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PHENOLOGICAL COMPARISON OF THE ONSET OF VESSEL FORMATION BETWEEN RING-POROUS AND DIFFUSE-POROUS DECIDUOUS TREES IN A JAPANESE TEMPERATE FOREST

by

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SUMMARY

Initiation of vessel formation and vessel maturation indicated by secondary wall deposition have been compared in eleven deciduous broad-leaved tree species. In ring-porous species the first vessel element formation in the current growth ring was initiated two to six weeks prior to the onset of leaf expansion, and secondary wall deposition on the vessel elements was completed from one week before to three weeks after leaf expansion. In diffuse-porous species, the first vessel element formation was initiated two to seven weeks after the onset of leaf expansion, and secondary wall deposition was completed four to nine weeks after leaf expansion. These results suggest that early maturation of the first vessel elements in the ring-porous species will serve for water conduction in early spring. On the contrary, the late maturation of the first vessel elements in the diffuse-porous species indicates that no new functional vessels exist at the time of the leaf expansion.

Key words: Diffuse-porous, phenology, ring-porous, vessel element formation, water conduction.

INTRODUCTION

For deciduous trees in temperate regions the maintenance of water transport at the time of leaf expansion is the most important precondition to guarantee the current year's growth. The pattern of water transport in a trunk is remarkably different between ring-porous wood and diffuse-porous wood (Zimmermann 1983). Ring-porous trees transport water mainly through large vessels in the outermost growth ring, while water is transported through vessels of several outer growth rings in diffuse-porous trees (Ladefoged 1952; Kozlowski & Winget 1963; Chaney & Kozlowski 1977; Ellmore & Ewers 1986).

Xylem differentiation patterns are also different between ring-porous and diffuse-porous species. In ring-porous species, xylem and phloem start to differentiate simultaneously (Munting & Willemse 1987). In some ring-porous species, first vessel elements in the current growth ring develop from overwintering xylem derivatives in the cambial zone (Tepper & Hollis 1967; Zasada & Zahner 1969; Imagawa & Ishida 1972b).

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Table 1. Height and diameter at breast height of sampled trees

Botanic name	Pore pattern*	Sample no.	Height (m)	Diameter (cm)**	Date*** L	V	W
Quercus serrata Thunb. ex Murky	R	169	15	30.8	4/29	4/6	5/4
		173	13	25.1	4/29	4/7	5/4
		180	11	21.2	4/23	4/7	5/5
		182	12	24.1	4/21	4/6	5/4
		185	12	23.4	4/21	4/13	4/28
Quercus acutissima Carruth.	R	165	12	24.3	4/29	4/6	5/20
		167	14	25.2	4/29	4/6	5/12
		171	13	28.9	4/23	3/30	5/14
		186	15	22.5	5/4	4/6	5/12
Castanea crenata Sieb. et Zucc.	R	162	13	23.7	4/21	4/6	4/29
		168	11	22.0	4/29	4/6	5/4
		175	11	21.8	4/23	3/30	4/30
		177	11	20.0	4/30	3/30	4/30
Zelkova serrata (Thunb.) Makino	R	176	14	21.2	4/23	3/30	4/23
		178	17	35.3	4/29	3/30	4/29
		179	15	22.6	4/23	3/30	4/30
		181	12	21.0	5/4	3/24	4/21
		213	10	19.8	4/13	3/24	4/28
		214	8	19.1	4/21	3/15	4/13
		215	10	20.3	4/28	3/24	4/21
Hovenia dulcis Thunb.	R	195	10	19.1	5/5	4/23	5/20
		196	16	23.1	5/5	4/23	5/20
		197	16	22.7	5/5	4/23	5/14
		198	14	26.0	5/5	4/23	5/20
Juglans mandshurica Maxim	D	192	13	23.0	4/14	5/14	5/26
var. *sachalinensis* (Miyabe et Kudo)		193	13	23.4	4/2	5/14	6/3
Kitamura		194	16	41.2	4/2	5/14	6/3
Magnolia obovata Thunb.	D	199	9	25.2	4/2	5/14	6/3
Prunus buergeriana Miq.	D	204	9	30.8	4/7	4/23	5/14
		206	12	28.0	4/7	4/30	5/14
		208	10	18.8	4/7	4/30	5/14
		209	10	20.1	4/7	4/30	5/14
		210	8	18.9	4/14	5/14	5/23
		211	8	20.4	41/4	4/30	5/14
		212	9	20.7	4/7	5/5	5/14
Prunus verecunda (Koidz.) Koehne	D	164	15	29.9	4/21	5/12	5/20
		166	10	21.4	4/21	5/12	5/12
		170	10	20.9	4/23	5/12	5/25
		183	13	22.4	4/28	5/19	5/25
		191	8	19.0	4/21	5/12	5/25
		205	14	25.7	4/23	5/20	5/26
Styrax japonica Sieb. et Zucc.	D	200	6	14.6	4/13	5/12	6/3
		201	8	17.4	4/13	5/14	6/3
		202	7	14.2	4/14	5/23	6/3
Cornus brachypoda C.A. Mey.	D	163	13	23.3	4/14	6/3	6/8
		172	8	22.7	4/13	6/3	6/8
		174	13	28.0	4/13	5/25	6/3
		187	8	17.5	4/13	5/25	6/3

* R = ring-porous; D = diffuse-porous
** Measured at breast height (1.3 m).
*** Sampling dates. L = leaf expansion; V = vessel initiation; W = secondary wall formation.

