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

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フェストロリウムとペレニアルライグラスの葉身の引張りとせん断特性

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## ABSTRACT

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 5 mm 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.

Key words: Festulolium, Grass leaves, Perennial ryegrass, Shearing, Tensile

## 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

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was higher than that from other cultivars (Munro *et al*.1992).Thus, there are many studies on feeding values and chemical compositions of various elements in festulolium and perennial ryegrass. However, few studies were reported with respect to biomechanical properties. In the agronomical field, biomechanical properties affect selective grazing by animals, digestibility of grasses, 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 research was to determine tensile and shearing properties of leaves in festulolium, compared with those of diploid and tetraploid cultivars of perennial ryegrass.

## MATERIALS AND METHODS

### Grass swards

The swards of the following 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 by a compound fertilizer equivalent to 65-49-65 kg ha$^{-1}$ of N-P$_2$O$_5$-K$_2$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 5 cm height immediately after taking samples. An additive compound fertilizer equivalent to 65-49-65 kg ha$^{-1}$ of N-P$_2$O$_5$-K$_2$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 side 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 length from a ligule side with scissors and had a midpoint marked. The weight was determined using a digital balance (± 0.0001 g) after absorbing water on leaf surface with paper towel. The width at midpoint was measured with scaled magnifier (± 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.

### Tensile tests

Tensile strength was measured using a 100 N loadcell of the breaking test machine (Aikoh Engineering Co.; M1334). Both ends of a leaf were seized with the original jaw clamps (Hongo *et al*. 2006). One clamp was connected with a loadcell and another clamp was moved downward at 10 ㎜ min$^{-1}$ for a low rate of deformation (Vincent 1992). The initial length of a sample between two clamps in the test machine was 27 ㎜. Tensile strength from a loadcell and elongation length from displacement transducer (NEC Sanei; 9E08-D3) were digitally recorded in a memory card (smart media) of the memory hicorder (Hioki; 8807) at a speed of 5 sec$^{-1000}$. A graph of applied force versus deflection was produced from the saved data. After the measurement, leaves were again immersed in distilled water.

The stiffness of leaves was estimated by the following equation:

$$E = stress/strain,$$

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).

### Shearing test

Shear strength was measured about 5 mm apart from a broken point in the tensile test using a pair of the scissors (Plus Co.; No.135) with sharp stainless blades. The principle structure was the same as the previous reports (Pereira *et al*. 1997: Lucas and Pereira 1990: Vincent 1992). Before a measurement, the surface of blades was rubbed with a swab including a lubricant oil to reduce friction (Vincent 1992). The travel rate of intersection points of the two blades was 20 ㎜min$^{-1}$. In each test, two passes were made: cutting of the specimen and an empty pass (Lucas and Pereira 1991). The work of fracture done was calculated deducting the work in the empty pass from the work done in the piece (Wright and Vincent 1996).

### Cross-sectional area

For a measurement of cross-sectional area, leaves were transversely sliced 3 mm in length using a razor blade. The section was vertically kept in touch with the side wall of a

plastic block. A cross section of leaf was photographed under a stereo-microscope (Nikon; SMZ-U) and the pictures were digitally saved in a memory card (smart media). Each picture was projected onto a monitor screen at a length magnification of 50-80 times. The contour line of cross-section was delineated with a cursor on a monitor screen using the commercial software of the computer graphics (Arcsoft Japan; Photo studio). The area inside this contour line was measured using the commercial software (Nagoya Daigaku; Lia32).

