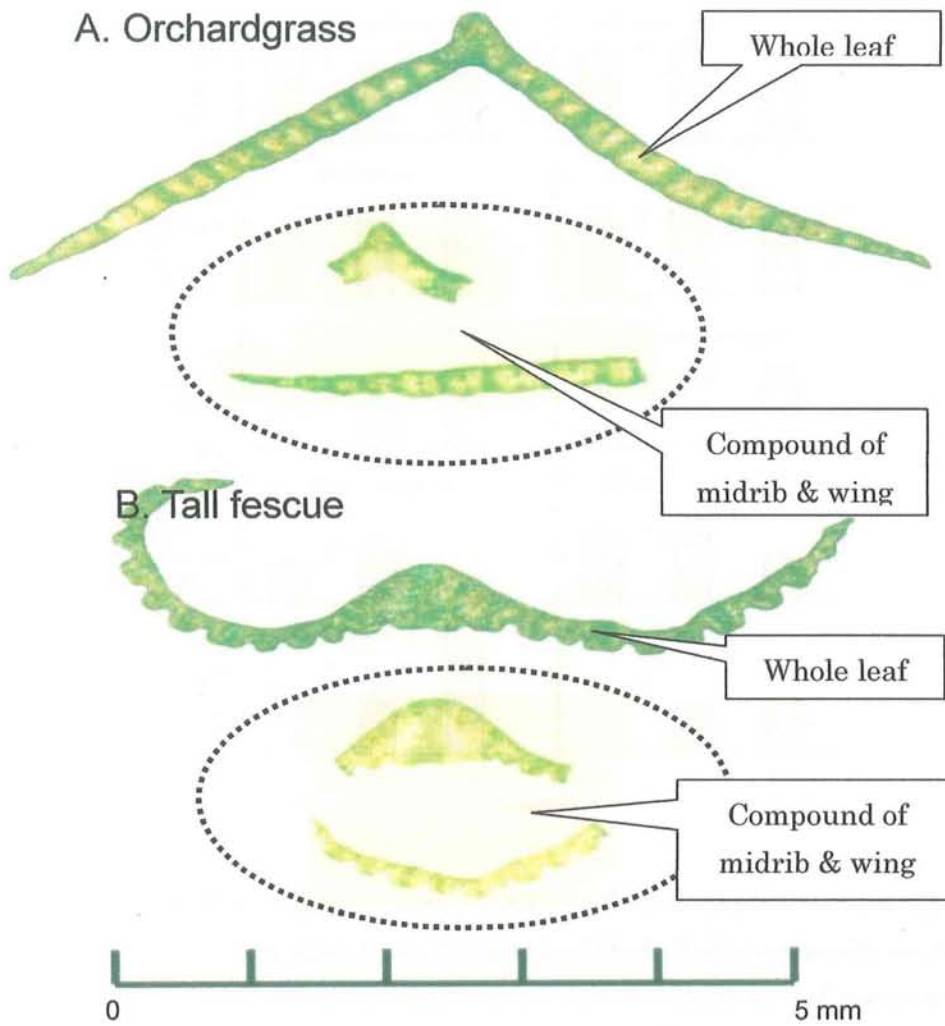


Fig. 3.3.4 Cross-sectional picture of leaf blades. Sampling for evaluation of importance of an interior angle of leaf blade in its biomechanical properties. Measurements were taken on a whole leaf versus a compound of its midrib and two leaf wings.



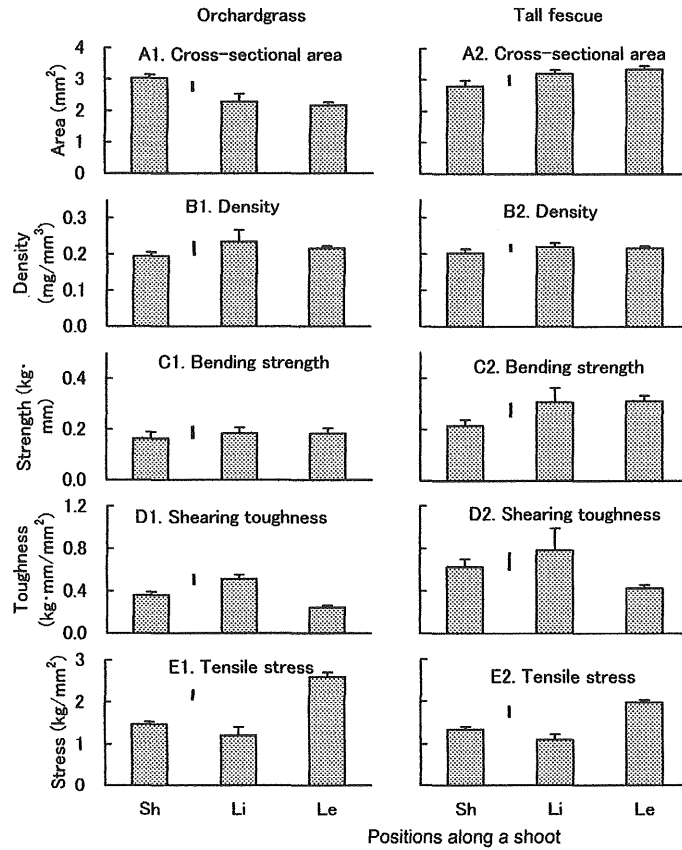


Fig. 3.3.5 Cross-sectional area, density and biomechanical properties at sheath (Sh), ligule (Li) and leaf blade (Le) in orchardgrass and tall fescue. Attached lines on bars show s.e. of mean and vertical lines show s.e.d. of the mean differences.

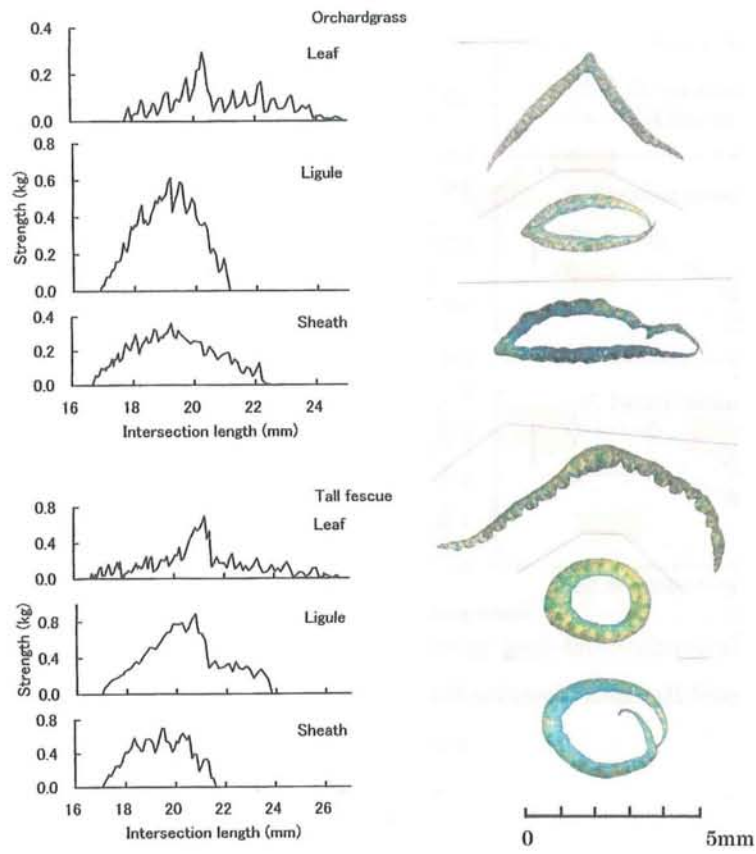


Fig. 3.3.6 Shearing force patterns and cross section at sheath, ligule and leaf blade in orchardgrass and tall fescue.

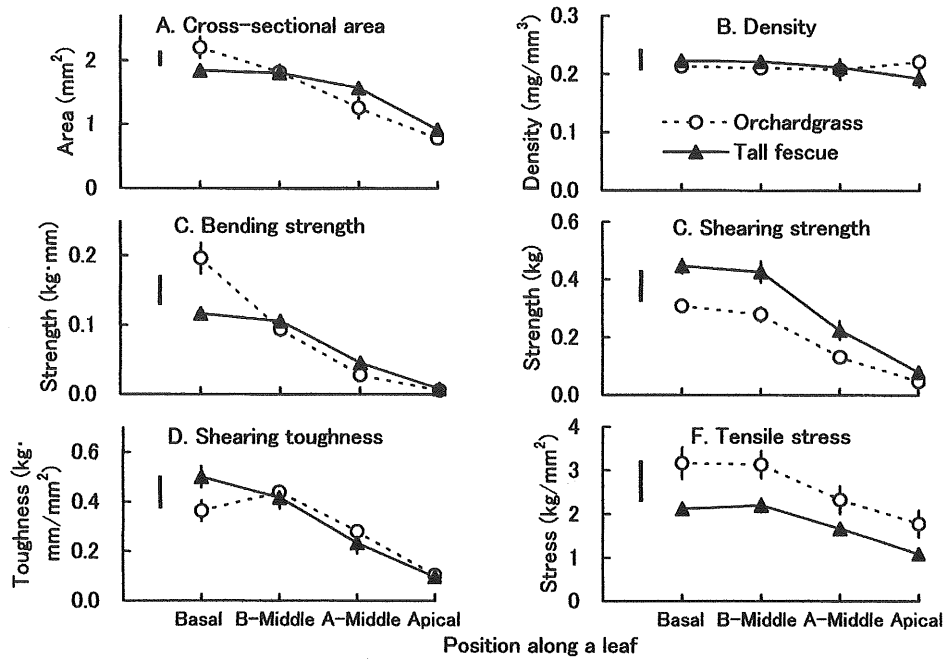


Fig. 3.3.7 Cross-sectional area, DM density and biomechanical properties at 4 positions along a leaf blade in orchardgrass and tall fescue. Vertical lines show s.e.d. of the mean differences.

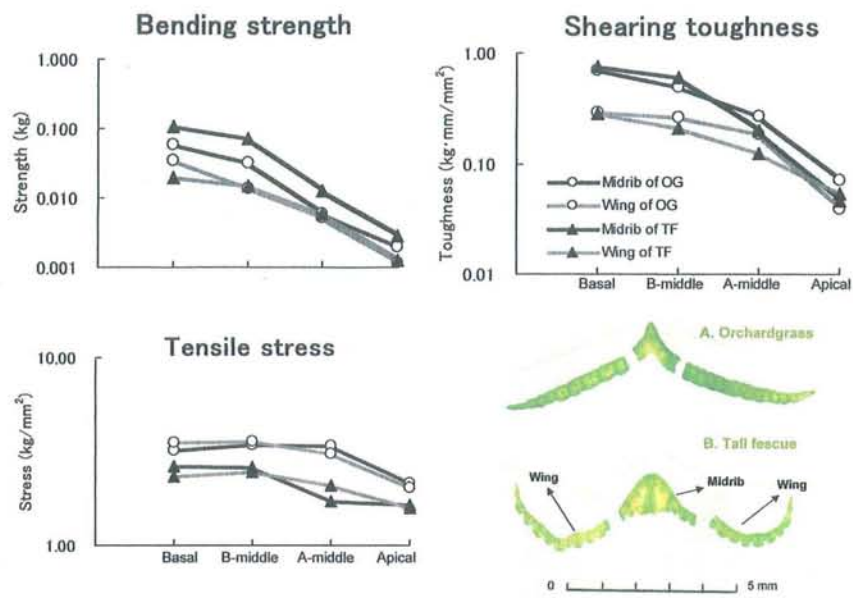


Fig. 3.3.8 Bending strength, shearing toughness and tensile stress of midrib versus wings of leaf blade of orchardgrass (OG) and tall fescue (TF) along with their cross-sectional picture.

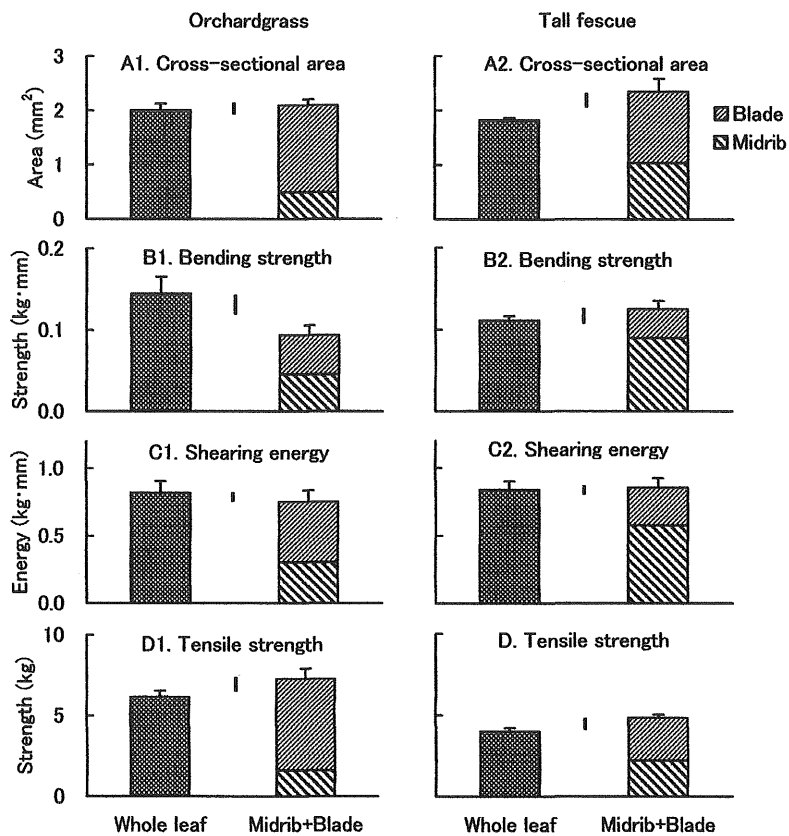


Fig. 3.3.9 Cross-sectional area and biomechanical properties of whole leaf and a compound of separated midrib and wings at a basal position of leaf blade in orchardgrass and tall fescue. Attached lines on bars show s.e. of mean and vertical lines show s.e.d. of the mean differences.

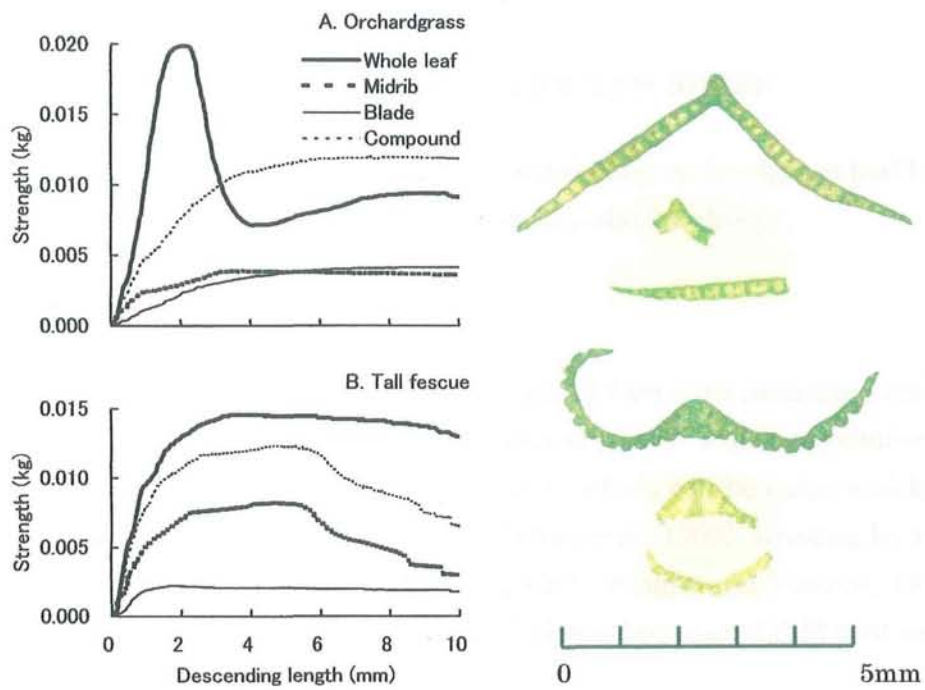


Fig. 3.3.10 Bending strength of whole leaf and separated midrib and wings of leaf blade in orchardgrass and tall fescue. A compound was obtained from bending strength of midrib plus wings of leaf blade.

## Chapter 4

### GRAZING BEHAVIOUR OF SHEEP

#### 4.1 Effect of biomechanical properties along orchardgrass leaf blade on biting force and impulse by sheep

##### 4.1.1. Introduction

Animals try to gather the maximum amount of food with minimum effort (Vincent, 1982). Grazing animals tend to make grazing choice which maximize intake rate (Illius *et al.*, 1992), and to choose plant parts which can be eaten quickly with ease (Kenney and Black, 1984; Hongo, 1998; O'Reagain, 1993). Grazing by ruminants is an action to break plant organs (Vincent, 1982; Wright and Vincent, 1996). Grazers typically remove only uppermost parts of plants because of different resistances to defoliation imposed by the physical structure of plant tissue (Illius *et al.*, 1995). There have been a plenty of works studied how sward canopy structure, through its linkage with herbage biomechanical properties, influenced on the grazing behaviour and/or bite dimensions by grazers, mainly looked at the effect of vertical distribution of sward components such as leaf, pseudo-stem, and dead material etc. MacAdam and Mayland (2003) studied the relationship between leaf strength and cattle preference for eight cultivars of tall fescue and found that both tensile and shear strength of leaf were negatively correlated with preference. However, there are few studies on effect of biomechanical properties at different sites of leaf blades of grasses on grazing behaviour. It is interesting to know how animals respond to different biomechanical characteristics within an individual leaf blade and what controls it.

The main objective of this study is to clarify the influence of biomechanical characteristics at basal or middle sites of orchardgrass leaf blades on grazing behaviour and analyze the three-directional biting forces and grazing impulse exerted by sheep.

#### 4.1.2. Materials and Methods

##### *Animals*

The experiments were conducted from 3rd to 8th August, 2005 at the Obihiro University of Agriculture and Veterinary Medicine in Hokkaido, Japan. Grazing trials were carried out using two Suffolk wethers aged 1.5 years. Animals were fed fresh grass and hay at maintenance levels. Two days before the commencement of grazing trials, animals were trained to be led with a halter and rope, and were accustomed to the hand-constructed sward. Two animals which were more familiar with an apparatus were selected.

##### *Artificial sward board*

The same sward board (Fig. 2.6) previously used by Hongo *et al.* (2004) and Hongo *et al.* (2007) was used for artificial construction of swards. The three-directional biting forces were digitally recorded in a memory card (smart media) at 5 sec<sup>-1000</sup>. One bite was distinguished on the trace by zero force for at least 0.2 sec between adjacent peaks. This definition was decided from the observation of the grazing behaviour of animals. Sequential peaks less than 0.2 sec apart were included into one bite. The number of bites was measured from discrete peaks.

