REGENERATION OF SCOTS PINE STEM AFTER WOUNDING

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ABSTRACT

Stem regeneration after wounding was studied in 110-year-old trees of Pinus sylvestris L. over a period of 30 years. The changes of cambial surface are shown as 3D models. For construction of the models ArcGIS and geodesic Surfer programs were applied. The trees responded to stem injury by increasing the cambial activity near the wound edge. The result was longitudinal rolls or spindles which gradually covered the wounded stem surface. The successively formed tree rings changed their orientation to perpendicular with respect to the wound surface. The disturbances of wood formation near the wound edge were manifested by oblique orientation of xylem rays with respect to annual ring boundaries. The spatial distribution of the xylem ray orientation is presented on the Surfer contour maps. Near the fusion of the wound spindles there were some areas consisting of irregularly oriented xylem cells. The cellular ordering of the xylem tissue in these areas was measured by applying digital image analysis software. Measurements shown on color-coded maps revealed that the tracheid orientation (seen on tangential sections) deviated between 0 and 90 degrees from the stem axis. In some areas a circular pattern of tracheid orientation was visible. Crooked and forked tracheids were also present. These results support the view that the adaptive growth occurring in the case of deep wounding is analogous to that observed when an inanimate body is in lateral contact with a tree stem. The intensive growth and accumulation of newly deposited tissue in the wound spindles seems to be the most effective mechanism for the tree stem regeneration to restore its biomechanical and transport functions. This could be considered as an illustration of Wolff’s law that the shape of an organ follows its function.

Keywords: 3D modeling, ArcGIS, cambium, Pinus sylvestris, stem regeneration, tree rings, wound, xylem rays.

INTRODUCTION

The processes connected with wound healing and regeneration of tissues and organs of living organisms play a key role in maintaining and restoring organ integrity. Tree stem injuries can be caused by various biotic and abiotic factors. The problem has often been studied and summarised in several reviews (e.g., Shigo 1984; Larson 1994; Liese &
The course of regeneration processes depends on the nature of the wound. In the case of a wound, when the cambium is directly injured, a barrier zone is formed to protect internal tissues (Moore 1978; Mulhern et al. 1979; Bauch et al. 1980; Schmitt & Liese 1990; Rioux & Oulette 1991). When a deep wound extends into the wood and a barrier zone cannot be formed, the process of healing concerns both cambium regeneration and injured surface covering. According to Kubler (1983), typical wound healing processes do not occur in deep wounds where the exposed wood dries up and becomes dead. On the dead surface the stem does not grow and the surface becomes covered by newly formed xylem, secondary phloem and cortical tissues which overgrow the wound from its edges. Tree stem regeneration processes often involve modification of anatomical structure of newly formed wood (Arbellay et al. 2012). These modifications may concern both the axial and the radial system of secondary xylem. In general, xylem rays are oriented orthogonally with respect to axial elements. This pattern is modified when the shape of annual ring boundaries becomes irregular. Xylem rays are commonly believed to play a role in radial transport and to act as a storage tissue. According to some authors, however, they may also be involved in biomechanical properties of trees (Burgert et al. 1999; Burgert & Eckstein 2001). The spatial orientation of xylem rays might be correlated with the spatial distribution of mechanical stresses and forces (Mattheck & Kubler 1995; Mattheck 1997; Hejnowicz 2002).

In spite of abundant empirical material, the mechanisms controlling tree stem regeneration involving complex biochemical and biophysical processes are still poorly understood. In addition, so far no quantitative 3D models have been proposed for simulating the process of tree stem regeneration after deep wounding. This study focuses upon such deep mechanical wounding and the regenerating processes in stems of adult Scots pine trees over a period of 30 years. The changes of cambial surface topography are presented as 3D models using ArcGIS and geodesic Surfer programs. For measurements of cellular ordering in the region of regenerating tissues the digital image analysis software OrientationJ was adopted.

MATERIAL AND METHODS

The studies were performed on three Scots pine trees (*Pinus sylvestris* L.), 110 years old, from the Jablonna Forest District (Central Poland). The trees were about 25 m high and 50 cm in stem diameter at breast height. About 30 years ago their stems had been injured at about 1.5 m above ground level. The mechanical wounding of the stems was caused unintentionally by heavy machines during some construction work in the forest. The length of the longitudinal stem wounds in the trees ranged between 40 and 100 cm. The stem fragments comprising the whole regenerating stem sector were subsequently sawn into successive smaller transverse discs (Fig. 1A). The thickness of the discs varied from 2 cm in the region where there was intensive change in the wound shape, to 20 cm in the area where there was only a slight change in the wound shape.