At the first stage of vessel formation, vessel elements enlarge rapidly in the tangential direction, followed by gradual radial expansion (Zasada & Zahner 1969; Wakuta et al. 1973). In *Kalopanax pictus*, which is a ring-porous species, vessel elements mature almost simultaneously with the break of winter buds (Imagawa & Ishida 1972a). In diffuse-porous species, however, phloem differentiation precedes xylem differentiation (Evert 1963; Davis & Evert 1970; Barnett 1992). In *Salix viminalis*, which is a diffuse-porous species, phloem development apparently precedes flowering and is followed by leaf flush and xylem differentiation (Senneby-Forsse 1986).

As introduced here, vessel formation patterns between ring-porous trees and diffuse-porous trees may be phenologically different in relation to the first water conduction. To clarify the correlation between the onset of vessel formation and leaf expansion, we investigated the initiation of the first vessel element formation in the current growth ring and its maturation in eleven deciduous broad-leaved tree species.

MATERIALS AND METHODS

Forty-eight normally growing trees of eleven deciduous broad-leaved tree species (Table 1) were selected in a secondary deciduous forest of the University Forest, Ishinomaki Senshu University, Miyagi Prefecture, Japan (N 141° 18", E 38° 27"): 24 trees of five ring-porous species, i.e., *Quercus serrata* Thunb. ex Murky (5 trees), *Quercus acutissima* Carruth. (4), *Castanea crenata* Sieb. et Zucc. (4), *Zelkova serrata* (Thunb.) Makino (7) and *Hovenia dulcis* Thunb.(4), and 24 trees of six diffuse-porous species, i.e., *Juglans mandshurica* Maxim. var. *sachalinensis* (Makino et Kudo) Kitamura (3), *Magnolia obovata* Thunb. (1), *Prunus buergeriana* Miq. (7), *Prunus verecunda* (Koidzumi) Koehne (6), *Styrax japonica* Sieb. et Zucc. (3) and *Cornus brachypoda* C.A. Mey. (4). All these trees reached the crown layer of the secondary forest. Their heights were 6–17 m, and the diameters at breast height were 14.2–41.2 cm (Table 1).

Small cubic wood samples (about 2 cm in diameter), containing outer bark, secondary phloem, cambium and one to several outermost annual rings of secondary xylem, were cut off from erect trunks around breast height (1.3 m high) using a chisel and a hammer. Sampling was done at five to nine days intervals from all trees of each species between March 7 and June 24, 1994. To minimize the influence of wound response by sampling, each cubic block was cut off at some distance away from the upper and lower areas of previously sampled points. As a result, 14 to 16 samples were taken from each tree. Sampling points of those wood blocks were distributed in an area of trunk between 80 and 180 cm high from ground level. Actually, visual effects such as tyloses formation, abnormal tissue formation or deposition of special substances in wood tissue caused by weekly wood block sampling were not observed, at least not till the time of the first vessel formation. At the time of each sampling, bud condition at the top of the tree crown was observed by means of a binocular telescope (× 20) to record when new leaves began to expand from winter buds.

Collected wood samples were fixed in FAA, embedded in celloidin, cut in the transverse plane at 20 μm thick with a sliding microtome and double-stained with saffranin O and fast green FCF. The onset of vessel formation in the current growth ring was marked when tangentially enlarged cells were recognized on the xylem side in the cambial zone. The maturation of the first vessel elements in the current growth ring, indicated by the deposition of secondary walls, was determined by means of the brightness of the vessel element walls and the fibrous tissues around them under a polarized microscope.

RESULTS

Onset of leaf expansion from winter buds

In the ring-porous species, the onset of leaf expansion from winter buds was observed from April 13 to May 5 (Table 1 & Fig. 1), and flowering began within a few days to several weeks after leaf expansion. In *Zelkova serrata* new leaves began to expand from April 13 to May 4, the earliest among the ring-porous species. Leaves of *Quercus serrata* and *Castanea crenata* began to expand from April 21 to April 30. In *Quercus acutissima* new leaves began to expand from April 23 to May 4. *Hovenia dulcis* was the latest among the investigated species in the onset of leaf expansion (May 5).