##### *Biting impulse*

With respect to three-directional biting forces, horizontal force was obtained as a compound of backward/forward and rightward/leftward forces, and total biting force as a compound of horizontal and vertical forces (Fig. 4.1.1). The duration time of each biting force was decided by changing pattern of vertical force, since horizontal forces were used in collecting and handling grass leaves during prehension (Hongo *et al.*, 2007). From this total biting force/time curve, biting impulse was calculated, equivalent to the area surrounded by the curve (Schmidt, 1977). Mean biting force was also calculated for the duration time.



### *Herbage grass*

A sward of orchardgrass (*Dactylis glomerata*), sown in May 1988, was fertilized and harvested regularly. It was mown on 30th June and 9th August 2005 and after each harvest applied with a compound fertilizer (10-18-12% of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) equivalent to 200 kg/ha. The third harvest was used for the grazing trials.

For the grazing trials, fresh grass was cut in the early morning. Undamaged, mature leaf blades of vegetative tillers were cut at the ligule. Excluding the tapered end, each leaf blade was clipped into middle and basal parts, to be representative of different levels of stratum in a sward, with a length of 10 cm with scissors to make different cutting treatments for comparison. Leaves were sprayed with water and stored in a polyethylene bag. Five nominal leaf densities for each cutting treatment were taken by attaching 5 (5L), 10 (10L), 15 (15L), 20 (20L) or 25 (25L) leaves per loadcell to an iron bolt, which was coated with rubber tubing, with cotton adhesive tape and further tied fast with 1-mm wire (Fig. 4.1.2). The bolt was then inserted into a nut on the upper end of loadcell and fixed before each grazing trial. One clump of leaves was used in each grazing trial (Fig. 2.7).

### *Grazing trials*

At a grazing trial, animals were led with a halter and rope up to sward board. The duration of time for building the swards for each grazing trial was about less than 5 min. During this period, animals were constrained with a rope.

The clump weight of leaves including an iron bolt was separately measured before and after each grazing trial. Leaves protruded 6 cm above the upper plate of the sward board. When most of the leaves were eaten, the animals were removed. Animals received three replicated clumps from each nominal leaf density and from both the basal and the middle leaf treatments for three days, which made 90 trials in total (3 days x 3 replications x 5 nominal leaf densities x 2 cutting treatments of leaf blade). After each grazing trial, a clump of leaves was removed from a loadcell. Residual lengths of all leaves were individually measured, and bite depth, the average depth of insertion of the mouth into sward canopy, was calculated from the original length of 10 cm. Sub-samples of about 100 g fresh leaves were dried in an oven at 70°C for dry matter (DM) determination. From these results, herbage DM

intake was determined. Water loss from the plant surface by evapotranspiration was ignored because of the short grazing time.

### *Leaf measurements*

Cross-sectional area, bending, tensile and shearing properties of leaf blades used in this experiment were measured in the same way described in chapter 2. Each measurement was replicated 6 times.

### *Statistical analysis*

In the statistical analysis, experimental days were treated as replicates. Variables of bite characteristics were analyzed using a paired t-test and an analysis of variance (Snedecor and Cochran, 1980).

## **4.1.3. Results**

### **4.1.3.1. Biomechanical properties of grass leaves**

#### *Fracture patterns in an individual leaf blade*

The patterns of fractures in both the basal and the middle leaves are shown in Fig. 4.1.3, with the displacement curves and pictures of cross-section from the same test specimen. The fracturing pattern in shear test included numerous small peaks and three distinguished peaks, of which the middle peak corresponded to the main vein of transverse section, and the two outers to the curled edges of leaf blade (Fig. 4.1.3A). On the other hand, there was one peak in the displacement curves which coincides with the breaking point by the tension (Fig. 4.1.3B). In the bending test, there was remarkable peak at about 2 mm of descending length in the basal leaf. This maximum force was created at the moment of flatter an angled leaf as shown in the cross-section (Fig. 4.1.3D). Measured values of fracturing and bending forces were substantially greater for the basal leaf than that for the middle leaf across all three morphologies of test.

### *Biomechanical properties of leaves*

The biomechanical properties such as tensile, shearing and three-point bending strengths and some morphological characteristics of leaf blades are shown in Table 4.1.1.

All measured values of biomechanical properties were significantly greater in the basal leaves than in the middle leaves. Bending, tensile and shearing forces were 8.5, 2.4 and 1.9 times higher in the basal than in the middle leaves, respectively. Correspondingly, bending and tensile stresses and shearing toughness were also 5.9, 1.7 and 2.0 times higher in the basal than in the middle part, respectively. There were a significant correlation between bending force and shearing work of fracture of leaf blades (Fig. 4.1.4).

Values in the morphological characteristics of leaf blades such as, width, DM weight and cross sectional area were significantly higher in the basal leaves than in the middle leaves, but plant density (DM weight per unit volume) showed similar values (Table 4.1.1).

#### 4.1.3.2. Grazing trials

##### *Bite parameters*

There were no significant differences in biting number (Fig. 4.1.5B) and biting size (Fig. 4.1.5C) between the basal and the middle leaves. However, the differences in these parameters between nominal leaf densities were significant.

Sheep penetrated deeper into stubbles made from the middle leaves. Mean biting depth was significantly ( $P < 0.001$ ) greater in the middle leaves ( $48 \pm 1.4$  mm) than in the basal leaves ( $36 \pm 1.4$  mm). However, there was no difference ( $P < 0.973$ ) in bite depth across five leaf densities (Fig. 4.1.5D).

### *Three-directional biting forces*

Three-directional biting forces were separately measured during the grazing trials. There were several peaks in the force of one bite, coinciding with a breakdown of leaves. The force exerted per bite did not significantly vary between treatment swards of middle ( $14.5 \pm 1.55$  N) and basal ( $15.3 \pm 1.26$  N) leaves of orchardgrass.

In terms of force direction, sheep foraged the basal leaves using significantly greater horizontal ( $P < 0.001$ ), backward/forward ( $P < 0.001$ ) and sideward ( $P < 0.027$ ) forces than did the middle leaves. Conversely, there was no significant difference in vertical forces ( $P < 0.712$ ). The significant difference in three-directional force component between nominal leaf densities was observed only for backward/forward direction ( $P < 0.025$ ) (Fig. 4.1.6).

Two types of force-pattern in individual bite were identified from the recorded data during the grazing trials (Fig. 4.1.7). Case A has two peaks, and case B has one. In case B, sheep broke the leaves with only one pull, whereas in case A, sheep used additional forces (horizontal forces), most probably by jerking head when prehended leaves was not broken with the first attempt. The fig. 4.1.7B shows that mean biting force in one-peak case was greater than that in the two-peak case. However, sheep used enough biting forces to harvest prehended grass with one peak (one pull) in most cases (in 134 bites out of 151 bites). In only 17 bites, sheep created two peaks in their bites (Table 4.1.2).

### *Sum of biting impulse*

Sums of biting impulses were similar between the basal and the middle leaves, whereas there was marginal differences between five leaf densities ( $P < 0.054$ ) (Fig. 4.1.8A). The grand mean of sum of biting impulse was also not significantly different ( $P < 0.644$ ) between the basal ( $0.27 \pm 0.045$  kg·s) and the middle leaves ( $0.24 \pm 0.052$  kg·s).

The concept of impulse is ordinarily most useful when the forces are large but act only for a short period. It is necessary to know only the momentum change, which is determined by the impulse. The relation between momentum and impulse has the advantage of eliminating the need for a detailed knowledge of how the forces change with time (Serway, 1982). In this study, the grand means of duration time per bite

and peak total force were  $0.16 \pm 0.005$  sec and  $1.9 \pm 0.16$  kg. It is suggested that the concept of impulse may be useful for a study of animal grazing.

#### *Grazed DM weight and biting impulse*

Grazed DM weights from the basal and the middle leaves were similar, and increased with increasing leaf densities (Fig. 4.1.5A).

To assess the benefit/cost ratio, DM intake per biting impulse was calculated. The ratio was not significantly different ( $P < 0.394$ ) between the two ( $1.11 \pm 0.107$  in the middle versus  $0.99 \pm 0.101$  g-DM/kg·s in basal leaves). There was no difference ( $P < 0.584$ ) between five leaf densities (Fig. 4.1.8B).

The benefit/cost ratio is an important parameter closely related with DM intake and growth rate (Phillips, 1993). The benefit factor may be expressed as DM weight, energy or nutrient contents, but there was no suitable parameter concerning to grazing cost used by animals. In this study, grazing cost was estimated by biting impulse. The DM intake per biting impulse was not affected by five leaf densities.

#### 4.1.4. Discussion

Values of biomechanical properties were remarkably greater in the basal leaves than in the middle leaves.

Depth of biting in the middle leaves was significantly deeper than in the basal leaves, but bite depth was not influenced by the nominal leaf densities. This shows that sheep responded to the difference between the two parts of leaf blades. Herbage biomechanical property may be the most possible explanation for the variation in bite depth between the two parts of leaf blade. The importance of the vegetation's biomechanical properties affecting biting depth has been widely recognized (Illius *et al.*, 1995, and Griffiths *et al.*, 2003b).

The results suggested that sheep could adjust their biting forces in connection with biomechanical property of prehensible grass leaves. Mean number of bites removed per stubble was only 3, at maximum (Fig. 4.1.5B), consequently sheep had almost

no opportunity for adequate appraisal by tasting. Sheep might have, most probably, obtained the information either from first bites or from touch stubbles with her nose, since there was greater difference in the bending strength between two parts of leaf blade (Table 4.1.1). In addition to this, there was a significant correlation between bending strength and shearing work of fracture (Fig 4.1.4). Therefore, it can be concluded that sheep may recognize different biomechanical characteristics of grass leaves in advance of prehension through bending strength. This is also supported by the fact that sheep were able to harvest grass leaves with only one peak in the pattern of biting force in most cases, in 134 bites out of 151 (Table 4.1.2).

Sheep used significantly greater horizontal forces to bite the basal leaves than the middle ones. The significance is particularly strong in case of backward/ forward force (Fig. 4.1.6B). However, the difference in biting resistance across leaf densities was significant for only mean backward/forward force used by sheep. This indicates the importance of difference in biting resistance between the basal and the middle parts of single leaf blade over the difference among leaf densities.

The horizontal biting forces were significantly higher in the basal leaves, suggesting that sheep may try to gain higher biting forces using additional horizontal forces, particularly in backward/forward directions. Therefore, a change of biting strategy may be explained by increasing involvement of teeth in biting to initiate a bigger crack on leaf blades and then be able to propagate with little effort (Vincent, 1990). This is also supported by our finding that the biting forces exerted by sheep in the trials surprisingly agreed the measured values in shear forces of corresponding number of leaves (Fig. 4.1.9). Additionally, the measured tensile strength in the test was far greater than the shear strength in the same leaf part, and it exceeded the biting force exerted by sheep as well.

However, Vincent (1990) emphasized the importance of tensile force in breaking grass by large grazers and he included sheep in the group by suggesting to note that the teeth are expressly not used and may even be absent (e.g., sheep upper front teeth). On the other hand, Hongo *et al.* (2004) found that changes in incisor dentition influenced biting force by comparing the grazing behaviour by sheep, after the loss of the temporary incisor and before their replacement with the permanent incisors, with that after the first pair of permanent incisors had completely developed. On that account, we are claiming that it is the shear force which will be the most important for severing grass leaves by sheep when grazing (Fig. 4.1.10).

#### 4.1.5. Conclusions

The strength of an orchardgrass leaf blade increased toward the basal side of the leaf blade. A significant positive correlation between bending strength and shearing work of fracture of a leaf blade showed that sheep can recognize the different biomechanical properties of the basal and the middle parts of the same leaf blade of orchardgrass and responded to it by changing biting strategy, even though the difference was not big to such an extent that it depresses DM intake.

The results from the present study confirm that herbage biomechanical property is one explanation for the determination of bite depth. Shear force plays an important role in sheep grazing to severe grass leaves.

#### 4.1.6 Summary

The grazing behaviour of sheep in response to different biomechanical characteristics of orchardgrass leaves was investigated using biting forces and associated impulses in grazing. Two Suffolk wethers were used in the grazing trials. Five, 10, 15, 20 or 25 leaves from two different sites (basal and middle) of leaf blades of orchardgrass (*Dactylis glomerata*) per loadcell were offered to animals, and three-directional biting forces were digitally recorded at 5 sec<sup>-1000</sup>. Biomechanical properties such as tensile, shear and three-point bending strengths were measured in sampled leaf blades, afterwards. Bending, tensile and shearing strengths were 8.5, 2.4 and 1.9 times higher in the basal than in the middle leaves, respectively. There was a significant positive relationship between bending strength and shearing work of fracture of leaf blades.

Sheep grazed the basal leaves with additional horizontal forces than they did the middle leaves, particularly in backward/forward direction. The sum of biting forces exerted by sheep during grazing trials agreed well with the sum of shearing strength of corresponding number of severed leaves, while the estimated values from tensile strength were more than ten times higher than the sum of biting forces exerted by sheep. The results showed that shearing properties of leaf blades may play an important role in sheep grazing behaviour. It suggests that sheep may recognize biomechanical properties of leaf blades prior to prehension through the bending strength of the leaves, so that they can adjust their bite parameters.

Table 4.1.1 Morphological and biomechanical features of the basal and the middle parts of orchardgrass leaves

	Basal leaf	Middle leaf	sed	Probability
Leaf width (mm)	6.2±0.24	5.6±0.22	1.30	<i>P</i> =0.075
Cross-sectional area (mm <sup>2</sup> )	1.74±0.124	1.20±0.070	0.572	<i>P</i> <0.001
DM density (mg·DM/mm <sup>3</sup> )	0.130±0.0049	0.133±0.0059	0.0310	<i>P</i> =0.651
Tensile force (N)	62.5±5.12	25.6±1.88	21.94	<i>P</i> <0.001
Tensile stress (MPa)	36.1±1.82	21.6±1.59	9.73	<i>P</i> <0.001
Bending force (N)	0.110±0.0216	0.013±0.0040	0.0883	<i>P</i> <0.001
Bending stress (MPa)	0.062±0.0105	0.010±0.0027	0.0435	<i>P</i> <0.001
Shearing work of fracture (10 <sup>-3</sup> J)	7.52±0.849	2.67±0.435	3.839	<i>P</i> <0.001
Shearing toughness (10 <sup>3</sup> J/m <sup>2</sup> )	4.30±0.323	2.21±0.291	1.749	<i>P</i> <0.001

Figures show mean±se. The mean total length of leaves was 571±26.2 mm.



Table 4.1.2 Proportions in biting force patterns

	Basal leaf			Middle leaf			Total		
	1-peak	2-peak	2-peak %	1-peak	2-peak	2-peak %	1-peak	2-peak	2-peak %
L5	9	1	10.00	11	0	0.00	20	1	4.76
L10	8	2	20.00	11	2	15.38	19	4	17.39
L15	13	4	23.53	12	2	14.29	25	6	19.35
L20	19	0	0.00	18	3	14.29	37	3	7.50
L25	15	2	11.76	18	1	5.26	33	3	8.33
Mean			12.33			10.26			11.26

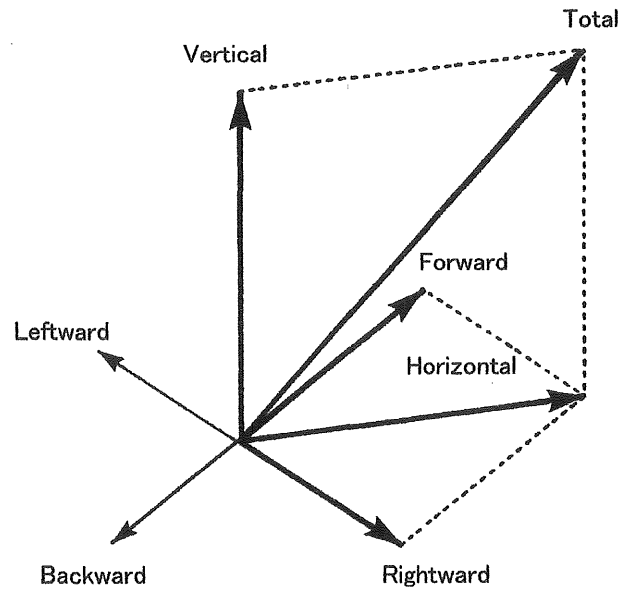


Fig. 4.1.1 Calculation method of total biting force from three-directional force component

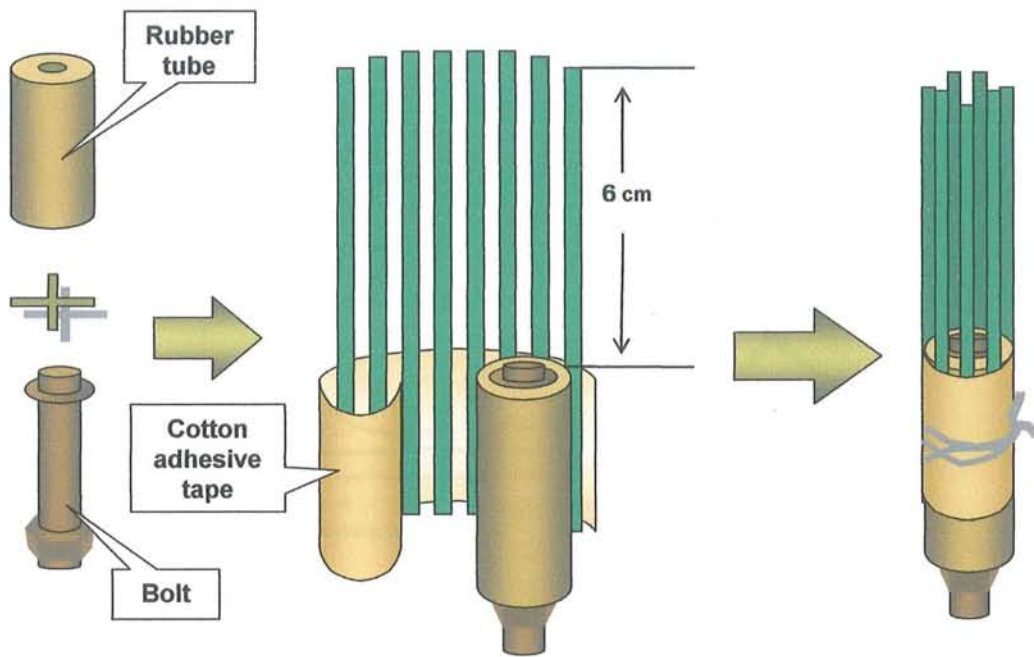


Fig. 4.1.2 Process of preparing a bolt attached with leaf blades of orchardgrass for making hand-constructed swards

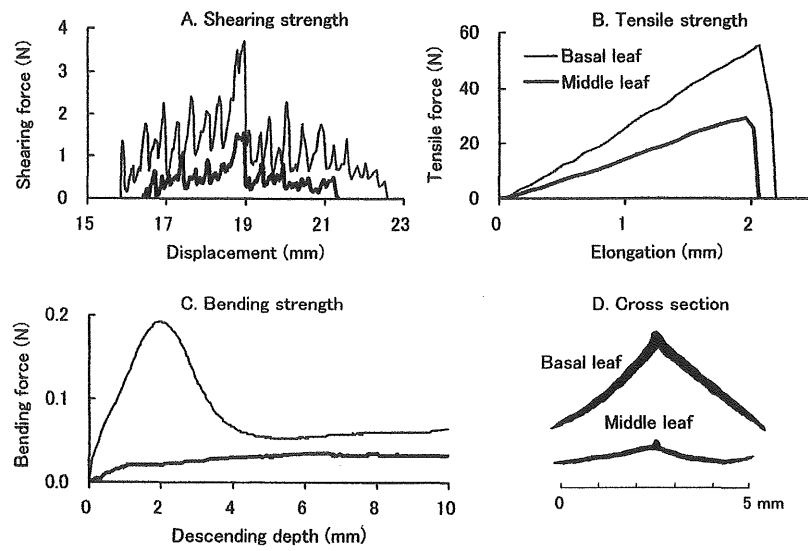


Fig. 4.1.3 Shearing, tensile and bending properties, and cross sections of the basal and the middle parts of orchardgrass leaves. Each parameter was measured in the same leaf.

