Springer Series in Wood Science

Editor: T. E. Timell

Springer
Berlin
Heidelberg
New York
Barcelona
Budapest
Hong Kong
London
Milan
Paris
Santa Clara
Singapore
Tokyo

Springer Series in Wood Science

Editor: T. E. Timell

M. H. Zimmermann
Xylem Structure and the Ascent of Sap (1983)

J. F. Siau
Transport Processes in Wood (1984)

R. R. Archer
Growth Stresses and Strains in Trees (1986)

W. E. Hillis
Heartwood and Tree Exudates (1987)

S. Carlquist
Comparative Wood Anatomy (1988)

L. W. Roberts/P. B. Gahan/R. Aloni
Vascular Differentiation and Plant Growth Regulators (1988)

C. Skaar
Wood-Water Relations (1988)

J. M. Harris
Spiral Grain and Wave Phenomena in Wood Formation (1989)

B. J. Zobel/J. P. van Buijtenen
Wood Variation (1989)

P. Hakkila
Utilization of Residual Forest Biomass (1989)

J. W. Rowe (Ed.)
Natural Products of Woody Plants (1989)

K.-E. L. Eriksson/R. A. Blanchette/P. Ander
Microbial and Enzymatic Degradation of Wood and Wood Components (1990)

R. A. Blanchette/A. R. Biggs (Eds.)
Defense Mechanisms of Woody Plants Against Fungi (1992)

S. Y. Lin/C. W. Dence (Eds.)
Methods in Lignin Chemistry (1992)

G. Torgovnikov
Dielectric Properties of Wood and Wood-Based Materials (1993)

F. H. Schweingruber
Trees and Wood in Dendrochronology (1993)

P. R. Larson
The Vascular Cambium: Development and Structure (1994)

M.-S. Ilvessalo-Pfäffli
Fiber Atlas: Identification of Papermaking Fibers (1995)

B. J. Zobel/J. B. Jett
Genetics of Wood Production (1995)

C. Mattheck/H. Kubler
Wood – The Internal Optimization of Trees (1995)

T. Higuchi
Biochemistry and Molecular Biology of Wood (1997)

T. Higuchi

Biochemistry and Molecular Biology of Wood

With 200 Figures and 25 Tables

Springer

Prof. Dr. TAKAYOSHI HIGUCHI
Wood Research Institute
Kyoto University (retired)
Momoyamacho, Yosai 22-8
Fushimi-ku, Kyoto 612
Japan

Series Editor:

T. E. TIMELL
State University of New York
College of Environmental Science and Forestry
Syracuse, NY 13210, USA

Cover: Transverse section of *Pinus lambertiana* wood. Courtesy of Dr. Carl de Zeeuw, SUNY College of Environmental Science and Forestry, Syracuse, New York

ISBN-13:978-3-642-64419-1 e-ISBN-13:978-3-642-60469-0
DOI: 10.1007/978-3-642-60469-0

Library of Congress Cataloging-in-Publication Data. Higuchi, Takayoshi, Biochemistry and molecular biology of wood/T. Higuchi, T. Umezawa. p. cm. – (Springer series in wood science) Includes bibliographical references and index. ISBN-13:978-3-642-64419-1 1. Wood – Anatomy. 2. Wood – Chemistry. 3. Plant molecular biology. I. Umezawa, T. (Toshiaki), 1957- . II. Title. III. Series. QK647.H53 1997 582'.0495 – dc20 96-28429

Softcover reprint of the hardcover 1st edition 1997

Cover design: Design & Production GmbH, Heidelberg

Typesetting: Best-set Typesetter Ltd., Hong Kong

SPIN: 10493637 31/3137/SPS – 5 4 3 2 1 0 – Printed on acid-free paper

Preface

Progress in wood chemistry has been related mainly to chemical wood pulping and bleaching and chemical utilization of wood and wood extractives. Methods of wood analysis were developed by Schorger (proximate analysis in 1917) and Dore (summative analysis in 1919), and standard methods based on Schorger's method, e.g., TAPPI standards (Technical Association of the Pulp and Paper Industry), have been widely used for chemical analysis of woods in many countries. Thus it is generally known that wood is composed of about 50% cellulose, 20–35% of lignin, 15–25% of hemicelluloses, and variable amounts of extractives. Chemical characterization and efficient utilization of these wood components have been studied in laboratories of wood chemistry and technology in universities and government institutions.

