Media Cybernetics Applications Note
A Tool for the Analysis of Annual Root Rings in Perennial Forbs

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Introduction

Annual rings in the roots of perennial forbs are an integrated record of a plant's life that is of great value to plant ecologists, population biologists and eco-physiologists. Discernible annual rings are characterized by both wide earlywood vessels (water transporting structures of plant roots or shoots) formed in spring and narrow latewood vessels formed in late summer and autumn.

Until now, analysis of root rings involved manual measurements performed along three separate radii of root cross-sections. Mean values of these measurements were used to describe the width of the respective rings. If radial growth was asymmetric around the root, this approach could lead to both large errors in the estimation of ring width and inconsistency of measurement between different observers.

To improve and extend analysis of annual rings the Visual Basic scripting language in Image-Pro® Plus was used to develop ROXAS © (*RO*ot *X*ylem *A*nalysis *S*ystem), a customized image analysis tool which performs automated analysis of root cross-sections[2]. The Image-Pro Plus scripting language enables us to create processing and measurement commands and parameters specific to root cross-section analysis such as mean ring width, ring area, number and size of vessels in each ring, vessel density, and the percentage of area occupied by vessels.

Methods

Image-Pro Plus Version 4.5.1.29 was used to automatically analyze root cross-sections photographed with a standard digital camera (Nikon CoolPix 990, 3.34 Megapixels) through the receptacle tube of a dissecting microscope (Leica MZ 8; Fig. 1). Image Pro's scripting language allowed full control over all the image processing and analysis commands to produce the parameters described in Figure 2, below.

In the flow of automatic analysis within ROXAS, the images are corrected for uneven illumination. Next, the contrast of vessels is enhanced using several image and edge enhancement procedures. Segmentation is performed and vessels are extracted from the resulting image using several morphometric parameters such as roundness, perimeter ratio, area/box, aspect, fractal dimension and caliper-diameter ratio (Fig. 3, 4).
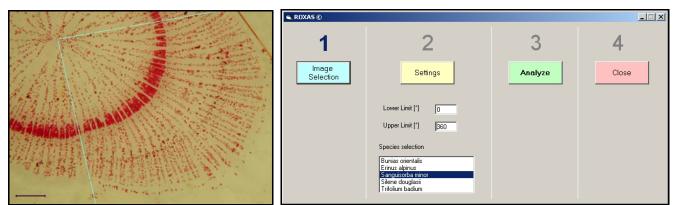

Fig. 1. (Left) Root cross-section of *Bunias orientalis* ($\varnothing$ = 30μm, bar = 1000μm). The lignified vessel walls are stained with Phloroglucinol-HCl. The analyzed section is indicated by the light blue lines. Fig. 2. (Right) In the main window of ROXAS © (*Ro*ot *X*ylem *A*nalysis *S*ystem) the plant species and the analyzed sector of the cross-section are specified. With selection of the plant species, a species-specific calibration set is loaded for analysis.

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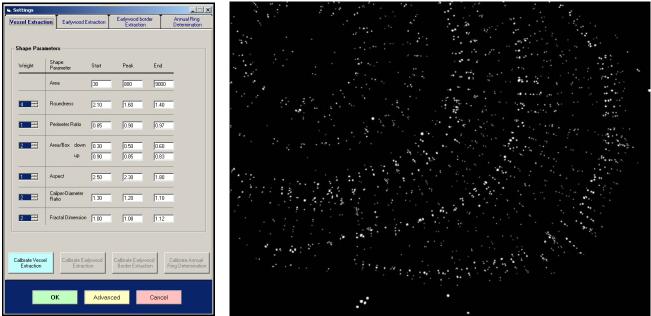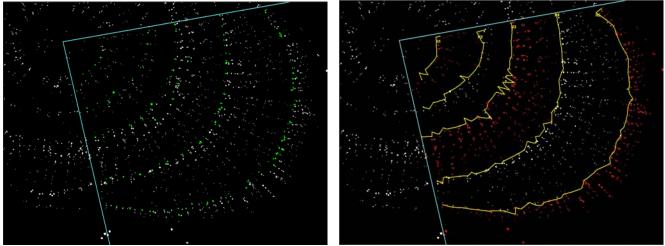
Fig. 3. (Left) In the "Vessel Extraction" tab, one of four calibration tabs in the "Settings"-window, several morphometric shape parameters that are used to distinguish vessels from other lignified cells or image noise can be changed or calibrated to new species. Fig. 4. (Right) Result of vessel extraction by ROXAS. Thresholded vessels are indicated by a white mask on a dark background.

The local neighborhood of each vessel is analyzed in an anisotropic way to search for vessels lying at the annual ring borders (Fig. 5). In the next step, ring identification algorithms connect the obtained vessels and form continuous ring borders. Following ring assembling, each vessel is attributed to the corresponding annual ring (Fig. 6).

Figures 5. (Left). Pattern recognition algorithms screen the local neighborhood of each vessel and extract the first (i.e. innermost) earlywood vessels (green objects) of each annual ring within the analyzed sector indicated by the two radial lines. Fig. 6. (Right). Vessels of consecutive rings alternately colored white and red to improve visual contrast. The yellow line indicates the ring border as recognized by ROXAS.
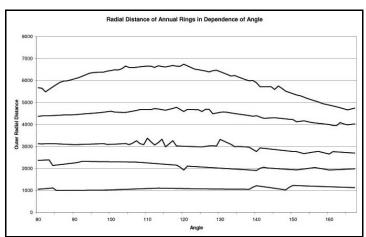
Fig. 7. The trajectory of each ring border is one core data output of automatic image analysis and is saved together with other calculated data into an MS Excel spreadsheet.

From the core data (represented by the size and position of each vessel and/or trajectories of annual rings), all the other anatomical parameters as described above are automatically deduced and saved into a MS Excel spreadsheet, (Fig. 7). Since the Image-Pro Plus scripting language is compatible with MS Visual Basic, the ROXAS application can easily export analysis data to MS Excel.

With this automated procedure, not only the age and the individual ring width of perennial forbs can be determined, but also many anatomical features related to annual root rings and vessels. With this additional data, new approaches to plant ecology are possible that were previously only accessible with laborious manual analysis.

For More Information
More information on this tool or possible future availability may be found on the herb-chronology web pages[3].

References
[2]International Journal of Plant Sciences: 166: 723-732
[3]http://www.geobot.ethz.ch/spotlights/herbchronology/method/analysis.

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