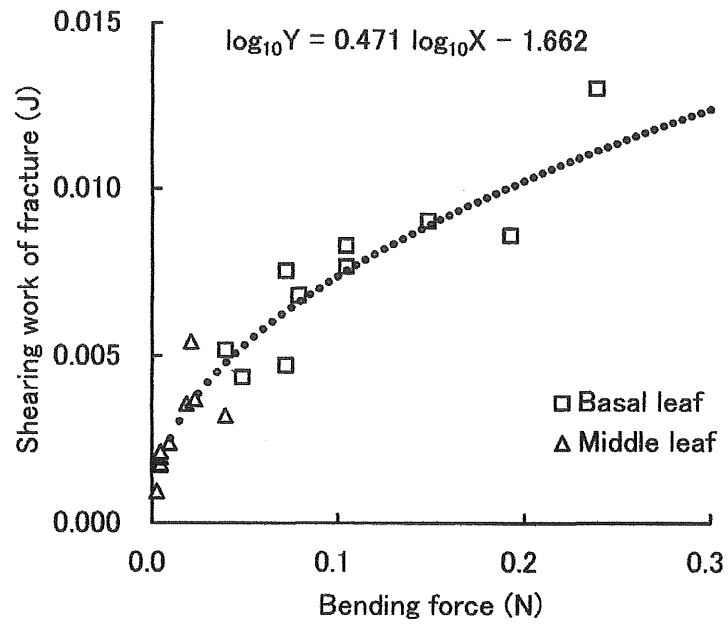


Fig. 4.1.4 Relationship between bending force and work of shearing fracture in orchardgrass leaves.

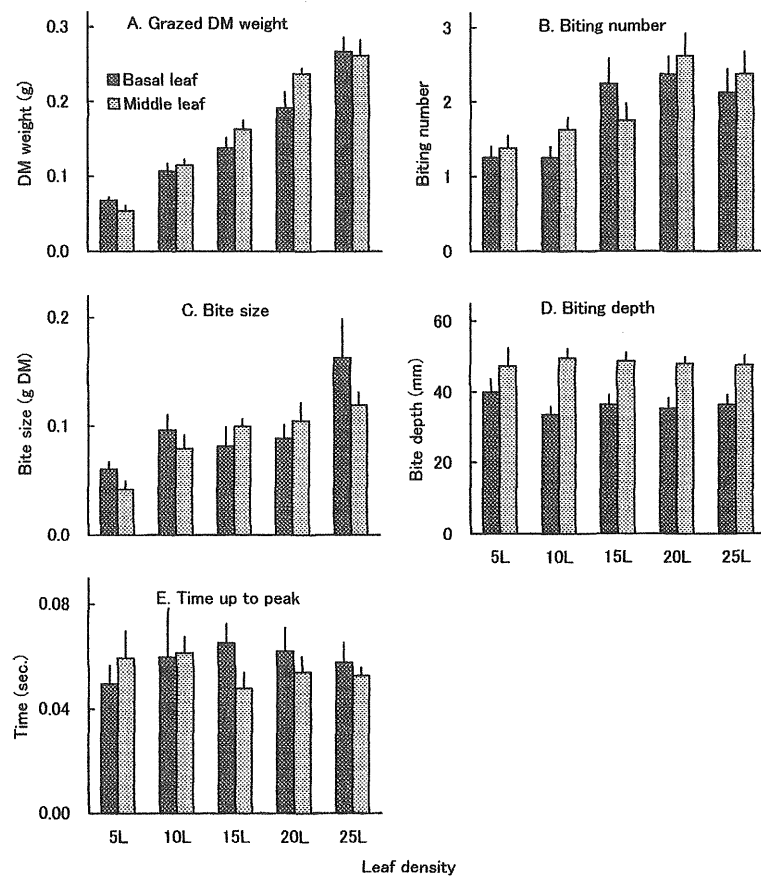


Fig. 4.1.5 Grazed DM weight, biting number, bite size, bite depth and time up to the peak biting force in grazing the basal and the middle parts of orchardgrass leaf blades. Attached lines on bars show s.e. of mean.

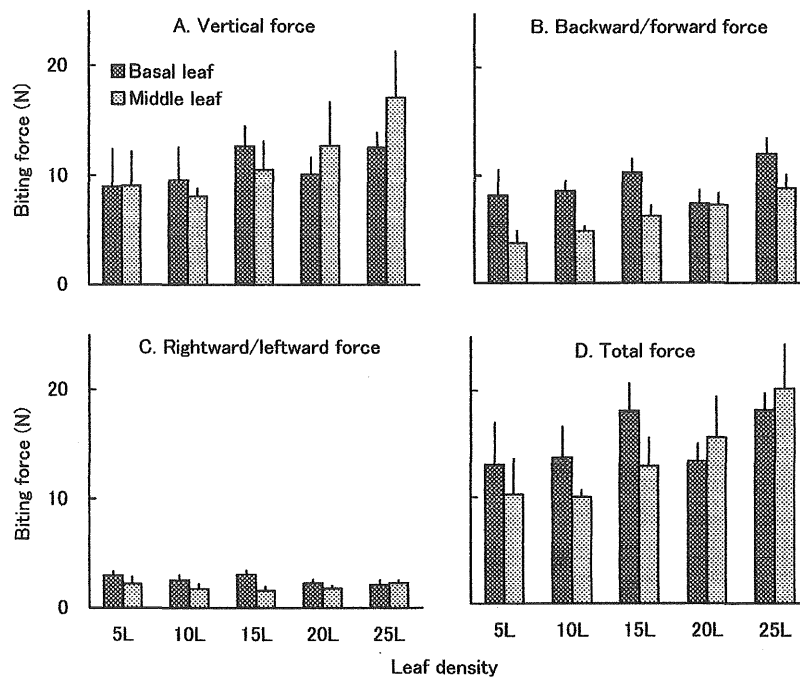


Fig. 4.1.6 Three-directional biting force component and total force in grazing the basal and the middle parts of orchardgrass leaf blades. Attached lines on bars show s.e. of mean.

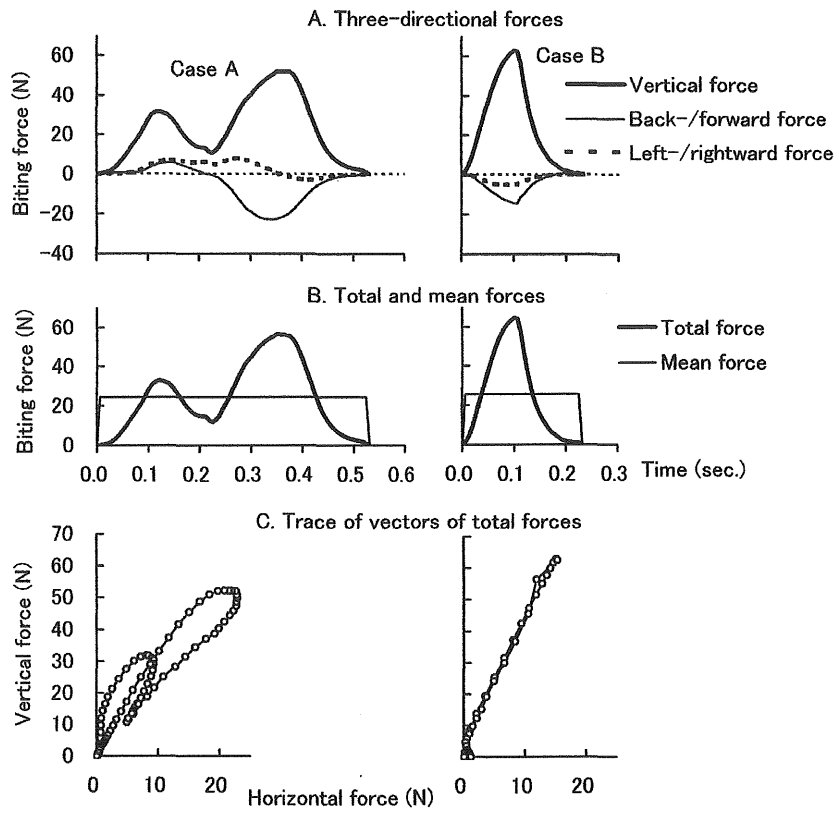


Fig. 4.1.7 Three-directional, total and mean forces, and trace of vectors of total forces at an interval of 0.006 sec.



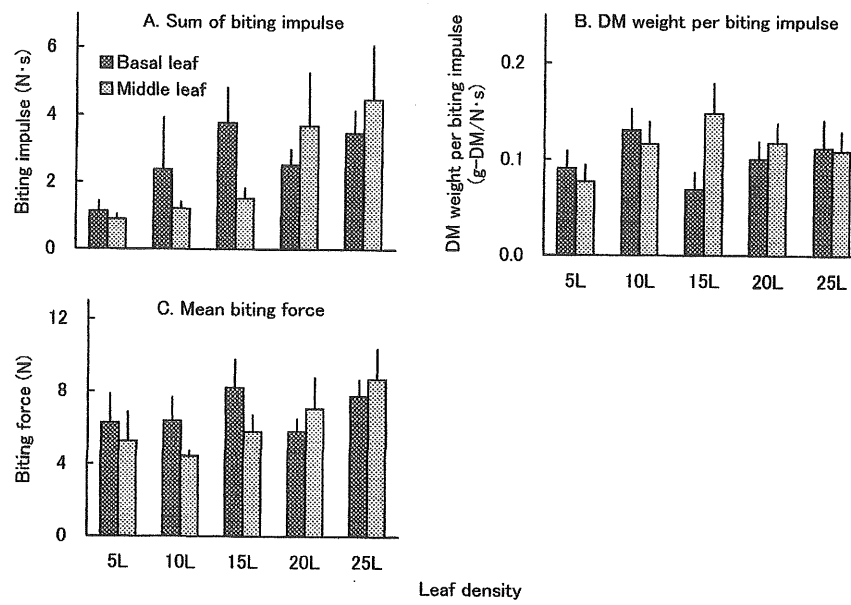


Fig. 4.1.8 Sum of biting impulse, DM weight per biting impulse, and mean biting force in grazing the basal and the middle parts of orchardgrass leaf blades. Attached lines on bars show s.e. of mean.

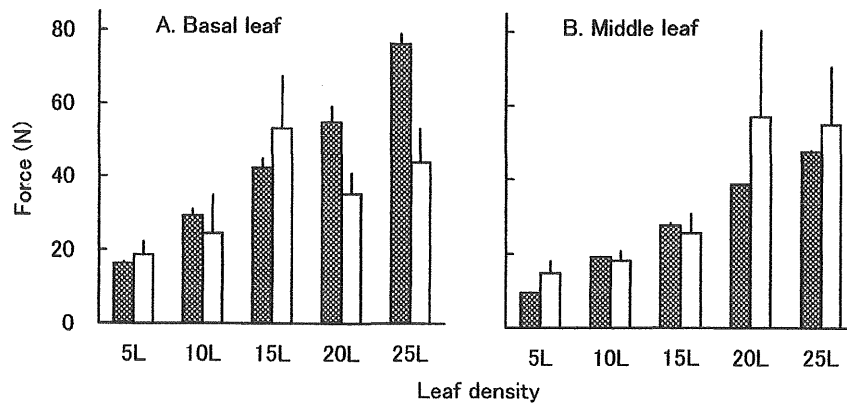


Fig. 4.1.9 Comparison of shearing forces estimated from grazed leaf number and shearing strength per leaf (solid bars) to the sum of peak biting forces created by sheep (open bars). Attached lines on bars show s.e. of mean.

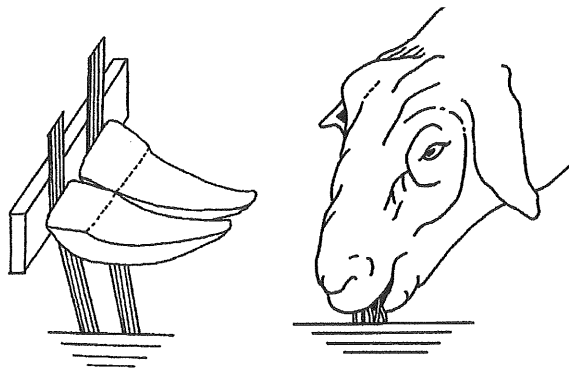


Fig. 4.1.10 Model of use of shearing action in sheep grazing by gripping grass leaves between incisors and dental pad.

## 4.2 Biting strategy of sheep in grazing grass leaf blades of diploid and tetraploid cultivars of perennial ryegrass

### 4.2.1 Introduction

Grazing by ruminants is an action to break plant parts away from the whole plant organs (Vincent, 1982; Wright and Vincent, 1996). Plant parts are severed by the lower incisors against the dental pad as the animal jerks its head slightly forwards and upwards (Hafez *et al.*, 1969). This biting behaviour is performed by head movement due to the action of dorsal neck muscles (Dyce *et al.*, 1987), and the activity of muscle produces biting force at the pointed ends of incisors.

The benefit/cost ratio is expressed as intake efficiency, which is calculated by intake amounts of DM weight, energy or nutrient contents against biting energy or force used by grazing animals. The benefit/cost ratio seems to be an important parameter closely related with DM intake and animal productivity such as growth rate and milk production (Barrett *et al.*, 2001; Phillips, 1993). Grazing ruminants may feed plant organs by lower biting cost, but there is no suitable parameter concerning to biting cost (Illius *et al.*, 1995).

Grazing ruminants appear to adjust the bite area to the force required to break plant parts (Laca *et al.*, 1993). An increase in number of leaves or stems per unit area should cause a reduction in bite area (Laca *et al.*, 1992a). There are upper limitations on the biting force that can exert per bite (Hodgson, 1985) and on the number of leaves which they can break per bite (Vincent, 1990).

These results suggested that two kinds of decision are required for grazing ruminant before prehension. The first concerns the decision to hold the number of leaves into their mouth. The second concerns the level of biting force, by which grazing ruminant will exert to break every plant materials simultaneously. The biomechanical characteristics of plants may be an important aspect of plants' resistance to grazing ruminant, and have a close relation with these two kinds of decision (Wright and Illius, 1995). To perform biting behaviour successfully, grazing ruminant must be thought to recognize total biomechanical strength of plant materials through various sense organs before prehension. Arnold (1966a) reported that lip-touch was important in determining the acceptability of some forage species. The sense of touch plays a major role in determining which items are rejected or preferred (Hafez *et al.*, 1969). A special sort of cutaneous sense is mediated by the

tactile hairs (Dyce *et al.*, 1987).

The diploid cultivar of perennial ryegrass (*Lolium perenne*) such as Aurora had been selected for higher concentrations of water-soluble carbohydrates, and for more small tillers than the tetraploid cultivars (Smith *et al.*, 2001; Davies *et al.*, 1991). Palatability was reported to be higher in Aurora than in tetraploid cultivars under rotational sheep grazing (Jones and Roberts, 1991). Thus, there are many studies on feeding values and chemical compositions of various elements in perennial ryegrass cultivars. However, few studies had been reported with respect to biomechanical characteristics and biting behaviour in diploid and tetraploid cultivars of perennial ryegrass.

There were several studies in biting forces used by grazing animals during prehension of plant materials (Hongo and Akimoto, 2003; Hongo *et al.*, 2004). These studies examined the relationship between peak biting force and DM intake. The objective of this study is to clarify biting strategy of sheep to harvest grass leaf blades and the effect of biting forces on DM intake with respect to an indicator of benefit/cost ratio.

#### 4.2.2 Materials and methods

##### Experimental design and animals

The experiments were carried out to follow the guideline of Obihiro University of Agriculture and Veterinary Medicine for proper conduct of animal experiment and related activity in academic research.

The experiments were composed of two sections: measurements of biting forces by sheep (grazing trial) and biomechanical properties of grass leaf blades. The experiments were conducted on 3 days (5<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup>) in August, 2005, at the Obihiro University of Agriculture and Veterinary Medicine in Hokkaido, Japan. Grazing trials were carried out using two Suffolk wethers (mean live weight of 71 kg) aged 2 years. Sheep were fed on fresh grasses of orchardgrass (*Dactylis glomerata*) and hay of timothy (*Phleum pratense*) at maintenance levels for a week. Two days before the commencement of grazing trials, sheep were trained to be led with a halter and rope, and became accustomed to the artificial sward board (described in chapter 2). Two animals were selected.

## Herbage grass

Two cultivars (diploid Aurora and tetraploid Prospero) of perennial ryegrass from Wales, UK were used: The swards of two cultivars (4 blocks of 4x5 m scale for each cultivar) were established in June 2002, and had been fertilized and harvested by the common methods. In the spring of 2005, the swards received a compound fertilizer (10-18-12% of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) equivalent to 65-117-78 kg/ha of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, and were monthly mown at 5 cm height. The fourth harvest was used for the grazing trials.

