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Tensile behaviour of grass

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The tensile behaviour of ryegrass (*Lolium perenne* L.), which was grown, harvested and tested under controlled conditions, is described. Whereas some of the grass leaf specimens behaved in a predominantly brittle manner, others evinced a semi-ductile mode such that a proportional limit could be identified. Results indicated that the tensile properties depended upon specimen location and the tensile test strain rate. The data showed that as strain rate was increased, the stiffness, toughness and strength increased, while ductility decreased. Comparison of test results as a function of water content did not reveal statistically significant differences in any of the mechanical parameters. Analysis of the leaf structure suggests that the epidermal cells play a major role as a load-bearing component.

1. Introduction

The agricultural, ecological and economic importance of grasses has been well-documented, but less information is available concerning the engineering properties of these biological materials. In this regard the mechanical behaviour of grass leaves is of particular interest because these structures are naturally occurring polymeric composites consisting of load-bearing elements held within a matrix [1]. One of the first modern studies to consider mechanical properties was conducted by Evans [2], who observed that the breaking load per dry weight of a standard length specimen of ryegrass, *Lolium perenne* L., was significantly correlated with both cellulose and fibre content. Using similar techniques to study other species, Wilson [3] found a significant relationship between leaf strength and cellulose content. Later work by Evans [4], Martens and de V. Booysen [5] as well as Theron and de V. Booysen [6] considered the importance of other factors, such as specimen location and water content, on mechanical behaviour. More recently, McRandal and McNulty [7] investigated the shear properties of perennial ryegrass (*Lolium perenne* L.) and measured strength, penetration resistance and energy absorption. In 1982, Vincent [1] reported the tensile and fracture toughness behaviour of notched specimens of perennial ryegrass. This innovative work proposed that the grass leaf could be modelled as a three-component composite system consisting of sclerenchyma fibres, vascular bundles and a matrix containing relatively large, thin-walled cells under turgor pressure. Experimental values of plant stiffness were represented by a Voigt model in which the fibre components accounted for 90 to 95% of the longitudinal stiffness while occupying only about 8 vol % of the composite. Data from the simple notch fracture tests indicated that the leaves were relatively notch insensitive. In a later paper, Vincent [8] described the dependence of measured modulus and fracture values upon the water content and concommitant changes in specimen dimensions.

Because of the large variability associated with measurements of biological materials, few attempts have been made to compare the data of different studies. A careful review of published experimental procedures indicates that details concerning material and testing parameters such as specimen age, size, location, and strain rate are often incomplete. In some cases, inappropriate methods were applied for determining basic mechanical quantities, such as breaking strength, and these limited the utility of the data [2]. Such factors could account for many of the discrepancies among reported mechanical property values. Therefore, information concerning the basic stress–strain properties of grass leaves should be of significant value to many workers. Accordingly, the purpose of this paper is to describe the tensile behaviour of ryegrass (*Lolium perenne* L.) which was grown, harvested and tested under well-defined, controlled conditions.

2. Materials and methods

We selected perennial ryegrass (*Lolium perenne* L.) as our experimental organism because of the availability of published data and the fact that these plants are easily cultured in large numbers under greenhouse conditions. Test specimens for water loss and three series of tensile experiments were sectioned from leaves from two plantings of healthy 7 w old plants (groups I and II). These plants, grown from seed in plantings 12 w apart, were maintained in the biology department greenhouse under controlled conditions of light, temperature and humidity. The age of the plants was measured from when the seedlings first appeared above the soil (growth medium) surface.

Water loss experiments were conducted on 20 randomly selected leaves (measuring +18 cm from the base to the tip of the leaf) from the group I plants.