In the last decade, biochemistry and molecular biology of microorganisms, animals, and plants have greatly progressed. At the same time wood has been recognized as a unique renewable ecomaterial produced by trees using solar energy. In addition, many desirable properties of wood and wood components as biomaterial that affects physiology and psychology in humans have recently attracted attention.

In order to elucidate the properties of wood and wood components produced by trees, characterization of genes encoding enzymes involved in the biosynthesis of wood components, differentiation of the cambium into phloem and xylem, and the mechanisms of the expression of these genes needed to be investigated. Research on the molecular biology of trees and wood has just begun in Europe, USA, Canada, New Zealand, and Japan, among other countries, and rapid progress is being made.

I became interested in wood biochemistry during my stay at Gifu University, where the first Wood Biochemistry Laboratory in Japan was founded in 1949, and at the Wood Research Institute, Kyoto University, through research on the biosynthesis of lignin and formation of heartwood.

I am very much indebted to Dr. Toshiaki Umezawa from the Wood Research Institute at Kyoto University, who contributed to the section on "Lignans" (Sect. 4.3.5). Dr. Umezawa was the first to synthesize the lignans, (–)-secoisolariciresinol, and (–)-matairesinol enzymatically from coniferyl alcohol. I wish to thank Dr. Stewart A. Brown (Trent University, Canada), Dr. Fernand Barnoud (Grenoble University, France), Dr. M. Shimada and Dr. F. Nakatsubo (Kyoto University), Dr. T. Yamasaki (Kagawa University), Dr. H. Ohashi and Dr. M. Tanahashi (Gifu University), and Dr. Y. Nakamura, Dr. H. Kuroda, Dr. H. Kutsuki, Dr. S. Kawai, and Dr. H. Fushiki for their assistance during my studies on lignin biochemistry. I also wish to thank Dr. T. Itoh

(Wood Research Institute, Kyoto University), Dr. D. Shibata and Dr. T. Hibino (Mitsui Plant Biotechnology Research Institute), Dr. H. Fukuda (Tokyo University), Dr. H. Ohashi (Gifu University), Dr. K. Fukazawa (Emeritus Professor at Hokkaido University), Dr. T. Minamikawa (Tokyo Metropolitan University), Dr. T. Hayashi and Dr. J. Sugiyama (Wood Research Institute, Kyoto University), Dr. K. Takabe (Kyoto University), Dr. H. Kuroda (Wood Research Institute, Kyoto University), Dr. R. Funada (Hokkaido University), and Dr. K. Fukushima (Nagoya University) for their support and help with this book. I thank the many scientists and publishers who kindly granted permission for me to use their original illustrations for this book. Finally, I would like to thank Dr. T. E. Timell, the editor of this series, and the staff of Springer-Verlag for their help and encouragement during the preparation of this book.

Kyoto, Japan
Autumn 1996

TAKAYOSHI HIGUCHI

Contents

1 Structure and Functions of Wood

Trees used as wood materials for timber, furniture, and pulp industries are softwoods (conifers), which are gymnosperms, and hardwoods, which are dicotyledonous angiosperms. Bamboos, other grasses, and palms are included in the monocotyledons.

Conifers are usually evergreen, with narrow, needlelike or scalelike leaves, and bear exposed seeds usually in cones, whereas hardwoods are mostly deciduous, have net-veined leaves, and bear seeds enclosed in fruits (Little 1993).

Bamboos are perennial grasses with woody stems or culms that occur mostly in tropical, subtropical, and temperate regions and are abundant in tropical Asia. Bamboos belong to the Bambusoideae, a subfamily of the Gramineae. There are about 750 species of bamboo in about 45 genera.

