

**Relationship between vessel formation and leaf phenology
in temperate broad-leaved trees**

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Chapter 1 General introduction

1.1 Introduction

In a temperate zone with four seasons, trees begin photosynthesis and water transport in spring after winter dormancy, and so spring growth is important for trees. Both leaves and vessels start to develop in the spring and hence it is a key factor for each species to decide when to form photosynthetic and water conducting organs. In this context, the association between leaf expansion and vessel formation has been discussed by various researchers (e.g., Ladefoged 1952; Lechowicz 1984; Begum et al. 2013; Panchen et al. 2014).

In temperate trees, woody tissue is characterized by one of three types of porosity (Wheeler et al. 1989); in phenological phase, ring-porous species form wider vessels in the early phase of annual ring formation and then form narrower vessels; diffuse-porous species form vessels with similar diameter across the annual ring; semi-ring-porous species are intermediate in character. In addition to these three types, radial-porous wood is characterized by vessels in radial alignment (Wheeler et al. 1989; Hayashi 1991; Itoh 1995). The differences in the size and distribution of pores lead to differences in the efficiency of water conduction.

The timing of vessel formation and leaf emergence differs between ring-porous and diffuse-porous trees growing in the same area (Wareing 1950, 1951; Ladefoged 1952; Suzuki et al. 1996, 2000; Frankenstein et al. 2005). Radial growth begins before or at the time of leaf appearance in ring-porous species, whereas it begins after leaf appearance in diffuse-porous species, revealed by terms of diameter growth (Friesner 1942; Komiyama et al. 1987; Komiyama 1991; Maruyama et al. 1992), cambial activity (Priestley and Scott 1936; Ladefoged 1952), and the first-formed vessels maturation (Suzuki et al. 1996, 2000). Studies of seasonal changes in vessel formation in evergreen species are very limited. The ring-porous evergreen species, *Castanopsis sieboldii*, and the radial-porous evergreen species, *Lithocarpus edulis*, have been reported to complete secondary wall deposition in the stems at the time of leaf appearance (Hirano 1998).

Many previous studies of wood anatomy have not considered the stages of leaf development or patterns of leaf appearance. Conversely, many ecological studies have not

investigated the process of vessel formation or vessel microstructure. To clarify the relationship between leaf phenology and vessel formation from the ecophysiological viewpoint, both traits need to be examined concurrently to determine how they relate to photosynthesis and water transport. Such studies in natural forests are very limited, as are studies of seasonal changes in vessel formation in individual trees over a year in both the twigs and stems of ring- and diffuse-porous species. Suzuki et al. (1996, 2000) examined seasonal vessel formation in stems only, and Ladefoged (1952) examined cell division, but not lignification, in different trees over the course of a year. In addition, it is important to specify the stage of vessel formation for discussing the difference in xylem formation between ring- and diffuse-porous species. Previous studies have reported that ring-porous species have overwintering cells (Tepper and Hollis 1967; Zasada and Zahner 1969; Imagawa and Ishida 1972), and that both ring- and diffuse-porous species initiate cambial cell division simultaneously after bud break, but overwintering cells in the cambial zone are enlarged prior to cell division in ring-porous species (Frankenstein et al. 2005).

I attempt to clarify how plant functions relating photosynthesis and water transport are coordinated developmentally for species with different porosity and vessel arrangement, comparing the timing of leaf phenology and vessel formation in twigs and stems from an ecophysiological viewpoint. Species with different types of porosity and vessel arrangement coexist throughout the temperate zone, and it is still unclear how porosity and vessel arrangement affects species' adaptation to their environment. Therefore, I also attempt to clarify this by determining how different aspects of plant function are coordinated developmentally.

In research of examining the relationship between leaf phenology and vessel formation, sampling is always problematic because frequent examination to trace the periodic process of vessel formation may cause considerable damage to the trees studied. Chapter 2 described the increment borer method and vessel lignification in diffuse-porous *Cercidiphyllum japonicum* and ring-porous *Zelkova serrata*, in detail. The degree of tree wounding, simplicity of collecting wood core samples, and quality of wood core samples obtained with an increment borer are investigated.