## Grazing trial

During the grazing trials, fresh grasses were cut in the early morning. Undamaged mature leaf blades of vegetative tillers were cut at the ligule. Each leaf blade was clipped 11 cm in length at the basal part with scissors. Leaf blade segments were stored in a polyethylene bag. Four nominal leaf densities were taken by attaching numbers of 10 (10L), 20 (20L), 30 (30L) or 40 (40L) leaf blade segments per loadcell to an iron bolt, which was coated with rubber tubing, with cotton adhesive tape and further tied fast with 1-mm wire. The bolt was then inserted into a nut on the upper end of loadcell and fixed before each grazing trial. One clump of leaf blade segments was used in each grazing trial. Two sheep were offered each clump of four densities on one day. Total clumps tested were 48 (2 cultivars x 2 sheep x 4 densities x 3 days).

The same artificial sward board and the recorder were used as previously reported (Hongo *et al.*, 2007). The sward board was composed of the three-dimensional loadcell (Fig. 2.3). The loadcell was used in order to detect biting forces exerted by sheep. Electrical signals of the loadcell were sent to a dynamic strain amplifier (NEC San-ei; AS2101). Amplified signals were digitally recorded at 0.006-second intervals as strain-time data using a memory hicorder (Hioki Co.; 8860 Type) with 16 channels.

At a grazing trial, sheep were led with a halter and rope up to sward board. The duration of time for building the swards for each grazing trial was about less than 5 min. During this period, sheep stood by with a rope.

The clump weight of leaf blade segments including an iron bolt was separately measured before and after each grazing trial. Leaves protruded 6 cm above the

upper plate of the sward board. When most of the leaves were eaten, sheep were removed. Sheep received three replicated clumps from each nominal leaf density. After each grazing trial, a clump of leaves was removed from a loadcell. Sub-samples of about 100 g fresh leaves were dried in an oven at 70°C for 48 hours for dry matter (DM) determination. From these results, herbage DM intake was determined. Water loss from the plant surface by evapotranspiration was ignored because of the short grazing time.

### **Biting parameters**

Biting forces of three-directional loadcell were saved as force/time data in a memory hicorder. For the composition of two forces, the resultant force was calculated by the vector addition method. At first, the horizontal force was obtained by the compound of backward/forward and rightward/leftward forces (Fig. 4.2.1A). Then, total biting force was obtained by the compound of horizontal and vertical forces (Fig. 4.2.1B). Peak biting force was the maximum value of total biting forces and mean biting force was obtained by averaging of every biting forces. The duration time of each bite was decided by changing pattern of vertical force, since horizontal forces were usually used in collecting and handling leaf blades during prehension. Traces of biting vectors were also obtained from both horizontal and vertical forces (Fig. 4.2.1C). Force/time figures were drawn on a monitor screen. The number of bites per point (loadcell), duration time per bite and time up to a peak biting force were obtained in a figure of total biting forces. Number of grazed leaves per bite and DM weight per bite were calculated by dividing total value by number of bites.

In order to assess the benefit/cost ratio in biting behaviour, the intake efficiency was defined as DM weight per mean biting force.

### **Length and weight of leaf blades**

After the grazing trials, 10 leaf blades with a similar size were chosen and clipped at the ligule side with scissors. At first, a total length of a leaf was measured. The basal segments of each leaf blade were cut into 10 cm in length from the ligule side with scissors and had a midpoint marked. The weight of both segments (basal segment 10-cm in length and the remaining part) was separately measured after

absorbing water on leaf surface with paper towel. The width of 10-cm segment was measured at midpoint with scaled magnifier under light pressure. Afterwards, 10-cm segments were immersed in water for at least 5 min, so that full turgor could be achieved before a measurement of biomechanical characteristics (Chan *et al.*, 1999). The remaining part was dried in an oven at 70°C for 48 hours for DM determination.

### ***Leaf measurements***

Cross-sectional area, bending, tensile and shearing properties of leaf blades used in this experiment were measured in the same way described in chapter 2. Each measurement was replicated 6 times.

### **Biting force per leaf blade**

From two parameters, total biting forces exerted by sheep and grazed leaf number per bite, an observed value of biting force per leaf was calculated.

### **Statistical analysis**

On each of three days, 16 clumps (2 sheep x 2 cultivars x 4 densities) were offered to sheep. In the statistical analysis, 3 experimental days were treated as replications and sheep as blocks. Variables of bite characteristics were analyzed using a paired t-test and an analysis of variance (Snedecor and Cochran, 1980). Linear regression was applied for testing the relationship between bending moment and mean shearing strength of leaf blade segment.

### **4.2.3 Results**

#### **Two patterns of biting forces**

Two patterns of biting forces were identified from the recorded data (Fig. 4.2.1B). In



Case 1, sheep broke leaf blades by a biting force with only one peak. In Case 2 with two peaks, however, sheep tried to break leaf blades by horizontally backward force, but could not severed leaf blades at the first attempt. Consequently, sheep changed horizontal force direction from backward to forward in order to break the prehended leaves (Fig. 4.2.1C). Grand mean of biting number per loadcell was  $2.19 \pm 0.12$  in all 48 clumps tested. In total, 105 bites were observed, of which 10 bites in Aurora and 12 bites in Prospero had two peak forces during a bite, corresponding to 26.3% and 17.9% of total bites (38 and 67 bites), respectively. The percentage values of bites with two peak forces were not significantly different among any treatments.

Most biting forces (78.4% of total bites in Aurora and 83.1% in Prospero) exerted by sheep were included in range of 3.5 to 22.1 N. However, sheep showed two extremely high biting forces (57 and 80 N) in the 40L treatment of Aurora. These two data were excluded from the subsequent calculations, because of exceptional high values.

### **Biting parameters**

The number of bites per point (loadcell), number of grazed leaves per bite, and DM weight per bite increased with increasing leaf densities (Fig. 4.2.2). Sheep used significantly more bites per loadcell ( $P < 0.001$ ) in grazing leaf blades of Prospero than those of Aurora. In contrast, the number of grazed leaf blades per bite was significantly lower ( $P = 0.002$ ) in grazing leaves of Prospero than those of Aurora. DM weight per bite was significantly higher ( $P = 0.027$ ) in Prospero than Aurora. Duration time per bite and time up to a peak biting force were not significantly different among any treatments. The grand means of duration time per bite and time up to a peak biting force were  $0.169 \pm 0.005$  and  $0.061 \pm 0.003$  sec, respectively.

### **Biting forces**

There was no significant difference between Prospero and Aurora with respect to three-directional biting force component (Fig. 4.2.3). Biting forces were significantly different among four leaf densities, mainly due to lower values at the 10L treatment. Sheep used similar biting forces at higher leaf densities (20L - 40L) in both Aurora and Prospero. The mean values at 20L - 40L treatments were  $11.9 \pm 0.9$  N in vertical

force,  $8.2 \pm 0.5$  N in horizontal force,  $14.9 \pm 0.9$  N in total force, and  $6.3 \pm 0.4$  N in mean force (42% of total force).

The intake efficiency was not significantly different among four leaf densities ( $P=0.112$ ) and between two cultivars ( $P=0.301$ ). These results were influenced by extremely low intake efficiency at the 20L treatment of Aurora, due to extreme high values of biting forces, especially in vertical direction. There was a tendency of increasing intake efficiency with increasing leaf density, excluding the result at the 20L treatment of Aurora. The grand mean of intake efficiency was  $14.4 \pm 1.0$  mg DM/N.

Biting angles composed of horizontal and vertical forces were not significantly different among any treatments. The grand mean of biting angles was  $53.1 \pm 2.0$  degrees.

#### **Fracturing patterns of leaf blades**

The fracturing force patterns of leaf blades in tensile, shearing and bending tests, and shapes of cross sections in two cultivars are shown in Fig. 4.2.4. Each result was obtained from the same test specimen.

In a tensile test, there was one peak in the strength/elongation curves (Fig. 4.2.4A). Elongation length was not significantly different between two cultivars. Mean values of elongation length were 1.6-1.7 mm (approximately 6% of the original length).

In a shearing test, the traveling length from a hinge to a moving cross-head of the two blades of the scissors was expressed as displacement length (Fig. 4.2.4B). The peak shearing force was observed at the central position along a displacement, corresponding to the fracture of the midrib in the cross section (Fig. 4.2.4D).

In a bending test, the remarkable peak force was observed at about 2 mm bending depth only in Prospero.

### **Morphological characteristics of leaf blades**

The length and width of leaf blades were significantly higher ( $P<0.001$ ) in Prospero than those in Aurora (Table 4.2.1). Cross-sectional area was about twice in Prospero than Aurora. Plant density was significantly different ( $P<0.001$ ).

### **Biomechanical characteristics of leaf blades**

Bending moment ( $P=0.029$ ), and tensile ( $P=0.006$ ) and maximum shearing strengths ( $P=0.002$ ) of leaf blades were significantly higher in Prospero than in Aurora (Table 4.2.1). However, there was no significant difference in tensile stress ( $P=0.156$ ) and shearing toughness ( $P=0.955$ ), which were the breaking strength normalized with respect to the cross-sectional area.

Mean biting force per leaf exerted by sheep was 0.42-0.64 N, compared with 0.26-0.34 N of mean shearing strength (Table 4.2.1). Peak biting force per leaf exerted by sheep was 0.94-1.46 N, compared with 0.89-1.31 N of maximum shearing strength.

### **Correlation between bending moment and mean shearing strength**

Mean shearing strength linearly correlated with bending moment (Fig. 4.2.5), although not statistically significant (d.f.=18,  $r=0.434$ ,  $P=0.056$ ). There was a considerable variation of mean shearing strength at lower bending strength.

## **4.2.4 Discussion**

### **Recording equipment**

In the previous studies (Hongo and Akimoto, 2003; Hongo *et al.*, 2004), biting forces were analogically recorded on a chart paper and only peak biting forces were measured. Analogical recording had serious limitations such as a mask of several small peaks within one biting peak. In this study, three-directional biting forces were digitally recorded at an interval of 0.006 sec. This method had a great

advantage for reproducing force-time curves at any time scale and measuring precise duration time.

### Breaking of leaf blades by shearing force

Wright and Vincent (1996) suggest that ruminants such as sheep and cattle commonly use tensile strength in severing prehended herbage. However, if sheep use tensile force to break leaf blades in this study, the estimated values of total biting force were 132 N (15.0 leaves per bite x 8.8 N of tensile strength) in Aurora and 162 N (12.3 leaves x 13.2 N) in Prospero, corresponding to 10.5 and 11.7 times, respectively, higher than observed total biting forces exerted by sheep. Therefore, tensile strength seems to be minor factor for breaking of leaf blades.

Since the number of grazed leaves per bite was similar at 30L and 40L treatments (Fig. 3B), the mean values at these treatments seem to be the upper limit of leaf number per bite. The mean values of grazed leaf number per bite were  $19.8 \pm 1.4$  in Aurora and  $12.7 \pm 1.6$  in Prospero. From the number of grazed leaves per bite and shearing strengths of a single leaf (Table 4.2.1), mean biting forces were estimated as to be 5.1 N and 4.3 N in Aurora and Prospero, respectively. These calculated values seem to be corresponding to observed mean biting forces ( $5.5 \pm 0.5$  N in Aurora and  $6.5 \pm 0.4$  N in Prospero) at 30L and 40L treatments observed in the grazing trials as shown in Fig. 4.2.3D. Similarly, the estimated values of total biting forces were 17.6 N in Aurora and 16.7 N Prospero, compared with observed values ( $13.3 \pm 1.1$  N in Aurora and  $16.4 \pm 1.0$  N in Prospero). These results suggest that sheep may break leaf blades mainly by shearing force. When ruminants hold grass leaves into their mouth, leaves may be bent and damaged by the lower incisors against the dental pad (Vincent, 1990). This action may directly relate with shearing force by the lower incisors.

Observed mean values of mean biting forces were  $5.5 \pm 0.5$  N in Aurora and  $6.5 \pm 0.4$  N in Prospero (Fig. 4.2.3D), corresponding to 0.8% and 0.9% of live weight of sheep in Aurora and Prospero, respectively, according to the calculation after converting Newton force into kilogram force. Similarly, observed mean values of total biting forces were  $13.3 \pm 1.1$  N in Aurora and  $16.4 \pm 1.0$  N in Prospero, corresponding to 1.9% and 2.4%, respectively. These results suggest that total biting forces which grazing animals can exert to sever individual mouthful of leaf blades may be controlled by

live weight of grazing animals.

### **Lever model in sheep grazing**

The result suggests that ruminants may sever plant organs by shearing force. This biting behavior is performed by head movement due to the action of dorsal neck muscles. In the musculoskeletal lever systems, the joint between the atlas and the skull act as fulcra (Dyce *et al.*, 1987). It is suggested that biting strategy of sheep may be shearing break-down by the application of the principle of the lever in order to break plant organs with a lower biting force and cost. The activity of muscle produces biting force at the pointed ends of incisors, resulted in successful cutting of plant materials. In this model, plant organs must be severed by the transverse force, like shearing force, and dimension of leaf severage may furnish bowl-shaped bite volume (Laca *et al.*, 1993; Woodward, 1998). It is considered that biting forces with two peaks (Fig. 4.2.1) are the most effective method to feed plant organs with the lowest cost. When ruminants move forward in a grazing position and jerk their head slightly forwards and upwards, the angle of incisors may keep a horizontal level, resulting in effective harvest of plant organs.

### **Control of leaf number into a mouth**

Before prehending bite, two kinds of decision are required for grazing ruminants. The first is the decision of the number of leaves to hold into their mouth, and the second concerns the level of biting force, by which grazing ruminants will exert to break every leaf blades simultaneously. In this study, biting forces were not variable among 4 treatments of leaf-density. Sheep usually used low total biting forces (13 – 17 N at 20L - 40L treatments). Therefore, the first decision factor of the leaf number to hold into their mouth seems to be most important for ruminant grazing. The similar results are reported that ruminants are capable of applying larger bite forces than they actually apply, and have to take a large number of bites per day (Parsons and Chapman, 1998; Tharmaraj *et al.*, 2003). One advantage of controlling the force applied in each prehending bite may be that it helps to maintain the harvesting process for a longer period of time by establishing a uniform force or a uniform momentum (work per unit time) of grazing. Usually, grazing animals tend

to select soft plant materials which require lower biting forces during grazing (Illius *et al.*, 1995).

In order to control the leaf number into a mouth, grazing ruminants seem to recognize total physical strength of leaf blades through various sense organs during prehension. Arnold (1966a) reported that lip-touch was important in determining the acceptability of some forage species. The sense of touch plays a major role in determining which items are rejected or preferred (Hafez *et al.*, 1969). A special sort of cutaneous sense is mediated by the tactile hairs (Dyce *et al.*, 1987). The walls of blood spaces surrounding the roots of these hairs contain numerous nerve endings. When the tips of the sinus hairs are touched, these nerve endings are stimulated and an impulse is sent to the central nervous system.

#### **Bending moment and shearing strength of leaf blades**

In a bending test, the remarkable peak force was observed at about 2 mm bending depth only in Prospero. This maximum bending force was created at the moment of fluttering an inner-angled cross section of Prospero as shown Fig. 4.2.4D.

There was a linear correlation between bending moment and mean shearing strength (Fig. 4.2.6), although statistically not significant. Shearing property is reported to be important during chewing of leaf blades by ruminant (Mackinnon *et al.*, 1988). It is suggested that sheep may recognize chewing easiness of leaf blades through bending strength prior to prehension and adjust the leaf number into a mouth.

#### **Biomechanical characteristics in diploid and tetraploid cultivars**

Bending, tensile, mean shearing and maximum shearing strengths of leaf blades were significantly higher in Prospero than in Aurora (Table 4.2.1). However, tensile stress and shearing toughness, which were the breaking strength normalized with respect to the cross-sectional area, showed similar values in Prospero and Aurora. These results suggest that higher biomechanical characteristics in Prospero may be supported simply by higher value of cross-sectional area, and the qualitative properties may be similar between diploid and tetraploid cultivars. For grazing

animals, it seems to have the advantage of feeding wider leaf blades of tetraploid cultivars because of higher plant weight per unit volume.

#### **Benefit/cost ratio**

The benefit/cost ratio, estimated as DM intake per mean biting force, is an important parameter closely related with DM intake and growth rate (Phillips, 1993). The benefit factor is usually expressed as DM weight, energy or nutrient contents, but there is no suitable parameter concerning to biting cost used by grazing animals (Illius *et al.*, 1995). In this study, biting cost was estimated by mean biting forces. Mean values of intake efficiency were  $13.3 \pm 1.5$  mg-DM/N in Aurora and  $15.4 \pm 1.4$  mg-DM/N in Prospero. Sheep seem to graze leaf blades by similar values of intake efficiency. Since there are few reports on this kind of research, further studies are needed.

#### **4.2.5 Conclusion**

Biomechanical properties of leaf blades were significantly different between two cultivars, and these differences influenced biting strategy of sheep. In order to obtain required amounts of nutrients and energy by the least cost, sheep are considered to adopt biting strategy harvesting plant organs by the application of the principle of the lever. It is suggested that sheep may decide biting forces by a sense of bending moment prior to prehension and adjust the leaf number into a mouth.

#### **4.2.6 Summary**

The biomechanical characteristics of leaf blades of diploid and tetraploid cultivars of perennial ryegrass (*Lolium perenne*), and their effects on biting behaviour of sheep were investigated using three-dimensional loadcell in order to clarify biting strategy of sheep and the effect of biting forces on DM intake. Ten, 20, 30 and 40 leaf blade segments per loadcell were offered to sheep. Sheep usually grazed leaves with low biting forces (3.5-22.1 N). The number of bites per point, number of grazed leaf blades per bite and DM weight per bite increased with increasing leaf densities.

Sheep used more bites in grazing leaf blades of Prospero than those of Aurora. In contrast, the number of grazed leaves per bite was lower in grazing leaf blades of Prospero than those of Aurora. The grand means of duration time per bite and time up to a peak biting force were  $0.169 \pm 0.005$  and  $0.061 \pm 0.003$  sec, respectively. Intake efficiency (DM weight per mean biting force) as an indicator of benefit/cost ratio was not significantly different among any treatments and the grand mean was  $14.4 \pm 1.0$  mg DM/N. There was apparent correlation between bending moment and mean shearing strength, suggesting that sheep may recognize biomechanical characteristics of all leaf blades prior to prehension through sensing bending strength and decide the level of creative biting force. From the number of grazed leaf blades per bite and mean shearing strength of a single leaf, mean biting forces were estimated (5.1 N in Aurora and 4.3 N in Prospero), compared with mean biting forces ( $5.5 \pm 0.5$  N in Aurora and  $6.5 \pm 0.4$  N in Prospero) observed in grazing trial. These results suggest that sheep may break leaf blades mainly by shearing force.