Abies, *Chamaecyparis*, *Cedrus*, *Cupressus*, *Cryptomeria*, *Juniperus*, *Larix*, *Picea*, *Pinus*, *Podocarpus*, *Pseudotsuga*, *Sciadopitys*, *Taxodium*, *Taxus*, *Tsuga*, *Thuja*, and *Thujopsis* (conifers) and *Acacia*, *Acer*, *Aesculus*, *Alnus*, *Betula*, *Castanea*, *Celtis*, *Eucalyptus*, *Fagus*, *Fraxinus*, *Juglans*, *Magnolia*, *Nothofagus*, *Populus*, *Quercus*, *Salix*, *Tilia*, *Ulmus*, and *Zelkova* (hardwoods) are some of the trees found in temperate and warm-temperate zones that are useful in the wood and pulp industries.

In addition, *Cassia*, *Diospyros*, *Dipterocarpus*, *Hevea*, *Mansonia, Ochroma*, *Pentacme, Shorea*, *Swietenia*, *Tectona*, *Terminalia*, *Tieghemella*, among others, are important tropical hardwoods used as wood-based material and furniture.

The tree is composed of a crown, a stem, and a root. Leaves of the crown produce sugars by photosynthesis from CO_2 absorbed by stomata and from water transported from the root. The synthesized sugars (mainly in the form of sucrose) are transported via the phloem to the meristematic tissues of the trunk and branches and are partly used as energy sources. They are converted to tree components such as proteins, cellulose, hemicelluloses, lignin, and wood extractives during tree growth.

The stem is a conductive organ for water and photosynthates; it is composed of phloem (bark) and xylem (wood) and supports the crown, along with the roots. The xylem is involved in transportation of water and minerals from the root, while the phloem transports photosynthesized and metabolic products. The root absorbs water and minerals from the soil and fixes the tree trunk in soil (Fig. 1).

The outermost layer of the tree trunk is composed of outer bark and phloem (inner bark), which covers the xylem. Between the phloem and the

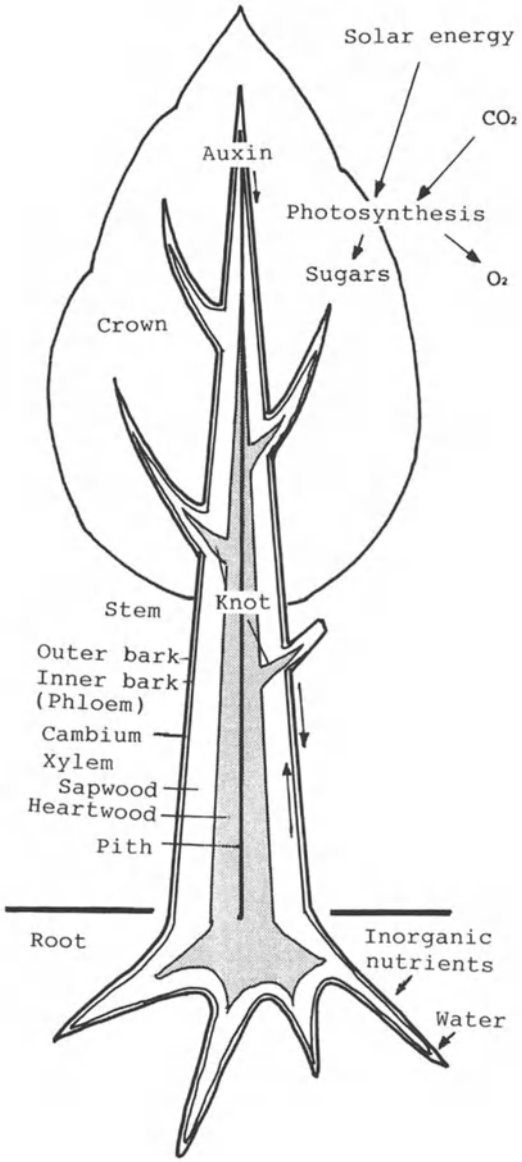

Fig. 1. Organization and functions of a tree (schematic). Water and inorganic nutrients are transported from root to upper tissues via outer sapwood, while photosynthesized sugars and auxins are transported from leaves and meristems to lower tissues and supplied to cambial tissues via phloem. (Courtesy of Dr. M. Fujita)

xylem, a cambial layer is located. Pith is present at the center of the trunk. The shapes and arrangements of the cells in the xylem are different at cross, radial, and tangential surfaces of the cut wood. Microscopic characteristics of tissues on the three surfaces of the cut wood have therefore been used to identify wood species (Saiki 1982; Fig. 2).