Chapter 3 attempts to clarify how ring porosity affects species' adaptation to their environment for species of ring- and diffuse-porous deciduous trees in a temperate forest where both types of species grow in the same forest stand, by determining the temporal relationship between leaf phenology and vessel formation in twigs and stems, relating photosynthesis and water transport.

To elucidate the functional linkage between intra-annual changes in vessel diameter and leaf emergence pattern, I examined the temporal relationships between vessel formation and leaf phenology throughout the early stage to the later stage of annual ring formation in ring-porous trees and compared them with those in diffuse-porous trees in temperate deciduous forests in Chapter 4.

In Chapter 5, seasonal relationship between leaf appearance and twig and stem vessel formation was studied in evergreen and deciduous tree species with different porosity and vessel arrangement in a temperate forest, where all species grew in the same forest stand. This chapter attempts to clarify how different leaf habits and variation in porosity and vessel arrangement link to species' adaptation to their environment.

Below, study sites and sample trees examined, and observation of leaf phenology were described.

1.2 Study sites and sample trees

1.2.1 Ashiu Forest Research Station

The study was conducted at the Kyoto University Forest in Ashiu, Kyoto Prefecture, central Japan at 35°18'N, 135°43'E. This is a cool temperate forest, and the dominant species are *Fagus crenata* and *Quercus mongolica* var. *grosseserrata*. The mean annual temperature over 30 years (1971–2000) at the meteorological station (356-m elevation) was 11.7 °C with mean minimum and maximum monthly temperatures of –0.2 and 24.2 °C, respectively. Mean annual precipitation was 2,353 mm, most of which occurred as winter snow (Forest Research Station of Graduate School of Agriculture, Kyoto University 2002). The study site is at 650–670 m

Table 1-1 Description of sample trees in Ashiu, including diameter at breast height, tree height, and number of trees during 2004 and 2005.

Porosity ^a	Species ^b	Family	Leaf emergence pattern ^c	Branching ^d	Transition between pore and non-pore zone	DBH ^e (cm)		Tree height (m)		Number of trees (trees)			
						Years: 2004	2005	2004	2005	twig		stem	
										2004	2005	2004	2005
Ring-porous	<i>Quercus mongolica</i> Fisch. ex Ledeb. var. <i>grosseserrata</i> (Blume) Rehder & E.H.Wilson	Fagaceae	Flush	Monopodial	Abrupt	28-47	28-39	13-22	13-22	1	3	5	4 ^f
	<i>Zelkova serrata</i> (Thunb.) Makino	Ulmaceae	Flush	Monopodial	Abrupt	21-30	21-30	13-15	13-15	1	4	5	5
	<i>Castanea crenata</i> Siebold & Zucc.	Fagaceae	Intermediate	Sympodial	Gradual	18-59	21-56	12-21	12-21	1	3	5 ^g	5
	<i>Fraxinus mandshurica</i> Rupr.	Oleaceae	Intermediate	Monopodial	Gradual	20-31	20-31	13-17	13-17	1	5	5	5
	<i>Hovenia tomentella</i> (Makino) Nakai	Rhamnaceae	Intermediate	Sympodial	Gradual	-	18-38	-	13-24	-	1	-	5
	<i>Aesculus turbinata</i> Blume	Hippocastanaceae	Flush	Monopodial		19-44	19-44	9-14	9-14	1	5	5	5
Diffuse-porous	<i>Fagus crenata</i> Blume	Fagaceae	Flush	Monopodial		41-50	41-50	14-21	14-21	1	3	5 ^h	5
	<i>Betula grossa</i> Siebold & Zucc.	Betulaceae	Intermediate	Sympodial		20-34	20-34	13-18	13-18	1	4	5	5
	<i>Cercidiphyllum japonicum</i> Siebold & Zucc.	Cercidiphyllaceae	Intermediate	Sympodial		19-65	19-74	9-28	9-31	1	3	5 ^h	6 ^h
	<i>Pterocarya rhoifolia</i> Siebold & Zucc.	Juglandaceae	Intermediate	Monopodial		23-34	17-34	17-20	18-20	1	4	5	5

^aPorosity classes were defined as in Hayashi (1991), except for *Hovenia tomentella*, which was determined by visual examination of pores.