Table 4.2.1 Morphological and biomechanical characteristics of leaf blades of two cultivars of perennial ryegrass, and biting force per a leaf blade exerted by sheep

Parameter	Aurora	Prospero	sed	Probability
<b>1. Morphological characteristics of leaf blades</b>				
Sample No.	10	10		
Leaf length (mm)	282±7	362±11	12	<i>P</i> <0.001
Leaf width (mm)	3.04±0.08	4.05±0.09	0.11	<i>P</i> <0.001
DM density (mg DM/mm <sup>3</sup> )	0.119±0.002	0.100±0.002	0.003	<i>P</i> <0.001
Cross-sectional area (mm <sup>2</sup> )	0.68±0.03	1.22±0.05	0.05	<i>P</i> <0.001
<b>2. Biomechanical characteristics of leaf blades</b>				
Sample No.	10	10		
Bending moment (N·mm)	0.053±0.009	0.152±0.046	0.045	<i>P</i> =0.040
Tensile strength (N)	8.8±0.8	13.2±1.2	1.4	<i>P</i> =0.006
Tensile stress (MPa)	13.1±1.1	10.9±1.1	1.5	<i>P</i> =0.156
Mean shearing strength (N)	0.26±0.03	0.34±0.04	0.05	<i>P</i> =0.108
Maximum shearing strength (N)	0.89±0.09	1.31±0.08	0.12	<i>P</i> =0.002
Shearing toughness (10 <sup>3</sup> J/m <sup>2</sup> )	1.11±0.19	1.09±0.17	0.24	<i>P</i> =0.955
<b>3. Biting force per leaf exerted by sheep</b>				
Sample No.	48	79		
Mean biting force (N)	0.42±0.04	0.64±0.06	0.08	<i>P</i> =0.198
Peak biting force (N)	0.94±0.10	1.46±0.13	0.17	<i>P</i> =0.004

Figures show mean±se.

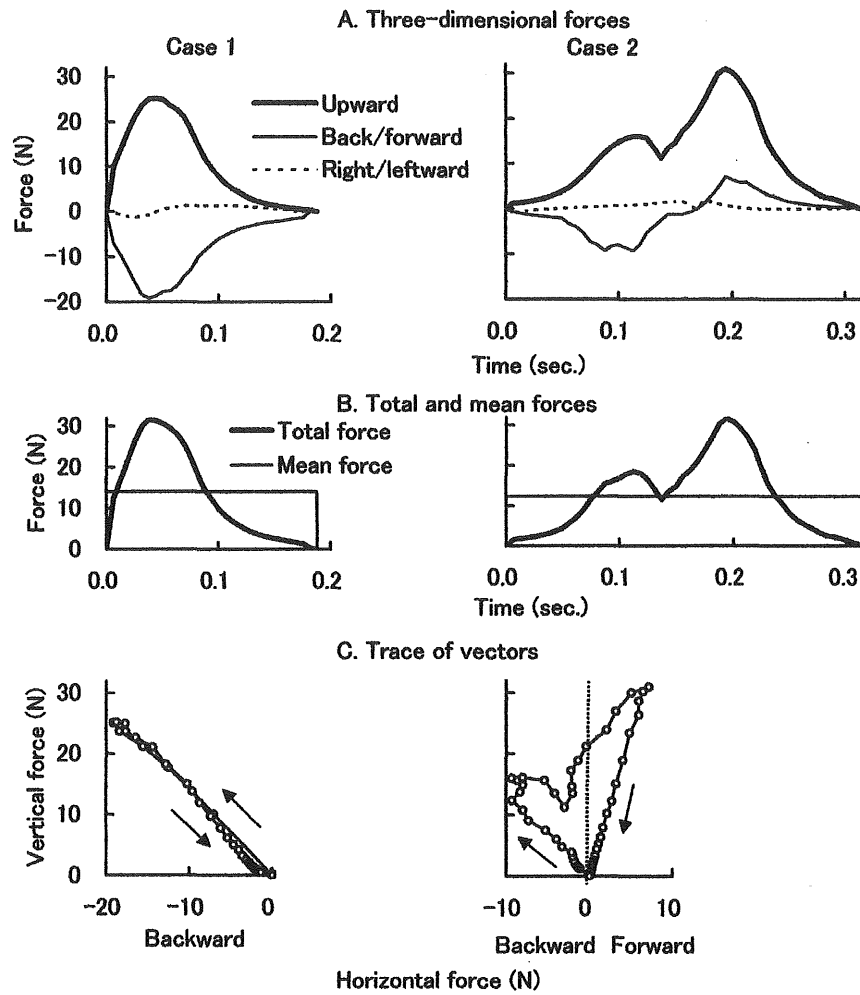


Fig. 4.2.1 Two patterns of biting forces and trace of vectors. Three-dimensional forces were obtained from a loadcell shown in Figure 2.6. Total force shows resultant forces of three-dimensional forces. Mean force was obtained from averaging biting forces. In trace of vectors of biting forces, measuring interval was a 0.006 second.

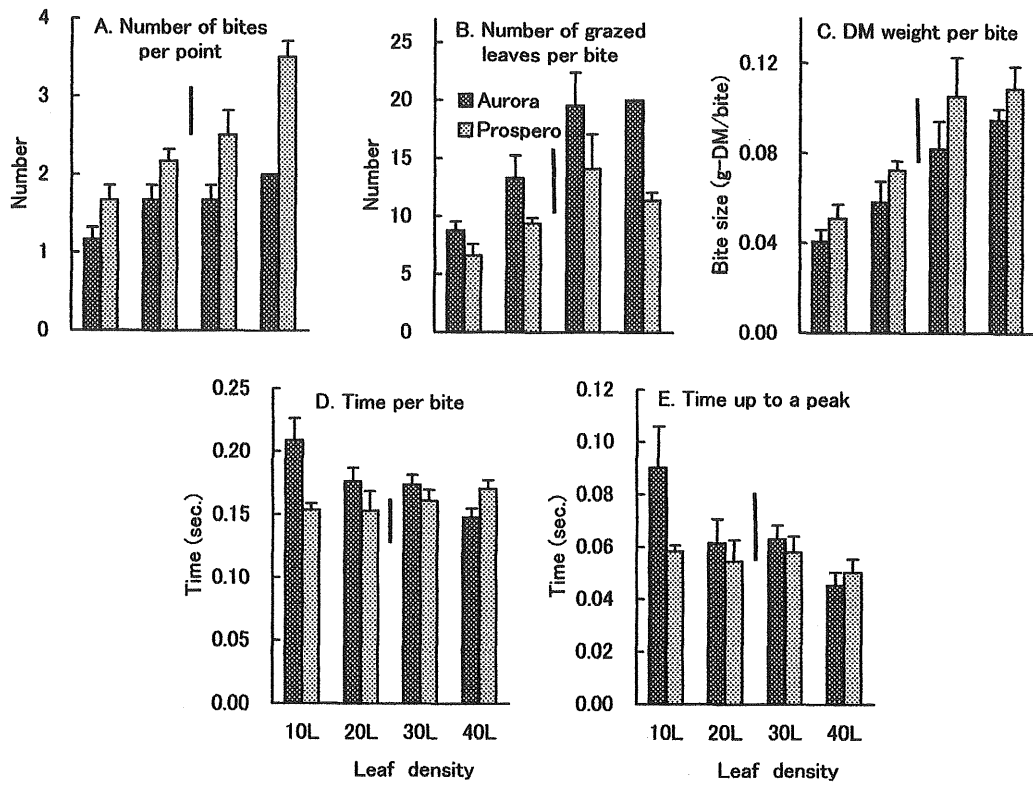


Fig. 4.2.2 Number of bites per point, number of grazed leaves per bite, DM weight per bite, time per bite, and time up to a peak force in grazing leaf blades of two cultivars of perennial ryegrass. Attached lines on bars show s.e. of mean and vertical lines show s.e.d. of the mean differences.

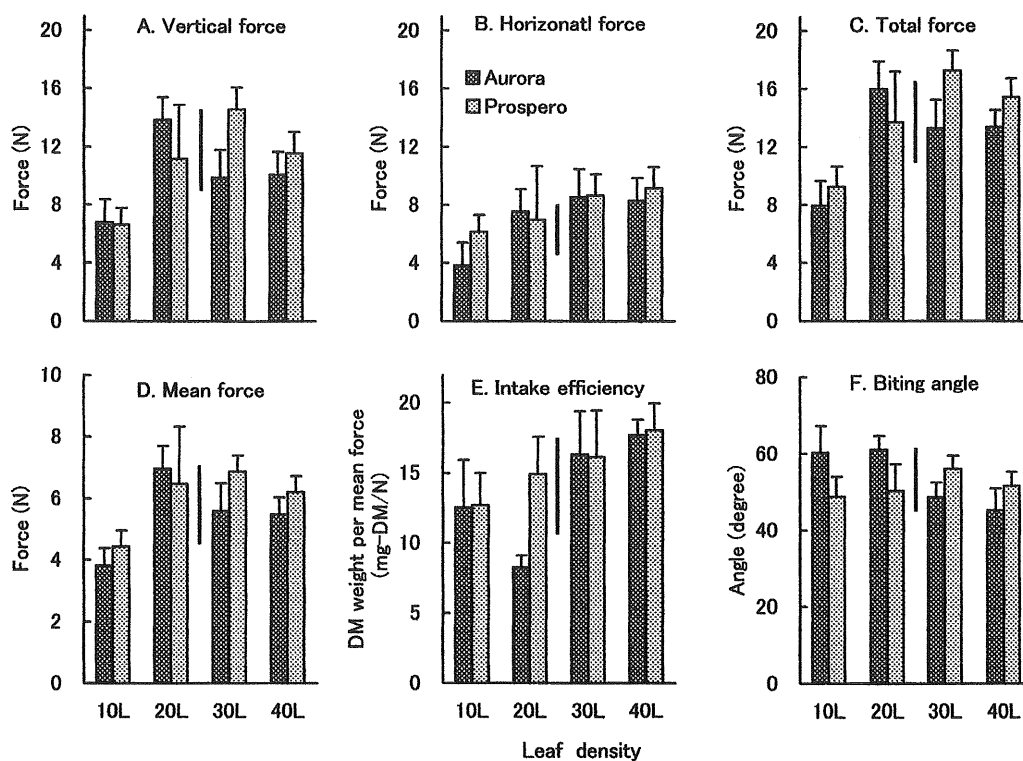


Fig. 4.2.3 Vertical, horizontal, total and mean biting forces, and intake efficiency (DM weight per mean biting force) and biting angle in grazing leaf blades of two cultivars of perennial ryegrass. Attached lines on bars show s.e. of mean and vertical lines show s.e.d. of the mean differences.

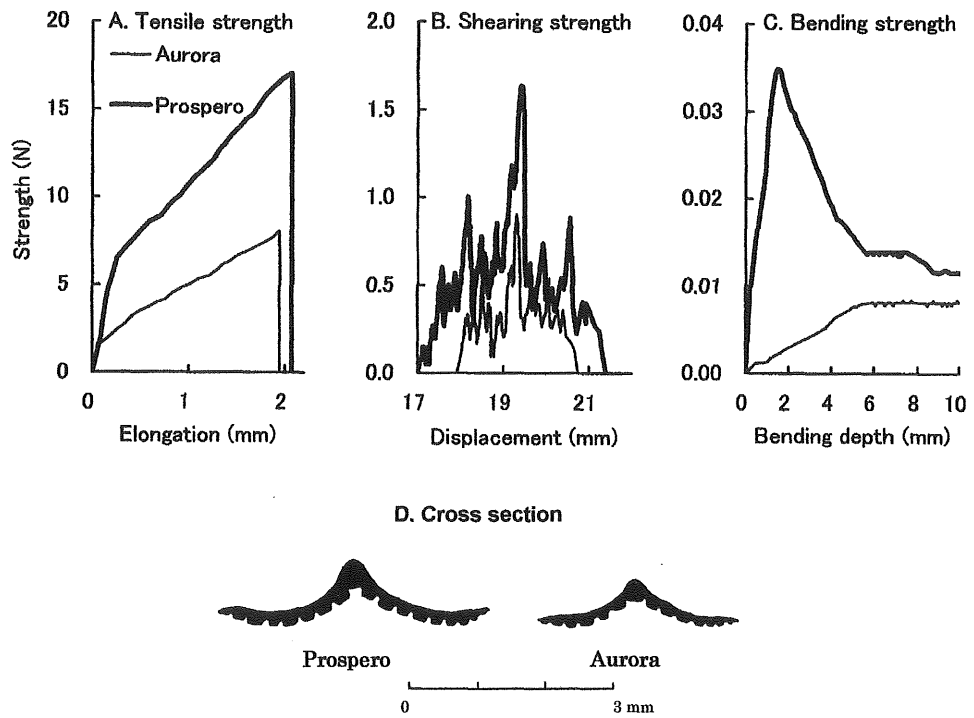


Fig. 4.2.4 Force/displacement patterns of tensile, shearing and bending strengths, and cross sections of leaf blades in two cultivars of perennial ryegrass. Each parameter was measured using the same leaf blade segment. The cross-sectional area was  $0.59 \text{ mm}^2$  in Aurora and  $1.06 \text{ mm}^2$  in Prospero.

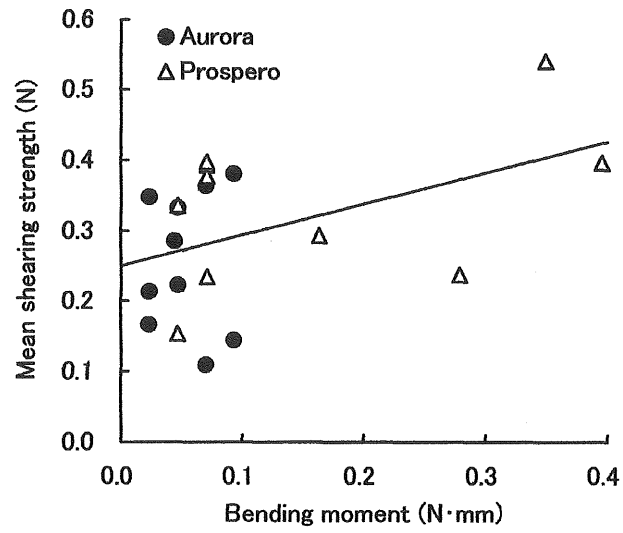


Fig. 4.2.5 Relationship between bending moment and mean shearing strength in leaf blades of two cultivars of perennial ryegrass. Correlation equation was as follows:

$$Y = 0.44X + 0.25 \quad (r=0.434, P=0.056)$$

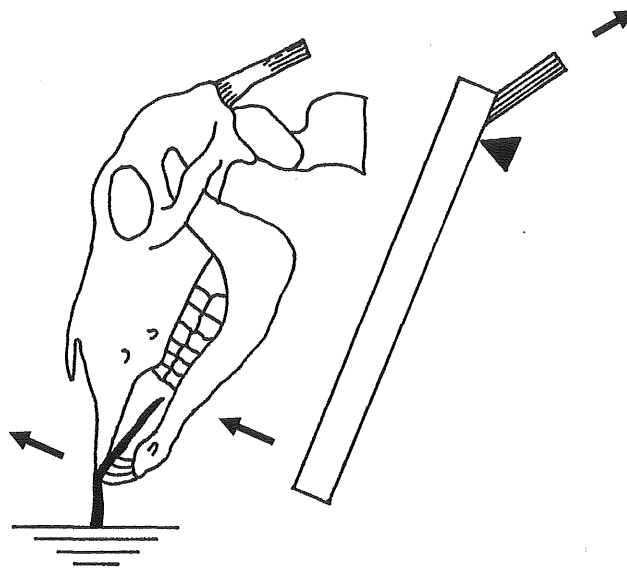


Fig. 4.2.6 A lever model of prehending bites in sheep grazing. The musculoskeletal system operates as a system of levers in which the joint between the atlas and the skull act as fulcra (Dyce *et al.*, 1987).

## Chapter 5

### GENERAL DISCUSSION

#### 5.1. Relationship between biomechanical properties and morphological characteristics of grass leaves

It is generally confirmed that, biological materials are arranged depending on the biological requirement (Atkins and Mai, 1985). The individual organism must be mechanically reliable if it is to survive and reproduce.

The results showed that biomechanical properties varied remarkably between different grass species. The variation was especially big in the case of tensile properties (Fig. 3.1.1 and Table 3.1.1). For example, tensile toughness of *Stipa pekinense* ( $4.12 \pm 0.324 \text{ kg} \cdot \text{mm}/\text{mm}^2$ ) was 13.7 times higher than that of *Digitaria adscendens* ( $0.30 \pm 0.031 \text{ kg} \cdot \text{mm}/\text{mm}^2$ ). Additionally, values in tensile properties as a whole were several times higher than those in shearing properties suggesting that harvesting grass leaves involving shearing might be more force-efficient way.

Behaviour under a tensile load depends only on material properties whereas a shearing load depends on structural properties as well (Vincent, 1990). Therefore tests in compression are more complex than tensile tests in which the structure of the material becomes more important (Gibson *et al.*, 1988). In the present study, tensile strength significantly ( $P < 0.001$ ) correlated with shear strength, though Kennedy and Doyle (1993) noted that tensile and shear strength were not necessarily positively correlated. But while some plants may be stronger in tension, fracture properties will be more dependent on the organization of the bundles of sclerenchyma which determines brittleness (Vincent, 1991).

Greater width between veins of leaf blades and associated increase in thickness indicate a higher ratio of mesophyll to structural tissue and therefore potentially higher cell contents availability and higher nutritive value. Leaf width and thickness were found as the leaf characteristic most associated with preference and suggested as a practical and convenient trait to use in breeding for increased grazing preference in grasses (Macadam and Mayland, 2003). Increased leaf width and thickness would result in increased leaf weight which would require more physical strength to sustain its weight and to keep leaf erectness. A high correlation between bending strength and leaf fresh weight found in the present study suggests



that heavier grass leaves require higher bending strength. There were extensive variations in within-plant support investments among grass species, and safety from mechanical failure under typical static loads varied from 15 to 75 (Table 3.1.1). In turn, increased leaf strength can add to the negative effect on grazing process.

Ligule of grass shoots was significantly tougher than leaf blades in shearing but developed less stress compared to leaf blade when subjected to tensile load in the present study. In other words, ligule would be easier to break when harvesting leaves by pulling. Wright and Illius (1995), studying fracture properties of five grass species, found that fracture in a tiller occurs at a zone of weakness at the intercalary meristem and argued that this is an evolutionary advantage to grass species which are commonly grazed. If that is the case, grass leaves would be harvested by grazers mostly at the base of the leaf blades which is not true. This might suggest that pulling is not a single way of harvesting by grazers.

Biomechanical properties of leaf blade in both orchardgrass and tall fescue decreased from the basal to the apical sites along the leaf blades. It is consistent with the finding of perennial ryegrass (Evans, 1967b), though the different result in perennial ryegrass was reported by Greenberg *et al.* (1989). The biomechanical properties per unit area of leaf blade also showed same pattern as above in our study.