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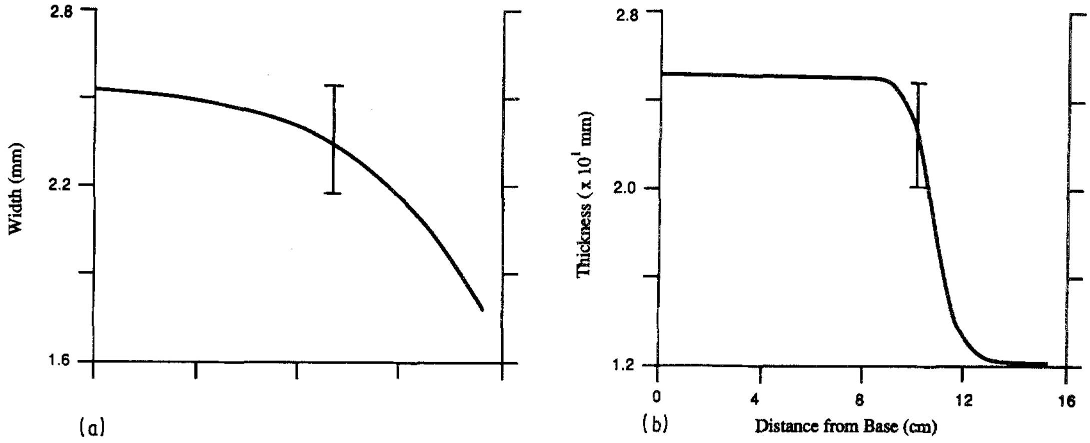

Figure 1 Dimensional changes in (a) the width and (b) thickness of the ryegrass leaf as a function of distance from the base measured along the longitudinal axis. Error bars indicate maximum standard deviation in a sample of 10 leaves.

Before testing, 1.3 cm pieces were removed from the tip and base, the remaining 16 cm leaf pieces were each divided into two 8 cm specimens (apical and basal). An additional 21 leaves were utilised in a series of tensile experiments to determine the effects of apical compared to basal location. In a subsequent series of tensile tests, 31 and 32 basal specimens (8 cm long) from the group II plants were used to evaluate the effects of engineering strain rate and water content, respectively.

Width and thickness measurements of the specimens as a function of distance from the leaf base were made on representative specimens from the group I and II plants. A micrometer was used in conjunction with a × 10 measuring eyepiece to obtain the values. The dimensions of the leaves were measured to ± 0.005 mm.

Weight loss measurements were made with a Mettler microbalance equipped with a personal computer for data acquisition. This allowed us to collect data continuously over a prolonged period of time. In this procedure an expanded leaf was removed from each of 10 group I plants, placed immediately in the balance and weight values continuously recorded under ambient conditions for a period of 1 h. The equilibrium water content of the group I and II leaves was determined by drying the tissue (6 d at 110° C) and calculating the percentage of dry weight.

All mechanical measurements were made in an Instron model 1130 electromechanical tester equipped for low load determination. After sectioning into 8 cm pieces, the specimens were secured between matching elastomeric-type specimen grips with rubber-coated steel platens. These grips were adjusted by means of springs which maintained a tension sufficient to prevent the specimen from slipping but not large enough to crush the tissue. All specimens had gauge lengths of 5 cm and were loaded in tension to failure at a cross-head velocity of either 2.5 (group I) or 0.5 and 5.0 (group II) cm min^{-1}. These are equivalent to engineering strain rates of 0.5, 0.1 and 1.0 min^{-1}, respectively. Loads were recorded on the instrument strip chart with a resolution of 0.09 N. Because the grass specimens were not suitable for use on a standard extensometer, deformations were obtained by recording the separation of two fiducial marks photographically, at regular intervals during the tests. Deformation values subsequently were measured on a projected image to ± 0.01 mm. The load and deformation data were converted to engineering stress and strain values using standard relationships and the results of the dimensional analyses. Owing to the tapered nature of the leaf (cf. Fig. 1), all specimens consistently fractured at the location where the cross-sectional area was relatively small. This was near but not immediately adjacent to the point at which the specimen left the grip mandrels.

