Visualization of Xylary Rings of Stems of *Artemisia tridentata* spp. *Wyomingensis*.

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Abstract Plants of the many subspecies of *Artemisia tridentata* are dominant shrubs of the Great Basin Desert of the United States. Many subspecies of *Artemisia tridentata* show extensive eccentric growth in which vascular cambium dies and no longer produces secondary xylem in stems. The purpose of this study was to create three-dimensional images of xylary rings from stem segments so that characteristics of individual xylary rings among successive segments could be accurately represented. Four stem segments from a branch were sized and aligned in MATLAB. Three xylary rings were given a unique color for visualization. All portions of images were removed so only the xylary rings were visible. Rings of the four segments were aligned to make a three-dimensional visualization. The images were analyzed to determine the locations of complete rings, locations of partial rings, percentages of arcs of rings of individual rings, and calculations of ring areas. Eccentric growth is localized. For example, on one stem segment all three rings were complete while the next segment 20 mm along the stem had two incomplete rings. The visualization and resulting data generated provide information about eccentric growth which, in turn, reflects the overall health and mechanical stability of stems.

Keywords Volume Visualization, Image Registration, *Artemisia tridentata*, Xylary Rings

1. Introduction

Creating a three-dimensional visualization of the rings in wood using images of slices taken at multiple resolutions and orientations requires both image registration and biologically specific methods for visualization. In this work, we present a procedure to cut, align, scale and view individual xylary rings from wood samples which combines mathematical methods for registration used in other fields such as medical imaging [1] and computer vision [2] with an application specific view of the contours of the rings in three dimensions.

The Central Basin and Range Ecoregion covers 343,000

km² throughout most of Nevada and a portion of Utah [3, 4]. Plants of *Artemisia tridentata* occupy most of the non-saline portions of the Great Basin Desert [5, 6, 7, 8]. The many subspecies of *Artemisia tridentata*, that occupy distinct areas, are a major contributor to community structure.

Stems normally produce circular annual rings of secondary xylem cells [9,10]. Stems of *Artemisia* species exhibit eccentric growth [11, 12, 13, 14]. Eccentric growth is characterized by a lack of complete annual or xylary rings. Incomplete xylary rings are caused by the death of the vascular cambium in specific areas but not all areas within a stem or root [11, 12, 13, 14]. Plants of subspecies of *Artemisia tridentata* produce eccentric xylem rings naturally. Previous results demonstrated that stem eccentricity began during the end of year one or the beginning of year two for young stems of *Artemisia tridentata* [14]. Incomplete xylem rings occur in all stems after the second year of growth [11, 12, 13]. This eccentric growth can lead to decreased growth, structural weakened stems and sometimes death of stem terminals.

To our knowledge, there has been no quantitative evaluation of the depths of annual rings. Previous research [11, 12, 13, 14] showed that eccentric growth occurs but no detailed evaluation of individual rings has been done. The purpose of this study is to use computer programs to create an accurate pictorial representation of three xylary rings in several stem segments in order to visualize rings among segments. Thus, images of three xylary rings were individually isolated and visualized from all other materials in the images. A three-dimensional visualization of the three xylary rings for the four segments was constructed to show a combined view of ring eccentricities along a stem.

2. Methods

2.1. Collection and Preparation of Stem Segments

A stem of *Artemisia tridentata* spp. *Wyomingensis* from a plant at Fremont Canyon (40.0° N, 112.1° W), UT, U.S.A. was selected for analysis. The stem selected was typical for

plants of the region. The stem was shipped to Manhattan College, Bronx, NY, U.S.A. A portion of the outer bark was removed so that a vertical line could then be drawn along the stem with a permanent marker. This line was used to insure that the orientation of stem segments after segments were sawed for eventual visual reconstruction. Stem segments (cross or transverse sections) were cut with a Dewalt DWHT20541 flush cut pull saw (www.dewalt.com, Towson, MD, U.S.A.). Average segment thicknesses were 7.8 mm. The thickness of each segment was determined with a digital caliper (Fisher model #14-648-17, Fisher Scientific Inc. Pittsburgh, PA, U.S.A.). Stem segment images were

obtained with a Leica EZ4 microscope (www.leica-microsystems.com, Wetzlar, Germany) with a Canon PowerShot ELPH100HS camera (www.canon.com, Ōta, Tokyo, Japan).