A midrib is strengthened vein down the middle of leaf blade. The greater part of leaf toughness may attribute to its midrib strength. Vascular bundles are comprised primarily of thick-walled and load-bearing cells (Greenberg *et al.*, 1989). Thick walled cells of midrib that are heavily lignified, and hence confer mechanical strength to the plant by way of attachment to chained bundles of vascular tissue only account for a small proportion of leaf cross-sectional area. Despite this, the fibre component accounts for 90-95% of the longitudinal stiffness of grass leaves (Vincent, 1982).

Long and narrow leaves of grasses may keep straight vertically by increased bending strength, which may be maintained by interior angles of leaves in a cross section. This maintenance method seems to be very effective for grass species to minimize metabolic investments in leaf-supporting structures (Chazdon 1986; Hongo *et al.*, 2007). A flat plate bending up (or down) has the greatest improvement in both beam stiffness and increase in section modulus (King and Vincent, 1996). The results from present study showed an interior angle of a leaf blade was

especially critical for leaf erectness in orchardgrass species whereas a thick midrib may take on the leaf-support role in tall fescue. Therefore, it might be desirable that the contribution of leaf interior angle to the uprightness of a leaf blade should be increased at the expense of contribution from the midrib volume.

The results from the experiment where biomechanical properties of different parts and organs of grass species were compared show wide variations in biomechanical properties despite the similar investment of DM density of plant materials. This fact proves the importance of the plant structural property over its material property in plant biomechanical property.

## **5.2. Grazing behaviour of sheep in response to grass biomechanical properties**

There were wide variations in biomechanical properties of leaf materials used in grazing trials (between the middle and the basal sites along an individual leaf blade of orchardgrass and between the diploid and tetraploid cultivars of perennial ryegrass). Leaf blade strength of orchardgrass in the basal site was severalfold of that in the middle site. Greater forces in both tensile and shearing tests were required to produce a fracture in the tetraploid cultivar of perennial ryegrass. Though, tensile stress and shearing toughness were greater in the diploid of perennial ryegrass, associated with the smaller cross-sectional area of the leaf blade. The leaf blade of orchardgrass was much stronger than that of both the cultivars of perennial ryegrass, even though the leaf strength at the apical site of the orchardgrass leaf blade was closer to that of the perennial ryegrass cultivars.

Sheep showed different responses in grazing of different leaf parts: the middle versus the basal parts of orchardgrass; and the diploid versus tetraploid cultivars of perennial ryegrass. Despite the fact that the force exerted per bite did not vary significantly, sheep can be considered using various biting strategies. The results suggest that biting strategy by grazing animals seems to vary depending on plant characteristics on offer, including the biomechanical property.

Three-directional biting force component was significantly different between two parts of orchardgrass leaf blade, whereas no variation was found between two cultivars of perennial ryegrass. The additional forces in the horizontal directions seemed to be a biting strategy used to overcome the greater biting resistance in the

base of orchardgrass leaf blade. This was done, most probably, by jerking the head to add involvement of incisal edge against the forage material to allow easier severance of orchardgrass leaves. Griffiths (2006) mentioned that faster rates of head acceleration of small-bodied ruminant play a major contributing role in the effort that animals exert in severing a bite. On the other hand, the difference in the leaf strength between two cultivars of perennial ryegrass was not large enough to require any change in the three-directional biting force component by sheep.

The average depth of insertion of the mouth into the sward canopy, commonly termed bite depth, has been widely accepted as the primary determinant of the short-term rate of herbage intake across a range of herbivore species (Mitchell *et al.*, 1991; Laca *et al.*, 1992b), at least for temperate forages. The sheep did not penetrate their mouth deeper into the basal leaves compared to the middle, indicating that leaf strength of the base of orchardgrass constrained sheep intake. In contrast, biting depth was similar between the two cultivars of perennial ryegrass.

However, sheep showed different behaviour when grazing diploid vs. tetraploid cultivars of perennial ryegrass. Sheep seemed to have a desire to have a greater intake from the tetraploid cultivar by putting more effort. Sheep made greater number of bites, and took therefore a greater DM intake from leaves of tetraploid cultivar. Biting term was also shorter when grazing the tetraploid than doing the diploid cultivar.

We assumed that these differences in sheep responses are associated with the different biomechanical characteristics of the leaf blades of the herbage grasses.

Animal grazing decisions occur over very short time and small spatial scales (Kotliar and Wiens, 1990). The appraisal of a patch is considered as one of the key phases of the decision-making process in grazing activity (Griffiths *et al.*, 2003a). Arnold (1967) has shown that touch, taste and smell are used in selective grazing. In grazing orchardgrass stubbles, sheep might have predicted the difference in bending forces between the top and the base stubbles as an appraisal key by touching with the muzzle. The bending strength of a leaf blade of orchardgrass was strongly related with both tensile and shearing forces required for fracture in the same specimen (Fig. 4.2.6A, C). But the relationship was weak in the case of perennial ryegrass cultivars (Fig. 4.2.6B, D), indicating that the difference in bending forces between the two cultivars may not give an adequate information about the biomechanical characteristics of stubbles on offer. It is difficult to explain

what source of information the sheep had used for distinguishing the tetraploid perennial ryegrass from the diploid ones. It is doubtful that sheep used the information from first bites within only a few bites. The mean number of bites removed was only 3 per stubble at maximum; consequently sheep had almost no opportunity for adequate appraisal by tasting. Greater reward from thick, wide leaf blades of the tetraploid perennial ryegrass may motivate sheep to take more bites.

In the present study, impulse per bite was not influenced by either biomechanical properties of forage or sward bulk density (leaf density level). It may suggest that there is a limit in the amount of force to be involved per bite by animal. In other words, biting cost may be similar for every bite. Sheep may adjust its biting tactics and biting parameters such as bite volume (depth, area), bite weight, bite number, biting rate etc. In the present case, the sheep made deeper bites in the middle leaves than those in the basal leaves, whereas the greater numbers of bites were taken in the leaves of tetraploid perennial ryegrass in comparison to those in the leaves of diploid cultivar.

### **5.3. Bite force and grazing efficiency**

Understanding the conceptual basis of bite depth with linkage with bite force has been the subject of ongoing research over the past decade (Griffiths, 2006). The original Summit Force theory implied that once a maximum force was attained, the bite dimensions would be moderated to maintain a constant bite force (Hodgson, 1985). The force exerted per bite (14.5-17.7 N) was relatively constant throughout the grazing treatments, in spite of that the biomechanical properties of leaves were so contrasting between the treatments in the present study. Nevertheless, sheep had no chance to change their bite area to maintain this constant bite force in our experiment, as we used only one clump of leaves for each grazing trial.

The benefit/cost ratio is an important parameter closely related with DM intake and growth rate of animals (Phillips, 1993). The benefit factor may be expressed as DM weight, energy or nutrient contents, but there was no suitable parameter concerning to grazing cost used by animals. In this study, grazing cost was estimated by biting impulse. The ratio of DM intake to the sum of grazing impulse by sheep did not vary significantly, reflecting an achievement of a balance of reward and effort (Fig. 4.1.8B, Fig. 4.2.4D). The ratio was not affected by the level of leaf

density, suggesting the ratio to be inherent to each animal species. The results from the present study suggest that a greater reward per bite may play an important role to motivate the sheep to put more effort in further grazing in a patch.

The interest in relating the tensile properties of plants to aspects of grazing behaviour, particularly prehension, has become popular recently (Illius *et al.*, 1995; Tharmaraj, 2000; Wright and Illius, 1995). Chewing during eating and rumination is considered to associate with a shearing action (John *et al.*, 1989; Inoue *et al.*, 1994). Henry *et al.* (1996) contended, however, that there was a lack of evidence to support the partitioning of the fracture mechanics between prehension and chewing. Our results showed that the bite force exerted by sheep was 17 times less, in average, than the sum of tensile strength of same number of leaf blades. This means that if sheep severed the leaves in only tension, they would not be able to sever even a single leaf blade from middle part of orchardgrass. The mean value of tensile strength of a single leaf from middle part of orchardgrass leaf blade was  $25.6 \pm 1.88$  N, whereas the force per bite exerted by sheep was  $14.5 \pm 1.55$  N. Instead, the sum of shearing strength of leaf blades was significantly closer to biting force by sheep. However, if sheep harvested leaves in pure shearing action, then biting rate, therefore intake rate would get much slower. It would be expected that bites of longer duration would utilize more muscular effort than those bites in rapid fracture (Griffiths, 2006). Consequently, effective combination of shearing action with tension would be efficient way of harvesting in sheep grazing. Our data provided sufficient evidence in confirmation of importance of shearing action in sheep grazing.

During the grazing trials, some stubble which could be harvested easily was depleted immediately. There might be an effect of sward depletion on the grazing behaviour by sheep. Further investigation is needed with elimination of the effect.

The study confirmed that the biomechanical characteristics of herbage grasses were important factors in animal grazing behaviour. Moreover, it showed that animal could respond to sward characteristics in a very short timescale and small spatial scale.

## SUMMARY

The objective of this study was to clarify biomechanical properties of leaf blades of herbage grasses and their effects on animal grazing behaviour, and to analyze the three-directional biting forces and biting impulse associated. It was hypothesized that the higher intake rate by grazing animal would be positively related to the ease to harvest grass leaves with low breaking cost.

The morphological characteristics such as cross-sectional area, DM weight, leaf length, width, DM density were measured in leaf blades of herbage grasses. Bending, tensile and shearing strengths as biomechanical properties were measured in leaf blades of herbage grasses. Seasonal change in biomechanical properties was studied in leaf blades of festulolium (*Festulolium loliaceum*), a hybrid between meadow fescue (*Festuca pratensis*) and perennial ryegrass (*Lolium perenne*) and cultivars of perennial ryegrass. Biomechanical properties at different parts of leaf blades were also examined in orchardgrass (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*).

Grazing trials were conducted to clarify the effects of biomechanical properties of grass leaf blades on sheep grazing behaviour. Two kinds of grass materials such as basal and middle parts of leaf blades of orchardgrass, and diploid and tetraploid cultivars of perennial ryegrass were used.

The following results were obtained:

- 1) Morphological and biomechanical properties of leaf blades varied broadly between 20 grass species. Tensile toughness in Hanegaya (*Stipa peginense*,  $4.12 \pm 0.324 \text{ kg} \cdot \text{mm}/\text{mm}^2$ ) was 13.7 times higher than that of Mehisiba (*Digitaria adscendens*,  $0.30 \pm 0.031 \text{ kg} \cdot \text{mm}/\text{mm}^2$ ). There was no consistent tendency of seasonal variation in the biomechanical properties of festulolium and cultivars of perennial ryegrass.
- 2) Biomechanical properties were measured at 3 positions (leaf blade, collar and sheath) along a shoot of orchardgrass and tall fescue. Shearing toughness at a collar was significantly higher than that at other organs. Tensile strength and stress at

leaf blade were significantly higher than those at other organs.

3) Biomechanical properties were measured at four positions from the base to the tip along a leaf blade of orchardgrass and tall fescue. The biomechanical properties apparently decreased from the basal to the apical positions along a leaf blade regardless of the sizes of cross-sectional area.

4) Biomechanical properties of separated midrib and remaining wing were measured at four positions along a leaf blade of orchardgrass and tall fescue. The midrib showed higher values of biomechanical properties than leaf wing at all four positions along a leaf blade. It is suggested that the midrib may provide structural support for leaf erectness in greater extent.

5) The role of interior angle in a cross section of a leaf blade on bending strength was examined in orchardgrass and tall fescue. Maintenance of interior angle in a leaf blade of orchardgrass was very important for a creation of high bending strength. The bulkier midrib in a leaf blade of tall fescue was considered to be a main component to support leaf blade without the contribution from the interior angle.

6) Bending, tensile and shearing strengths were 8.5, 2.4 and 1.9 times, respectively, higher at the basal part than the middle part of a leaf blade of orchardgrass. Similarly, those strengths were 2.9, 1.5 and 1.5 times, respectively, higher in tetraploid than diploid cultivar of perennial ryegrass. Tensile stress and shearing toughness were greater in the diploid cultivar than tetraploid cultivar.

7) The biting forces used by sheep were similar throughout grazing treatments. When sheep grazed the basal parts of leaf blades of orchardgrass, sheep used additional horizontal forces, particularly with backward/forward direction. There was no significant difference between the two cultivars of perennial ryegrass in the three-directional biting forces used by sheep. Sheep tended to graze leaf blades of the tetraploid of perennial ryegrass with greater number of bites during shorter period, resulted in greater DM intake compared to the diploid cultivar of perennial ryegrass.

8) Observed biting forces used by sheep agreed well with the estimated values calculated from shearing strength of the corresponding number of grazed leaves. In contrast, the estimated values from tensile strength were more than ten times higher than biting forces exerted by sheep. The results suggest that shearing force may play an important role in grazing grass leaf blades by sheep. Sheep seem to recognize biomechanical properties of leaf blades prior to prehension and to adjust biting force and the number of leaves into her mouth through the bending strength of the leaves.



## Effect of Biomechanical Properties of Herbage Grasses on Grazing Behaviour of Sheep

(イネ科牧草のバイオメカニクスの特性がヒツジの採食行動におよぼす影響)

### 要 約

イネ科牧草葉身のバイオメカニクスの特性と、これらの特性が放牧家畜の採食行動におよぼす影響を明らかにし、さらに、3方向のバイト強度と関連するバイト力積を測定することを目的として本研究を行った。放牧家畜による高い摂取速度は、破断強度が低いイネ科牧草葉身の食べやすさと正の相関がある、という仮説を立てて実験を行った。

葉身の形態上の特徴として、断面積、DM重、葉身の長さ、幅、密度を2イネ科牧草葉身について測定した。バイオメカニクスの特性として、曲げ、引張り、せん断強度をイネ科牧草葉身について測定した。また、メドウフェスク(*Festuca pratensis*)とペレニアルライグラス(*Lolium perenne*)の雑種であるフェストロリウム(*Festulolium loliaceum*)とペレニアルライグラスの品種について、バイオメカニクスの特性の季節変動を調査した。さらに、オーチャードグラス(*Dactylis glomerata*)とトールフェスク(*Festuca arundinacea*)の葉身の異なる部位におけるバイオメカニクスの特性も測定した。

イネ科牧草葉身のバイオメカニクスの特性がヒツジの採食行動に及ぼす影響を明らかにするために、採食実験を行った。実験には、オーチャードグラス葉身の基部と中間の部位、および、ペレニアルライグラスの2倍体と4倍体の葉身を使用した。

以下のような結果が得られた。

1)葉身の形態およびバイオメカニクスの特性は、測定したイネ科植物20種の間で大きな差が見られた。ハネガヤ(*Stipa pekinense*,  $4.12 \pm 0.324 \text{ kg} \cdot \text{mm}/\text{mm}^2$ )の引張り靱性はメヒシバ(*Digitaria adscendens*,  $0.30 \pm 0.031 \text{ kg} \cdot \text{mm}/\text{mm}^2$ )より13.7倍も高い値を示した。フェストロリウムとペレニアルライグラス品種のバイオメカニクスの特性の季節変動について一定の傾向は認められなかった。

2)オーチャードグラスとトールフェスクの苗条の3部位(葉身、葉襟、葉鞘)におい

て、バイオメカニクス的な特性を測定した。せん断靱性は他の部位よりも葉襟において有意に高かった。引張り強度とストレスは他の部位よりも葉身において有意に高かった。

3) オーチャードグラスとトールフェスクの1枚の葉身について、基部から先端までの4部位においてバイオメカニクス的な特性を測定した。基部から先端にかけてバイオメカニクス的な特性は明らかに低下し、断面積の大きさはほとんど影響しなかった。

4) オーチャードグラスとトールフェスクの1枚の葉身について、主脈とそれ以外の部分に分け、基部から先端までの4部位においてバイオメカニクス的な特性を測定した。4部位すべてにおいて、主脈のバイオメカニクス的な特性は高かった。また、主脈は葉身の直立性を支える基本的な器官となっていることが示唆された。

5) オーチャードグラスとトールフェスクの葉身の断面において、内向きの角度が曲げ強度におよぼす影響を調査した。オーチャードグラスの葉身では、内向きの角度を維持することにより、大きな曲げ強度を生みだしていた。一方、トールフェスクの葉身では、内向きの角度を維持しておらず、非常に大きな主脈自体が、葉身を支えていると考えられた。

6) オーチャードグラス葉身の曲げ、引張り、せん断強度は、中間部に比べて基部の方がそれぞれ8.5、2.4、1.9倍高い値を示した。同様に、それらの強度は、2倍体に比べて4倍体の方がそれぞれ2.9、1.5、1.5倍高い値を示した。引張りストレスとせん断靱性は、4倍体に比べて2倍体の方が高い値を示した。

7) ヒツジが使ったバイト強度は、各種の処理区においてほぼ同様の値を示した。ヒツジは、オーチャードグラス葉身の基部を食べる時、水平方向の強度、特に、手前または向側の強度、を大きく使って採食した。ヒツジが使用した3方向のバイト強度について、ペレニアルライグラスの2品種の間には有意な差はなかった。ヒツジがペレニアルライグラスの4倍体を採食する時、より短い時間でバイト数を多くする傾向が見られ、このような方法により結果として2倍体より大きなDM採食を達成していた。

8) 採食する時にヒツジが使った観察されたバイト強度は、ヒツジが1バイトで採食した葉数と1枚の葉のせん断強度から計算で求めた推定値とよく一致した。一方、同様の方法で引張り強度を用いて計算すると、推定値はヒツジが使った観察されたバイト強度

の 10 倍以上も大きい値となった。このような結果から、ヒツジはイネ科牧草葉身を採食する時にせん断の力を主に使っていると推察された。ヒツジは、まず葉身の曲げ強度を感知することで、葉身を破断する前に葉身のバイオメカニクス的な特性を認識し、バイト強度と口に入れる葉数を調整しているものと推察された。