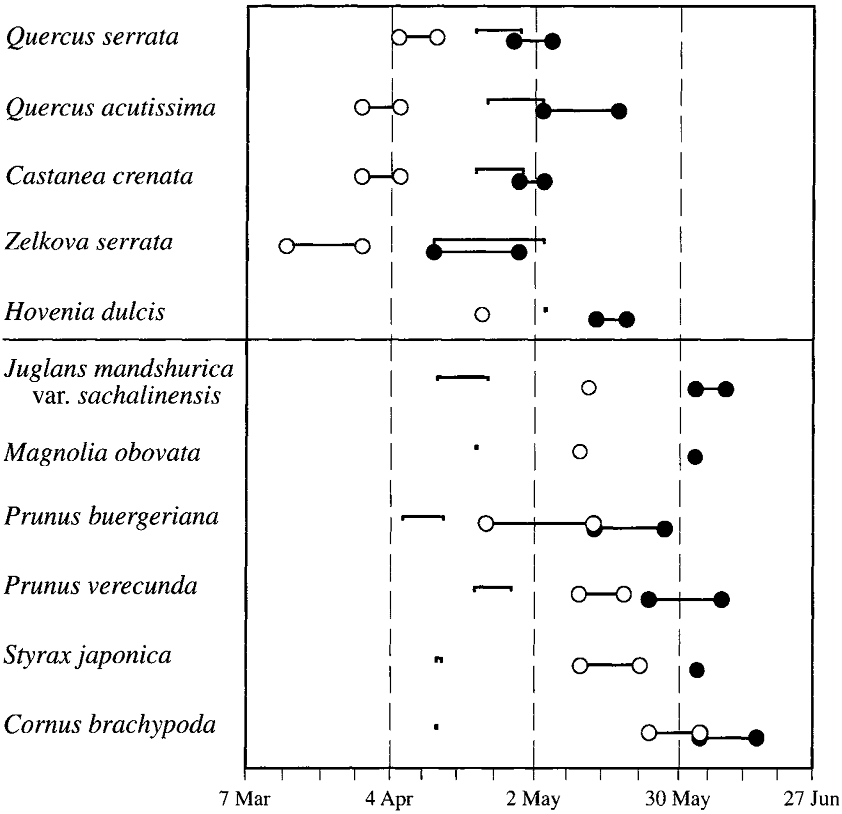


Fig. 1. Phenology of the onset of leaf expansion (▬), the initiation of first vessel element formation (●–●), and the completion of secondary wall deposition on the first vessel elements (○–○). Horizontal bars indicate the range of variation in each species.

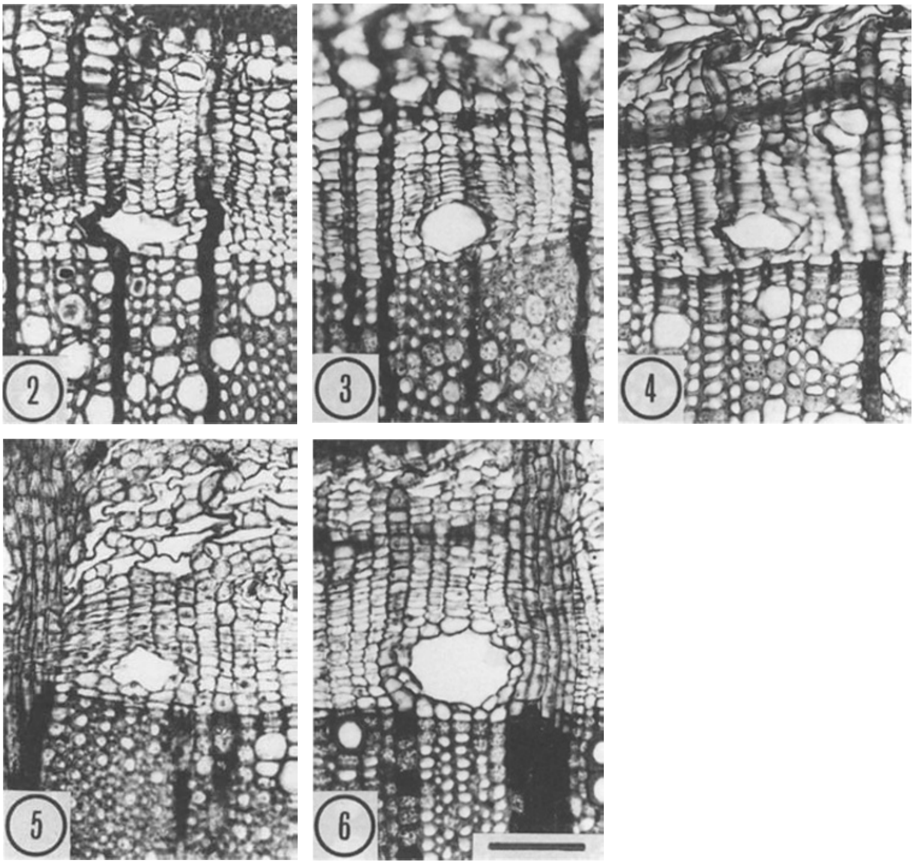


Fig. 2–6. Light microphotographs of cross sections showing enlarging first vessel elements in current growth rings of five ring-porous species. – 2: *Quercus serrata* (April 6); a vessel element is located just to the outside of the smooth ring boundary and begins to enlarge tangentially. – 3: *Q. acutissima* (April 6); a vessel element begins to enlarge. – 4: *Castanea crenata* (March 30); a vessel element begins to enlarge tangentially. – 5: *Zelkova serrata* (March 15); a vessel element begins to enlarge tangentially. – 6: *Hovenia dulcis* (April 23); a vessel element enlarges. — Scale bar is 100 μm.

The diffuse-porous species started leaf expansion slightly earlier than the ring-porous species (April 7 to April 28, Table 1 & Fig. 1), and flowering began within a few to several weeks after the leaf expansion. *Prunus buergeriana* was the earliest among the investigated species; new leaves began to expand from April 7 to April 14. Both in *Styrax japonica* and *Cornus brachypoda*, new leaves began to expand on April 13 or April 14, followed by *Juglans mandshurica* var. *sachalinensis* (April 14 to April 28). *Magnolia obovata* started the leaf expansion on April 21. *Prunus verecunda* was the latest among the diffuse-porous species (April 21 to April 28).