The cambium is derived from a procambium located slightly below the growing point of a tree stem. The procambium is arranged as a concentric

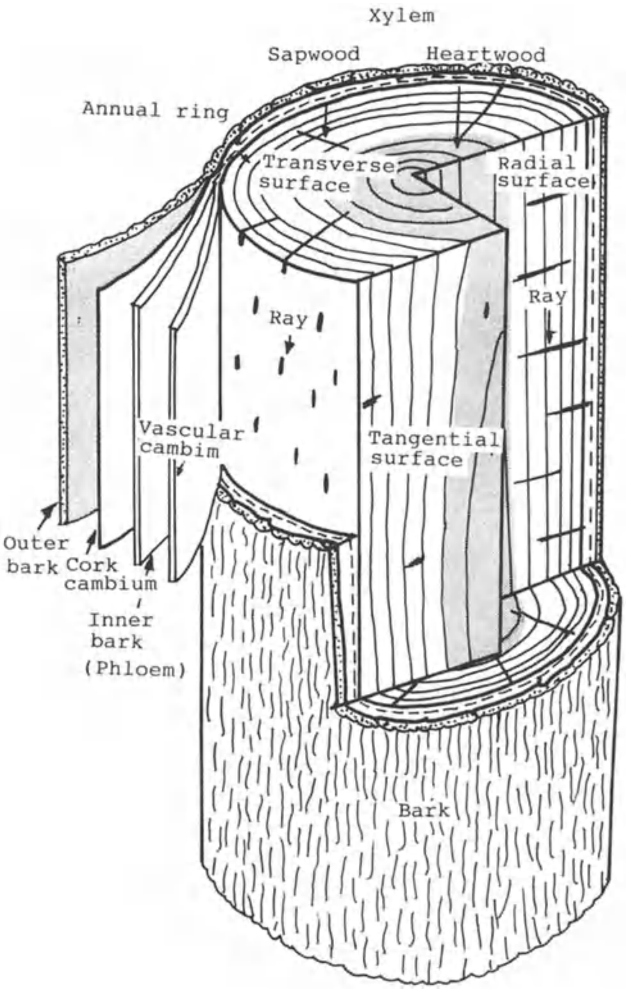

Fig. 2. Planes of wood and their designation: transverse surface or cross section, radial surface, and tangential surface. (Courtesy of Dr. M. Fujita)

circle on the cross surface of the growing tip of the tree and becomes a vascular bundle, a permanent tissue, as the tree grows. The vascular bundle is composed of two different tissues, fascicular phloem and fascicular xylem, and between the two tissues there is a thin layer of meristematic cells, the fascicular cambium.

During tree growth, fascicular cambial cells are divided tangentially to produce xylem tissue on the inner side and phloem tissue on the outer side. In conjunction with this process, interfascicular cambium is formed in the fundamental tissue between the vascular bundles, connecting the neighboring fascicular cambia to form a vascular cambium. Thus the formation of the vascular cambium, which covers pith and primary xylem, is completed as a secondary meristematic tissue (Shimaji 1976a; Fig. 3).