Images of four stem branch segments designated segments #36, #38, #40 and #42 were selected for ring analysis. Segment #36 had the smallest diameter (youngest stem segment) while segment #42 had the largest diameter (oldest) among the segments analyzed (Table 1). The first step was to trace xylary rings and draw ten sectors (each 36°) starting with 0° at the black arrow using Microsoft (www.microsoft.com) Paint Program (Figure. 1).

Table 1. Characteristics of the four stem segments of *Artemisia tridentata* ssp. *Wyomingensis*.

Stem segment number	Stem area (mm ²)	Largest number of rings in segment	Smallest number of rings in segment
36	202	20	6
38	141 ¹	20	5
40	199	22	1
42	274	24	1

¹A large portion of stem segment #38 was eccentric so the area was much smaller than other segments.

Figure 1. Image of segment #46 with the xylary rings and angles labeled from a stem of *Artemisia tridentata* ssp. *Wyomingensis*. Note the black mark used to indicate zero degree angle. The first step was to find the biological center. The next step was to trace the xylary rings. The inner most ring was produced first. Twenty-seven xylary rings are present in the segment. The arrow indicates the 0° angle that was used to assist in the orientation process to align all the images of the study. Other lines to help orientation were drawn every 36°.

2.2. Dimensional Ring Visualization

Xylary rings 3, 7 and 10 within each segment were selected for visualization. To illustrate a 2-dimensional visualization of the rings from the original images each ring was filled with a specific color with Microsoft Paint. Rings 3, 7 and 10 were free-form selected and filled with green, blue and red colors, respectively (Figure 2B, 3B).

Figure 2. Images of segment #36 of *Artemisia tridentata* spp. *Wyomingensis*. A) Image with rings and transects marked. The zero degree transect is at the black arrow. B) Image in which ring 3 is colored green, ring 7 is blue and ring 10 is red.

2.3. Scaling and Alignment of Stem Segments for 3-Dimensional Visualization

Images were processed using MATLAB

(www.mathworks.com, Natick, MA, U.S.A.) including the image processing toolbox. The first step was to convert the green, blue and red colors to gray color so that the rings could be placed into a single matrix with the entries based upon gray color intensity. Next, all images were scaled to the highest resolution among the images (154 pixels per millimeter).

Figure 3. Images of segment #42 of *Artemisia tridentata* spp. *Wyomingensis*. A) Image with rings and transects marked. The zero degree transect is at the black arrow. B) Image in which ring 3 is colored green, ring 7 is blue and ring 10 is red.

The next step was to rotate all scaled images horizontally to align the zero degree angle points as identified by the black arrow (Fig. 4). Then, images were cropped to align all biological centers of all segments on a 4000 X 4000 matrix.

In summary, images were scaled to have the same resolution and aligned so that both the zero degree angle lines and the biological centers of all images were identical. This series of steps was the image registration part of the process which was required before the 3D visualization could be done. This type of registration uses an affine transformation [1].

Figure 4. Rotation of image of segment #46 of *Artemisia tridentata* spp. *Wyomingensis*. Images were rotated to have the zero degree angle in the horizontal axis so all images could be aligned.

Figure 5. Image of rings 3, 7 and 10 only for segment #38 of *Artemisia tridentata* spp. *Wyomingensis*.

3. Dimensional Ring Visualization Procedure

To create the 3-dimensional visualization with the scaled and rotated images, the three selected rings for each segment were filled with black pixels. Next, colors of each image were inverted making the rings white. Thereafter, image properties were set to black and white making the rest of the image black. Finally, the color was inverted again so that only the rings were visible as black (Figure 5). Through this procedure we isolated the xylary rings of interest. The four images were then loaded into MATLAB again and were

processed using the scaling and alignment described previously. A three-dimensional matrix of the combined images was created with each image as a different slice in the vertical axis. The three-dimensional matrix was visualized using contours of the regions in each of the slices (Figure 6). Isolating the contours of the rings allowed us to view the 3D volume without obstruction across planes. These contour images were saved and imported into Microsoft Paint so that rings 3, 7 and 10 could be colored green, blue, and red, respectively (Figure 7). This final visualization provided the representation most useful for interpretation in a biological context.

