BOT. GAZ. **145**(1):50–54. 1984.

0006-8071/84/4501-0006$02.00

LENGTH, WIDTH, AND PATTERN OF REGENERATIVE VESSELS ALONG STRIPS OF VASCULAR TISSUE

RONI ALONI AND M. H. ZIMMERMANN

Department of Botany, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel; and Cabot Foundation, Harvard University, Petersham, Massachusetts 01366

The effect of stem girdling on vessel differentiation was studied in *Acer rubrum* by cinematographic analysis and by vessel-length distribution measurements. Partial girdling, presumably an obstruction to the flow of auxin, increased the rate of vessel differentiation in the bottleneck area, resulting in numerous small regenerative vessels. The regenerative vessels that were formed along an intact, longitudinal strip of cambium were short and narrow, presumably because of the local high auxin concentration. Numerous short and narrow vessels are a safer water-conducting system than wide and long vessels.

Introduction

We have proposed a new hypothesis, ascribing the general increase in vessel size and decrease in vessel density from leaves to roots to a gradient of decreasing auxin concentration from leaves to roots (ALONI and ZIMMERMANN 1983). The second point of the six-point hypothesis suggests that local structural or physiological obstructions of the flow of auxin, e.g., at nodes where two flows of auxin merge, raise the auxin concentration locally (fig. 1*A*). This local increase in auxin concentration is expected to result in an increase in vessel density and decrease in vessel size. Branch junctions appear to be examples of such local obstructions. Vessel diameters in a branch are similar to those of the main stem at a level of equal diameter; i.e., they gradually increase with increasing distance from the leaves. However, there is a sharp drop in vessel diameter in the branch just above its point of attachment to the main stem (ZIMMERMANN 1978).

To test the effect of local obstructions on vascular differentiation, two experiments were designed to simulate branch insertions (fig. 1*A*). In the first, partial girdling was performed, leaving a longitudinal bridge of cambium and bark (fig. 1*B*). In the second, an oblique cambium and bark bridge was left (fig. 1*C*). The effect of these surgical modifications on vessel differentiation in the region of the bark bridges was studied.

A second aim of this study was to describe more thoroughly vessel regeneration in tissue formed either from an existing or from a newly formed cambium after injury. Earlier studies on xylem regeneration emphasized wound vessel members, i.e., those portions of regenerative vessels that could be distinguished with clearing and sectioning methods (SINNOTT and BLOCH 1945; JACOBS 1952; ALONI and JACOBS 1977*a*, 1977*b*; SACHS 1981). However, vessel lengths were never measured in such experiments, as it was difficult to detect vessel ends with the methods existing at that time. Here we used the latex-paint perfusion method of ZIMMERMANN and JEJE (1981) to determine vessel-length distributions quantitatively.

Manuscript received May 1983; revised manuscript received August 1983.

Address for correspondence and reprints: Dr. RONI ALONI, Department of Botany, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel.

Material and methods

Stump sprouts of *Acer rubrum* L. (1, 2, and 3 yr old) were selected from a recent forest clearing of the Harvard Forest. At the beginning of July 1982, 70 sprouts were partially girdled (fig. 1*B*), leaving a vertical strip of cambium and bark tissue across the girdled area, and on 30 sprouts that were all 3 yr old, an oblique bridge was left (fig. 1*C*). The bridges were ca. 0.5 cm wide and 3–5 cm long. The girdled stems were collected and studied during July, August, and September 1982. Xylem differentiation was studied in cross sections of the region of the girdle, taken at successive 0.2-, 0.5-, or 1-cm intervals along the girdled portions of the stems. In addition, a sequential series of 1,600 cross sections 50 μm thick (every second section was used) was prepared from a 16-cm portion of a 3-yr-old stem with an oblique bridge, 1 mo after it was girdled. This series was photographed sequentially with a 16-mm cine camera through the shuttle microscope, and the resulting film was analyzed with a Vanguard film analyzer (ZIMMERMANN 1975).

