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SHORT COMMUNICATION

Lignified and nonlignified fiber cables in the lacunae of *Typha angustifolia*

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Abstract The leaves of *Typha* are noteworthy in terms of their mechanical properties. We determined the mechanical properties of the fiber cables within the leaf. We found that in vegetative plants, the lignified fiber cables isolated from the leaf sheath and nonlignified fiber cables isolated from the leaf blade of *Typha angustifolia* differ in their diameter, swelling capacity, Young's modulus, tensile strength, and break load. These differing properties are related to their contributions to stability in the two regions of the leaf.

Keywords Biomechanics · Cattails · Fiber cables · Lignin · *Typha*Handling Editor: Alexander Schulz

Introduction

The tall leaf blades of cattails are remarkable in their ability to remain upright and support their own weight (Schulgasser and Witztum 2004) as well as withstand strong winds (Teale 1949; Rowlatt and Morshead 1992). We have found that the nonlignified fiber cables in the air-filled lacunae of leaf blades that are strong under tension probably serve to protect the flexible leaf blades from buckling during high winds (Witztum and Wayne 2014, 2015). In order to further explore the biomechanical features of mechanically resistant leaves composed of a minimum amount of material, we investigated the physical parameters of the cables in the leaf sheath and the leaf blade of vegetative plants. Here, we report that in the leaves of *Typha angustifolia*, these nonlignified fiber cables in the air-filled lacunae of the leaf blade are continuous with lignified fiber cables in the lacunae in the leaf sheath below the leaf blade. We also report that the nonlignified fiber cables in the leaf blade above the leaf sheath attachment are stiffer (higher Young's modulus) than the thicker lignified fiber cables in the leaf sheath.

Materials and methods

Vegetative plants of *Typha angustifolia* L. were collected from various localities in Ithaca, NY, USA. Fiber cables and free hand transverse and longitudinal sections of leaves of vegetative plants were stained with phloroglucinol-HCl (McLean and Ivimey Cook 1941) and observed on a stereomicroscope using darkfield and incident illumination. Except where noted, sections were obtained from the leaf blade 20–40 cm above the highest attachment point of the sheath or from the leaf sheath 20–40 cm below highest attachment point of the sheath. Young's moduli, tensile strengths, and the break loads of the fiber cables were measured with an Instron 3342 with a 100-N load cell using the Bluehill 2 software. We isolated fiber cables by pulling them from the leaves 20–40 cm from the point of highest attachment of the sheath with the help of a dissecting needle. To perform the Instron analysis, freshly removed cables were mounted lengthwise on cardboard frames 5 cm×

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2 cm over an elongated window 3-cm long with super glue (Original Super Glue Gel; Pacer Technology, Rancho Cucamonga, CA, USA) and covered with a small additional piece of cardboard. The top and bottom of the window frame were attached to the clamps, and the two long sides of the frame were cut to allow pulling on the 3-cm fiber cable segment at a rate of 20 mm/min until the cable broke. Curves of stress versus strain until the cable broke were generated. We also performed Instron analysis without the cardboard frames and super glue. The two methods gave identical results, indicating that the super glue had no effect on the results. The diameters of the fiber cables were measured prior to the test with a compound microscope (Olympus BH-2; SPLAN40; AmScope MU300 camera and ToupView (3.7) image capturing software) using NIH Image J (http://imagej.nih.gov/ij/), and the cables were tested for the presence or absence of lignin with phloroglucinol-HCl. All data are given as the mean± standard deviation. *T* tests were performed with Excel.

The cut ends of leaves were immersed in 1 % (w/v) neutral red for hours and days in order to determine the longitudinal continuity or discontinuity of the xylem in the lignified and nonlignified fiber cables in the leaf sheath and leaf blade. The presence or absence of neutral red was observed in transverse sections. The presence or absence of tracheary elements in the fiber cables was determined with polarized light microscopy.

Results

The fiber cables in the air-filled lacunae of the leaf blade are not lignified (Fig. 1a, c), and those in the lacunae of the leaf sheath are lignified (Fig. 1b, d). The fiber cables in the air-filled lacunae of the leaf blade are continuous with the fiber cables embedded in the spongy parenchyma cells in the lacunae of the leaf sheath. However, not all of the fiber cables in the lacunae of the leaf sheath extend into the leaf blade. Thus, there are fewer fiber cables in the air-filled lacunae of the leaf blade (Fig. 1c) than embedded in the spongy parenchyma cells in the lacunae of the leaf sheath (Fig. 1d). The outer layer of all the fiber cables is composed of a layer of cells that contain prismatic calcium oxalate crystals (Witztum and Wayne 2014). These prismatic crystals differ from the needle-like raphides found in the root (Seago and Marsh 1989).