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## REFERENCES

- AKIN, D.E. and BURDICK, D. (1975). Percentage of tissue types in tropical and temperate leaf blades and degradation of tissues by rumen microorganisms. *Crop Science* 15, 661-668.
- ALLDEN, W.G. and WHITTAKER, I.A.McD. (1970). The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. *Australian Journal of Agricultural Research* 21, 755-766.
- ARNOLD, G.W. (1964). Some principles in the investigation of selective grazing. *Proceedings of Australia Society of Animal Production* 5, 258-271.
- ARNOLD, G.W. (1966a). The special senses in grazing animals. I. Sight and dietary habits in sheep. *Australian Journal of Agricultural Research* 17, 521-530.
- ARNOLD, G.W. (1966b). The special senses in grazing animals. II. Smell, taste, and touch and dietary habits in sheep. *Australian Journal of Agricultural Research* 17, 531-542.
- ARNOLD, G.W. (1967). The use of in vitro methods associated with sampling techniques to measure intake and digestibility under grazing conditions. In: O.Paladines (Editor), *Metodos in vitro para determinar el Valor Nutrivo de los Forrajes*. Montevideo.
- ARNOLD, G.W. (1981). Grazing behaviour. In: Morley, F.H.W. (ed.) *World Animal Science, B1, Grazing Animals*. Amsterdam Elsevier, pp. 79-104.
- ARNOLD, G.W. (1987). Grazing behaviour. In: Snaydon, R.W. (ed.) *Managed grasslands. Ecosystems of the world: 17A*. Amsterdam Elsevier, pp. 129-136.
- ARNOLD, G.W. and DUDZINSKI, M.C. (1966). The behavioural response controlling the food intake of grazing sheep. In: *Proceedings of Tenth International Grassland Congress, Helsinki*, pp. 367-370.
- ARNOLD, G.W. and DUDZINSKI, M.C. (1978). Ethology of free-ranging domestic animals. Elsevier, Amsterdam, pp. 198.
- ARNOLD, G.W., BALL, J., McMANUS, W.R. and BUSH, I.G. (1966). Studies on the diet of the grazing animals. I. Seasonal changes in the diet of sheep grazing pastures of different availability and composition. *Australian Journal of*

- Agricultural Research* 17, 543-556.
- ATKINS, A.G. and MAI, Y.W. (1985). *Elastic and Plastic Fracture – Metals, Polymers, Ceramics, Composites, Biological Materials*. Chichester: Ellis Horwood Limited. pp. 51-56.
- BARRETT, P.D., LAIDLAW, A.S., MAYNE, C.S. and CHRISTIE, H. (2001). Pattern of herbage intake rate and bite dimensions of rotationally grazed dairy cows as sward height declines. *Grass and Forage Science* 56, 362-373.
- BENVENUTTI, M.A., GORDON, I.J., POPPI, D.P., CROWTHER, R. and SPINKS, W. (2008). Foraging mechanics and their outcomes for cattle grazing reproductive tropical swards. *Applied Animal Behaviour Science* 113, 15-31.
- BERNAYS, E.A. (1986). Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science* 231, 495-496.
- BLACK, J.L. and KENNEY, P.A. (1984). Factors affecting diet selection by sheep. II. Height and density of pasture. *Australian Journal of Agricultural Research* 35, 565-578.
- BOOTH, D.A. (1990). Sensory influences on food intake. *Nutrition reviews* 48, 71-77.
- BRISKE, D.D. (1986). Plant response to defoliation: morphological considerations and allocation priorities. In: Joss, P.J., Lynch, P.W. and Williams, O.B. (eds) *Rangelands: A Resource Under Siege*. Cambridge University Press, Sydney, pp. 425-427.
- BRISKE, D.D. (1991). Developmental morphology and physiology of grasses. In: Heitschmidt, R.K. and Stuth, J.W. (eds) *Grazing Management: An Ecological Perspective*. Timber Press, Portland, Oregon, pp. 85-108.
- BRISKE, D.D. (1996). Strategies of plant survival in grazed systems: A functional interpretation. In: Hodgson, J. and Illius, A.W. (eds) *The Ecology and Management of Grazing Systems* CAB International, Wallingford, UK, pp. 37-67.
- BRISKE, D.D. and RICHARDS, J.H. (1995). Plant responses to defoliation: a physiological, morphological, and demographic evaluation. In: Bedunah, D.J. and Sosebee, R.E. (eds) *Wild Land Plants: Physiological Ecology and Developmental Morphology*. Society for Range Management, Denver, Colorado,

pp. 635-710.

- BROUGHAM, R.W. (1959). The effects of season and weather on the growth of ryegrass and clover pasture. *New Zealand Journal of Agricultural Research* 2, 282-296.
- BROUGHAM, R.W. (1960). The effect of frequent hard grazings at different times of the year on the productivity and species yield of a grass-clover pasture. *New Zealand Journal of Agricultural Research* 3, 125-136.
- BROWN, R.H. and BLASER, R.E. (1968). Leaf area index in pasture growth. *Herb. Abstr.* 38, 1-9.
- BURLISON, A.J., HODGSON, J. and ILLIUS, A.W. (1991). Sward canopy structure and the bite dimensions and bite weight of grazing sheep. *Grass and Forage Science* 46, 29-38.
- CARLQUIST, S. (1961). *Comparative plant anatomy*. Holt, Rhinehart and Winston, New York, NY.
- CARLQUIST, S. (1969). Wood anatomy of Lobelioideae (Capaulnaceae). *Biotropica* 1(47) 47-72.
- CARLQUIST, S. (1975). *Ecological strategies of xylem evolution*. University of California Press, Berkeley, CA.
- CASLER, M.D., PITTS, P.G., ROSE-FRICKER, C., BILKEY, P.C. and WIPFF, J.K. (2001). Registration of 'Spring Green' festulolium. *Crop Science* 41, 1365-1366.
- CASLER, M.D., PETERSON, P.R., HOFFMAN, L.D., EHLKE, N.J., BRUMMER, E.C., HANSEN, J.L., MLYNAREK, M.J., SULC, M.R., HENNING, J.C., UNDERSANDER, D.J., PITTS, P.G., BILKEY, P.C. and ROSE-FRICKER, C.A. (2002). Natural selection for survival improves freezing tolerance, forage yield, and persistence of festulolium. *Crop Science* 42, 1421-1426.
- CHACON, E. and STOBBS, T.H. (1976). Influence of progressive defoliation of grass sward on eating behaviour of cattle. *Australian Journal of Agricultural Research* 27, 709-727.
- CHACON, E., STOBBS, T.H. and SANDLAND, R.L. (1976). Estimation of herbage consumption by grazing cattle using measurements of eating behaviour. *Journal of the British Grassland Society* 31, 81-87.



- CHAFFEY, N. (2000). Physiological anatomy and function of the membranous grass ligule. *New Phytologist* 146, 5-21.
- CHAN, T.K., LIM, S.H., TAN, H.T.W. and LIM, C.P. (1999). Variation of bending capacity along the lamina length of a grass, *Imperata cylindrica* var. *major* (Gramineae). *Annals of Botany* 84, 703-708.
- CHAZDON, R.L. (1986) The costs of leaf support in understory palms: economy versus safety. *The American Naturalist* 127, 9-30.
- CHOONG, M.F., LUCAS, P.W., ONG, J.S.Y., PEREIRA, B., TAN, H.T.W. and TURNER, I.M. (1992). Leaf fracture toughness and sclerophylly their correlations and ecological implications. *New Phytologist* 121, 597-610.
- CHURCH, D.C. (2nd) (ed.) (1979). *Digestive Physiology and Nutrition of Ruminants. Vol. 1-Digestive Physiology*. D.C. Church, Corvallis, Oregon.
- COLEMAN, S.W. (1992). Plant-animal interface. *Journal of Production Agriculture* 5, 7-13.
- COLEMAN, S.W., FORBES, T.D.A., and STUTH, J.W. (1989) Measurements of the plant-animal interface in grazing research. In: G.C. Marten (ed.) *Grazing research: Design, methodology and analysis. CSSA Spec. Publ. 16*, ASA, CSSA, Madison, WI. pp. 37-51.
- CURLL, M.L. and WILKINS, R.J. (1982). Frequency and severity of defoliation of grass and clover by sheep at different stocking rates. *Grass and Forage Science* 37, 291-297.
- DAVIS, I.F. (1964). Diet selected by sheep grazing on annual pasture in southern Victoria. *Proceedings of the Australian Society of Animal Production* 5, 249-250.
- DAVIES, D.A., FOTHERGILL, M. and JONES, D. (1991). Assessment of contrasting perennial ryegrasses, with and without clover, under continuous sheep stocking in the uplands. 3. Herbage production, quality and intake. *Grass and Forage Science* 46, 39-49.
- DEMARQUILLY, C., BOISSAU, J.M. and CUYLLE, G. (1966). Factors affecting the voluntary intake of green forage by sheep. In: *Proceedings of Ninth International Grassland Congress* São Paulo. pp. 877-885.
- DIAZ, S., NOY-MEIR, I. and CABIDO, M. (2001). Can grazing response of

- herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology* **38**, 497.
- DYCE, K.M., SACK, W.O. and WENSING, C.J.G. (1987). *Textbook of Veterinary Anatomy*. W.B. Saunders Company, Philadelphia.
- EASTON, H.S. (1989). Variability of leaf shear strength in perennial ryegrass. *New Zealand Journal of Agricultural Research* **32**, 1-6.
- EDMOND, D.B. (1963). Effects of treading perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens*) pastures in winter and summer at two soil moisture levels. *New Zealand Journal of Agricultural Research* **6**, 265-276.
- ENNOS, A.R. (1989). The mechanics of anchorage in seedlings of sunflower *Helianthus annuus* L. *New Phytologist* **113**, 185-192.
- ENNOS, A.R. (1991). The mechanics of anchorage in wheat *Triticum aestivum* L.I. The anchorage of wheat seedlings. *Journal of Experimental Botany* **42**, 1601-1606.
- EVANS, P.S. (1964). A study of leaf strength in four ryegrass varieties. *New Zealand Journal of Agricultural Research* **7**, 1-16.
- EVANS, P.S. (1967a). Leaf strength studies of pasture grasses. I. Apparatus, techniques and some factors affecting leaf strength. *Journal of Agricultural Science, Cambridge* **69**, 171-174.
- EVANS, P.S. (1967b). Leaf strength studies of pasture grasses. II. Strength, cellulose content and sclerenchyma tissue proportions of eight grasses grown as single plants. *Journal of Agricultural Science, Cambridge* **69**, 175-181.
- FAHN, A. (1990). *Plant anatomy*, (4th)(ed.) Pergamon press, Great Britain.
- FORBES, T.D.A. (1988). Researching the plant-animal interface: the investigation of ingestive behaviour in grazing animals. *Journal of Animal Science* **66**, 2369-2379.
- FRENCH, M.J. (1988). *Invention and Evolution: Design in nature and Engineering*. Cambridge University Press, Cambridge.
- GHEQUIERE, M., EMILE, J.C., JADAS-HECART, J., MOUSSET, C., TRINEAU, R. and POISSON, C. (1996). First in vivo assessment of feeding value of *Festulolium* hybrids derived from *Festuca arundinacea* var. glaucescens and

- selection for palatability. *Plant Breeding* 115, 238-244.
- GIBB, M.J., HUCKLE, C.A., NUTHALL, R. and ROOK, A.J. (1997). Effects of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. *Grass and Forage Science*, 52 309-321.
- GIBSON, L.J., ASHBY, M.F. and EASTERLING, K.E. (1988). Structure and mechanics of the iris leaf. *Journal of Material Science* 23, 3401-3048.
- GIVNISH, T.J. (1978). Ecological aspects of plant morphology: leaf form in relation to environment. *Acta Biotheoretica* 27, 83-142
- GOODMAN, A.M. and ENNOS, A.R. (1997). The responses of field grown sunflower and maize to mechanical support. *Annals of Botany* 79, 703-711.
- GORDON, I.J. and LASCANO, C. (1993). Foraging strategies of ruminant livestock on intensively managed grasslands: potential and constraints. In: *Proceedings of 17<sup>th</sup> International Grassland Congress*, Hamilton, New Zealand, pp. 681-689.
- GORDON, J.E. (1976). The new science of strong materials, or why you don't fall through the floor. 2<sup>nd</sup> edn. Penguin, London.
- GORDON, J.E. (1978). Structures, or why things don't fall down. Penguin books, Middlesex, U.K.
- GREENBERG, A.R., MEHLING, A., LEE, M. and BOCK, J.H. (1989). Tensile behaviour of grass. *Journal of Materials Science* 24, 2549-2554.
- GRIFFITHS, W.M. (2006). Plant-animal mechanics and bite procurement in grazing ruminants. In: Herrel, A., Speck, T. and Rowe, N. (eds.) *Ecology and Biomechanics: a mechanical approach to the ecology of animals and plants*. CRC Press, Boca Raton, Florida, USA. pp. 101-122.
- GRIFFITHS, W.M. and GORDON, I.J. (2003). Sward structural resistance and biting effort in grazing ruminants. *Animal Research* 52, 145-160.
- GRIFFITHS, W.M., HODGSON, J. and ARNOLD, G.C. (2003a). The influence of sward canopy structure on foraging decisions by grazing cattle. I. Patch selection. *Grass and Forage Science* 58, 112-124.
- GRIFFITHS, W.M., HODGSON, J. and ARNOLD, G.C. (2003b). The influence of sward canopy structure on foraging decisions by grazing cattle. II. Regulation of bite depth. *Grass and Forage Science*. 58 125-137.

- GURNEY, C. and HUNT, J. (1967). Quasi-static crack propagation. *Proceedings of the Royal Society of London (series A)* **299**, 508-524.
- HAFEZ, E.S.E., CAIRNS, R.B., HULET, C.V. and SCOTT, J.P. (1969). The behaviour of sheep and goats. In: *The Behaviour of Domestic Animals* (eds. Hafez, E.S.E.), Bailliere, Tindall and Cassell Ltd, London, 296-348.
- HALYK, R.M., and HURLBUT, L.W. (1968). Tensile and shear strength characteristics of Alfalfa stems. *Trans. ASAE* **11**, 256-257.
- HENDRICKSON, R. and MINSON, D.J. (1980). The feed intake and grazing behaviour of cattle grazing a crop of *Lablab purpureus* cv. Rongai. *Journal of Agriculture Science, Cambridge* **95**, 547-554.
- HENRY, D.A., MACMILLAN, R.H. and SIMPSON, R.J. (1996). Measurement of the shear and tensile fracture properties of leaves of pasture grasses. *Australian Journal of Agricultural Research* **47**, 587-603
- HENRY, D.A., SIMPSON, R.J., and MACMILLAN, R.H. (2000). Seasonal changes and the effect of temperature and leaf moisture content on intrinsic shear strength of leaves of pasture grasses. *Australian Journal of Agricultural Research* **51**, 823-831.
- HOBBS, R.J. and HUENNEKE, J.K. (1992). Disturbance, diversity, and invasion: implications for conservation. *Conservation Biology* **6**, 324-337.
- HODGSON, J. (1979). Nomenclature and definitions in grazing studies. *Grass and Forage Science* **34**, 11-18.
- HODGSON, J. (1981). Variations in the surface characteristics of the sward and the short-term rate of herbage intake by calves and lambs. *Grass and Forage Science* **36**, 49-57.
- HODGSON, J. (1982). Influence of sward characteristics on diet selection and herbage intake by the grazing animal. In: Hacker, J.B. (ed.) *Nutritional Limits to Animal Production from Pastures*. Commonwealth Agricultural Bureau, Farham Royal, UK, pp. 153-166.
- HODGSON, J. (1985). The control of herbage intake in the grazing ruminant. *Proceedings of the nutrition Society* **44**, 339-346.
- HODGSON, J. (1990). *Grazing Management: science into practice*. Longman

Scientific and Technical, Longman Group UK, Ltd.

- HONGO, A. (1998). Selective grazing in pure leaf and leaf/culm mixtures of herbage grasses by sheep. *Journal of Agriculture Science, Cambridge* 131, 353-359.
- HONGO, A. and AKIMOTO, M. (2003). The role of incisors in selective grazing by cattle and horses. *Journal of Agriculture Science, Cambridge* 140, 469-477.
- HONGO, A., ZHANG, J., TOUKURA, Y. and AKIMOTO, M. (2004). Changes in incisor dentition of sheep influence biting force. *Grass and Forage Science* 59, 293-297.
- HONGO, A., TOUKURA, Y., CHOQUE, J.L., ARO, J.A. and YAMAMOTO, N. (2007). The role of a cleft upper lip of alpacas in foraging extremely short grasses evaluated by grazing impulse. *Small Ruminant Research* 69, 108-114.
- HUGHES, N.R.G., B. de VALLE, C., SABATEL, V., BOOCK, J., JESSOP, N.S. and HERRERO, M. (2000). Shear strength as an additional selection criterion for quality in *Brachiaria* pasture ecotypes. *Journal of Agricultural Science, Cambridge* 135, 123-130.
- HUGHES, T.P., SYKES, A.R., POPPI, D.P. and HODGSON, J. (1991). The influence of sward structure on peak bite force and bite weight in sheep. *Proceedings of the New Zealand Society of Animal Production* 51, 153-158.
- ILLIUS, A.W. (1989). Allometry of food intake and grazing behaviour with body size in cattle. *Journal of Agricultural Science, Cambridge* 113, 259-266.
- ILLIUS, A.W., CLARK, D.A. and HODGSON, J. (1992). Discrimination and patch choice by sheep grazing grass-clover swards. *Journal of Animal Ecology* 61, 183-194.
- ILLIUS, A.W., GORDON, I.J., MILNE, J.D. and WRIGHT, W. (1995). Costs and benefits of foraging on grasses varying in canopy structure and resistance to defoliation. *Functional Ecology* 9, 894-903.
- INOUE, T., BROOKES, I.M., BARRY, T.N. and JOHN, A. (1989). Effects of selection for shear strength on the voluntary intake and digestion of perennial ryegrass fed to sheep. *Proceedings of the New Zealand Society of Animal Production* 49, 221-224.
- INOUE, T., BROOKES, I.M., JOHN, A., KOLVER, E.S. and BARRY, T.N. (1994).

- Effects of leaf shear breaking load on the feeding value of perennial ryegrass (*Lolium perenne*) for sheep. I. Effects on leaf anatomy and morphology. *Journal of Agricultural Science, Cambridge* 123, 137-147.
- JAMIESON, W.S. and HODGSON, J. (1979). The effect of variation in sward characteristics upon the ingestive behaviour and herbage intake of calves and lambs under a continuous stocking management. *Grass and Forage Science* 34, 273-282.
- JANIS, C.M. (1988). Correlation of relative muzzle width and relative incisor width with dietary preference in ungulates. *Zoological Journal of the Linnean Society* 92, 267-284.
- JONES, E.L. and ROBERTS, J.E. (1991). A note on the relationship between palatability and water-soluble carbohydrates content in perennial ryegrass. *Irish Journal of Agricultural Research* 30, 163-167.
- JOHN, A., INOUE, T., BROOKES, I.M., HUNT, W.F. and EASTON, H.S. (1989). Effects of selection for shear strength on structure and rumen digestion of perennial ryegrass. *Proceedings of the New Zealand Society of Animal Production* 49, 225-228.
- KAY, R. (1975). The functional adaptations of primate molar teeth. *American Journal of Physical Anthropology* 43, 195-216.
- KENNEDY, P.M. and DOYLE, P.T. (1993). Particle-size reduction by ruminants: Effect of cell wall composition and structure. In: Jung, H.G., Buxton, D.R., Hatfield, R.D. and Ralph, J. (eds) *Forage Cell Wall Structure and Digestibility*. ASA/CSSA/SSSA, Madison, USA, pp. 499-534.
- KENNEY, P.A., BLACK, J.L. and COLEBROOK, W.F., (1984). Factors affecting diet selection by sheep. III. Dry matter content and particle length of forage. *Australian Journal of Agricultural Research* 35, 831-838.
- KING, M.J. and VINCENT, J.F.V. (1996). Static and dynamic fracture properties of the leaf of New Zealand flax *Phormium tenax* (Phormaceae: Monocotyledones). *Proceedings of the Royal Society of London, B, Biological Sciences* 263, 521-527.
- KOTLIAR, N.B. and WIENS, J.A. (1990). Multiple scales of patchiness and patch structure: a hierarchical framework for the study of heterogeneity. *Oikos* 59, 253-260.