^bScientific names are based on those reported by Hayashi et al. (1987) except for *Hovenia tomentella* (Meyer and Walker 1984).

^cThe type of leaf emergence pattern of each species was determined by seasonal observation of tree specimens, and was based on the types reported by Kikuzawa (1983).

^dBranching of each species was determined by seasonal observation of tree specimens, and was based on the types reported by Kikuzawa (1983) and Yagi (2004).

^eDiameter at breast height.

^fOne *Quercus mongolica* var. *grosseserrata* tree had Japanese oak wilt in autumn 2005, although leaves and vessels continued to form until late summer.

^gOne *Castanea crenata* tree had heartwood decay, but leaves and vessels continued to form in 2004.

^hNo current-year vessels were observed in one *Cercidiphyllum japonicum* tree and one *Fagus crenata* tree in 2004, and in one *Cercidiphyllum japonicum* tree in 2005.

elevation, with mean temperatures about 2 °C cooler than at the meteorological station in 2004. The minimum temperatures were –12.3 °C in 2004 and –14.7 °C in 2005 (Tyozidani Meteorological Reference Materials for Business Use, Unpublished data). The study area comprised 20 m on either side of a 1-km portion of a path in a flat-bottomed, south-facing valley. All sample trees were thus exposed to similar weather conditions.

Sample trees (Table 1-1) were selected from canopy trees of four ring-porous species, *Castanea crenata*, *Fraxinus mandshurica*, *Quercus mongolica* var. *grosseserrata*, and *Zelkova serrata*, and five diffuse-porous species, *Aesculus turbinata*, *Betula grossa*, *Cercidiphyllum japonicum*, *Fagus crenata*, and *Pterocarya rhoifolia*. In 2005, I added another ring-porous species, *Hovenia tomentella*. Scientific names are based on those reported by Hayashi et al. (1987) except for *H. tomentella* (Meyer and Walker 1984). Porosity classes were defined from Hayashi (1991), except for *H. tomentella*, which was determined by visual examination of pores. Study trees were selected from individuals with relatively straight stems and many leaves, 18–74 cm in stem diameter at breast height and 9–31 m tall. All species occur naturally in Ashiu, except for *Fraxinus mandshurica*, which is non-native and was planted. *Z. serrata* trees were planted individuals.

1.2.2 Kamigamo Experimental Forest Station

The study was conducted in a secondary forest of deciduous and evergreen trees in Kamigamo Experimental Forest Station (35°04'N, 135°46'E, 109–225 m above sea level), Kyoto University, Kyoto Prefecture, Japan. The mean annual temperature of a 30-year period from 1976 to 2005 was 14.7°C with the highest temperature observed in August (31.8°C) and the lowest in January (–0.9°C). The mean annual precipitation was 1,523 mm. Environmental data was obtained from the Forest Research Station of Graduate School of Agriculture, Kyoto University (2007).

Sample trees (Table 1-2) with six different types of porosity and vessel arrangement from nine species were selected: the ring-porous deciduous *Quercus serrata*; diffuse-porous deciduous *Liquidambar styraciflua*; semi-ring-porous deciduous *Acanthopanax sciadophylloides*; ring-porous evergreen *Castanopsis cuspidata*; diffuse-porous evergreen

Cinnamomum camphora, *Ilex pedunculosa*, and *Symplocos prunifolia*; and radial-porous evergreen *Quercus glauca* and *Quercus myrsinaefolia*. Scientific names used as reported by Hayashi et al. (1987). Classes of porosity and vessel arrangement in this study were defined as suggested by Hayashi (1991) and Itoh (1995, 1998), except of *L. styraciflua* that was determined by visual examination of pores. I characterized *Acanthopanax sciadophylloides* as semi-ring-porous species following the classification described by Itoh (1998), although Hayashi (1991) determined *Acanthopanax sciadophylloides* as ring-porous species. I observed that vessels of the pore-zone were narrower in *Acanthopanax sciadophylloides* compared to other ring-porous species; in addition, *Acanthopanax sciadophylloides* often had the same porosity as of diffuse-porous species. Study trees were selected from individuals with a diameter at breast height ranged between 12 and 56 cm, a height between 7 and 29 m, and relatively straight stems. *L. styraciflua* and *Q. myrsinaefolia* individuals were planted trees.