*Statistical analysis*

Variables of biomechanical properties were analyzed using a paired t-test and an analysis of variance (Snedecor and Cochran 1967). The regression analysis was also carried out.
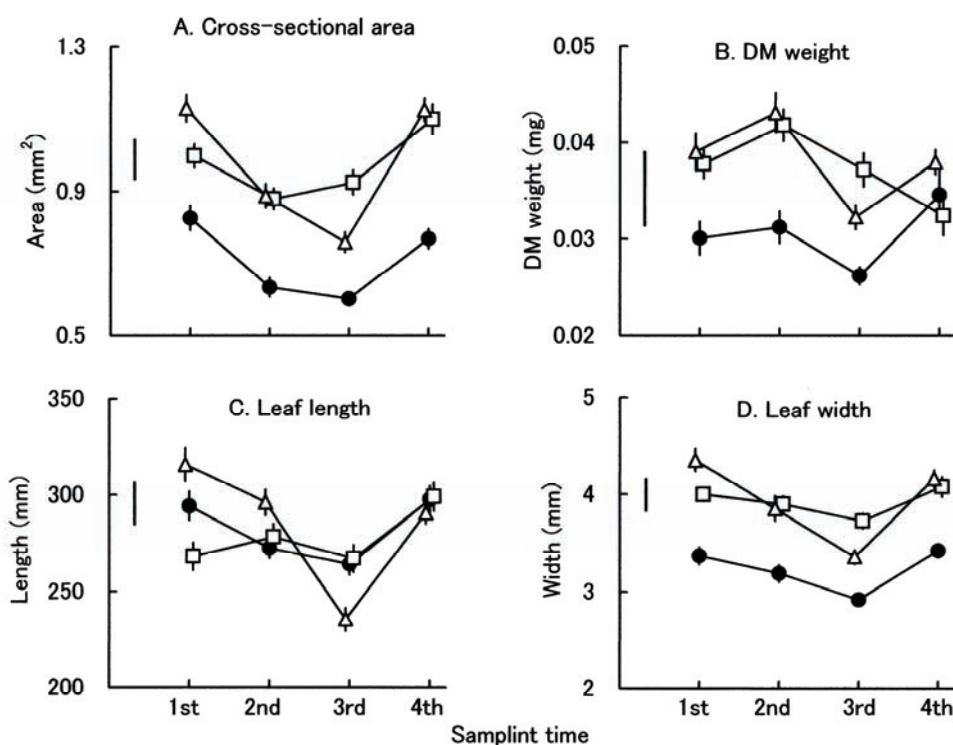


Fig. 1  Cross-sectional area, DM weight, leaf length and leaf width at four sampling times in diploid (●) and tetraploid (□) cultivars of perennial ryegrass and festulolium (△). Attached lines with symbols show s.e. of mean and vertical lines show s.e.d. of the mean differences.

## 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. 1). Mean values of four samples were 0.70±0.016 ㎟, 0.98±0.019 ㎟ and 0.97±0.021 ㎟ 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 samples, but not significantly different ($p<0.179$) at the 4th sample.

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±0.04 ㎜, 3.9±0.04 ㎜ and 3.9±0.06 ㎜ in diploid and tetraploid cultivars of perennial ryegrass and festulolium, respectively.
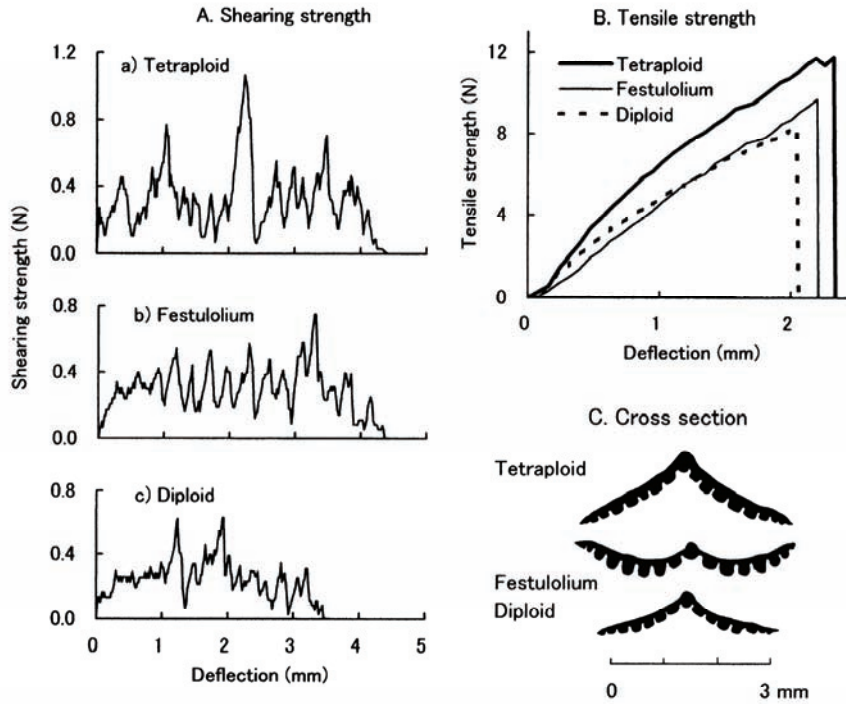
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Fig. 2 Shearing and tensile fracture patterns and cross sections of diploid (Aurora) and tetraploid (Prospero) cultivars of perennial ryegrass and festulolium. Each measurement was obtained from the same leaf.