In both ring- and diffuse-porous species there is no significant relationship between tree height and the date of leaf expansion. In all diffuse-porous species, and *Hovenia dulcis*, the date of leaf expansion was fairly stable within the species without any rela-

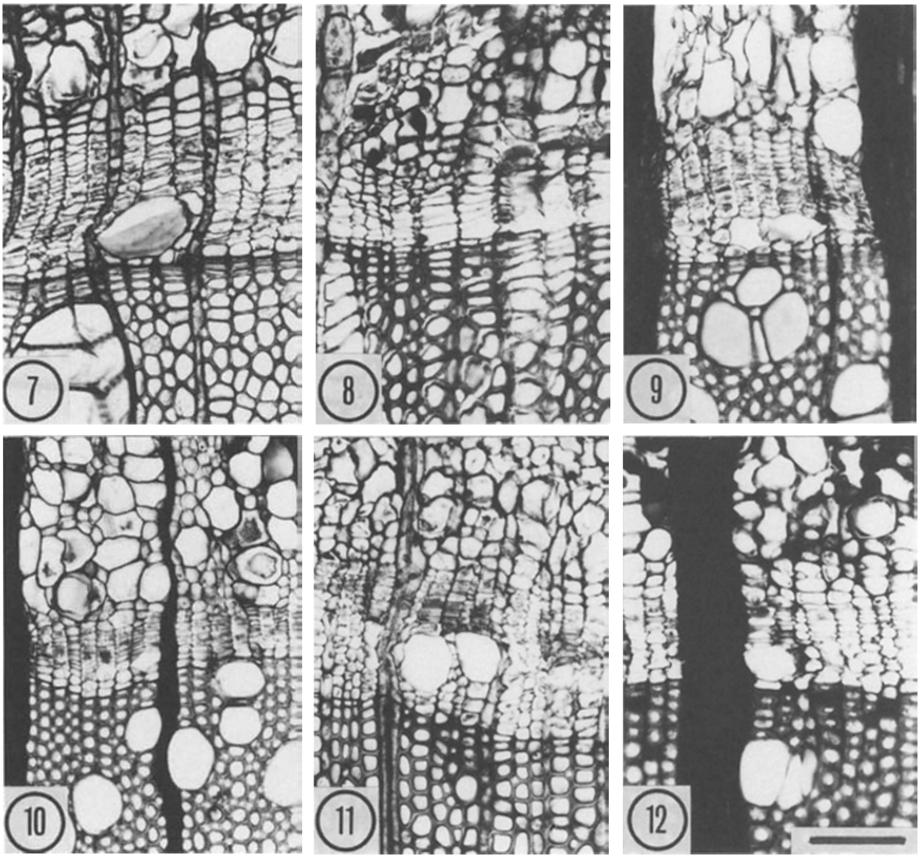


Fig. 7–12. Light microphotographs of cross sections showing enlarging first vessel elements in current growth rings of six diffuse-porous species. – 7: *Juglans mandshurica* var. *sachalinensis* (May 14); a vessel element is enlarging. – 8: *Magnolia obovata* (May 12); four vessel elements beginning to enlarge are located just to the outside of the growth ring boundary. They are slightly larger than the other cells in the xylem side of the current growth ring. – 9: *Prunus buergeriana* (May 14); two vessel elements, one of them enlarges tangentially and the other is round. – 10: *Prunus verecunda* (May 19); a small vessel element is located just to the left of a ray and enlarges tangentially. – 11: *Styrax japonica* (May 12); two round or ovate vessel elements are located 2 or 3 cells to the outside of the growth ring boundary. – 12: *Cornus brachypoda* (June 3); an ovate vessel element, located just to the outside of the growth ring boundary, is attached to the right side of a ray tissue. — Scale bar is 100 μm.

tion to tree height. In other ring-porous species, taller trees of some species (for example, *Quercus serrata*) might expand leaves later than smaller trees, while smaller trees of *Castanea crenata* expanded leaves later than the taller tree, and in *Zelkova serrata* the date of leaf expansion was quite variable without any relation to tree size.

Initiation of the first vessel formation

The ring-porous species developed the first vessel elements several weeks earlier than the diffuse-porous species (Table 1 & Fig. 1). In *Zelkova serrata* the first vessel elements began to enlarge from March 15 to March 30 (Fig .5), which was the earliest among the investigated species. *Quercus acutissima* began to form the first vessel elements from March 30 to April 6 (Fig. 3), and followed by *Castanea crenata* (March 30 to April 6; Fig. 4) and *Q. serrata* (April 6 to April 13; Fig. 2). In *Hovenia dulcis* the first vessel elements began to enlarge on April 23 (Fig. 6), the latest among the ring-porous species.

At the same time as in *Hovenia dulcis, Prunus buergeriana* began to form the first vessel elements (April 23 to May 14; Fig. 9). It was the earliest among the diffuse-porous species. In *Magnolia obovata* (Fig. 8), *Juglans mandshurica* var. *sachalinensis* (Fig. 7), *Prunus verecunda* (Fig. 10), and *Styrax japonica* (Fig. 11), the first vessel elements began to enlarge on May 12. *Cornus brachypoda* was the latest among the investigated species in the initiation of the first vessel elements (May 25 to June 3; Fig. 12).

In all five ring-porous species and two diffuse-porous species, *Prunus buergeriana* and *P. verecunda*, the first vessel elements were located a few cells to the outside of the boundary between previous and current annual rings (Fig. 2–6, 10, 11). However in the other four diffuse-porous species, the first vessel elements tended to be located just to the outside of the growth ring boundary (Fig. 7–9, 12). The cells at the phloem side in the diffuse-porous species were larger than those in the ring-porous species.

