Mechanical Adaptations of Cleavers (Galium aparine)

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INTRODUCTION

The mechanical design of terrestrial plants has received much attention (Haberlandt, 1914; Ennos, 1993; Speck, 1994; Speck and Rowe, 1999; Vincent, 1994); studies have investigated the design of upright species (Ennos et al., 1993; Niklas and Paolillo, 1998) and the mechanics of climbing woody species (Gartner, 1991; Rowe and Speck, 1998; Spatz et al., 1999; Isnard et al., 2003). It is recognized that in self-supporting plants the mechanical tissue is generally arranged around the periphery of the stem; for example, collenchyma, vascular tissue and sclerenchyma tend to be located away from the centre of the stem to ensure a high degree of flexural rigidity. In contrast non-self-supporting plants tend to have a centrally arranged mechanical tissue to facilitate twisting and climbing growth habits (Usherwood and Speck, 1999; Faerber et al., 2001). The mechanical design of plant roots has to a lesser degree been reported (Coutts, 1983; Ennos and Fitter, 1992; Ennos, 1994, 2000; Gartner, 1994; Goodman and Ennos, 1996; Niklas, 1999). In self-supporting plants a rigid element is required to prevent overturning, usually achieved by a lignified central stele in monocots and secondary thickening of dicot roots, whereas, in prostrate and climbing plants, a large number of fibrous roots with centrally arranged mechanical tissue is desirable in order to resist uprooting yet allow roots to remain flexible.

Not only is the gross arrangement of mechanical tissue within a stem or root important in determining its mechanical behaviour, but also differences in cell wall ultrastructure are known to have a significant impact. Polarized light microscopy and X-ray diffraction have been used to investigate the orientation of cellulose microfibrils in the cell walls and this can be used to explain differences in mechanical behaviour (Cave, 1966; Preston, 1974). Cellulose microfibrils are believed to bear the tensile stresses within the cell walls and are the principal tension support members in plants (Niklas and Paolillo, 1997). Orientation of the cell wall microfibrils has been used to help explain mechanical behaviour in self-supporting plants such as softwoods Pinus radiata, Picea abies (Cave and Walker, 1994; Färber et al., 2001), hardwoods Quercus robur, Fagus sylvatica (Lichtenegger et al., 1999), and more recently, in two neotropical woody climbers, Bauhinia guianensis and Condylomacarpus guianensis, which switch from a self-supporting growth phase to a non-self-supporting phase after contacting a support (Hoffmann et al., 2003).

However, no study has investigated the mechanical design of both the stems and roots of semi-self-supporting herbaceous annuals. Therefore, cleavers (Galium aparine), a fast-growing herbaceous annual with a scrambling-ascending growth habit, was chosen for this study. It is an important arable weed, commonly found in hedgerows, and mature plants often use upright species for support (Froud-williams, 1985; Taylor, 1999). The upper regions
of the stem have a characteristic square-shaped cross-section with long internodes separating whorls of elliptical to oblanceolate leaves (Puntieri and Pysek, 1998). Both the stems and leaves, which occur in whorls at the node, are covered in hooks; these are thought to aid attachment to their support (Fritsch and Salisbury, 1961) and allow the plant to climb without twining. However, plants are not rigidly fixed to their support and are often blown from supporting vegetation in strong winds (Heathcote, 1987). Hooks are also present on the mericarps which easily become entangled in animal fur thus aiding dispersal (Malik and Vanden Born, 1988; Gorb and Gorb, 2002).

The inspiration for this study resulted from observations of the contrasting form of the lower and upper stem of G. aparine. In particular, it was observed that the lower stem appeared to be highly extensible and relatively elastic — returning to its original length after stretching using the hands. This is rather unusual in terrestrial plants and it is only in aquatic species of the buttercup family (e.g. Ranunculus fluitans) and marine macro-algae (e.g. Nereocystis luetkeana), which have a central core of mechanical tissue, where similar mechanical behaviour has been investigated (Koehl and Wainwright, 1977; Usherwood et al., 1997).

Therefore this study aims to characterize the mechanical behaviour of the stem and roots of cleavers and relate this to the arrangement of structural tissue, the net microfibrillar orientations in the cell walls, and plant growth habit. To achieve this, a series of experiments was carried out on field-grown plants at the University of Lincoln, UK and the mechanical behaviour of the stem and roots was tested in tension and the orientation of the cell wall microfibrils investigated using polarized light microscopy.