The proportions of the various structural elements in the leaf were determined via light microscopy. Cross-sectional specimens from a 20 w old plant were prepared by standard dehydration, embedding and sectioning procedures [9], stained with Safranin and counterstained with Fast Green, and examined at magnifications of × 100 and × 400. Selected sections were then photographed and area fractions subsequently measured by means of a Sigmascan digitizer. Volume fraction values of the components were assumed proportional to the measured cross-sectional quantities.

3. Results

The width and thickness profiles of representative leaf specimens are presented in Fig. 1. The maximum standard deviation from these data was 10%. The measurements indicated that the width decreased monotonically from the base to the tip while the thickness remained constant to a distance of ~ 10 cm from the base, decreased significantly over the next 3 cm and again became constant. These results demonstrated that cross-sectional areas must be carefully determined if accurate values for strength parameters are to be obtained. This is particularly important in apical sections because thickness decreases abruptly towards the leaf tip.

Because dehydration can affect the mechanical properties of biological tissue [10], experiments were conducted to determine the rate of water loss from the grass specimens during the tensile test procedures. These results (cf. Fig. 2) indicated that after a 4 wt % loss during the first 15 min, the dehydration rate

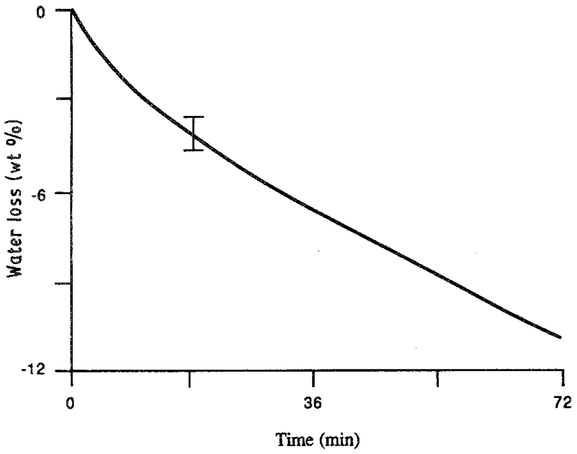

Figure 2 Evaporative water loss from ryegrass specimens under ambient conditions. Error bar indicates maximum standard deviation in a sample of 20 leaves.

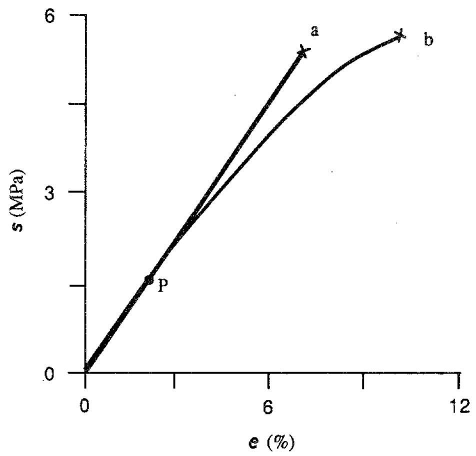

Figure 3 Engineering stress–strain curves showing representative (a) brittle and (b) semi-ductile behaviour during tensile testing. Point P identifies the proportional limit.

became constant with a maximum loss of ~10 wt % after 1 h. The tensile tests were completed within 4 min, and the maximal water loss (2 wt %) during this interval was not considered to affect mechanical behaviour significantly. On the other hand, these data show that the magnitude of water loss which occurs during longer term tests, e.g. creep and stress relaxation, might obscure the true nature of the material behaviour.

Stress–strain curves representing the two types of tensile behaviour we observed are presented in Fig. 3. Some of the specimens behaved in a predominantly brittle manner (curve a), while others evinced a semi-ductile mode (curve b). In the semi-ductile mode, a proportional limit (s_p and e_p) was defined as the point at which the curve departed from the initial straight line behaviour [11]. Otherwise, the two curves were treated similarly with values of stiffness (E) as well as stress and strain at fracture (s_f and e_f) calculated from standard engineering relationships [11]. Toughness (U_T) in each case was calculated as the energy per unit volume based upon the total area under the stress–strain curve [11].