On each saw-cut disc, the annual ring boundaries were marked for the year of wounding and for subsequent 5-year periods (Fig. 1B). These lines were the basis for
reconstructing the shape of the stem cambium just before wounding and during the period of stem regeneration. For the construction of a numerical model, these lines were considered as contour lines. On each disc with the contour lines, a reference net was marked in a constant position with respect to the stem pith. The reference net consisted of 6 points forming two adjoining squares, and sides of the squares were 4 cm. This reference net was used for the construction of a system of X, Y coordinates. These coordinates made it possible to arrange consecutive discs with respect to the constant Z (axial) axis.

Photographs of successive discs were taken. Using the ArcGIS (Esri) program, the photographic image of each disc was calibrated and subsequently the contour lines (X, Y data) were vectorised. Each contour line was assigned to a certain number of years (0, 5, 10 etc.) coinciding with number of years that had taken place after wounding had been done. The third Z value was the distance from the base of the whole stem fragment. As a result, data files for creating 3D surface plots of the cambial surface at various periods after wounding (0, 5, 10, etc. years) were made with the Surfer 8 (Golden Software, Inc.) program. The surface plots are considered to be models of the stem cambium during the regeneration of the cambium after wounding. In some of the stem discs, the curvature of the tree ring boundaries was analysed using the AutoCAD (Man and Machine Software) program.

Material for the anatomical study was selected from some of the above-mentioned stem discs, from regions near the edge of the wound. Microscopic examinations were carried out on transverse sections cut with a sliding microtome. An optical microscope was used to observe the sections that were mounted in glycerin. Callose-like substances in compression wood tracheids were identified with an OLYMPUS fluorescent microscope using aniline-blue, phosphate buffer at pH 8.0 and toluidine blue.

Measurements of the angle between the xylem rays and the annual ring boundaries were performed on the transverse microtome sections. The wounded stem region was divided into a number of microscopic sections. Digital photographs of each section were made and subsequently assembled using Photoshop (Adobe) software. The ray angle with respect to the previous annual ring boundary was measured in the selected points on photographs for which X, Y coordinates were determined. The three characteristics (angle, X and Y) were taken for preparing maps of spatial distribution of ray angle using the Surfer 8 program by local polynomial interpolation.

Figure 1. – A: Segment of wounded stem of Scots pine tree cut into transverse discs used for analysis of annual ring boundaries in wood formed during the period of stem regeneration (Tree No. 1). – B: Scheme of transverse stem disc in the wounded area; black lines indicate the boundaries of annual rings of wood formed at various periods of time after wounding (5–30 years); compare curvature of the boundary lines of annual rings in the wood fragment taken from the wound spindles with the radii of curvature of the concentric circles which may illustrate annual increments in younger stem (Tree No. 1); scale bar = 50 mm. – C & D: Three-dimensional models of regenerating stem wood surfaces at various periods of time (5, 10 … 30 years) after stem wounding (Tree No. 1); surfaces of relevant annual increments arranged jointly in stack C or separately (D).
Analysis of the cellular ordering in the wound region was performed by measurements of the local cellular orientation in tangential sections using the digital image processing tool, OrientationJ, which is an ImageJ plug-in (Fonck et al. 2009; Rezakhanlou et al. 2011). The angles of the oriented structures in the image are shown on the color-coded maps. The OrientationJ plug-in used in the present study is available online at http://bigwww.epfl.ch/demo/orientation.

RESULTS

The arrangement of tree rings near the wound boundaries, shown in 5-year intervals as seen on the transverse sections of stem discs, is illustrated in Figure 1B. On the basis of the data from all the discs, 3D models of stem regeneration over a period of 30 years following mechanical wounding were created (Fig. 1C, D). In the 3D models, the contour lines seen in the transverse sections represent the location of the cambial surface at the end of the growing season in a given year. The exemplary 3D model presented on Figure 1C, D concerns one tree (Tree No. 1) but analogous models (not shown) with similar results were performed for each of the three investigated trees. Analyses of all of the studied materials indicated that trees responded to the injury by increasing cambial activity near the wound boundaries. Characteristic longitudinal folds (wound spindles) with wider annual rings were formed. Numerous samples revealed that successively formed tree rings changed their orientation to perpendicular with respect to the wound surface. In many cases, the change of tree ring orientation proceeded very effectively within a short distance from the wound edge.

