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X-ray μCT imaging technique reveals corm microstructures of an arctic-boreal cotton-sedge, *Eriophorum vaginatum*

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ABSTRACT

X-ray computed tomography (CT), a non-destructive imaging technique, has recently been effectively applied to botanical research. In this study an X-ray μCT technique was developed to allow for anatomical study of the overwintering corms of *Eriophorum vaginatum*, an ecologically important sedge species in arctic tussock-tundra and boreal peatlands. Using a GE Medical MS8X-130 X-ray μCT scanner, optimal imaging parameters included scanning isolated corms at 80 kVp and 100 μA with a 3500 ms exposure time and an isotropic voxel size of 10 μm. A Gaussian blur image filter with a blur radius (σ) of two pixels was applied to the optimal dataset to improve visual detection and contrast of tissues while removing 99.2% of image noise. Using the developed X-ray μCT technique several undocumented anatomical characteristics of the corm were identified including the vascular connection between a parent corm and branching cormel and the 3D shape of sclereid clusters. The 3D structure of sclereid clusters was determined whereby the perimeter of their lance shape is greatly reinforced by sclereids with thicker secondary cell walls as compared to those of the interior of the cluster. The structure of sclereid clusters and their association with leaf traces suggests they may be stabilizing the corm-leaf connection to protect vascular tissues from physical damage. The proposed X-ray μCT technique is an excellent tool for determination of the 3D structure of *E. vaginatum* corms and may be used to detect alterations in tissue structure and chemistry in response to environmental change in this and other Cyperaceous species.

1. Introduction

X-ray computed tomography (CT) is a non-destructive imaging technique that utilizes the variability in the density and chemical composition of samples to form images (Hounsfield, 1973). Originally designed as a medical diagnostic tool, X-ray CT scanners form images by exposing a sample to a polychromatic X-ray beam. X-rays are differentially absorbed by the sample which produces a 2-dimensional (2D) "view", with many views produced at many angles to the sample and being combined mathematically into a 3D image. This 3D image represents the radiodensity of the sample and can provide a structural map of its internal components. X-ray CT has proved useful in quantifying the internal structure of samples from a variety of disciplines including biology (Stock et al., 2003), histology (Cnudde et al., 2008), palaeontology (Sutton, 2008), geology (Ketcham and Carlson, 2001), thermochronology (Evans et al., 2008), hydrology (Wildenschild et al., 2002), soil science (Elliot and Heck, 2007a, 2007b; Jassogne et al., 2007; Torrance et al., 2008), and materials science (Wang, 2007; Tondi et al., 2009).

The use of X-ray computed tomography (CT) for botanical research is of interest due to the potential advantages of this imaging method over classical means of sectioning and microscopy. Sample processing prior to image acquisition, such as dehydration, embedding, or sectioning is not required meaning digital sections can be obtained in less time and without structural alterations, such as tissue compression or tearing, that are prevalent in physically sectioned samples (Steppe et al., 2004). As X-ray CT imaging is non-destructive, repeated scanning or additional sampling techniques can be applied to the same sample (Dutilleul et al., 2005). Imagery from X-ray CT scans can also be viewed and re-viewed at virtually any sectioned slice angle in 2D or 3D. Alternatively objects of interest can be segmented from the images as digital surfaces, or isosurfaces, making it easier to analyse complex structures or samples difficult to section using classical techniques (Evans et al., 2008). Resolutions near the micron level can be achieved for cm-sized samples using X-ray μCT (Cnudde et al., 2008; Evans et al., 2008) while <1 μm resolution can be achieved for mm-sized samples using X-ray sub-micronCT (Van den Bulcke et al., 2009a). Recent botanical applications of X-ray CT techniques have included obtaining taxonomically significant anatomy of pyritized fossil fruit (DeVore et al., 2006), determination of wood anatomy (Steppe et al., 2004) and wood water content (Fromm et al., 2001),

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assessing fungal decay of wood (Van den Bulcke et al., 2009b), quantification of canopy growth of tree seedlings (Dutilleul et al., 2005, 2008), characterization of plant roots in soil (Pierret et al., 2005; Lontoc-Roy et al., 2006; Hamza et al., 2007), and quantification of the spatial distribution of leaf trichomes on *Arabidopsis thaliana* (Kaminuma et al., 2008).

Eriophorum vaginatum, a "cotton-sedge", is ecologically significant to boreal peatlands and arctic tussock-tundra where it is often the dominant, herbaceous vascular species (Polozova, 1970; Wein, 1973; Chapin et al., 1979, 1993; Lavoie et al., 2003), being forage for muskoxen, caribou, deer, and sheep (Wein, 1973; Duffy et al., 2001). *E. vaginatum* readily colonizes disturbed sites and thrives in open areas with nutrient poor substrates comprised of peat or wet, fine-grained soils, often overlaying permafrost (Polozova, 1970; Chapin et al., 1979; Lavoie et al., 2003). The elevated tussock growth form of *E. vaginatum* creates its own microenvironment (Chapin et al., 1979), which is thought to provide varied microsites for the establishment of other successive species (Polozova, 1970; Lavoie et al., 2003). *E. vaginatum* can survive tundra fires (Wein, 1973; Racine et al., 1987), extensive, repeated, simulated herbivory (Archer and Tieszen, 1983), and periodic drought (Wein, 1973). *E. vaginatum* can grow in soils with a wide range in pH, from ~3 to 8 (Wein, 1973; Stevens, 2006) and has been grown successfully on peat amended kimberlite, a waste product from diamond mining (Stevens, 2006). Moreover, this species can endure anthropogenic stressors such as ^{137}Cs fallout from the Chernobyl accident (Jones et al., 1998), crude oil spills on tundra (Racine, 1994), and in peatlands that have been aerially polluted by Pb, Fe, and Cu–Ni smelting (Markert and Thornton, 1990; Huopalainen et al., 2000). Hence, *E. vaginatum* is of interest on an ecological basis, as a potential species for revegetation of vacuum-mined peatlands, and for pollutant biostabilization in industrially damaged wetlands.