Completion of secondary wall deposition in the first vessel elements

In the ring-porous species, the first vessel elements and the fibrous tissue encircling them completed secondary wall deposition three to seven weeks after the initiation of vessel element formation (Fig. 1, 13–22). In *Zelkova serrata*, the first vessel elements completed secondary wall deposition from April 13 to April 30 (Fig. 19, 20). It was the earliest among the investigated species, and followed by *Quercus serrata* (Fig. 13, 14) and *Castanea crenata* (Fig. 17, 18). In *Q. acutissima* the first vessel elements completed secondary wall deposition from May 4 to May 20 (Fig. 15, 16). This was later than in *Q. serrata*, in spite of the earlier initiation of vessel elements in *Q. acutissima*. *Hovenia dulcis* was the latest among the ring-porous species; the first vessel elements completed secondary wall deposition from May 14 to May 20 (Fig. 21, 22).

In the diffuse-porous species, the first vessel elements completed secondary wall deposition one to five weeks after their initiation (Fig. 1, 23–34). In *Prunus buergeriana* the first vessel elements completed secondary wall deposition from May 14 to May 26 (Fig. 27, 28), followed by *P. verecunda* (Fig. 29, 30). In *Styrax japonica* (Fig. 31, 32), *Magnolia obovata* (Fig. 25, 26), *Juglans mandshurica* var. *sachalinensis* (Fig. 23, 24) and *Cornus brachypoda* (Fig. 33, 34), the first vessel elements completed secondary wall deposition from June 3 to June 9. Especially in *Styrax japonica* and *Juglans mandshurica* var. *sachalinensis*, the area of fibrous tissue having completed secondary wall was wider than those in the other investigated species.

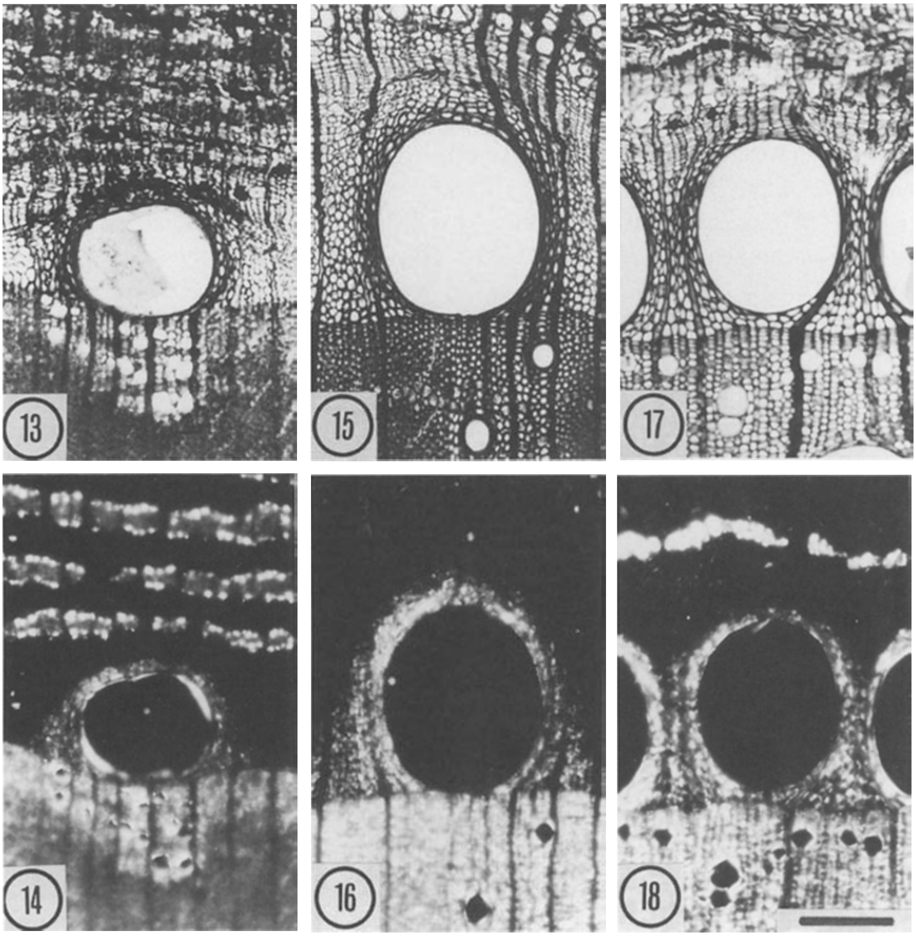
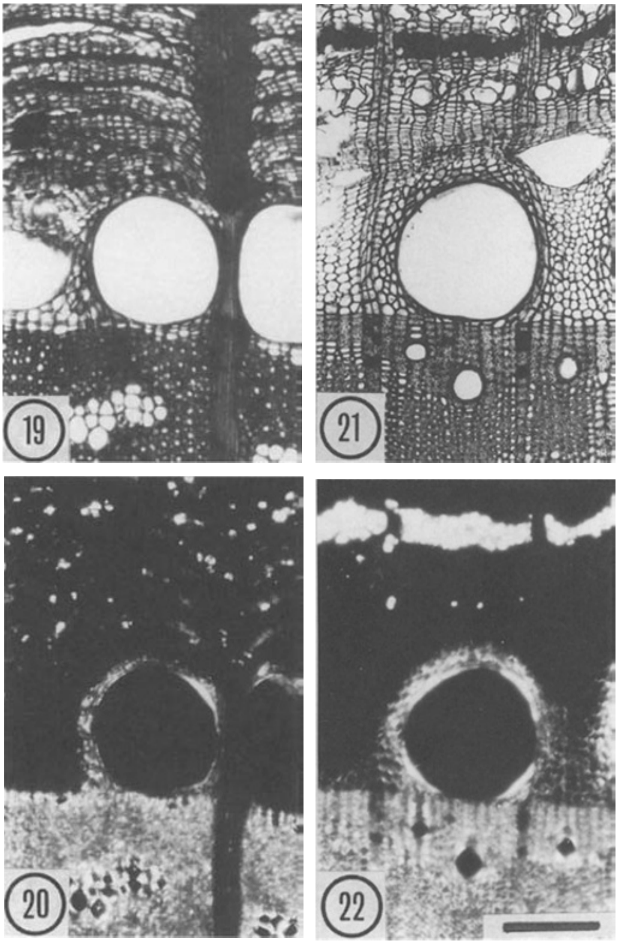