### Fracture patterns

The force-deflection patterns of shearing and tensile fractures are shown in Fig. 2. Shearing strength reached a 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).

Shearing strength and 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. 4). The grand mean was $1.40 \pm 0.023$ MNmkg$^{-1}$ in density-specific stiffness and $0.080 \pm 0.0013$ MNmkg$^{-1}$ in density-specific strength.
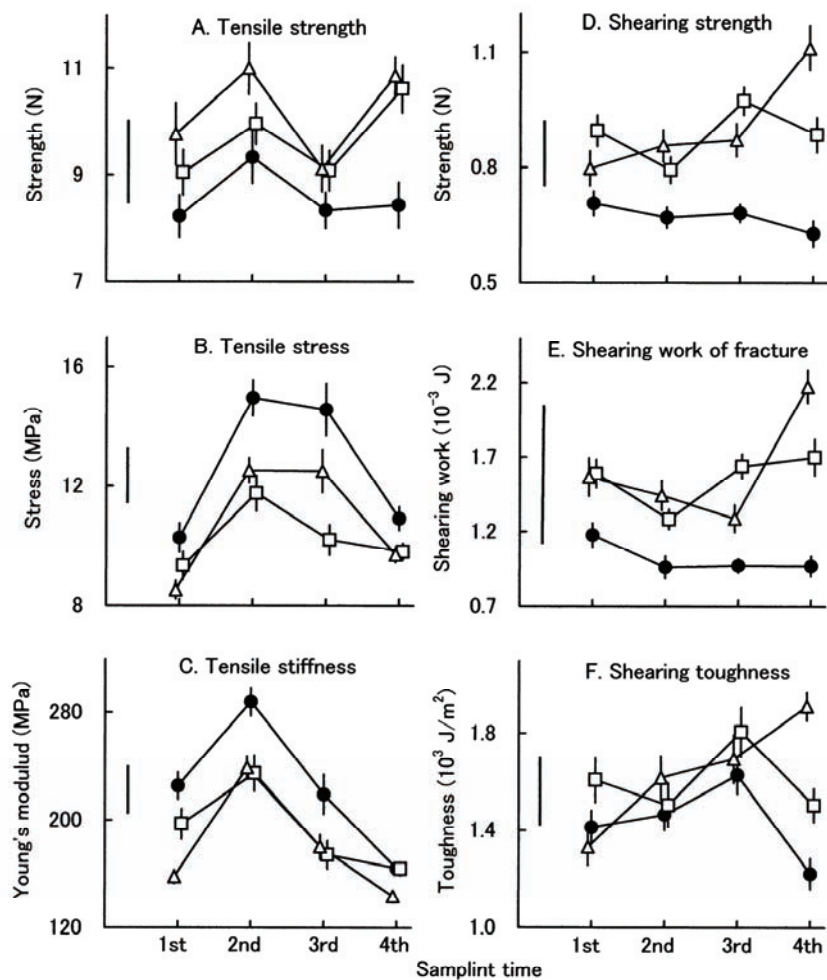
Fig. 3 Tensile and shearing properties at four sampling times in diploid (●) and tetraploid (□) cultivars of perennial ryegrass and festulolium (△). Attached lines with symbols show s.e. of mean and vertical lines show s.e.d. of the mean differences.
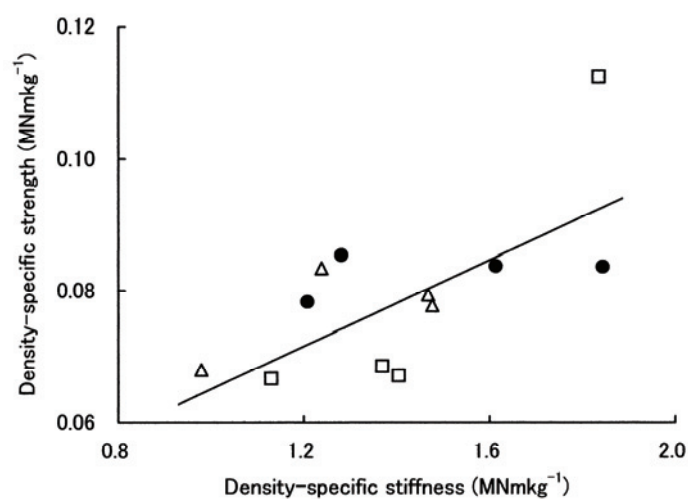