Vessel lengths were studied in 30 stems into which a very dilute suspension of water-soluble latex paint was infiltrated (ZIMMERMANN and JEJE 1981). The

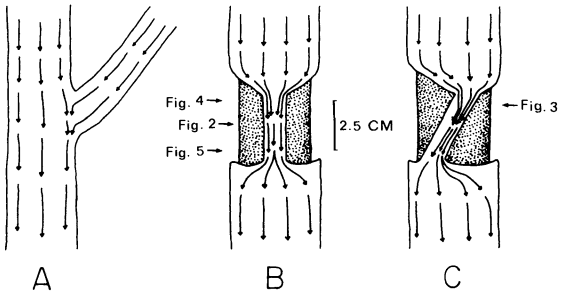


FIG. 1.—Diagrams of the assumed patterns of auxin flow, indicated by arrows, through the following obstructions: *A*, a branch junction; *B*, a longitudinal bridge of cambium and bark; *C*, an oblique bridge of cambium and bark. The assumed increase in auxin concentration in the constriction area is shown by converging arrows. The highest concentration of auxin is expected along the lower edge of the oblique bridge (*C*). The xylem tissue exposed by the partial girdling is indicated by stippling. The positions of figs. 2–5 are marked.

stem was cut above, below, or in the middle of the girdling area, and the paint was applied to all freshly cut surfaces (at *A*, *B*, and *C* in fig. 7). Paint-containing vessels were counted and measured under the light or dissecting microscope, and vessel-length distributions were calculated from these counts (ZIMMERMANN and JEJE 1981). Over 4,000 cross sections were studied.

Results

Effect of partial girdling on development and pattern of vascular differentiation

Partial girdling affected the cross-sectional appearance of those vessels that had started, but not yet completed, differentiation before the experiment was begun. Many of these vessels appeared polygonal (almost star shaped) in cross section and were used to identify the portion of xylem initiated before girdling (fig. 2). These vessels were filled with latex paint and may therefore be considered to have been functional. They were easy to see along the bridges of cambium and bark and up to 5 cm above and below the girdled area.

In the xylem tissue that developed immediately after girdling, vessel elements completed their differentiation before the surrounding cells (fig. 2). In the xylem that was produced later and was initiated around the incisions made in the vascular cambium immediately after injury, there were fewer vessels, which were arranged in radial groups (figs. 2–5, right side).

The first regenerative vessel elements formed after girdling appeared at the upper portion of the bridges of cambium and bark (fig. 1*B*, *C*) 7 days after injury. Differentiation of these elements continued both acropetally and basipetally from this point. Two weeks after girdling, the regenerative vessels did not conduct latex farther than 0.5 cm. However, some of these regenerative vessels, in stems collected 1 mo or more after girdling, conducted latex along 2–3 cm (figs. 6, 7).

Length, width, and density of regenerative vessels around an injury

Regenerative vessels formed immediately after partial girdling were shorter and narrower than vessels formed in the intact plant and vessels formed in the girdled plants at a later date (fig. 6). By applying paint at three different locations, we determined that the shortest regenerative vessels differentiated at the upper end of the bridge. They made contacts with longer regenerative vessels above and below this point (fig. 7).