Figure 6. Image of the contours of rings 3, 7, and 10 for four segments from a stem of *Artemisia tridentata* spp. *Wyomingensis*. The values on the x and y axes indicate pixel ranges. The values on the z axis indicate segments #36 (upper), #38, #40, and #42 (lower), respectively. The three dimensional image was rotated to best show the rings.

Figure 7. Three-dimensional color visualization of four stem segments of *Artemisia tridentata* spp. *Wyomingensis*. Ring 3 is green, ring 7 is blue and ring 10 is red. The biological center is a black dot inside ring 3. The black arrow indicates the zero degrees transect. Note this view can be used to show relationships among rings for the four segments and the arc coverage of each ring. The three-dimensional image was rotated to best show the rings.

3. Results

The composite three-dimensional view shows entire rings and eccentric rings for rings 3, 7 and 10 (Fig.7). As stated previously, segment #42 was the oldest segment. For segment #42, ring 3 (green) was complete, but rings 7 (blue) and 10 (red) were incomplete (eccentric). Ring 7 had about 342 degrees of arc, while ring 10 was about 216 degrees of an arc. For segment #40, ring 3 was complete and rings 7 and 10 were eccentric (incomplete). For segment #40, the gaps for rings 7 and 10 were remarkably different from those in segment #42 in terms of direction of ring discontinuities. For segment #38, all rings were eccentric and incomplete at several regions. Segment #36, the youngest segment, showed the most incomplete rings. Specifically, rings 3, 7 and 10 had about 180, 144, and 97 degrees of an arc, respectively. These images document the eccentric nature of stem segments of *Artemisia tridentata* using a 3-dimensional visualization.

4. Discussion

The purpose of this study was to describe the methods to accurately create a 3-dimensional pictorial representation of xylary rings of several stem segments of an eccentric stem of *Artemisia tridentata* spp. *Wyomingensis*. The technique used to align and scale the images before the 3-dimensional visualization are used in other imaging situations where objects are imaged at different resolutions and with different orientations and where there are markers in the images that guide the alignment (the zero degree line and the biological center). The class of techniques used for this paper area type affine transformation of images which are used in a variety of fields such as medical imaging [1] and computer vision [2] to co-register images. This type of registration both translates and scales images.

One potential application of this study would be to create a predictive model of xylary ring growth. Data of the (1) locations of complete rings, (2) locations of partial rings, (3) percentages of individual arcs, and (4) percentages of individual arcs of rings, and (4) calculations of ring areas. These parameters provide information about the overall health and mechanical stability of the stems. Since arc lines were visible, arc locations and percentages were determined (Figs. 5B and 6B). For segment #36, ring areas of rings 3, 7 and 10 were 4.2 mm², 21.0 mm², and 50.2 mm², respectively, while for segment #42, ring areas were 8.0 mm², 23.3 mm², and 20.4 mm², respectively. Ring 10 for segment #36 was more than twice the area of ring 10 for segment #42, while the area of ring 3 for segment #42 was more than twice the area for segment #36. Therefore, ring locations, ring arcs, and ring areas were quite different for the two segments. These techniques can be used to fully explore changes in xylary ring growth. Simple changes in areas of rings can be calculated but these areas do not reflect differences in arcs of rings.

This study produced an accurate pictorial representation of three xylary rings in four stem segments that were separated by approximately 19.7 mm each. Since the images could be correctly aligned and the images of xylary rings could be separated and visualized, therefore differences in characteristics of successive rings at various arc locations could be compared accurately. Percentages of arcs and the alignment characteristics among successive segments clearly show the nature of eccentric growth of *Artemisia tridentata* over short distances. Eccentric growth is very localized since it solely depends upon whether the vascular cambium remained alive and produced secondary xylem or died over short distances (less than 50 µm) [14]. Current and past results [14] show that the presence/absence of cambium function at one location had little or no effect on cambial presence/absence at nearby locations. Since the death of the vascular cambium is permanent, the woodiness of *Artemisia* is very irregular and in turn may lead to reduced growth of stems [11, 12, 13, 14]. Moreover, since extensive eccentric growth of stems of *Artemisia* occurs in main stems at nodes with flowering branches at stem terminals each year [14] eccentric growth may limit the heights of individual main stems [15].

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