**MATERIALS AND METHODS**

**Biological materials**

To characterize the morphology and mechanical properties of the stem, material was collected from plants growing at the University of Lincoln’s Riseholme campus, Riseholme, Lincoln, UK. Thirty mature plants were collected by carefully untangling the stem from surrounding plants to avoid loading the basal area of the stem; any plants which were damaged were discarded. All the plants were just starting to flower and were categorized as in the first generative-reproductive phase. This is when flowers are produced and fruits develop, but the main shoot continues to grow; the second is when terminal inflorescences are produced, elongation growth ceases, the fruit ripens and the mother plant dies (Groll and Mahn, 1986; Taylor, 1999).

Due to difficulties in excavating intact root systems from field soils (Fitter and Stickland, 1992), 32 plants were grown outside, in 5-L pots, to provide root material. Seeds were collected in the previous year from the same site. Three seeds were placed in the centre of each pot and were germinated in a sandy loam. Seedlings were thinned to one seedling, selecting plants of uniform size, once the seedlings had produced the first whorl of leaves after 10 d. Once the plants had developed two leaf-whorls, the pots were placed outside in four rows and a fence was erected using 20-mm square plastic mesh netting and garden canes spaced every 2 m to provide support for the growing shoots. Once the plants reached the first reproductive phase, root systems were taken to the laboratory for analysis.

**Morphology**

Shoot systems from mature plants growing around the university’s campus were cut just below the soil surface and taken to the laboratory for morphological and mechanical analysis. The basal region of the stem was placed between damp sponges to prevent water loss and the length and basal diameter of the stem was measured using a ruler and dial callipers, respectively.

Root material was collected from the pot plants and the soil was carefully washed away from the root systems by submerging them in buckets of water and using a hose to gently remove the soil. The number and basal diameter of the major first-order lateral roots, defined as roots with a diameter >0.5 mm, 40 mm from the insertion at the taproot, were measured.

**Anatomical observations**

Transverse basal sections, of the stem and first-order lateral roots, were cut using a single-sided razor blade and examined under the light microscope. Fresh sections were stained with toluidine blue and phloroglucinol to determine the distribution of lignified tissues. These were photographed and tracings were made to depict the location of mechanical support tissue. Sections were differentiated into the parenchyma, vascular tissue, sclerenchyma bundles and the pith.

**Orientation of wall microfibrils**

To investigate the orientation of cellulose microfibrils in the cell wall, longitudinal sections were taken of the stem, approx. 50 mm from the stem base, and first-order lateral roots, approx. 50 mm from where they joined the tap root. Samples, 5 mm long, were taken from five plants and were fixed using a mixture of ethanol (70 %), glacial acetic acid and formalin (18 : 1 : 1). Fixed specimens were then dehydrated, embedded in paraffin wax and sectioned (in a plane tangential to the epidermis) with a sledge microtome at 15-μm increments.

To determine the net orientation of the cellulose wall microfibrils the longitudinal sections were dewaxed, mounted in DPX and examined under polarized light. Single cell walls were viewed in polarized light with crossed polarization filters and a quartz-sensitive tint plate (red plate) (Paolillo, 1995; Niklas and Paolillo, 1997). The sample was rotated on the stage of the microscope to the position of maximal extinction and the angle between the longitudinal axis of the cell and the direction of the polarizer was measured using a graduated rotating stage with a vernier scale, allowing measurement to the nearest 0.1°. The red plate developed blue–yellow shifts in colour and a rose
Mechanical tests

Mechanical tests were carried out on basal samples (120 mm) of the stem and first-order lateral roots and the diameter was measured, at the midpoint, using callipers. The basal region of the first-order lateral roots was defined as the region closest to the insertion at the tap root. Stem and root samples were kept moist, before testing, by placing them between wet sponges. Samples were tested within 1 h of harvesting using a Universal Testing Machine (model 4443; Instron, High Wycombe, UK). Basal sections of the stem and root were clamped in the jaws of the Instron and stretched at a rate of 40 mm min\(^{-1}\); the distance between the clamps was 80 mm. A sandwich of neoprene rubber and sandpaper was used to prevent slipping.

To calculate the mechanical properties, 30 stems and 30 roots were tested to failure. During the mechanical tests an interfaced computer plotted a graph of force (N) vs. extension (m) and the following properties were calculated:

The breaking stress \(\sigma_{\text{max}}\) (strength) is given by the formula:

\[
\sigma_{\text{max}} = 4F_{\text{max}}/\pi D^2
\]

where \(D\) is the diameter of the sample and \(F_{\text{max}}\) is the maximum load the sample could withstand before breaking.