As summarized in Table I, the tensile properties varied with distance from the region of actively dividing cells (meristematic zone) of the leaf. The principal meristematic zone is located at the base of the leaf [12]. The data (cf. Table I) were obtained from apical and basal sections from the group I plants. In general, the apical sections (distal to the meristematic zone) were stiffer, tougher and had greater fracture strengths than did the basal sections. Nearly all apical sections behaved in a semi-ductile manner with a well-defined proportional limit while approximately 85% of the basal sections showed brittle behaviour. In addition, the stress and strain at the proportional limit for the remaining basal sections were higher than for the corresponding apical section. Statistical analysis of the data showed significant differences in values of the stiffness, proportional limit and fracture strength as a function of specimen location. Despite the lower fracture strains in the apical sections, differences in the toughness values approached statistical significance as well.

Results describing the influence of engineering strain rate on the mechanical properties are presented in Table II. The data showed that as strain rate was increased, the stiffness, toughness and strength increased, while ductility decreased. Differences in the stiffness and toughness parameters were significant at the 0.05 level. Also, the fraction of specimens which showed brittle behaviour increased with increased strain rate. In order to document this correlation better, an additional set of experiments was conducted in which mechanical response was characterized as a function of strain rates ranging from 0.05 to 5.0 min^{-1}. These results showed that the behaviour was semi-ductile for strain rates below 0.5 min^{-1} and predominantly brittle for strain rates greater than 2.5 min^{-1}.

In order to confirm previously reported effects of water content upon grass leaf mechanical properties [8], a set of specimens was soaked in water until equilibrium and then tested. As indicated in Table III, soaking produced an increase in the water content but only by ~10%. When the results of the tensile tests on these specimens were compared with those from the

TABLE I Dependence of mechanical properties on specimen location

Property	Apical section	Basal section	Significance
E (MPa)	75.2 ± 22.1 (18)*	49.0 ± 17.2 (21)	$p < 0.01$
s_p (MPa)	1.5 ± 0.9 (16)	4.3 ± 0.6 (3)	$p < 0.05$
e_p (%)	4.0 ± 2.2 (16)	9.9 ± 2.7 (3)	$p < 0.05$
s_f (MPa)	5.5 ± 1.1 (20)	3.9 ± 0.6 (19)	$p < 0.01$
e_f (%)	9.2 ± 3.5 (19)	9.7 ± 4.3 (20)	ns†
U_T ($10^2\,\text{J}\,\text{cm}^{-3}$)	22.8 ± 9.6 (19)	19.7 ± 10.1 (20)	ns

*Number of measurements in parentheses.
† Not significant.

TABLE II Dependence of mechanical properties on strain rate

Property	0.1 min^{-1}	1.0 min^{-1}	Significance
E (MPa)	43.4 ± 18.6 (16)*	61.4 ± 16.6 (15)	$p < 0.05$
s_p (MPa)	2.9 ± 1.0 (15)	3.3 ± 0.5 (4)	ns†
e_p (%)	6.9 ± 4.7 (16)	4.9 ± 3.3 (4)	ns
s_f (MPa)	4.3 ± 1.0 (16)	5.0 ± 1.0 (14)	ns
e_f (%)	12.9 ± 8.5 (15)	9.5 ± 2.6 (15)	ns
U_T ($10^2\,\mathrm{J\,cm^{-3}}$)	26.8 ± 10.0 (15)	35.2 ± 2.9 (14)	$p < 0.05$

*Number of measurements in parentheses.
†Not significant.

in situ watered material (cf. Table IV), no significant differences in any mechanical parameters were observed.