Some of the lignified fiber cables embedded in the spongy parenchyma cells in the lacunae of the leaf sheath contain one or two files of tracheary elements (data not shown). Dye uptake experiments show that neutral red is transported through the lignified bundles that traverse the air-filled lacunae in the leaf sheath that contain tracheary elements but not through the nonlignified fiber bundles that traverse the air-filled lacunae in the leaf blade (data not shown). The fiber cables that are capable of transporting neutral red do not extend into the leaf blade.

We tested Niklas' (1992) hypothesis that lignin acts as a waterproofing agent around the cellulose microfibrils. We found that the nonlignified cables swell 47±19 (n=5) percent while the lignified fiber cables only swell 6±6 (n=6) percent upon wetting. The difference in the swelling factor between the nonlignified fiber cables in the leaf blade and the lignified fiber cables in the leaf sheath is significantly different (p<0.01).

The nonlignified fiber cables isolated from the air-filled lacunae of the leaf blade differ significantly from the lignified fiber cables isolated from the lacunae of the leaf sheath in terms of their geometrical and mechanical properties (Table 1). The nonlignified fiber cables are significantly thinner, and the break load is significantly less than the diameter and break load of the lignified fiber cables. On the other hand, the Young's modulus and the tensile strength are significantly greater in the thin nonlignified fiber cables in the air-filled lacunae of the leaf blade than in the thicker lignified fiber cables embedded in the spongy parenchyma cells in the lacunae of the leaf sheath.

The stress-strain curve of nonlignified fiber cables before the cable breaks is linear (Fig. 1e). By contrast, the stress-strain curve of the lignified fiber cable before the cable breaks is nonlinear (Fig. 1f), indicating that the mechanical properties of the nonlignified fiber cables in the distal portion of the leaf differ from the mechanical properties of the fiber cables in the proximal part of the leaf.

Discussion

The nonlignified fiber cables in the air-filled lacunae of the leaf blade that are strong in tension and contribute to a tensegrity structure (Witztum and Wayne 2014) are continuous with lignified fiber cables embedded in spongy parenchyma in the lacunae of the leaf sheath. There is no apparent discontinuity of the fibers and tracheids within the fiber cables in the leaf sheath itself—any discontinuity is only at the point of leaf sheath transitioning into blade. Since the leaf of *Typha* probably grows from an intercalary meristem (Kaul 1974), the nonlignified fiber cables in the air-filled lacunae of the leaf blade are probably older than the lignified fiber cables embedded in the spongy parenchyma cells in the lacunae of the leaf sheath. Since older