- KRUEGER, W.C., LAYCOCK, W.A. and PRICE, D.A. (1974). Relationships of taste, smell, sight, and touch to forage selection. *Journal Range Management* 27, 258-262.
- LACA, E.A., UNGAR, E.D., SELIGMAN, N.G., RAMEY, M.R. and DEMMENT, M.W. (1992a). An integrated methodology for studying short-term grazing behaviour of cattle. *Grass and Forage Science* 47, 81-90.
- LACA, E.A., UNGAR, E.D., SELIGMAN, N.G. and DEMMENT, M.W. (1992b). Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. *Grass and Forage Science* 47, 91-102.
- LACA, E.A., DEMMENT, M.W., DISTEL, R.A. and GRIGGS, T.C. (1993). A conceptual model to explain variation in ingestive behaviour within a feeding patch. *Proceedings 17<sup>th</sup> International Grassland Congress*, 710-712.
- LAREDO, M.A. and MINSON, D.J. (1973). The voluntary intake, digestibility, and retention time by sheep of leaf and stem fractions of five grasses. *Australian Journal of Agricultural Research* 24, 875-888.
- LEES, G.L., HOWARTH, R.E., GOPLEN, B.P. and FESSER, A.C. (1981). Mechanical disruption of leaf tissues and cells in some bloat-causing and bloat-safe forage legumes. *Crop Science* 21, 444-448.
- LEES, G.L., HOWARTH, R.E. and GOPLEN, B.P. (1982). Morphological characteristics of leaves from some legume forages: relation to digestion and mechanical strength. *Canadian Journal of Botany* 60, 2126-2132.
- LEIGH, J.H. and MULHAM, W.E. (1966a). Selection of diet by sheep grazing semi-arid pastures on the Riverine Plain. 1. A bladder saltbush (*Atriplex vesicaria*)-cotton bush (*Kochia aphylla*) community. *Australian Journal of Experimental Agriculture and Animal Husbandry* 6, 460-467.
- LEIGH, J.H. and MULHAM, W.E. (1966b). Selection of diet by sheep grazing semi-arid pastures on the Riverine Plain. 2. A cotton bush (*Kochia aphylla*)-grassland (*Stipa variabilis*-*Danthonia caespitosa*) community. *Australian Journal of Experimental Agriculture and Animal Husbandry* 6, 468-474.
- LESNIEWSKA, A., PONITKA, A., SLISARKIEWICZ-JARZINA, A., ZWIERZYKOWSKA, E., ZWIERZYKOWSKI, Z., JAMES, A.R., THOMAS, H.

- and HUMPHREYS, M.W. (2001). Androgenesis from *Festuca pratensis* x *Lolium multiflorum* amphidiploid cultivars in order to select and stabilize rare gene combinations for grass breeding. *Heredity* **86**, 167-176.
- LUCAS, P.W. (1979). The dental-dietary adaptations of mammals. *Neues Jahrbuch Fuer Geologie und Palaeontologie monatscefte* **8**, 486-516.
- LUCAS, P.W. and PEREIRRA, B.P. (1990). Estimation of fracture toughness of leaves. *Functional Ecology* **4**, 819-822.
- LUCAS, P.W. and PEREIRRA, B.P. (1991). Thickness effect in cutting systems. *Journal of Material Science Letter* **10**, 235-236.
- LUCAS, P.W., CHOONG, M.F., TAN, H.T.W., TURNER, I.M. and BERRICK, A.J. (1991). The fracture toughness of the leaf of *Calphyllum inophyllum* L. (Guttiferae). *Philosophical Transactions of the Royal Society of London B334*, 95-106.
- MACADAM, J.W. and MAYLAND, H.F. (2003). The relationship of leaf strength to cattle preference in tall fescue cultivars. *Agronomy Journal*. **95**, 414-419.
- MACKINNON, B.W., EASTON, H.S., BARRY, T.N. and SEDCOLE, J.R. (1988). The effect of reduced leaf shear strength on the nutritive value of perennial ryegrass. *Journal of Agricultural Science, Cambridge* **111**, 469-474.
- MANNETJE, L.'t. (1974). Relations between pasture attributes and liveweight gains on a subtropical pasture. In: *Proceedings of the 12th International Grassland Congress, Moscow* **3**, 299-304.
- MARTENS, P.O. and BOOYSEN, P. de V. (1968). A tensilmeter for the measurement of the tensile strength of grass leaf blades. *Proceedings of the Grassland Society of South Africa* **3**, 51-56
- McARTHUR, C. and SANSON, G.D. (1988). Tooth wear in eastern grey kangaroos (*Macropus giganteus*) and western grey kangaroos (*Macropus fuliginosus*), and its potential influence on diet selection, digestion and population parameters. *Journal of Zoology* **215**, 491-504.
- McNAUGHTON, S.J. (1984). Grazing lawns: animals in herds, plant form and coevolution. *The American Naturalist* **124**, 863-886.
- McNAUGHTON, S.J. (1985). Ecology of a grazing ecosystem: the Serengeti.



*Ecological Monographs* 55, 259-294.

- MILNE, J.A. HODGSON, J. THOMPSON, R., SOUTER, W.G. and BARTHAM, G.T. (1982a). The diet ingested by sheep grazing swards differing in white clover and perennial ryegrass content. *Grass and Forage Science* 37, 209-218.
- MILNE, J.A. HODGSON, J., SPENCE, A.M. and BALDWIN, B.A. (1982b). Effect of ablation bulbs on the diet ingested by grazing sheep. *Applied Animal Ethology* 8, 261-268.
- MINSON, D.J. (1972). The digestibility and voluntary intake by sheep of six topical grasses. *Australian Journal of Experimental Agriculture and Animal Husbandry* 12, 21-27.
- MINSON, D.J. 1982. Effect of chemical composition on feed digestibility and metabolizable energy. *Nutrition Abstracts Reviews Series B* 52, 591-615.
- MINSON, D.J. (1987). Plant factors affecting intake. *In: Managed Grasslands, B. Analytical Studies* (Ed. R.W. Snaydon), Elsevier Science, B.V., Amsterdam. pp. 137-144.
- MINSON, D.J., HARRIS, C.E., RAYMOND, W.F. and MILFORD, R. (1964). The digestibility and voluntary intake of S22 and H.I. ryegrass, S170 tall fescue, S48 timothy, S215 meadow fescue and Germinal cocksfoot. *Journal of the British Grassland Society* 19, 298-305.
- MITCHELL, R.J., HODGSON, J. and CLARK, D.A. (1991) The effect of varying leafy sward height and bulk density on the ingestive behaviour of young deer and sheep. *Proceedings of the New Zealand Society of Animal Production* 51, 159-165.
- MOORE, J.E. and MOTT, G.D. (1973). Structural inhibitors of quality in tropical grasses. *In: Matches, A. G. (ed.) Anti-Quality Components of Forages*, CSSA Special Publication No. 4, USA.
- MOULIA, B. (2000). Leaves as shell structures: double curvature, auto-stresses, and minimal mechanical energy constraints on leaf rolling in grasses. *Journal of Plant Growth Regulation* 19, 19-30.
- MUNRO, J.M.M., DAVIES, D.A., EVANS, W.B. and SCURLOCK, R.V. (1992). Animal production evaluation of herbage varieties. 1. Comparison of Aurora with Frances, Talbot and Melle perennial ryegrass when grown alone and with

- clover. *Grass and Forage Science* 47, 259-273.
- NGUYEN, H.T., SLEPER, D.A. and MATCHES, A.G. (1982). Inheritance of forage quality and its relationship to leaf tensile strength in tall fescue. *Crop Science* 22, 67-72.
- NIKLAS, K.J. (1992). *Plant Biomechanics. An Engineering Approach to Plant Form and Function*. The University of Chicago Press, Chicago and London.
- NIKLAS, K.J. (1993) Influence of tissue density-specific mechanical properties on the scaling of plant height. *Annals of Botany* 72, 173-179.
- NIKLAS, K.J. (1998). The mechanical roles of clasping leaf sheaths: Evidence from *Arundinaria testae* (Poaceae) shoots subjected to bending and twisting forces. *Annals of Botany* 81, 23-34.
- NIKLAS, K.J., MOLINA-FREANER, F. and TINOCO-OJANGUREN, C. (1999). Biomechanics of the columnar cactus *Pachycereus pringlei*. *American Journal of Botany* 86, 767-775.
- O'REAGAIN, P.J. (1993). Plant structure and acceptability of different grasses to sheep. *Journal of Range Management* 46, 232-236.
- PAOLILLO, D.J.Jr. and NIKLAS, K.J. (1996). Effects of Rht-dosage on the breaking strength of wheat seedling leaves. *American Journal of Botany* 83, 567-572.
- PARSONS, A.J. and CHAPMAN, D.F. (1998). Principles of grass growth and pasture utilisation. In: *Grass for Dairy Cattle* (eds. Cherney, J.H. and Cherney, D.J.R.). CAB International, Wallingford, 283-309.
- PENNING, P.D., PARSONS, A.J., ORR, R.J. and TREACHER, T.T. (1991). Intake and behaviour responses by sheep to changes in sward characteristics under continuous stocking. *Grass and Forage Science* 46, 15-28.
- PEREIRA, B.P., LUCAS, P.W. and TEOH, S. (1997). Ranking the fracture toughness of thin mammalian soft tissues using the scissors cutting test. *Journal of Biomechanics* 30, 91-94.
- PHILLIPS, C.J.C. (1993). Cattle behaviour. Farming Press Books, Ipswich, pp. 75-111.
- PRACHE, S., ROGUET, C., LOUAULT, F. and PETIT, M. (1997) Diet selection of dry ewes between vegetative and reproductive tillers during the grazing down of

- a cocksfoot sward. *Proceedings of the 5th Research Conference of the British Grassland Society*, Reading, pp. 55–56.
- ROARK, R.J. (1965). *Formulas for Stress and Strain* (4th ed.). McGraw-Hill Book Company, New York.
- ROARK, R.J. and YOUNG, W.C. (1975). (5th) (eds) *Formulas for Stress and Strain*, McGraw-Hill, London.
- ROSENTHAL, J.P. and KOTANEN, P.M. (1994). Terrestrial plant tolerance to herbivory. *Trends in Ecology and Evolution* **9**, 145-148.
- SANSON, G., READ, J., ARANWELA, N., CLISSOLD, F. and PEETERS, P. (2001). Measurement of leaf biomechanical properties in studies of herbivory: Opportunities, problems and procedures. *Austral ecology* **26**, 535-546.
- SCHEIRS, J., DE BRUYN, L., and VERHAGEN, R. (2001). A test of the C<sub>3</sub>-C<sub>4</sub> hypothesis with two grass miners. *Ecology* **82**, 419-421.
- SCHMIDT, P.W. (1977). Impulse. In: McGraw-Hill (Ed.), *Encyclopedia of Science and Technology*. McGraw-Hill Book Company, New York, pp. 51-52.
- SCHWENDENER, S. (1874). *Das Mechanische Prinzip im Anatomischen Bau der Monocotylen*. Verlag W. Engelmann, Leipzig.
- SCOTT, J.D. (1956). The study of perennial buds and the reaction of roots to defoliation as a basis of grassland management. In: Neale, G.J. (ed.) *Proceedings of the 7th International Grassland Congress*, Palmerston North, New Zealand. Wright and Carmen Ltd., E. Wellington, New Zealand. pp. 479.
- SERWAY, R.A. (1982). *Physics for Scientists and Engineers* (3rd ed.). Saunders College Publishing, Philadelphia, pp. 219-253.
- SILK, W.K., WANG, L.L. and CLELAND, R.E. (1982). Mechanical properties of the rice panicle. *Plant Physiology* **70**, 460-464.
- SMITH, K.F., SIMPSON, R.J., CULVENOR, R.A., HUMPHREYS., M.O., PRUD-HOMME, M.P. and ORAM, R.N. (2001). The effects of ploidy and a phenotype conferring a high water-soluble carbohydrate concentration on carbohydrate accumulation, nutritive value and morphology of perennial ryegrass (*Lolium perenne* L.). *Journal of Agricultural Science, Cambridge* **136**, 65-74.

- SNAYDON, R.W. (1981). The ecology of grazed pasture. In: Morley, F.H.W. (ed.) *World Animal Science, B1, Grazing Animals*. Amsterdam Elsevier, pp. 13-31.
- SNEDECOR, G.W. and COCHRAN, W.G. (1980) *Statistical Methods*. (7th ed.) Iowa State University Press, Ames, 83-237.
- SPATZ, H.C., BEISMANN, H., EMANNS, A. and SPECK, T. (1995). Mechanical anisotropy and inhomogeneity in the tissues comprising the hollow stem of the giant reed *Arundo donax*. *Biomimetics* 3, 141-145.
- SPECK, T. (1994). Bending stability of plant stems: Ontological, ecological, and physiological aspects. *Biomimetics* 2, 109-128.
- SPEEDING, C.R.W., LARGE, R.V. and KYDD, D.D. (1966). The evaluation of herbage species by grazing animals. In: Hill, A.G.G. (ed.) *Proceedings of the 10th International Grassland Congress*. Valtioneuvoston Kirjapaino, Helsinki, Finland, pp. 479-483.
- STOBBS, T.H. (1973). The effect of plant structure on the intake of tropical pastures I. variation in the bite size of grazing cattle. *Australian Journal of Agricultural Research* 24, 809-819.
- SUN, D. and LIDDLE, M.J. (1993). Trampling resistance, stem flexibility and leaf strength in nine Australian grasses and herbs. *Biological Conservation* 65, 35-41.
- TAYLOR, ST C.S., MURRAY, J.I. and ILLIUS, A.W. (1987). Relative growth of incisor arcade breadth and eating rate in cattle and sheep. *Animal production* 45, 453-480.
- THARMARAJ, J. (2000). Effects of resistance to prehension and structure of pastures on grazing behaviour and intake of dairy cows. Ph.D. thesis, University of Melbourne, Australia.
- THARMARAJ, J., WALES, W.J., CHAPMAN, D.F. and EGAN, A.R. (2003). Defoliation pattern, foraging behaviour and diet selection by lactating dairy cows in response to sward height and herbage allowance of a ryegrass-dominated pasture. *Grass and Forage Science* 58, 225-238.
- THERON, E.P. and BOOYSEN, P. de V. (1966). Palatability in grasses. *Proceedings of the Grassland Society of South Africa* 1, 111-120.

- UNGAR, E.D. (1996). Ingestive behaviour. In: Hodgson, J. and Illius, A.W. (eds) *The Ecology and Management of Grazing Systems*. CAB International, Wallingford, UK, pp. 185-218.
- VAVRA, M. and GANSKOPP, D. (1998). Grazing behaviour in ungulates: Current concepts and future challenges. *Annals of Arid Zone* 37(3), 319-335.
- VINCENT, J.F.V. (1982). The mechanical design of grass. *Journal of Materials Science* 17, 856-860.
- VINCENT, J.F.V. (1983). The influence of water content on the stiffness and fracture properties of grass leaves. *Grass and Forage Science* 38, 107-114.
- VINCENT, J.F.V. (1990). Fracture properties of plants. *Advances in Botanical Research*. 17, 235-287.
- VINCENT, J.F.V. (1991). Strength and fracture of grasses. *Journal of Materials Science* 26, 1947-1950.
- VINCENT, J.F.V. (1992). (ed.) *Biomechanics-materials: A practical approach*. Oxford University Press, Oxford.
- VOISEY, P.W. (1976). Engineering assessment and critique of instruments used for meat tenderness evaluation. *Journal of Texture Studies* 7, 11-48.
- WADE, M.N., PEYRAUD, J.L., LEMAIRE, G. and COMERON, E.A. (1989). The dynamics of daily area and depth of grazing and herbage intake of cows in a five day paddock system. *Proceedings 16<sup>th</sup> International Grassland Congress*, Nice, France, pp. 1111-1112.
- WAINWRIGHT, S.A., BIGGS, W.D., CURREY, J.D. and GOSLINE, J.M. (1976). *Mechanical design in organisms*. Princeton University Press, Princeton.
- WESTOBY, M. (1986). Mechanism influencing grazing success for livestock and wild herbivores. *The American Naturalist* 128, 940-941.
- WILLOUGHBY, W.M. (1958). A relationship between pasture availability and animal production. *Proceedings Australian Society Animal Production* 2, 42-45.
- WILSON, D. (1965). Nutritive value and the genetic relationships of cellulose content and leaf tensile strength in *Lolium*. *Journal of Agricultural Science, Cambridge* 65, 285-292.

- WILSON, J.R., AKIN, D.E., MCLEOD, M.N., and MINSON, D.J. (1989) Particle size reduction of the leaves of a tropical and a temperate grass by cattle. II. Relation of anatomical structure to the process of leaf breakdown through chewing and digestion. *Grass and Forage Science* 44, 65-75.
- WOODWARD, S.J.R. (1998). Bite mechanics of cattle and sheep grazing grass-dominant swards. *Applied Animal Behaviour Science* 56, 203-222.
- WRIGHT, W. (1992). The fracture properties of grass and their relevance to feeding in herbivores. PhD Thesis, University of Reading, UK.
- WRIGHT, W. and ILLIUS, A.W. (1995). A comparative study of the fracture properties of five grasses. *Functional Ecology* 9, 269-278.
- WRIGHT, W. and VINCENT, J.F.V. (1996). Herbivory and the mechanics of fracture in plants. *Biological Reviews of the Cambridge Philosophical Society* 71, 401-413.
- YATES, J.J., EDYE, L.A., DAVIES, J.G. and HAYDOCK, K.P. (1964). Animal production from a *Sorghum almum* pasture in south-east Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry* 4, 325-335.
- ZANG, J.M. (2004). Effect of physical strength of forage grasses on grazing behaviour of sheep. PhD Thesis, Iwate University, Japan.
- ZANG, J.M., HONGO, A. and AKIMOTO, M. (2004). Physical strength and its relation to leaf anatomical characteristics of nine forage grasses. *Australian Journal of Botany* 52, 799-804.