Value-adding application of micro-CT to highly rigid plant tissues: *Phragmites australis* (Cav.) Trin. Ex Steud. knot sections

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As part of a study on stem rigidity of Common Reed (*Phragmites australis* Cav. Trin ex Steud.), grown in natural habitats and in constructed wetlands, authors tried to prepare anatomical sections of node regions of dried material used for the assessment of mechanical parameters. Sections should especially show location and number of vascular bundles, usually associated with mechanical properties of plant tissues, and are known for their complex anatomy in nodular regions. Nodes, but also internodes of *Phragmites australis* were extremely hard and brittle in our material which made serial cross sections of acceptable quality a futile undertaking, despite us trying different standard methods. Therefore, we switched to a method for visualizing the inner nodal structures with a method not applied to reed so far. Applying Microcomputer Tomograph (Micro XCT Type Skyscan) techniques revealed detailed information on location and association of vascular bundles, the septum and the process of leaf bundle development. We report here about the different conventional cutting techniques which failed to produce high quality cross sections and show first results of the micro-CT technique.


Keywords: *Phragmites australis*, knot sections, transverse sections, micro-tomography.

Introduction

Mechanical robustness studies on Common Reed (*Phragmites australis* Cav.) Trin. ex Steud.) were performed intensively in the context of reed die-off related to eutrophication of lakes (Binz-Reist 1989, OstenDorp 1995, 1999). An extensive study on mechanical properties of reed was carried out by Hosner (1990), who compared the strength of plants from artificial wetlands, and from natural locations. As part of an ongoing study the same set of mechanical parameters was assessed for reed sampled from two artificial wetlands receiving street run-off, and from natural localities. Mechanical strength parameters, especially flexural rigidity and breaking strength, were determined for reed internodes located in the centre of the full stem length. One aspect never studied before is the detailed histological differentiation of reed stems in the close vicinity of knots, the spatial continuity of vascular bundles, and the quantitative determination of reinforcing structures in mechanically weaker and stronger stems.
As there is only a limited amount of information available in the relevant literature (Esau et al. 2009, Bresinsky et al. 2008, Nultsch 2001), authors tried to shed light on those little known anatomical features.

**Methods**

Since comparability of mechanical strength measurements affords to work with fully dried material when following the same approach as all relevant studies (Hosner 1990, Ostendorp 1995, 1999), the histological cuts had to be carried out with dried stems also. The high amount of ligneous material in the vascular bundles, and the original high mechanical strength of reed stems especially in knot sections, were a specific aspect to be recognised prior to the start of this study. Techniques applied were:

Sliding carriage microtome (OM-E, Reichert):

40–60 µm slices were prepared, stained with Phloroglucin/HCl and Safranin-Astrablue, and studied with a transmission light microscope (Olympus CX 4, Olympus Austria GmbH). It was impossible to prepare complete full-diameter slices, neither in the internode section nor in the knot as such. Perfect cuts were obtained only for rather small sectors. As the distribution of the vascular tissue is not totally even across the whole stem, quantitative relationships of number and outline of bundles and of non-vascular tissue could not be calculated with the accuracy needed to describe the whole stem cross section.

Various preparations prior to cutting had been carried out: dry stems, and stems immersed for several days in water, in 45% ethanol and in Strasburger’s Mixture (ethanol, water, glycerol 1:1:1), and cutting under steam. None of these procedures resulted in better results than cutting the dry stems.

A reflected-light microscope was used to study stem cross sections, which were either untreated or embedded in Agar Low Viscosity Resin (Agar Scientific, Great Britain), the latter either untreated or polished with very fine sanding-cloth (1200). None of these methods were satisfactory as the primary cutting of the stem as well as the sanding resulted in smudging the cellular details, especially where small cellular lumina occur in the vascular tissue.

Resin-embedded stems were also cut with the sliding microtome (steel knife) and rotary microtome (glass knife), but both methods revealed unsatisfying results.

Cutting trials were finalised with etching resin-embedded material with ethanol/NaOH-mixture and consecutive cutting of the protruding vascular tissues. Results were disappointing, too, despite the strong mechanical fixation of the plant structures in the resin.

Since cutting methods failed in producing satisfactory results dry and wet knot sections of reed were scanned with a dental x-ray instrument (Galileos, Sirona, Bensheim, Germany). Unfortunately the contrast between different tissues was too low for interpretation.