The breaking strain \(\epsilon_{\text{max}}\) (extensibility) is given by:

\[
\epsilon_{\text{max}} = \delta_{\text{max}}/L
\]

where \(\delta_{\text{max}}\) is the extension at which the sample broke and \(L\) is the original length. Values of \(\delta_{\text{max}}\) and \(F_{\text{max}}\) were only considered if the sample failed away from the clamps.

The Young’s modulus (E) or stiffness of the stem and root material was calculated by taking the initial computer-fitted slope \(dF/d\delta\) from the force vs. extension graph and using the following formula:

\[
E = 4LdF/\pi D^3d\delta
\]

Statistical analysis

A Kolmogorov–Smirnov Test (Sokal and Rohlf, 1995) was used to test the normality and similarity of the shapes of underlyng distributions before proceeding with the analysis. Analysis of variance was used to test for significant differences in the net orientation of the cell wall microfibrils between the cell types. All values in the text are means ± the standard deviation.

RESULTS

Morphology

The stems of \(G.\) aparine ranged from 1·05 to 2·57 m in length with a basal diameter of 0·88 ± 0·30 mm. They were anchored by a fibrous root system with between 10 and 26 major first-order laterals with a basal diameter of 0·87 ± 0·11 mm. It was noted, but not quantified, that the stem base was flexible allowing bending back on itself without showing visible signs of fracture.

Anatomical observations

Examination of transverse sections under the light microscope showed that in the lower stem (base+100 mm) there was a central core of structural tissue with only a small central lumen and narrow cortex (Fig. 1A). The vascular tissue was largely composed of secondary xylem with vessels, fibres and sclerified parenchyma; staining with phloroglucinol showed that the cell walls were lignified. The vessel members were arranged in an irregular manner (Fig. 1A). In contrast, further up the stem (typically >150 mm from the soil surface), the transverse section showed a simple four-angled arrangement with four distinct non-lignified sclerenchyma bundles arranged in the corners of the stem (Fig. 1B). The vascular tissue was in a typical cylinder arrangement with pith composed of parenchyma cells.

In the first-order lateral roots, structural material was arranged centrally; staining with phloroglucinol showed that this central core of vascular tissue was lignified (Fig. 1C).

Mechanical properties

Stem. The relationship between the stress and the strain had two distinct phases; first, an initial phase, where the structure behaved in a hookean (linearly elastic) manner up to approx. 5 % strain followed by yielding to 7 % and then a second linear phase and eventual failure. Results showed that the basal region of the stem was highly extensible, strong, but also compliant; breaking strains of approx. 24 % were recorded (Table 1).

Roots. Similar results were obtained for the roots – again two distinct phases could be identified from the stress : strain plot. First, an initial linear phase up until approx. 6 % strain then the root yielded to 7 %, followed by a second linear phase up until failure at approx. 28 % strain. The results indicate that the basal region of the first-order lateral root was highly extensible, strong but also compliant (Table 2).
between the orientation of the cell wall microfibrils in the different cell types of the stem (Table 3; \( P < 0.05; n = 5 \)); microfibrils in the walls of fibres and narrow vessel members diverged from the longitudinal by approx. 13 % more than those from the wide vessel members.

However, in the root, there were no significant differences in the net orientation of the wall microfibrils between the narrow and wide vessel members (\( P > 0.05; \) Table 3); microfibrils diverged from the longitudinal by approx. 6°.

**DISCUSSION**

In some ways, the mechanical behaviour and structure of the lower stem of mature cleavers was similar to that of climbing terrestrial species, which do not have to resist overturning forces generated by the wind. The mechanical tissue was arranged centrally in the stem. Indeed, this centralized arrangement of mechanical tissue has also been associated with aquatic species, such as the long-leaved water crowfoot (\( R. fluitans \)) and giant bull kelp (\( N. luetkeana \)), which typically experience tensile forces generated by water flow (Koehl and Wainwright, 1977; Usherwood et al., 1997). This contrasts with self-supporting plants where the structural tissue is arranged more distally to improve the rigidity of stems.