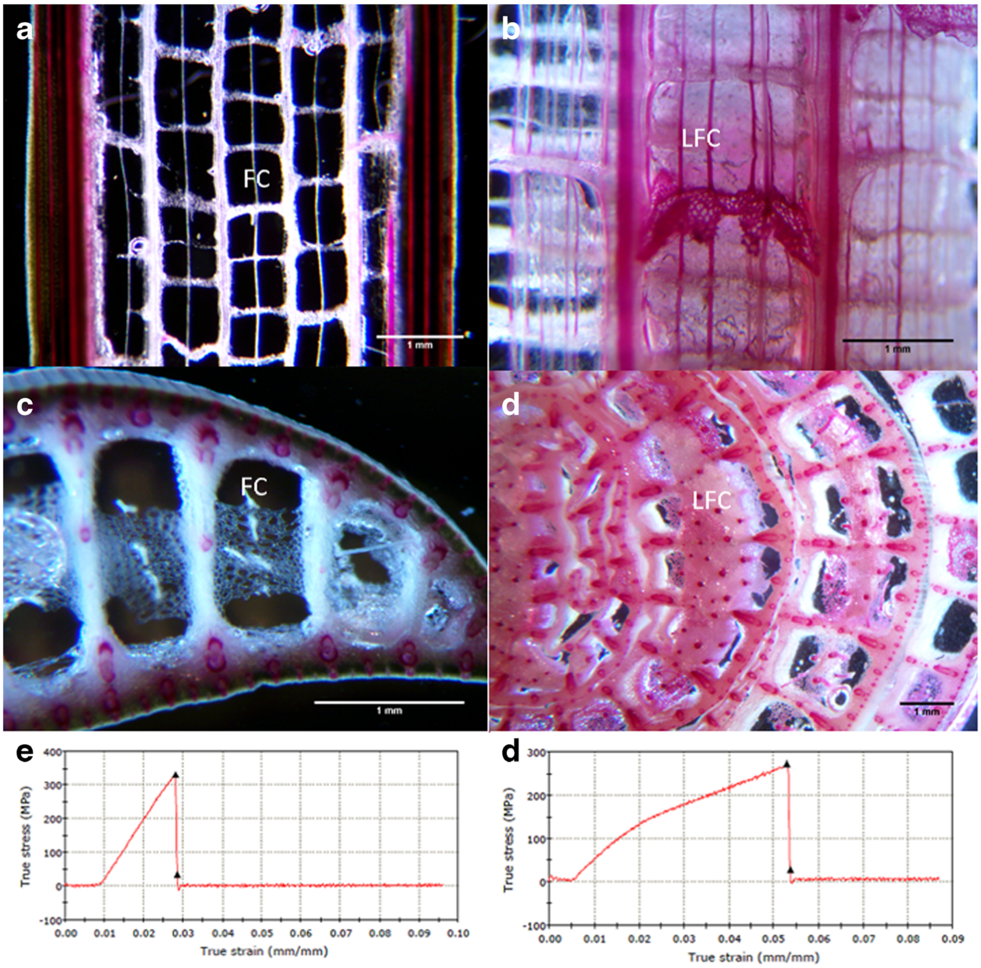

Fig. 1 Fiber cables in the leaf of *Typha angustifolia*. **a** Longitudinal section of leaf blade treated with phloroglucinol-HCl that gives a red reaction with lignin. **b** Longitudinal section of a leaf sheath treated with phloroglucinol-HCl that gives a red reaction with lignin. **c** Transverse section of leaf blade treated with phloroglucinol-HCl that gives a red reaction with lignin. **d** Transverse section of leaf sheaths treated with phloroglucinol-HCl that gives a red reaction with lignin. **e** Stress-strain relation of a nonlignified fiber cable (FC) isolated from the leaf blade. **f** Stress-strain relation of a lignified fiber cable (LFC) isolated from the leaf sheath

tissues are typically more lignified than younger tissues, this reversal may point to a difference in function of the two ends of the fiber cable. The older and more distal fiber cables that are nonlignified have a greater Young's modulus and tensile strength than younger and more proximal lignified fiber cables. This gives the nonlignified fiber cables the mechanical properties useful for withstanding the back and forth motion caused by wind.

The lignified fiber cables swell less than the nonlignified fiber cables upon being submerged in water, indicating that lignin may waterproof the cellulose microfibrils. Lignin also restricts the swelling of wood pulp (Eriksson et al. 1991). It may be significant that the lignified fiber cables are in the base of the leaf that is often submerged. Lignin however is also strong in compression (Célino et al. 2014) and may form a composite structure with the cellulose of the fiber cells and the water-filled spongy parenchyma that may distribute the compressive force produced by the weight of the more distal parts of the leaf.

The shape of the stress-strain curves of the fiber cables isolated from the two regions of the leaf may be indicative of the fact that the mechanical properties of

Table 1 The mechanical properties of nonlignified and lignified fiber cables isolated from the leaf blade and leaf sheath, respectively

Region of leaf	Diameter µm	Young's modulus MPa	Tensile strength MPa	Breaking load N
Blade (*n*=19) nonlignified	18.55±3.34	19547±7356	490.29±186.54	0.1287±0.0327
Sheath (*n*=31) lignified	55.52±18.47	13537±4304	303.18±87.86	0.7540±0.4102
Significance	$p<0.001$	$p=0.005$	$p<0.001$	$p<0.001$

the nonlignified fiber cables depend on cellulose, and the mechanical properties of the lignified fiber cables depend on both lignin and cellulose. The nonlinear shape of the curves generated using lignified fiber cables may be a result of creep (Niklas 1992).

The fiber cables isolated from the leaf sheath contain lignin, while the fiber cables in the leaf blade do not. The tensile strength and Young's modulus of cellulose are greater than that of lignin (Gibson 2012). In general, lignified fibers isolated from coconut and bamboo have a smaller Young's modulus and tensile strength than nonlignified fibers isolated from flax, ramie, hemp, and cotton (Célino et al., 2014). Consequently, the differing shapes of the stress-strain curves of fiber cables isolated from the leaf blade and leaf sheath are a reflection of the composition of the fiber cables.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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