A schematic diagram representing the cross-sectional structure of the leaf is shown in Fig. 4. The structure of the grass leaf is symmetric about the midvein and includes sclerenchymatous fibres, vascular bundles, and epidermal cells which comprise the "fibre" portion, along with a matrix consisting of thin-walled parenchyma cells found primarily in leaf mesophyll. The fibres, vascular bundles, and epidermal cells are the load-carrying elements and are clearly distinguishable at light microscopic magnification. The vascular bundles are comprised primarily of thick-walled, load-bearing cells. The epidermal cells, also load-bearing, have wavy edges and are tightly interlocked [12, 13], even though their walls are less thick than those of the fibres or vascular elements. The fibres are composed of elongate sclerenchyma located between the lower epidermis and the vascular bundle.

4. Discussion

Vincent has described the mechanical behaviour of grass leaves in terms of a three-component Voigt model in which the sclerenchymatous fibres account for 90 to 95% of the leaf stiffness [1]. The data from the tensile tests of the group I material indicated that the mechanical properties of grass leaves depended upon the leaf section (apical compared to basal) from which the specimens were taken. Although previous investigators have reported that apical regions were weaker, these conclusions were based upon breaking forces rather than stresses [4, 5]. Given the stiffer, stronger and tougher nature of the apical sections, we suspected that the proportion of load-carrying elements increases from the base to the apex. To test this hypothesis, serial transverse sections were examined and the volume fractions of the respective components determined. The results of this analysis are summarized in Table V. The data show that the volume fraction corresponding to the total of the epidermis and vascular bundle components increased with increasing distance from the leaf base. The volume fractions described in Table V differ significantly from those reported by Vincent [1]. This is probably due to variations in material parameters such as leaf age, the distribution among a smaller number of components or to the use of a different procedure for calculating fractional quantities. As grass leaves age, sclerenchyma fibres may increase somewhat, but their contribution to the overall load-bearing capabilities is limited by the small volume fraction (< 1%) of the fibres. Based upon the structural analysis of mature leaves (cf. Fig. 4 and Table V), we believe that the epidermal cells of the leaf act in a fibre-like manner and significantly participate in the load-bearing function. These cells typically, and in this case, are thick-walled, tightly bound together and lack intercellular spaces. In order to assess the quantitative contribution which each of these components makes to the overall mechanical characteristics of the leaf, the tensile properties of each component should be independently measured.

In general, increases in strain rate cause increases in the strength and decreases in the ductility parameters of polymeric materials [11]. The results reported in Table II conform to this pattern. In addition, the data show stiffness decreased at the slower strain rate. This behaviour, which results from the viscoelastic nature of biological materials such as grass [14], can be observed qualitatively in the tensile response of a Maxwell element as a function of strain rate [15].

When the relationship between mechanical properties and strain rate was examined (Table II), the data showed that a proportional limit was associated with lower strain rates. In order to determine the significance of this observation, additional tests were conducted over a wider range of strain rates, and the percentage of specimens showing this semi-ductile mode was calculated (cf. Table VI). A transition was found between the two modes for $0.1 > \dot{e} > 1.0\,\mathrm{min}^{-1}$.

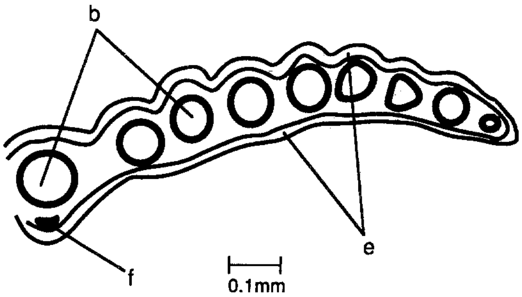

Figure 4 Schematic drawing showing representative cross-section of a 20 w old leaf. The leaf structure is composed of vascular bundles (b), fibres (f), epidermis (e) and matrix.