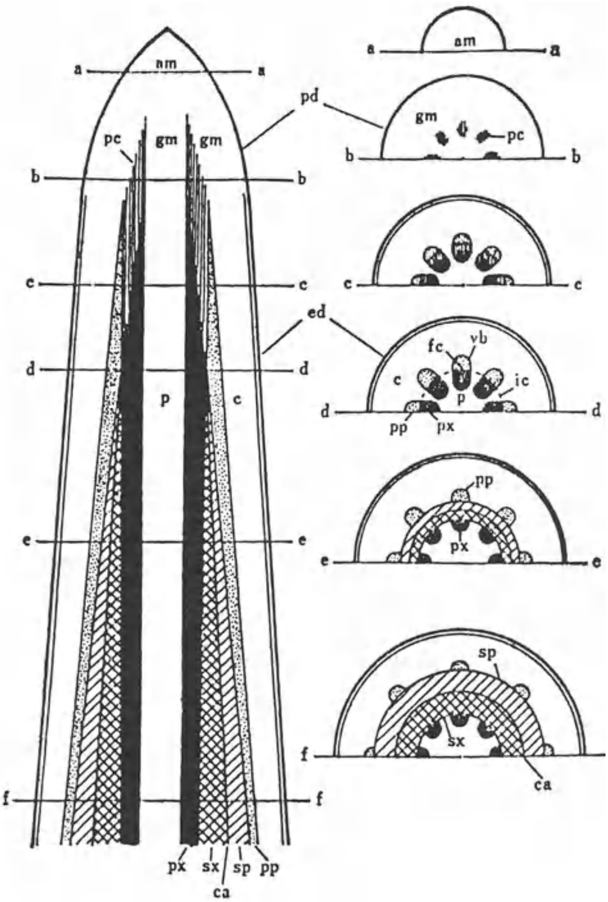

Fig. 3. Schematic model of a developing tree stem. *am* Meristematic tissue of growing point; *pd* dermatogen; *gm* fundamental meristem; *pc* procambium; *ed* epidermis; *p* pith; *c* cortex; *vb* vascular bundle; *fc* fascicular cambium; *ic* interfascicular cambium; *pp* primary phloem; *px* primary xylem; *sp* secondary phloem; *sx* secondary xylem; *ca* cambium. *a*, *b*, *c*, *d*, *e*, *f* denote transverse surfaces of a developing tree stem, respectively. *a* Growing point (apical meristem); *b* tissue consisting of procambium and fundamental meristem; *c* tissue consisting of vascular bundle and ground tissue; *d* tissue consisting of epidermis, cortex, and vascular bundles differentiating to fascicular and interfascicular cambia, and pith; *f* mature xylem tissue consisting of secondary xylem and secondary phloem. (Shimaji 1976a)

The vascular cambium produces xylem tissue at the inner side and phloem tissue at the outer side, followed by enlargement of the circumference of the cambium. The xylem and the phloem newly formed by the division of the cambial cells are called secondary xylem and secondary phloem, respectively.

Figure 4 shows the cross surface of wood of a 3-year-old *Pinus resinosa* and the mode of cell division and growth of the cambial cells.