Measurements of tree ring boundary curvature in the wound region were performed on selected transversely cut discs. The greatest tree ring boundary curvature was found in the folds at both edges of the wound. The curvature radius ranged from 1 to 4 cm close to the wound boundary and increased to 5–30 cm farther away. In some discs in the region near the lateral edge of the stem wound the curvature of the boundary lines of annual rings in the wood fragment taken from the wound spindles was comparable with the radiuses of curvature occurring in consecutive annual increments in the stem region formed during early growth of the tree (Fig. 1B).

Microscopic observations of the transverse stem sections revealed that in the areas of newly formed xylem near the lateral wound there were some regions where xylem rays were not oriented perpendicular to annual ring boundaries (Fig. 2A–C), as is common in normal wood. Two examples of spatial distribution maps of the orientation of xylem rays in two regions near the stem wound are presented in Figure 2. On the map of the region of narrower rings above the wound, the ray orientation was less variable and ranged between 90 and 100 degrees (Fig. 2A). The most oblique orientation of rays, up to 136°, was observed close to the lateral edges of the wound in the region of concrescence of two wound spindles coming from lateral wound edges (Fig. 2B).

Microscopic examinations revealed some characteristic properties of the xylem tissue formed during stem regeneration. The greater the distance from the wound edges, the more similar the xylem tissue became to that formed in intact Scots pine stems. Those tracheids successively formed during the growing season were arranged in regular radial
Figure 2. Maps of spatial distribution of ray orientation with respect to annual ring boundaries of previous annual ring in two positions of the stem wound (A and B) indicated on Fig. 2C as A' and B', respectively (Tree No. 1). Figures on contour lines denote values of the angles (degrees). — Scale bar = 1 mm.
Figure 3. Cellular organization in the pine stem wound region. – A: Transverse section of an annual ring of wood close to the stem wound edges (Tree No. 2); radial rows of tracheids and xylem rays are oriented obliquely with respect to annual ring boundaries. Figures denote the values of angles between the direction of radial rows of tracheids and the ring boundaries and radial number of tracheids in the annual ring in three positions near the wound edge (position 1 is proximal and 3 distal). – B: Transverse section of wood in the wound spindles consisting of tracheids with thick rounded cell walls similar to compression wood. Some of the tracheids showed fluorescence after staining for the presence of callose-like substances (inset). – C–E: Tangential sections of wood at the edge of the stem wound (Tree No. 3); C & D: Color-coded maps of local predominant cellular angle orientations obtained by digital image processing software OrientationJ for ImageJ plug-in (B, C); the maps show the angle of cellular orientations (degrees) with respect to the stem axis. Irregular pattern of tracheids and radial xylem rays seen in the region of the lateral stem wound edge; arrows indicate areas with a circular pattern of tracheids (C) and with crooked tracheids (D); E: Tracheid with forked ends (arrow). — Scale bars = 200 μm in A, C; 100 μm in D; 80 μm in E; 60 μm in B.
rows oriented, in general, perpendicularly with respect to annual ring boundaries. The radial number of tracheids in annual wood increments varied significantly and ranged from 10 to over 100. Closer to the wound both radial rows of tracheids and xylem rays were oriented obliquely with respect to annual ring boundaries (Fig. 3A). In some regions, there were rings with a highly different radial number of tracheids within small tangential distances (few millimeters) of the same ring. Annual rings closer to the wound edges were characterised by a relatively wider portion of latewood. The tracheids seen in transverse sections sometimes had thick rounded cell walls similar to those found in compression wood (Fig. 3B). In addition, some of these tracheids showed fluorescence which appeared after staining for the presence of callose-like substances.

Near the region of the fusion of two wound spindles there were some areas consisting of irregularly oriented xylem cells adjoining the tissue of more regularly organised xylem cells (Fig. 3C–E). The cellular orientation with respect to stem axis ranged in the tangential sections in these regions between 0 and –90 and +90 degrees (Fig. 3C, D). There were also some regions consisting of crooked tracheids. In some areas a circular pattern of tracheid orientation was visible. Branched tracheids were present as well. (Fig. 3E).

DISCUSSION

The present study shows that the history of the stem regeneration process inscribed in the wood structure can be effectively reconstructed by numerical 3D models using the programs ArcGIS and Surfer. These programs are commonly used in geodesy for terrain modeling. The application of numerical models in studying tree stem regeneration allows for a more detailed analysis of the dynamics of long-term processes and changes in the geometry of the cambial surface.