Fig. 13–22. Light (Fig. 13, 15, 17, 19, 21) and polarized-light (Fig. 14, 16, 18, 20, 22) microphotographs of cross sections showing the maturation of the first vessel elements in the current growth ring of ring-porous species. – 13, 14: *Quercus serrata* (May 5). – 15, 16: *Quercus acutissima* (May 4). – 17, 18: *Castanea crenata* (April 30). – 19, 20: *Zelkova serrata* (April 28). – 21, 22: *Hovenia dulcis* (May 20). — Scale bars are 200 μm.

Relation between the onset of leaf expansion and vessel element development

In the ring-porous species, first vessel elements began to enlarge two to six weeks prior to the leaf expansion, and completed secondary wall deposition nearly at the same time as leaf expansion (Fig. 35). However, in the diffuse-porous species the first vessel elements began to form two to seven weeks after leaf flushing, and completed secondary wall deposition four to nine weeks after leaf expansion (Fig. 35).



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DISCUSSION

The correlation between the development of first vessel elements and the expansion of new leaves is clearly different between the ring-porous and the diffuse-porous species. In the ring-porous species, the first vessel elements in the current growth ring began to enlarge one to six weeks prior to leaf expansion. In the diffuse-porous species, on the contrary, new leaves began to expand two to seven weeks prior to the initiation of vessel element formation. Fahn and Werker (1990) reported that the cambial activity in diffuse-porous species started at the base of winter buds and then spread downward slowly. In our study, it was clear that the first vessel elements in the diffuse-porous species start to form clearly later than leaf expansion. Therefore the delay of vessel formation *vis-à-vis* leaf expansion in the diffuse-porous species may be due to the late cambial reactivation in the lower part of the trunk, where we obtained wood samples.