Regarding computer tomography, high spatial resolution of animal tissue was practised since many years (Schäfers 2003) and this triggered our interest in this methodology.

As a courtesy we started cooperation with the Department of Theoretical Biology (Head of the Department: Gerhard Müller), where we tried a new Micro Computer Tomograph (MicroXCT Type Skyscan 1174, Skyscan, Belgium). The RTW 50/800 x-ray source fea-
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tures an anode voltage of 50 kV and an x-ray current intensity of 800 µA. The probe is rotated in steps of 0.3°. Pixel size is 15.003 µm, which relates to the horizontal and vertical distance of cross sections. 981 cross sections were recorded per reed stem sample. X-rays were metered by a fixed-mount szintillator cristal (XRadia) and then recorded with a VDS 1.3 MpFW camera (pixel size 18.58 µm, integrated in Skyscan 1174). The very detailed reconstruction of the stem sections was carried out with the respective Xradia software (Version 1.5), installed on a Dell Precision 490 Computer.

Results

All the findings reported here refer only to the stem section used for our mechanical robustness studies, which comprise the central node and adjacent internode regions, and cannot necessarily be transferred to other stem sections.

The longitudinal cut CT-image of the node section shows a convoluted transverse solid septum, protruding upwards, which is reinforced by supporting elements (Fig. 1). These elements are built of vascular bundles, which merge with the septum from above and from below. Below the convoluted septum a small swelling of the vertical inner side of the hollow stem is found.

Directly above the septum a second perpendicular swelling of the tubular stem is found, which consists of numerous bundles merging in a horizontal direction, which is known as the ‘transversal ring of vascular bundles’ (Fig. 2), typical for grasses. About 0.5 mm above this structure another thickening of the stem tube is detected, which also may support stem rigidity.

In the CT-images of the cross sections the vascular bundles are distributed over the intermodal region of the stem in a rather even pattern, but closer to the knot the arrangement of the bundles changes into a horse-shoe like shape, while the number of bundles stays the same over the whole knot section. Even where bundles are connected with leaf sheaths or other structures the bundles just branch and no new bundles are added to the stem. Peripheral bundles, as well as inner bundles, show fixed location, except for small lateral dislocations, but bundles never cross over.

In the CT images the highly sclerenchymatous parenchyma is seen near the outer part of the stem, but in the knots it is distributed over the whole cross section.
The horse-shoe like structures are located c. 2 to 3 mm under the tip of the convoluted septum. They consist of 8 bundles, 4 on each side, which are clearly detected in the CT, and smaller bundles in the bend, which cannot be singled out. In this location emerges the leaf sheath, and new vascular strands are built thereon.

Immediately up from the development region of the leaf, these structures are vanishing, and the bundles spread out in an even pattern over the whole cross section.

**Discussion**

Micro-CT imagery is a highly practicable method to study plant parts in a quick and non-destructive way, when conventional cutting methods fail. Depending on the size of the object data are compiled within a few hours and create longitudinal and horizontal cuts, as well as 3-D images, simultaneously. Preparing the same number of cuts with conventional microtome techniques takes much longer, destroys the object, and can result in dislocation of tissues. A most important feature is the possibility of studying plant parts which are mechanically too rigid to be penetrated by regular microtome techniques, as was the case in this study. Quantification, and colouring, of tissues of different density is also possible with appropriate software, which will be tried in future experiments.

Despite these advantages conventional microtome techniques do not become totally obsolete, as slices for light transmission microscopy display some more detail, like individual cells in low density, soft parenchyma, and e.g. phloem tissue can be distinguished from small xylema cells by staining. Yet, CT methodology opens up a new and non-destructive way of studying plant structures. In the case of reed our study offered a first glance on the internal composition of vascular tissue in the highly rigid stem nodes.

**Acknowledgements**

Authors acknowledge the kind support by Gerhard MÜLLER, Head of the Department of Theoretical Biology, University of Vienna, who offered instrument time at the microCT. Waltraud KLEPAL assisted with glass knife cuttings, and Daniela GRÜBER with EM preparation techniques. Christopher BRANDL helped with field collections of plant material and technical support. Norbert EXLER established contact with Nicola TESCHLER at the Natural History Museum, Vienna, who scanned our probes with the dental x-ray system. Irene LICHTSCHEIDL supported us with excellent inverted light microscope facilities.

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**Manuscript received:** 2010 04 13

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