Table 1-2 Description of sample trees in Kamigamo

Leaf habit	Vessel arrangement ^a	Species ^b	Family	DBH ^c (cm)	Tree height (m)	Number of trees (trees)	
						twig	stem
Deciduous	Ring-porous	<i>Quercus serrata</i> Thunb.	Fagaceae	22-36	8-14	3	5
	Diffuse-porous	<i>Liquidambar styraciflua</i> L.	Hamamelidaceae	22-56	22-29	4	6
	Semi-ring-porous	<i>Acanthopanax sciadophylloides</i> Fr. et Sav.	Araliaceae	16-29	7-17	5	6
Evergreen	Ring-porous	<i>Castanopsis cuspidata</i> (Thunb.) Schottky	Fagaceae	12-56	7-26	3	3
	Diffuse-porous	<i>Cinnamomum camphora</i> Sieb.	Lauraceae	29-45	15-19	3	3
		<i>Ilex pedunculosa</i> Miq.	Aquifoliaceae	19-24	8-11	4	5
		<i>Symplocos prunifolia</i> Sieb. et Zucc.	Symplocaceae	19-24	7-9	5	5
	radial-porous	<i>Quercus glauca</i> Thunb.	Fagaceae	20-44	11-17	4	5
		<i>Quercus myrsinaefolia</i> Blume	Fagaceae	22-53	15-20	4	5

^aClasses of vessel arrangement were defined as suggested by Hayashi (1991) and Itoh (1995, 1998), except of *Liquidambar styraciflua* that was determined by visual examination of pores. We characterized *Acanthopanax sciadophylloides* as semi-ring-porous species following Itoh (1998) classification, though Hayashi (1991) determined *Acanthopanax sciadophylloides* as ring-porous species. We observed that vessels of the pore-zone were narrower in *Acanthopanax sciadophylloides* compared to other ring-porous species and sometimes its porosity was similar to diffuse-porous species.

^bScientific names are based upon the report of Hayashi et al. (1987).

^cDiameter at breast height.

1.3 Observation of leaf phenology

Leaves of each sample tree were examined using binoculars (Nikon 8 × 30, 8.8° WF; Tokyo, Japan) and photographed *in situ* on the trees and sampled twigs. I distinguished between flush leaves and successive leaves (Kikuzawa 1983; Miyazawa and Kikuzawa 2004). Flush

leaves (i.e., early leaves) appear almost simultaneously in a short period immediately after budbreak in early spring, whereas successive leaves (i.e., late leaves) appear gradually over a longer period.

The type of leaf emergence pattern, i.e., flush, succeeding, or intermediate was determined for each species by seasonal observations and using the criterion defined by Kikuzawa (1983). I observed only flush and intermediate emergence patterns (Table 1-1). Species with flush-type emergence had flush leaves only, whereas species with intermediate-type emergence had both flush and successive leaves.

The following phenological parameters were observed: leaf appearance was defined as the time at which the lamina separated from the shoot axis (Kikuzawa 1983), and full leaf expansion was defined as the time at which leaf area was judged to have increased (Suzuki et al. 2000). The following terms refer to the whole-tree level in this paper, unless indicated otherwise, unlike the shoot-level phenology of earlier studies (e.g., Marks 1975; Bicknell 1982; Kikuzawa 1983). “The beginning of leaf appearance” indicates that at least one flush leaf had appeared; “completion of leaf appearance,” almost all flush leaves had appeared; “full leaf expansion,” most of the flush leaves had fully expanded (also used when successive leaves of *H. tomentella* had fully expanded after the end of shoot elongation); and “the end of shoot elongation,” the point at which new successive leaves stopped appearing. At the end of shoot elongation, species with sympodial branching dropped their shoot-tips, and species with monopodial branching produced winter buds, but no new leaves appeared. The dates of each phenological stage for individual trees were recorded as the first date on which each stage was observed. Leaf appearance stages clearly differed from the full leaf expansion stage in the intensity of greenness.