Evidently *E. vaginatum* existence and persistence in adverse environments depends on the vitality of its overwintering corms (Chapin et al., 1979; Archer and Tieszen, 1983; Racine et al., 1987; Racine, 1994). To elucidate the survival mechanisms of corms, a detailed understanding of corm anatomy and tissue relationships is required. We present a method to non-destructively image the corm tissues of *E. vaginatum* using X-ray μCT. The specific objectives were (i) to develop an X-ray μCT technique to non-destructively image live, hydrated corms of *E. vaginatum* and (ii) to apply this technique to reveal tissue architecture of the corm. This study investigates tissue structure relationships within the corm of *E. vaginatum* with implications on species survival.

2. Materials and methods

2.1. Plant material

Tussocks of *E. vaginatum* were collected during the summer of 2007 from a wetland near Cartier, Ontario, Canada (46°39′42″N, 81°31′14″W). The site was an open, black-spruce dominated peatland with *Picea mariana* (Mill.) B.S.P., *Ledum* sp., *Chamaedaphne calyculata* (L.) Moench., *E. vaginatum*, *E. virginicum* L., *Kalmia angustifolia* L., *K. polifolia* Wangenh., *Vaccinium oxycoccus* L., *Sphagnum* spp., and *Carex oligosperma* Michx. being the predominant species present. *E. vaginatum* tussocks were approximately 30 cm in diameter and 50 cm tall (from peat surface to top of leaves). The tussocks were complex networks of tillers comprised of tightly branching corms, adventitious roots, leaves, and vertical rhizomes.

2.2. Light microscopy

To identify corm tissues and their structural relationships using conventional light microscopy, living corms from collected tussocks were hand-sectioned with razor blades and observed with an Olympus CX-41 light microscope equipped with an Olympus Q-Colour 3 digital camera (Olympus America Inc., USA) for image capture, with images being adjusted for brightness and contrast as required using Adobe Photoshop CS3 Extended v10.0.1 (Adobe Systems Incorporated, USA).

2.3. X-ray μCT scanning

Sample preparation was not required for X-ray μCT scanning other than to size them appropriately to fit into the scanning tubes. For low resolution scans, a tussock was representatively sub-sampled and leaves were trimmed to 0.5 cm above corms. Any peat and loose decaying material was removed using tap water to simplify image interpretation after scanning. For high resolution scans of corms, individual tillers were removed from the tussock and living corms were isolated from all other material to maximize the number of corms scanned, however, corm branching architecture was preserved. Samples were stabilized within the scanning tubes where required by using radiolucent foam pieces placed outside of the scanned region.

Plant material was scanned using a GE Medical (formerly Enhanced Vision Systems, London, Ontario, Canada) MS8X-130 X-ray μCT system which uses a tungsten target, microfocus X-ray tube as the X-ray source. The detector in this X-ray μCT scanner has been rotated 90° to allow samples of wider dimensions to be scanned (Elliot, personal communication). Corms were scanned at 80 kVp, 100 μA using two high-pass filters (Cu) to reduce beam hardening effects on images, which appear in X-ray CT images as a brightened edge near the exterior of the scanned region and a darkened centre (Ketcham and Carlson, 2001). Beam filtration using a high-pass filter reduces beam hardening artefacts by pre-hardening the X-ray beam, which reduces the number of low energy photons interacting with the object (Kalender, 2005). Three isotropic voxel resolutions of 50, 20, and 10 μm (Table 1) were used to determine their effect on corm tissue differentiation and image noise. Attempts to maximize soft tissue contrast at lower kVp and at 6 μm voxel resolution, near the limit of this system, resulted in images of poorer quality than the optimal dataset of 10 μm voxel resolution, even after image filtering (data not shown). Each scan combined 720 views of the plant material over 360° sample rotation and was reconstructed into signed, 16-bit image volumes using GE Medical proprietary software. X-ray absorbance of image volumes was calibrated to Hounsfield Units (HU) using air and water standards contained in microcentrifuge-tubes and scanned with the corm samples. Representative corm volumes incorporating several corm internodes, i.e. between 132 and 382 axial image slices, were cropped from the original image volumes using the ROI tool within GE-HC SliceView v.1.1. (http://sourceforge.net/project/showfiles.php?group_id=69063). For comparison with hand-sections of live plant material, image orientation is referred to such that the axial *Z* image plane is synonymous with cross-sections of corms while the *X* and *Y* orthogonal planes (coronal and sagittal) are synonymous with longitudinal sections, as the corm can be considered radially symmetrical.

Table 1
Parameters for X-ray μCT scans of *E. vaginatum* corms at 80 kVp, 100 μA.

Voxel resolution (μm^3)	Sample tube diameter (mm)	Exposure time (ms)	Distance from source (mm)	Detector binning
50	64	1700	300	2 × 2
20	28	1700	120	2 × 2
10	12	3500	120	1 × 1