The mechanical properties of the tissues were similar to those reported by authors studying young climbing plants; for example, Köhler et al. (2000) reported that the central core of young Aristolochia macrophylla stems had a Young’s modulus of 222 MPa similar to that of the lower stem of \( G. aparine \) reported in this study (Table 1), whereas the whole axis of \( A. macrophylla \) was almost three times stiffer (Köhler et al., 2000). However, the results do show that the mechanical behaviour of cleavers differs from other terrestrial species in a fundamental way; the lower stem is highly extensible, experiencing strains of up to 24 % before failing. It is unusual for the stems of terrestrial plants to withstand such high strains; for example, Köhler et al. (2000) reported breaking strains of only 7 % in the stems of young non-self-supporting lianas (\( A. macrophylla \)). It is
only in aquatic species, that similar breaking strains have been reported; a study investigating the mechanics of a freshwater species, *R. fluitans*, reported breaking strains of approx. 15 % (Usherwood et al., 1997). However, even higher breaking strains of up to 38 % have been measured in the stipes of marine macroalgae such as the giant bull kelp (*N. luetkeana*) (Koehl and Wainwright, 1977). The functional significance of these findings may be that the stems of *G. aparine* are better able to cope with sudden tugs, in a similar way to the stems of aquatic plants cope with changes in flow rate in their aquatic environment. Indeed, *G. aparine* is commonly found attached to self-supporting species including other herbs and crop plants (Malik and Vanden Born, 1988) and so may have evolved in such away as to minimize the effects of a support which sways in the wind. *Galium aparine* is known to be dispersed by animals; the hooked mericarps are frequently found attached to the fur of animals. It is possible that an extensible stem may also help prevent the plant from being uprooted by passing mammals.

The mechanism by which the basal region of the cleaver stem permits the very high breaking strains is unclear; there is no evidence to suggest that a higher microfibrillar angle in the vessels or fibres is responsible. Orientations of microfibrils in the stems were approx. 8°. This is within the typical range of values reported for self-supporting plant species (Cave and Walker, 1994; Niklas and Paolillo, 1997) and also the neotropical woody climber, *Bauhinia guianensis* (Hoffmann et al., 2003). In contrast, microfibril angles in *Condylocarpon guianense*, another neotropical liana, were found to be much higher, ranging between 27 and 31° (Hoffmann et al., 2003) and in other climbing plants such as *Aristolochia* species approx. 20° (Köhler et al., 2000). However, these values are all substantially lower than the high microfibril angle (60°) found in the cortical cells of the marine macroalga *N. luetkeana* which is thought to permit the very high breaking strains of 38 % (Koehl and Wainwright, 1977).

Surprisingly, the anatomy of the stem does not shed light on the mechanism behind the high breaking strains; one might expect collenchyma tissue, which is often found in young developing plants, and is known to be responsible for flexible support tissues (Esau, 1977), to be predominant in the stems of mature *G. aparine* stems. However, the stem of cleavers showed no obvious distribution of collenchyma tissue; the cross-section of the basal stem part was dominated by lignified vascular tissue (Fig. 1A) and the cortex consisted of six or seven layers of parenchyma cells. A possible explanation for the high breaking strains may be that lower levels of lignification are responsible; however, further experiments would be required to determine the chemical composition of the cell walls.

The high breaking strains recorded for the roots was less surprising; breaking strains of approx. 28% have been reported for the roots of wheat (Easson et al., 1995), but only 7 % for the roots of sunflower seedlings (Ennos, 1989) and approx. 10 % for the roots of tomatoes (Gartner, 1994).

There are several ways to test the mechanism by which the stems of *G. aparine* withstand such high breaking strains and its importance to the survival of the plant. One is to compare the orientation of microfibrils in both extended and relaxed stems; it should be possible using trigonometry to calculate the theoretical gain in length resulting from the steepening of the spiral angle during loading. A second test would be to investigate whether subjecting the stem to high strains affects its water conductivity and to determine at what point this becomes detrimental to the growth of the plant. If the flow rate was significantly reduced and the plant suffered a higher mortality then it may be that the plant never experiences such high strains during growth and that this is merely a secondary effect of another character. However, if this is a mechanism to help withstand high extensions one would expect the plant to be able to withstand high strains without any detrimental effects on growth and reproductive success.

It is important that future work determines the mechanism by which these high breaking strains occur and also characterizes how the mechanical properties vary along the stem. Clearly more work needs to be done on a range of climbing and prostrate annual growth forms to determine if stems with high breaking strains are more widespread among other herbaceous semi-self-supporting terrestrial species.

In conclusion, this study has shown that, in cleavers, the lower stem is highly extensible; breaking strains of approx. 24 % were recorded. No other terrestrial plants have been shown to have such extensible stems. This may be related to the ‘cable-like’ arrangement of the structural material in the lower stem, which was dominated by a central core of vascular tissue. The highly extensible basal stem may have evolved as a mechanism to help prevent the plant from being uprooted.

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**LITERATURE CITED**


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