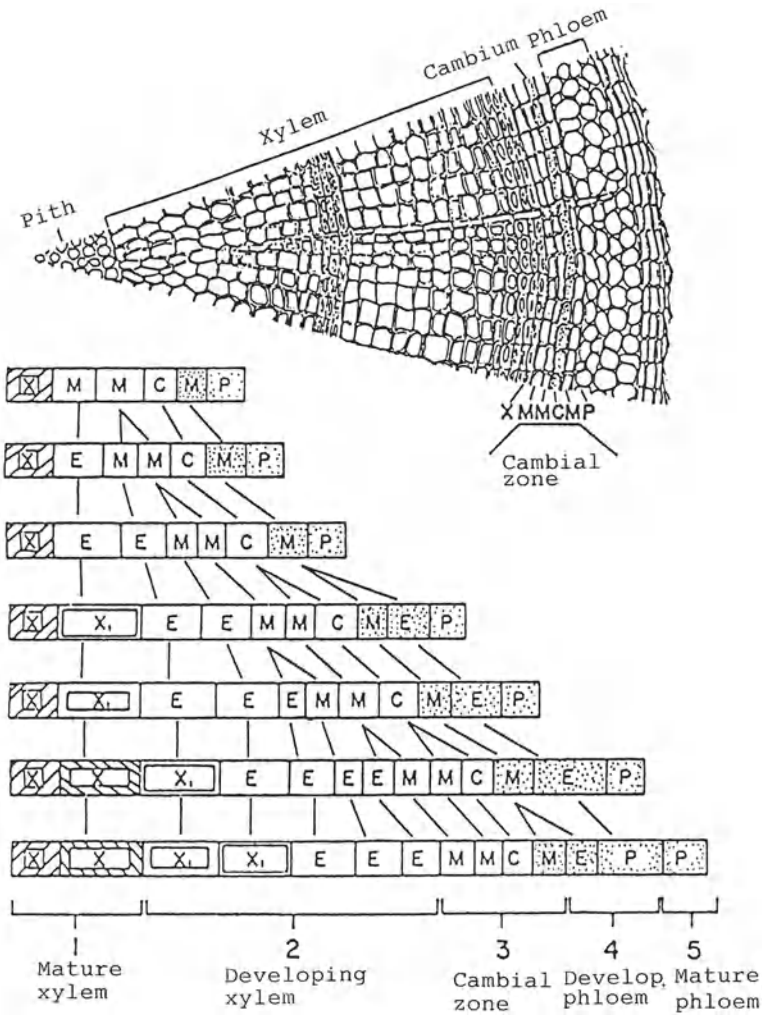

Fig. 4. Transverse surface of wood of a 3-year-old red pine, and the mode of cell growth of the cambial zone. *1* Mature xylem; *2* developing xylem; *3* cambial zone; *4* developing phloem; *5* mature phloem. *C* Cambial initial; *M* xylem mother cell (*left of C*); phloem mother cell (*right of C*); *X* mature xylem cell; *P* mature phloem cell; *E* growing cell

Since cambial cells divide continuously during growth, the secondary xylem continuously increases in thickness, a feature typical of woody plants, especially trees.

Cambial initials are composed of fusiform initials and ray initials. The former divide both in radial and tangential directions. In radial division of xylem and phloem mother cells, one of the divided cells always remains a fusiform initial, while another cell is transformed into a xylem or phloem

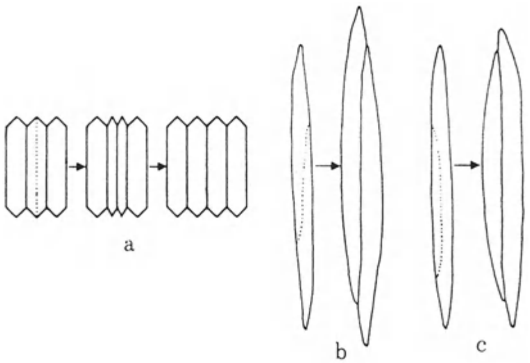

Fig. 5a–c. Pattern of the increase in fusiform initials. **a** Radial division; **b** pseudotransverse division; **c** lateral division. (Shimaji 1976b)

mother cell. The xylem and phloem mother cells divide further to give xylem on the inner side and phloem on the outer side, respectively.

In tangential division, fusiform initials first divide longitudinally, after which the initial cells formed divide radially (radial division) to give xylem tissues (stratification of hardwoods; Fig. 5a).

The risk of harmful mutations from errors occurring during the mitotic cycles of permanent initials that divide hundreds or even thousands of times in long-lived species has been stressed by Gahan (1988). Such a risk would be avoided if the initiating role could possibly pass to one of the nondetermined derivatives, although the molecular mechanism of this has not been elucidated.

In a different type of division the initial cell first divides up and down at the slightly S-shaped surface (pseudotransverse division; Fig. 5b), and the two cells formed grow contiguously in a tangential direction, following which both cells continuously divide (lateral division) in the radial direction as fusiform initials (Fig. 5c).

Enlargement of the cambial circle occurs mainly by an increase in the number of fusiform initials, but ray initials also increase, accompanied by the enlargement of the cambium. Bailey (1923) found that the diameters of fusiform initials (16 μm) and ray initials (14 μm) of a 1-year-old *Pinus strobus* increased to 42 and 17 μm, respectively, in a 60-year-old tree, while the number of fusiform initials ($n = 720$) and ray initials ($n = 70$) in the cambium of the 1-year-old tree increased to 23 000 and 8800, respectively, in the 60-year-old tree, indicating that the increase in the circumference of the cambium is largely due to the increase in the number of initials, especially fusiform initials.