Chapter 2 Examination of wood sampling method with an increment borer:

An investigation of seasonal changes in vessel formation

2.1 Introduction

To examine the relationship between leaf phenology and vessel formation, the periodic process of vessel formation is traced. The sampling is always problematic because frequent examination to trace the periodic process of vessel formation may cause considerable damage to the trees studied.

In anatomical studies, two methods, periodical sampling of wood blocks (cambial block method) and cambial marking, have been used. In the cambial marking method, a small wound is inflicted periodically on the cambium with a pin, knife, or other tools; wood blocks including marking positions are then collected. By this method, the radial increment of the xylem between the marked periods is recognized. This technique was originally developed by Wolter (1968) and investigated extensively by Kuroda et al. (1984a, b, 1985). It has been used to measure radial increments of tropical trees (Shiokura 1989; Nobuchi et al. 1995; Callado et al. 2001; Mukogawa et al. 2003) and temperate trees (Nobuchi et al. 1993). Because the markings cause only small wounds, more marks can be made within a small area of a stem. However, there is a time lag between the date of marking and the time for the tissue around the mark to form completely. Consequently, the analysis of anatomical features becomes more complicated.

In the cambial block method, samples are collected by cutting off small cubic wood blocks containing bark, phloem, cambium, and xylem using a chisel or knife. This method has been used to measure the growth dynamics of tropical trees (Fujii et al. 1999) and to trace cambial activity and vessel formation (Wareing 1951; Ladefoged 1952; Imagawa et al. 1970, 1972a, b; Wakuta et al. 1973; Suzuki et al. 1996, 2000; Farrar et al. 1997). It has the advantage that cambial activity can be observed, although it takes more time and effort for sampling and processing, and sample trees receive larger wounds.

For periodical and frequent sampling of a tree, a less destructive and more convenient sampling method is necessary. I used an increment borer to collect wood core samples to reduce damage and sampling effort. The increment borer is a common tool in tree ring research. The

method has been used for analyses of dendrochronology (Carrer et al. 2007), stem growth (Nishizawa 1955; Toda et al. 1995), histochemistry (Nobuchi et al. 1976, 1979, 1986), and seasonal changes in vessel formation (Ladefoged 1952; Fonti et al. 2007). Increment borers are not usually used in analyses of cambial activity, because the cambial zone is often destroyed. However, for the analysis of the time course of vessel formation from the viewpoint of water conduction, sampling with an increment borer is considered useful. Ladefoged (1952) used increment boring to trace seasonal changes in vessel formation, but did not provide a detailed description.

This paper describes the increment borer method in detail. The degree of tree wounding, simplicity of collecting wood core samples, and quality of wood core samples obtained with an increment borer were investigated.

2.2 Materials and methods

2.2.1 Sampling

Cercidiphyllum japonicum (diameter at breast height [DBH] = 19-65 cm, tree height [H] = 9-28 m) and *Zelkova serrata* (DBH = 21-30 cm, H = 13-15 m) were used as diffuse- and ring-porous wood species, respectively. Five trees of each species were selected at the Ashiu Forest Research Station (Chapter 1). The descriptions of sample trees are listed in Table 2-1.

Table 2-1 Description of sample trees and the dates of vessel formation.