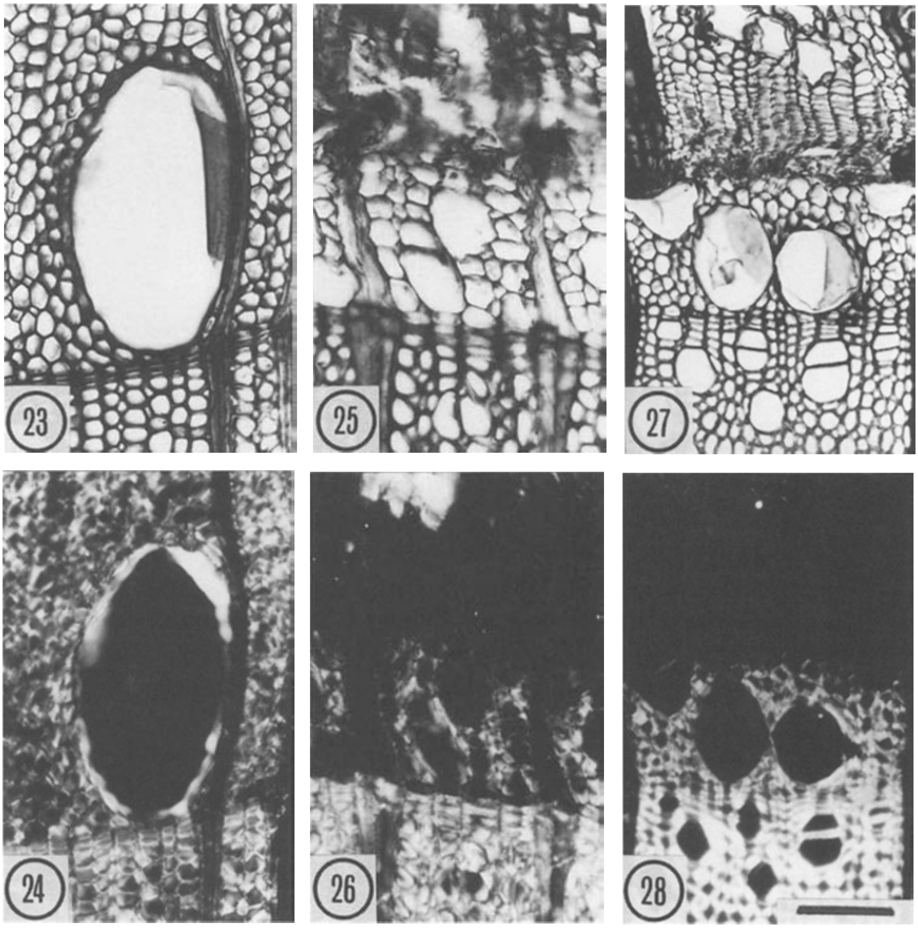
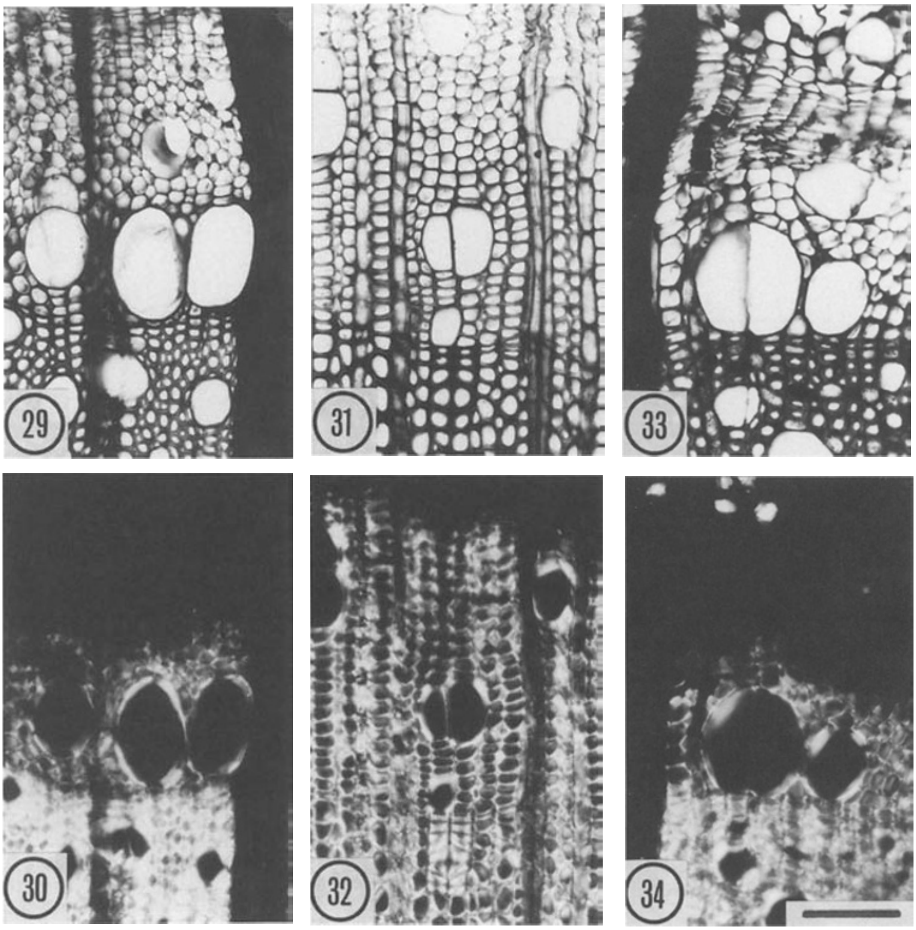


Fig. 23–34. Light (Fig. 23, 25, 27, 29, 31, 33) and polarized-light (Fig. 24, 26, 28, 30, 32, 34) microphotographs of cross sections showing the maturation of the first vessel elements in the current growth ring of diffuse-porous species. – 23, 24: *Juglans mandshurica* var. *sachalinensis* (June 3). – 25, 26: *Magnolia obovata* (June 3). – 27, 28: *Prunus buergeriana* (May 26). – 29, 30: *Prunus verecunda* (May 25). – 31, 32: *Styrax japonica* (June 3). – 33, 34: *Cornus brachypoda* (June 15). — Scale bars are 100 μm.

It has been reported that the cambium of ring-porous species becomes active at the time of bud opening (Tepper & Hollis 1967). In our study, however, first vessel elements in the ring-porous species began to form two to six weeks earlier than leaf flushing. The existence and activity of overwintering cells seem to be important in the formation of first vessel elements before leaf expansion. Overwintering cells are the cells that have divided from the cambium at the end of the last growing season, and overwinter without differentiating into xylem or phloem. In the ring-porous species in our study the first vessel elements in the current growth ring may develop from overwintering cells, as shown in *Fraxinus americana* (Tepper & Hollis 1967), in *Quercus*



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rubra (Zasada & Zahner 1969) and in six ring-porous species (Imagawa & Ishida 1972b). Diffuse-porous species are also known to possess overwintering cells. However, these cells are considered to contribute only to phloem production (Evert 1963; Barnett 1992).