The increase in ray initials occurs by a process of repeated tangential divisions of short fusiform initials formed by pseudolateral (Fig. 6a,b) and lateral divisions (Fig. 6c) of fusiform initials. In this process, short fusiform initials are rapidly contracted to form simple ray initials. Furthermore, short fusiform initials are generally divided at cross surfaces to give ray initials (Shimaji 1976b).

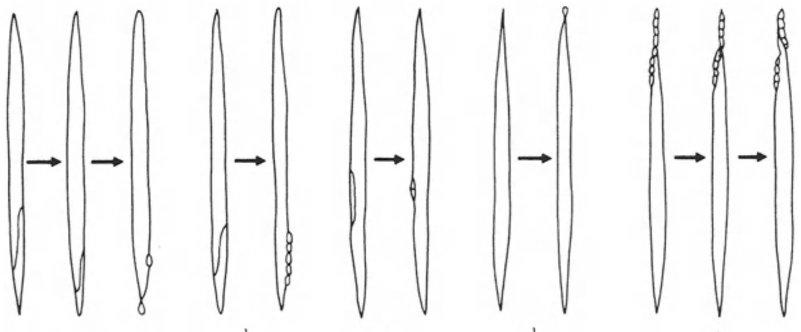

Fig. 6a–e. Pattern of the increase in ray initials. **a**,**b** By pseudolateral division; **c** by lateral division; **d** from a tip of fusiform initial; **e** by intrusive growth of a fusiform initial. (Shimaji 1976b)

In addition, the tip of a long fusiform initial can be divided to form ray initials (Fig. 6d), and by intrusive growth of a fusiform initial a group of ray initials is divided into two groups of initials that increase by division (Fig. 6e).

Newly formed constituent cells in the ray initials groups increase by repeated lateral divisions. The ray initials and fusiform initials are interwoven and contribute to the strength of the whole cambial fabric and also to that of the vascular tissues with their horizontal and vertical systems. The relative ratio between the two types of initials is therefore roughly maintained throughout the life of the tree.

The production of new rays by transverse divisions of the fusiform initials and then splitting and uniting of preexisting rays is considered to arise in response to complex axial and radial flows of developmental signals such as the polar auxin flow (Catesson 1994).

In temperate and warm-temperate zones, cambial activity has a certain periodicity, and active cell divisions and growth occur in the spring to summer seasons. Xylem produced in spring and early summer is called earlywood and is composed of relatively large xylem cells with thin walls. Xylem produced from late summer to autumn is called latewood. These cells are relatively small, and the cell walls are thicker than those of earlywood. Xylem composed of earlywood and latewood in temperate and warm-temperate zones therefore appears as annual rings of concentric circles on the cross-section of a stem.

Cell-length variations with season, age, and systematic position influence wood properties. Occasionally, false annual rings are formed in defoliated trees by disease or insect attack.

Wood produced in tropical rain forests generally has no annual rings, but sometimes contains false growth rings produced under different growth conditions in wet and dry seasons.

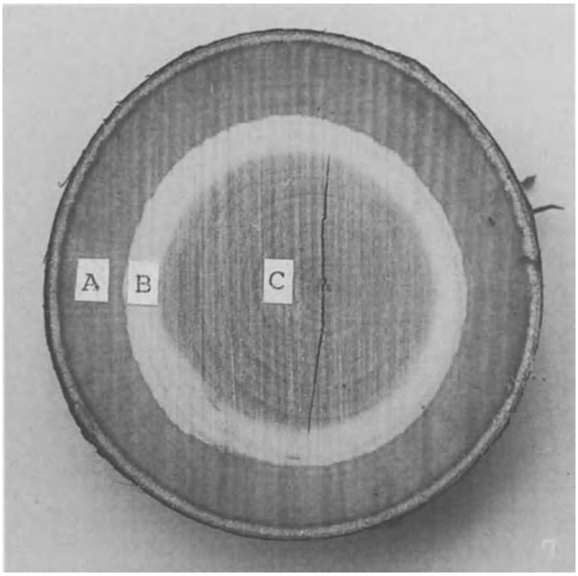

Fig. 7. A cross section of wood of *Prunus sargentii*. *A* Sapwood; *B* transition wood; *C* heartwood. (Courtesy of Dr. S. Ishida)