The present results support earlier suggestions of Kubler (1983) that there is distinction between the process of wound healing and tree stem regeneration after wounding. The term “wound” mostly corresponds to an injury of living cells and tissues. Consequently, an actual “wound” in a tree stem should refer to an area where the continuity of the cambium, phloem and living cortical cells is disrupted as well as the surface of the exposed wood. During regeneration processes, the wound area of the exposed wood is covered by growing xylem, secondary phloem, callus and cortical tissues. This indicates that the stem wound itself is not healing, but the wound area is being covered by newly formed tissues. The differential intensity of wood production on the lateral sides of wound boundaries observed in this study may contribute to the concept of tree shape optimisation and “the axiom of uniform stress” described by Mattheck and Kubler (1995) and Mattheck (1997). According to this concept, the force flow running down the stem has to be deflected around the wound sides which induces additional stresses. The annual rings thicken locally on both sides of the wound and the additional deposited material increases the area for force flow which then reduces the stresses. From this point of view the adaptive growth occurring during stem regeneration would be analogous to that observed when an inanimate body in lateral contact with a tree stem becomes increasingly enveloped by newly formed wood. The contact stresses
are then reduced by enlarging the contact area. Thus, the results indicate that intensive growth and accumulation of newly deposited material in the wound spindles seems to be a very effective mechanism of tree stem regeneration restoring its biomechanical and transport functions. The adaptive growth during tree stem regeneration could also be considered as an illustration of Wolff’s law (Wolff 1892; D’Arcy Thompson 1917; Mattheck & Kubler 1995; Murray 2003), originally elaborated for animal bone structure. Wolff’s law states that new material is deposited differently in specific regions of an organ, depending on stress distribution, which indicates that in living systems the shape of an organ follows its function.

Change in the cambial surface orientation was expressed in the form of a strong curvature of annual ring boundaries in the wound spindles. As shown, an originally tangential system of annual rings was transformed into a perpendicular one over a period of 20 years after wounding. This reorientation of the cambial surface was due to an unequal number of tracheids in radial rows within annual rings associated with the oblique orientation of tracheid rows and xylem rays. Since in conifer wood the number of tracheids in a radial file reflects the number of periclinal divisions in the cambial zone, the observed differences indicate unequal rates of cambial periclinal divisions. A similar wood structure was described by Bannan (1957) in irregularly shaped *Thuja occidentalis* stems. In the present study, elementary cellular events in cambium like anticlinal division, elimination and intrusive growth of cambial initials were not studied in tangential sections. However, some approximate information concerning these events could be deduced from microscopic examination of transverse sections in which radial rows of tracheids may appear or disappear. Frequency of the two events could serve (with some caveats) as indices of anticlinal divisions and the elimination of cambial fusiform initials, respectively. It was interesting to note that in many regions near the wound edge, preliminary observations of transverse sections did not reveal the symptoms of an increased frequency of anticlinal divisions or the elimination of cambial initials in spite of great differences in cambial periclinal divisions resulting in the formation of wound spindles.

It is interesting to note that curvature of annual rings in the wound spindle coincides with the curvature of tree rings of small diameter formed during early growth of the tree. However, the fact that latwood to earlywood ratio was increasing in rings close to the wound it indicates that woundwood resembles mature rather than juvenile wood.

Xylem rays were oriented obliquely to the annual ring boundaries mainly in those regions of newly formed wood that could be considered to be the most modified as compared to normal wood. This occurred in regions where the cambial surface had a high curvature, in growth increments with a high portion of latwood with tracheids having thick rounded cell walls (as seen in transverse section) in which callose-like substances were revealed. This may suggest that the rays were obliquely oriented mostly in these regions which resembled compression wood and were probably under greater mechanical stress which is considered as one of the factors inducing compression wood (Timell 1986). Seemingly, such a situation could be explained by the Mattheck model (Mattheck & Kubler 1995; Mattheck 1997) of force flow around the wounds.
The regions of fusion of two wound spindles coming from the lateral wound edges were characterised both by an oblique orientation of rays and irregularly oriented xylem cells. The cellular ordering was clearly visible on the color-coded maps of the local angular orientation of tracheids. These irregularities may have been the result of specific interactions between mechanical stresses and other influences from adjoining tissues. The circular pattern of tracheids observed in the present study resembled the patterns described both in conifers and angiosperms, e.g., near buds (Hejnowicz & Kurczyńska 1987), in forked stems (Lev-Yadun & Aloni 1990), near mechanical wounds (Aloni & Wolf 1984), and around injuries caused by insects (Liphschitz & Mendel 1987). Some authors (Hejnowicz & Kurczyńska 1987; Lev-Yadun & Aloni 1990; Kurczyńska & Hejnowicz 1991) have reported that deviations from the polar transport of auxin (Sachs & cohen 1982) is involved in the control of these phenomena.

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REFERENCES


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