Species	Porosity	Tree number	DBH ^{*1} (cm)	H ^{*2} (m)	The dates of vessel formation				
					22 or 23 Apr	6 or 7 May	22 May	3 June	17 June
<i>Cercidiphyllum japonicum</i>	Diffuse-porous	Cj -242	19	9	a ^{*3}	b ^{*3}	c ^{*3}		
		Cj -243	32	18	a	a	b	c	
		Cj -244	65	28	a	a	a	b	c
		Cj -245	62	27	a	a	b	c	
		Cj -246	42	24	a	a	a	a	a
<i>Zelkova serrata</i>	Ring-porous	Zs -202	30	15	b ^{*4}	c ^{*4}		d ^{*4}	e ^{*4}
		Zs -203	22	13	a ^{*4}	c		d	e
		Zs -204	21	14	b	c		d	e
		Zs -205	30	13	a	b	c,d	e	
		Zs -206	24	14	b	c		d	e

^{*1}DBH: Diameter at breast height. ^{*2}H: Tree height. ^{*3}Vessel formation of *Cercidiphyllum japonicum*. a; No vessels are evident. b; The first-formed vessels adjacent to the annual ring border (the first-formed vessels) are enlarged. c; All first-formed vessels take shape with the progression of their lignification. ^{*4}Vessel formation of *Zelkova serrata*. a; The first-formed wide vessels adjacent to the annual ring border (the first-formed wide vessels) are enlarged. b; Some first-formed wide vessels are lignified and others are not. c; All first-formed wide vessels take shape with the progression of their lignification. d; The first-formed narrow vessels in the non-pore zone are enlarged but not yet lignified. e; All first-formed narrow vessels in the non-pore zone are lignified.

Cylindrical wood core samples (7 mm in diameter, 20 mm in length) were collected with an increment borer (Mattson, Mora, Sweden; Fig. 2-1) near breast height (1.3 ± 0.2 m above ground) facing south whenever possible, but from the upper side of slope when trees were growing on a steep slope, and fixed with 3% aqueous glutaraldehyde. The holes caused by sampling were about 8 mm in diameter and about 25 mm in depth (Fig. 2-2). The horizontal distance between holes was set at least 20 mm apart. This distance is sufficient to avoid the influence of previous wounding, based on a previous study (Okada et al. 1993) in which the discoloration caused by wounding was 5 mm in the tangential direction 4 months after boring. No visual influence of the wound response after sampling, such as abnormal tissue formation, was observed.

Sample collection was carried out biweekly (on 22 or 23 April, 6 or 7 and 22 May, 3 and 17 June 2004). All holes were plugged with silicon resin immediately after each sampling.

Fig. 2-1 Collecting a wood core sample with an increment borer near breast height.

Fig. 2-2 Sampling hole (diameter: ~8 mm, depth: ~25 mm).

Fig. 2-3 Transverse sections of wood around the outer annual ring in a diffuse-porous tree, *Cercidiphyllum japonicum*. (a) No vessels are evident (Cj-243, 23 April 2004). (b) The vessels first formed next to the annual ring border (the first-formed vessels) are enlarged, but not yet lignified (gray arrow; Cj-243, 22 May 2004). (c) All first-formed vessels are lignified (black arrow; Cj-243, 3 June 2004).

Fig. 2-4 Transverse sections of wood around the outer annual ring in a ring-porous tree, *Zelkova serrata*. (a) The wide vessels first formed next to the annual ring border (the first-formed wide vessels) are enlarged, but not yet lignified (gray arrow; Zs-203, 22 April 2004). (b) Some first-formed wide vessels are lignified (black arrow) and others are not (gray arrow; Zs-204, 22 April 2004). (c) All first-formed wide vessels are lignified (black arrow; Zs-203, 6 May 2004). (d) The first-formed narrow vessels in the non-pore zone are enlarged, but not yet lignified (gray arrow; Zs-203, 3 June 2004). (e) All first-formed narrow vessels in the non-pore zone are lignified (black arrow; Zs-203, 17 June 2004).

Fig. 2-5 Transverse sections of wood around the outer annual ring with the phloroglucinol-hydrochloric acid reaction in a diffuse-porous tree, *Cercidiphyllum japonicum* and a ring-porous tree, *Zelkova serrata*. (a) The vessels first formed next to the annual ring border (the first-formed vessels) are enlarged, but not yet lignified (gray arrow; Cj-243, 22 May 2004). (b) All first-formed vessels are lignified (black arrow; Cj-243, 3 June 2004). (c) The first-formed wide vessels are enlarged, but not yet lignified (gray arrow; Zs-203, 22 April 2004). (d) All first-formed wide vessels are lignified (black arrow; Zs-203, 6 May 2004).