After a variable number of years, the central portion of the stem is inactivated; it becomes filled with resinous materials and species-characteristic phenolics and develops a dark color. This part is called heartwood; all cells in the heartwood are dead. However, in some species of *Picea*, *Abies*, *Tsuga*, and *Populus*, the heartwood is not dark and is difficult to identify. The xylem outside the heartwood contains living cells (mainly ray parenchyma cells) and is called sapwood. The water content in sapwood is generally higher than in heartwood. Heartwood generally contains a considerable amount of extractives. In the heartwood of some hardwoods such as white oak, tyloses develop in the vessels. The innermost sapwood is gradually transformed into heartwood during growth, and this xylem tissue is called intermediate or transition wood. Transition wood is generally pale in color and contains extractives in the parenchyma cells and tyloses in the vessels (Higuchi 1992; Fig. 7).

1.1 Microscopic Structure

1.1.1 Softwoods (Conifers)

In softwoods, about 95% of the constituent cells are long, fibrous tracheids which are arranged longitudinally in the stem (Table 1).

Tracheids serve to transport water from the roots to growing points and leaves; they also have a mechanical function in supporting the tree. Earlywood

Table 1. Volume percentages of cell elements in Japanese gymnosperm woods. (Sudo 1976)

Species	Tracheids	Parenchyma	Rays
Pinus densiflora	95.87		3.43
P. thunbergii	97.03		1.89
Picea jezoensis	95.22		4.25
Abies sachalinensis	95.80		4.20
A. firma	93.86	0.29	5.81
Thuja standishii	97.44		2.56
Thujopsis dolabrata	96.61	0.81	3.21
Chamaecyparis obtusa	97.09	0.58	2.33
C. pisifera	96.52	0.39	3.09
Pseudotsuga japonica	94.71		4.73
Cryptomeria japonica	97.20	0.80	2.00
Larix leptolepis	95.16		4.58
Sciadopitys verticillata	98.61		1.39
Taxus cuspidata	96.98		3.02
Ginkgo biloba	92.74	0.25	7.01

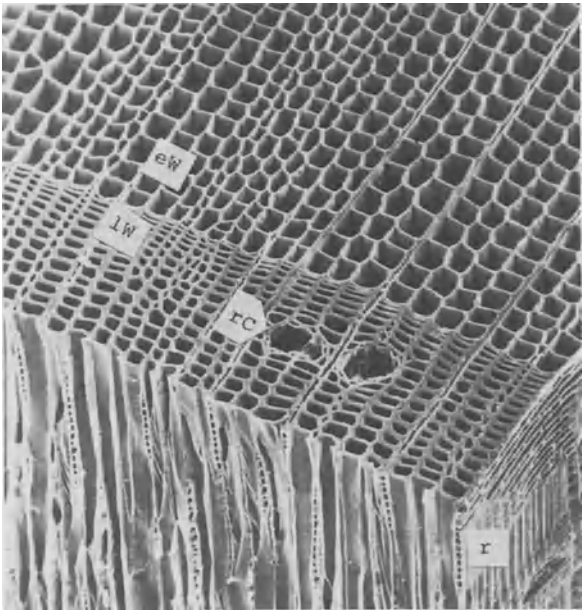

Fig. 8. Scanning electron micrograph of the wood of *Picea ezoensis*. Tracheid size changes abruptly from early wood (*ew*) to latewood (*lw*). Ray (*r*) on the radial surface, and resin canals (*rc*) on the transverse surface. (Courtesy of Dr. H. Saiki)

tracheids are mainly involved in water transport, while latewood tracheids have a mechanical function. Pits, spiral thickening, and spiral checking are present in the tracheids. Pits are passages for water between neighboring cells. Bordered pits occur between tracheids, while simple pits occur between parenchyma cells. Half-bordered pits connect a parenchyma cell with a tracheid (Saiki 1982; Fig. 8).