Fig. 4 Relationship between density-specific stiffness and density-specific strength at four sampling times in diploid (●) and tetraploid (□) cultivars of perennial ryegrass and festulolium (△).
Y = 0.032 X + 0.035 (r = 0.666, $p < 0.007$).

# DISCUSSION

Grass leaves are probably the simplest of all plants 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±2.2 ㎜ in total leaf length and 3.7±0.03 ㎜ 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. 2), which may be maintained under high turgor (Moulia 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. 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±5.7 and 180±4.5 versus 224±6.4 MPa). Reversely, cross-sectional area was significantly higher in tetraploid perennial ryegrass and festulolium than in diploid perennial ryegrass (0.98±0.019 and 0.97±0.021 versus 0.70±0.016 ㎟). 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.

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## 要　　約

　フェストロリウム(*Festulolium loliaceum*)の 2 品種 (Ba11356 and Ba11358)、ペレニアルライグラス (*Lolium perenne*)の 2 倍体の 2 品種(Ba11353 and Aurora)と 4 倍体の 2 品種(Ba10855 and Prospero)を用いて、葉身の引張りとせん断特性を調査した。帯広市にある造成後 3 年目の牧草地から 2004 年 7 月から 9 月までの間に 4 回の牧草サンプルを採取した。引張り強度は専用試験器にとりつけた 100 N ロードセルを用いて測定した。せん断強度は、引張り強度を測定した部位より約 5 mm 離れたところで、ハサミ型試験器で測定した。

　葉身の断面積、DM 重、幅はペレニアルライグラス 4 倍体とフェストロリウムの方がペレニアルライグラス 2 倍体より有意に高かった。せん断強度は変位のほぼ中央でピークに到達したが、これは主脈のせん断に一致した。せん断による破断パターンで、葉身断面の内側にある小隆起をせん断する時に多数の小さいピークを観察した。引張り強度、せん断強度、せん断による仕事量はペレニアルライグラス 4 倍体とフェストロリウムの方がペレニアルライグラス 2 倍体より有意に高かったが、引張り応力と耐変形性は逆にペレニアルライグラス 4 倍体とフェストロリウムの方がペレニアルライグラス 2 倍体より有意に低かった。断面積の小さい細いか薄い葉身は、縦方向の耐変形性が高くなる傾向を認めた。以上のように、フェストロリウムの形態と生体力学的特性ははペレニアルライグラス 4 倍体と極めて類似していた。

キーワード：フェストロリウム、イネ科葉身、ペレニアルライグラス、せん断、引張り