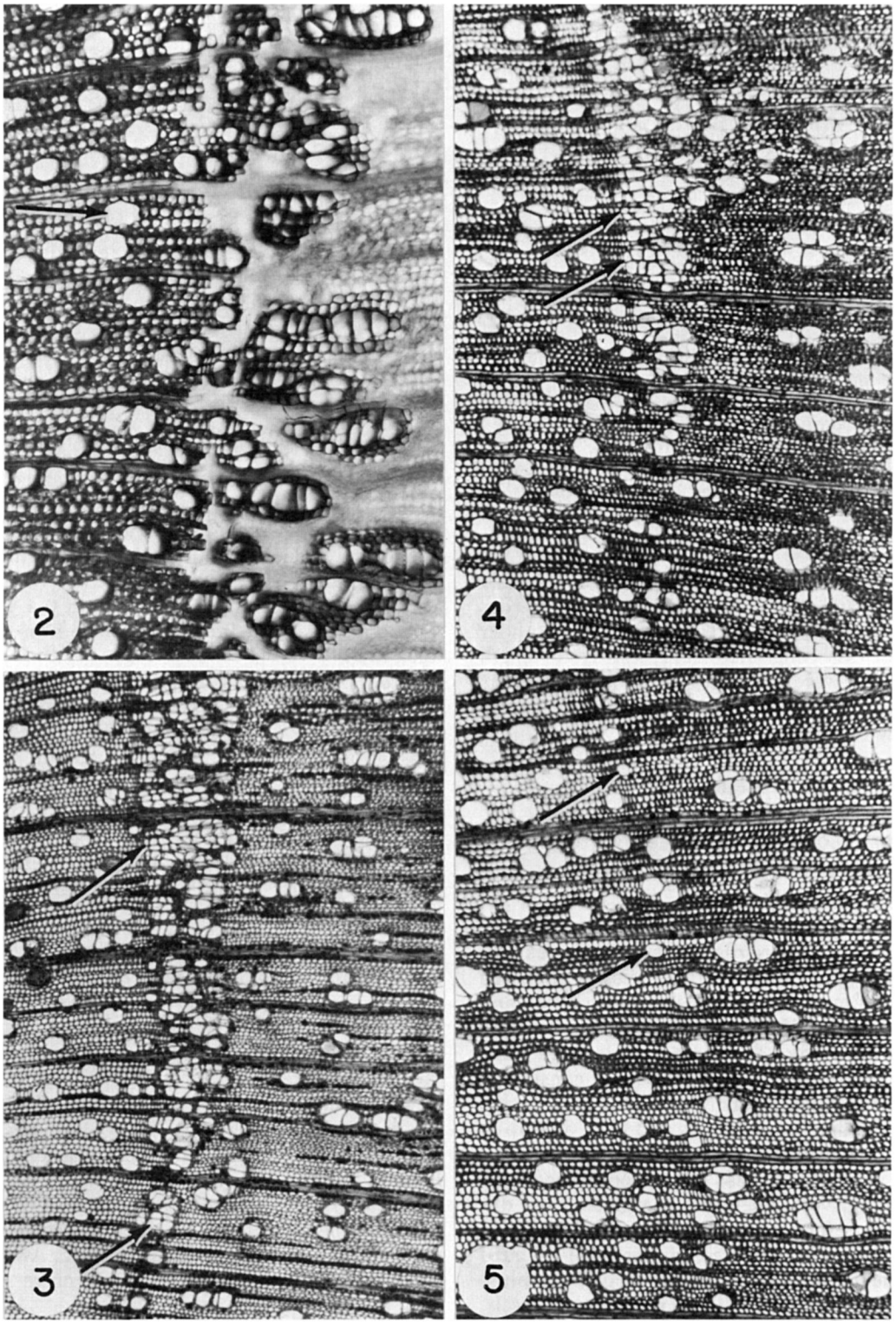
Regenerative vessel diameters increased basipetally from 12.0 ± 0.6 μm in the upper to 31.8 ± 0.8 μm in the lower portions of a longitudinal bridge made on a 3-yr-old sprout (no. = 20 in both cases). Above the girdled area, regenerative vessel diameters increased acropetally to 22.4 ± 0.4 μm (fig. 6) and more with increasing distance from the girdled area.

Progressing basipetally, there was also a decrease in density of the regenerative vessels (figs. 4, 5). In addition, there was a gradual transition from clusters of numerous narrow vessels in the upper (figs. 3, 4) to few and wide solitary regenerative vessels in the lower portions (fig. 5). The biggest clusters of regenerative vessels (fig. 3) occurred at the upper end of the oblique bridge at its lower edge. Here one would expect the highest concentration of auxin (fig. 1*C*).

Discussion

Our findings are in accordance with the hypothesis that auxin controls the size and density of vessels along the plant axis (ALONI and ZIMMERMANN 1983). The two experimental arrangements simulated the pattern of the vessel system found in branch junctions (ZIMMERMANN 1978). The results suggest that obstructions in the flow of auxin, which are expected to increase the concentration of auxin locally, caused rapid vessel differentiation in the constriction area (the strips of bark and cambium left intact) and resulted in numerous short and narrow vessels immediately after girdling. Away from such constrictions, slow differentiation resulted in few and large regenerative vessels.