Plant growth hormones are also important to control the reactivation of cambial activity. Digby and Wareing (1966) reported that in *Ulmus glabra* (ring-porous) tryptophan, which is the precursor of IAA, was extracted from upper, middle and bottom parts of the trunk several weeks before bud swelling. In *Populus trichocarpa* (diffuse-porous), in contrast, the substance was not detected at the same time, and extracted

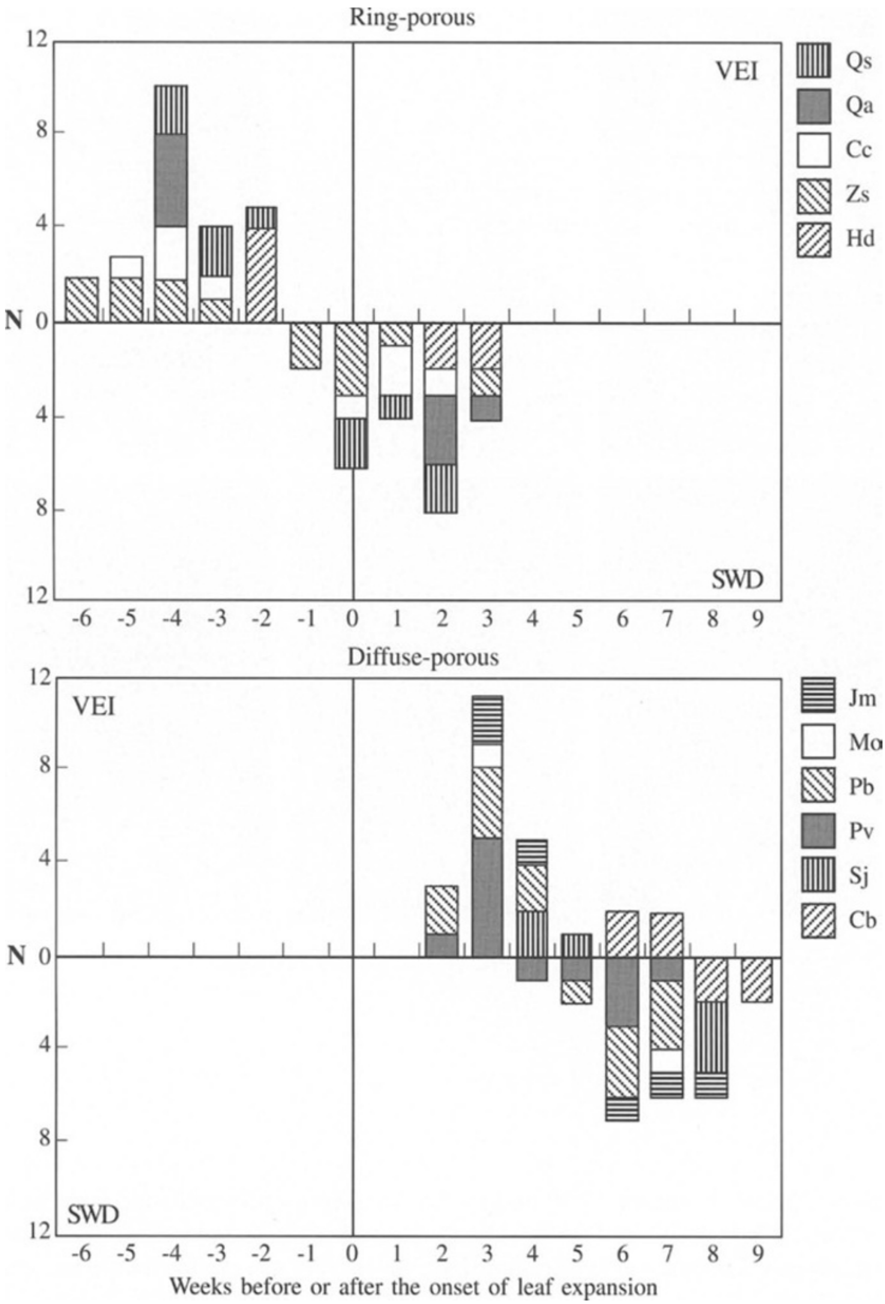

Fig. 35. Relation of the initiation of vessel element formation (VEI) and the completion of secondary wall deposition in the first vessel elements (SWD) with the onset of leaf expansion. N = number of individual trees. Qs = *Quercus serrata*; Qa = *Quercus acutissima*; Cc = *Castanea crenata*; Zs = *Zelkova serrata*; Hd = *Hovenia dulcis*; Jm = *Juglans mandshurica* var. *sachalinensis*; Mo = *Magnolia obovata*; Pb = *Prunus buergeriana*; Pv = *Prunus verecunda*; Sj = *Styrax japonica*; Cb = *Cornus brachypoda*.

only from the upper part of the trunk at the time of bud swelling. Thus the different manner of correlation between the initiation of vessel element formation and leaf expansion of the ring-porous species against the diffuse-porous species may also be induced by the different distribution of hormonal substance through the trunk of trees.

Yata et al. (1970) reported that in the differentiating vessel elements of *Populus nigra* var. *italica* the disintegration of end walls occurred soon after the completion of secondary wall deposition in the lateral walls. Thus we can regard the vessel elements which have finished secondary wall deposition ready for water conduction. In the diffuse-porous species in our study, new leaves expanded more than one month before the first vessel elements completed secondary wall deposition. Thus the first vessel elements in the diffuse-porous species cannot contribute to the initial uptake of water when new leaves begin to expand. While in the ring-porous species, the first vessel elements completed secondary wall deposition one week before to three weeks after the leaf expansion. This result suggests that the first vessel elements in the ring-porous species contribute to water transport soon after leaf expansion. It has been reported that ring-porous species transport water mainly through the wide vessels in the earlywood of the current growth ring, whereas in diffuse-porous species water passes through the vessels of several outermost growth rings (Kozlowski & Winget 1963; Chaney & Kozlowski 1977; Ellmore & Ewers 1986). Our results support these observations and phenologically confirm the difference in the vessel formation patterns between ring-porous and diffuse-porous woods.

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