TABLE III Influence of treatment on water content

Protocol	Dry matter (wt %)
Soaked for 3 h at 4° C	1.0 ± 0.1
Normal watering *in situ*	10.1 ± 0.5

TABLE IV Dependence of mechanical properties on water content

Property	Soaked*	Normal†	Significance
E (MPa)	56.6 ± 20.7 (16)‡	61.4 ± 16.6 (15)	ns§
s_p (MPa)	2.6 ± 0.6 (7)	3.3 ± 0.5 (4)	ns
e_p (%)	5.0 ± 1.6 (7)	4.9 ± 3.3 (4)	ns
s_f (MPa)	4.6 ± 0.8 (16)	5.0 ± 1.0 (14)	ns
e_f (%)	10.0 ± 3.0 (16)	9.5 ± 2.6 (15)	ns
U_T (10^2 J cm^{-3})	31.7 ± 11.0 (15)	35.2 ± 9.8 (16)	ns

*1% dry matter.
†10% dry matter.
‡Number of measurements in parentheses.
§Not significant.

Because the toughness corresponds to the energy required to fracture a material, the area under the stress–strain curve provides a measure of the toughness in terms of the energy absorbed per unit volume [11]. As a rule, maximum toughness corresponds to an optimum combination of strength and ductility parameters. The higher values of stiffness and fracture strength in the samples tested at 1.0 min^{-1} compensated for the lower fracture strain values relative to the specimens tested at 0.1 min^{-1}. Greater toughness was observed in the specimens which did not have a well-defined proportional limit. Because more brittle tensile behaviour is generally associated with lower toughness values, the maximum toughness for this biomaterial may occur at strain rates between 0.1 and 1.0 min^{-1}.

Fracture toughness testing is generally complex, therefore efforts have been made to determine critical fracture toughness parameters from more easily obtained tensile measurements [11]. In their study of titanium alloys, Hahn and Rosenfield [16] related critical strain to the crack opening displacement at fracture and showed that the critical value of the stress intensity factor, i.e. the fracture toughness (K_C), could be estimated from tensile test quantities according to

$$K_C \approx (0.25 E s_Y l^* e_f)^{0.5} \qquad (1)$$

where s_Y is the tensile yield strength, l^* is the plastic zone width at the onset of cracking and e_f is the true fracture strain in uniaxial tension. They demonstrated that l^* can be approximated by n^2 where n is the material strain-hardening exponent. Using data from Table II and assuming a range for n from 0.1 to 0.5, substitution into Equation 1 gives a K_C from 0.31 to 1.57×10^5 Pa m$^{-3/2}$. These values are of the same order of magnitude as those reported by Vincent [1] and support his contention that the values for K_C and specific fracture energy of grass are quite low.

The mechanical properties of biological materials appear to be highly sensitive to water content [17, 18]. This results from the plasticizing effects of water and from fluid exchanges induced by the application of particular stress states. In general, the addition of a plasticizer to a polymeric material results in a decreased strength and stiffness [19]. Vincent [8] studied the influence of water on the mechanical behaviour of ryegrass and reported that values of unnotched breaking strength as well as longitudinal and transverse modulus decreased with increasing water content. He presented water content (% H_2O) in 50% increments relative to dry weight over a total range of 0 to 300%. Similar trends were found in our study, but no statistically significant patterns could be established (cf. Table IV). Discrepancies between these findings may relate to differences in the range of water content, specimen locations and/or tensile strain rates utilized.

Knowledge of biomechanical behaviour and the degree to which it is influenced by internal and external factors, has significance for several areas within basic and applied biological science because such understanding provides new insights into patterns of plant growth and development.

TABLE V Variation of structural characteristics with location

Distance from base (cm)	Component volume fraction (%)			
	Fibres	Bundles	Epidermis	Matrix
3.8	1	11	37	51
14.0	1	17	35	47

TABLE VI Tensile behaviour as a function of strain rate

Engineering strain rate (min^{-1})	% brittle mode (cf. Fig. 3)
0.05	0 (6)*
0.10	0 (16)
0.50	10 (11)
1.0	27 (15)
5.0	78 (9)

*Total number of specimens tested in parentheses.

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