Our results show that the number of regenerative vessels decreased, while their diameter increased basipetally along the intact longitudinal strips of vascular tissue. The same pattern was found in the intact vessel system in the leaf of *Saccharum* (COLBERT and EVERT 1982) and along the stem of *Populus* (MEICENHEIMER and LARSON 1983). Our



FIGS. 2–5.—Cross sections of *Acer rubrum* showing the patterns of vessel differentiation in the bridges of cambium and bark 2 wk (fig. 2) and 1 mo (figs. 3–5) after girdling. All photographs are presented in the same orientation; the most recently formed wood is on the right side. Fig. 2, Early stage of differentiation in the middle of a longitudinal bridge (fig. 1*B*) of a 2-yr-old sprout. Vessels that appear polygonal in cross section (arrow) had started differentiation before girdling. In the xylem formed after girdling, vessel differentiation precedes that of other elements. Many of the recently formed vessels are arranged in radial groups; × 87. Fig. 3, Tangential gradients in the size and in the density of regenerative vessels (arrows) formed in the upper portion of an oblique bridge near its lower edge (fig. 1*C*). Many small regenerative vessels are in the upper part of the figure, while fewer and wider ones are in the lower part; × 52. Figs. 4, 5, Sections taken from the same 3-yr-old sprout, showing the increase in the diameter of the regenerative vessels (arrows) and decrease in their density along a longitudinal bridge (fig. 1*B*); both × 65. Fig. 4, Many narrow, regenerative vessels were formed in the upper portion of the bridge. Fig. 5, Fewer and wider regenerative vessels differentiated 2.5 cm below the section shown in fig. 4.

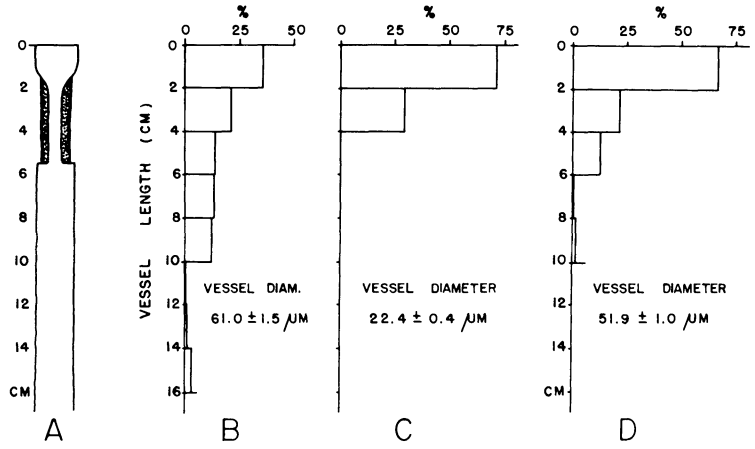


FIG. 6.—Effect of the longitudinal bridge of cambium and bark (*A*), on the vessel-length distribution and average vessel diameter ± SE (no. = 20) of regenerative vessels formed in the upper part of the bridge (*C*) measured at the cut surface (at 0 cm) in a 3-yr-old stump sprout of *Acer rubrum*. Also shown are the same features of the vessels formed in the earlywood of the third year (i.e., before girdling) (*B*) and in the latewood formed after girdling (*D*). The small horizontal lines at the bottom of diagrams *B* and *D* indicate greatest lengths. The proportion of width to length in this drawing is shown correctly.

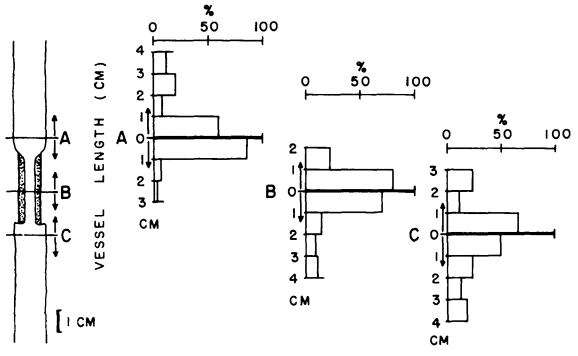


FIG. 7.—Pattern of vessel-length distribution of regenerative vessels around an injury in 1-yr-old sprouts of *Acer rubrum*. Changes in vessel-length distribution in three stems to which latex paint had been applied to the two cut surfaces: above (*A*), in the middle (*B*), and below the longitudinal bridge (*C*). Vessel lengths are shortest in the upper portion of the bridge (immediately above *B*) and increase up and downward. The small horizontal lines in the graphs indicate greatest lengths. Diameters of the sprouts in the girdled area were 0.7–0.8 cm. The width of the sprout appears somewhat enlarged. The percentage scale represents the number of paint-containing regenerative vessels at the cut surfaces.

results with *Acer* show an inverse relation between vessel density and vessel size, confirming the results reported for *Phaseolus* (ALONI and ZIMMERMANN 1983). Our experiments showed a positive correlation between vessel length and vessel diameter, a correlation that has already been reported throughout entire intact specimens of *Acer rubrum* (ZIMMERMANN and POTTER 1982).

