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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.

Wert-steigernde Anwendung von micro CT auf hoch formbeständige Pflanzengewebe: *Phragmites australis* (Cav.) Trin. Ex Steud. Knotenabschnitte.
Im Zuge einer Festigkeitsstudie an Schilf (*Phragmites australis* Cav. Trin ex Steud.) von natürlichen Standorten und Pflanzenkläranlagen wurde versucht, anatomische Schnitte vom Untersuchungsmaterial, den getrockneten Stängeln, im nodalen und internodalen Bereich herzustellen. Ziel der Untersuchung war die Aufklärung der komplexen Anordnung der Gefäßbündel in den Nodien und im nahe gelegenen Internodialbereich, wo sich die mechanischen Eigenschaften der Stängel konzentrieren. Beide Stängelbereiche waren extrem hart und brüchig, wodurch Serienschnitte entsprechender Qualität mit konventionellen Methoden nicht hergestellt werden konnten. Es wurde daher eine neue Methode eingesetzt, die auf Schilf-Stängel bisher noch nicht eingesetzt worden war. Eine Microcomputer Tomographie (Micro XCT Type Skyscan) Methode war in der Lage, detailierte Information über Lage und Verbindung der Gefäßbündel, das Septum und die Entwicklung der Blattspurstränge zu geben. Wir berichten kurz über die konventionellen, aber nicht erfolgreichen Schneidetechniken und zeigen erste Resultate der Micro-Computer Tomographie.

Keywords: *Pragmites 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.

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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.

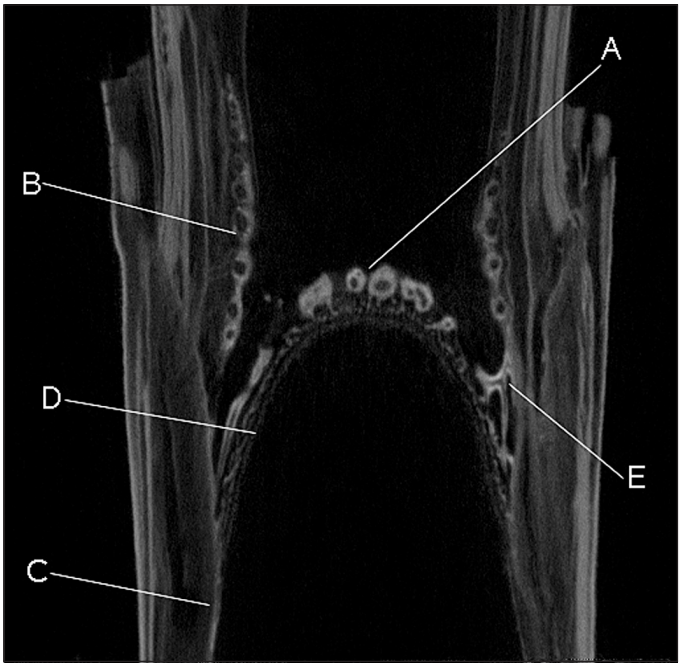

Fig. 1: Radial longitudinal section of common reed, node and adjacent stem region. A: vascular bundles on top of the septum. B: circular bundles. C: swelling of the inner side of the stem.D: supporting elements. E: zone of vertical bundles merging with the upper side of the septum. – Abb. 1: Radialer Längsschnitt an Schilf, Knoten und anschließender Stängel-Bereich. A: Gefäßbündel am höchsten Punkt des nodalen Septums. B: zirkuläre Bündel. C: Verdickung an der Stängel-Innenseite. E: Zone vertikaler Bündel im Bereich der Vereinigung mit der Septum-Oberseite.

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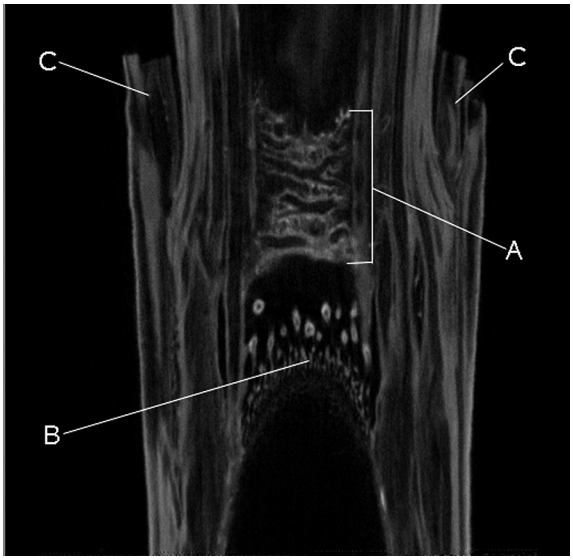

Fig. 2: Tangential longitudinal section of common reed, node and adjacent stem section. A: circular bundles. B: section of bundles located on the upper side of the septum. C: Protrusion of the leaf sheath. This section shows that quantification of the different types and locations of the vascular strands is easily possible with this technique. – Abb. 2: Tangentialer Längsschnitt an Schilf, Knoten und anschließender Stängel-Bereich. A: Zirkuläre Bündel. B: Abbild von Bündeln, die an der Oberseite des Septums aufliegen. C: Austrittsstelle der Blattscheide. Dieser Schnitt zeigt, dass die Quantifizierung unterschiedlicher Typen und Positionen von Gefäßbündeln mit dieser Beobachtungstechnik leicht vorgenommen werden können.

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.

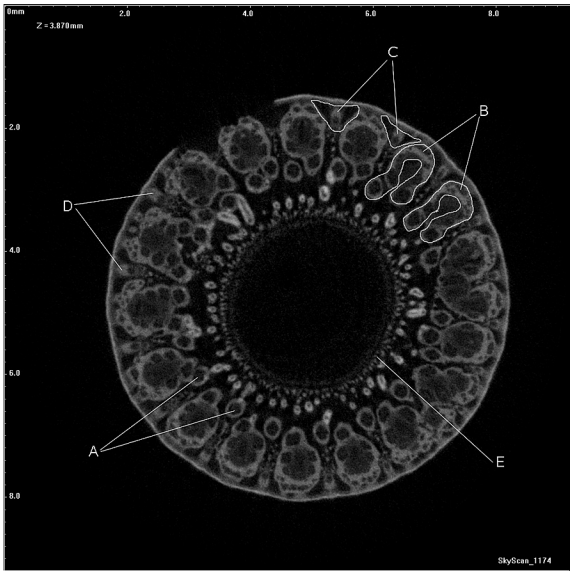

Fig. 3: Transversal section of a reed stem c. 2 mm below the tip of the convoluted septum. A: regular mono-cotyledon bundles. B: horse-shoe like structures consisting of 8 bundles, 4 on each side. C: bundles leading towards the leaf sheath. D: small secondary bundles. E: inside limit of septum. – Abb. 3: Querschnitt eines Schilf-Stängels ca. 2 mm unterhalb der Spitze des aufgewölbten Septums. A: reguläre, monokotyle Gefäßbündel. B: Hufeisen-förmige, aus 8 Bündeln aufgebaute Strukturen, jeweils 4 an jeder Seite. C: Bündel, die in Richtung Blattscheide verlaufen. D: kleine, sekundäre Bündel. E: Innere Grenze des Septums.

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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.

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