The optical shuttle method (ZIMMERMANN and TOMLINSON 1966) and the paint-perfusion technique (ZIMMERMANN and JEJE 1981) were useful tools for studying the three-dimensional structure of the regenerative vessel system in secondary xylem. After studying the vessel network in films of serial sections, we easily distinguished older from regenerative vessels in sections perfused with paint even above and below the girdle.

Work with stems of both *Cucumis* and *Coleus* showed that regenerative vessels do not contact the damaged vessels above and below the wound but are contiguous only with newly formed vessels from the cambium after the injury (BENAYOUN et al. 1975; ALONI and JACOBS 1977*a*, 1977*b*). Our study shows that regenerative vessels formed in *A. rubrum* after partial girdling are both very short and very narrow, compared with the nonregenerative vessels formed before and some time after wounding. However, the length and width of regenerative vessels increase with increasing distance from the girdled area. From a functional point of view, the formation of numerous small regenerative vessels has an adaptive value in case of repeated injury. Numerous small vessels are much safer water conductors than few large ones, although their combined conductance is smaller (ZIMMERMANN 1983).

Acknowledgments

This study was conducted at the Harvard Forest while RONI ALONI was a Bullard Research Fellow of Harvard University. Miss MONICA MATTMULLER assisted us in every phase of this work; her important help is greatly appreciated. We thank Professor P. B. TOMLINSON and Dr. FRANK EWERS for their critical manuscript reviews.

LITERATURE CITED

ALONI, R., and W. P. JACOBS. 1977*a*. Polarity of tracheary regeneration in young internodes of *Coleus* (Labiatae). Amer. J. Bot. **64:**395–403.

———. 1977*b*. The time course of sieve tube and vessel regeneration and their relation to phloem anastomoses in mature internodes of *Coleus*. Amer. J. Bot. **64:**615–621.

ALONI, R., and M. H. ZIMMERMANN. 1983. The control of vessel size and density along the plant axis—a new hypothesis. Differentiation **24:**203–208.

BENAYOUN, J., R. ALONI, and T. SACHS. 1975. Regeneration around wounds and the control of vascular differentiation. Ann. Bot. **39:**447–454.

COLBERT, J. T., and R. F. EVERT. 1982. Leaf vasculature in sugarcane (*Saccharum officinarum* L.). Planta **156:**136–151.

JACOBS, W. P. 1952. The role of auxin in differentiation of xylem around a wound. Amer. J. Bot. **39:**301–309.

MEICENHEIMER, R. D., and P. R. LARSON. 1983. Empirical models for xylogenesis in *Populus deltoides*. Ann. Bot. **51:**491–502.

SACHS, T. 1981. The control of the patterned differentiation of vascular tissues. Pages 152–255 *in* H. W. WOOLHOUSE, ed. Advances in botanical research. Vol. **9.** Academic Press, London.

SINNOTT, E. W., and R. BLOCH. 1945. The cytoplasmic basis of intercellular patterns in vascular differentiation. Amer. J. Bot. **32:**151–156.

ZIMMERMANN, M. H. 1975. The study of vascular patterns in higher plants. Pages 221–235 *in* I. F. WARDLAW and J. B. PASSIOURA, eds. Transport and transfer processes in plants. Academic Press, New York.

———. 1978. Hydraulic architecture of some diffuse-porous trees. Can. J. Bot. **56:**2286–2295.

———. 1983. Xylem structure and the ascent of sap. T. TIMELL, ed. Springer series in wood science. Vol. **1.** Springer, Berlin, Heidelberg, and New York.

ZIMMERMANN, M. H., and A. A. JEJE. 1981. Vessel-length distribution in stems of some American woody plants. Can. J. Bot. **59:**1882–1892.

ZIMMERMANN, M. H., and D. POTTER. 1982. Vessel-length distribution in branches, stems and roots of *Acer rubrum* L. IAWA Bull. **3:**103–109.

ZIMMERMANN, M. H., and P. B. TOMLINSON. 1966. Analysis of complex vascular systems in plants: optical shuttle method. Science **152:**72–73.