Fig. 2-6 Double-stained transverse section containing phloem and cambium (arrow; cambium, arrowhead; sieve tube) (Cj-244, 7 May 2004).



Fig. 2-1

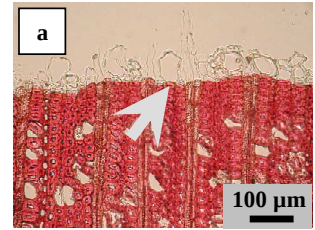
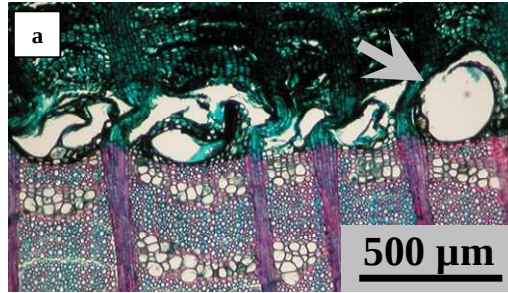


Fig. 2-2

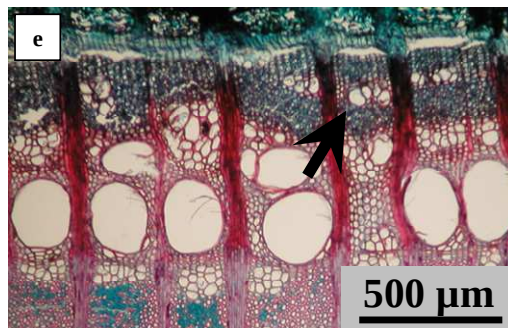
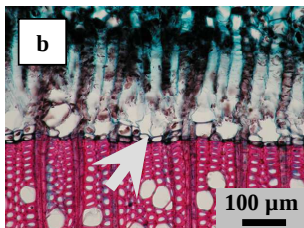
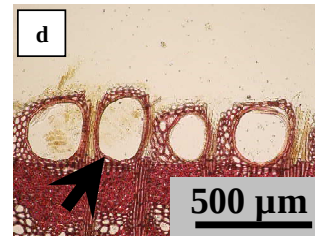
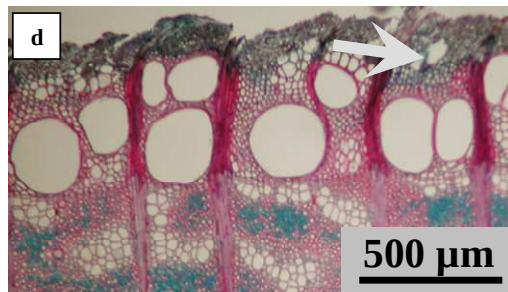
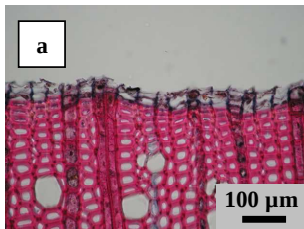
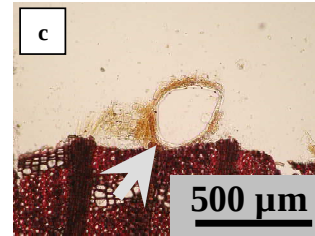
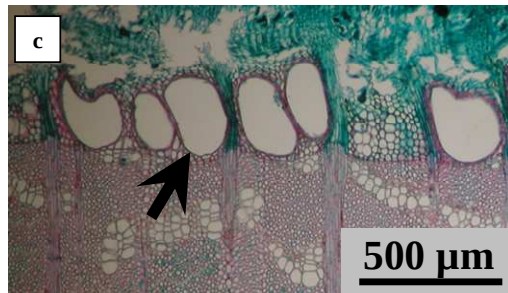
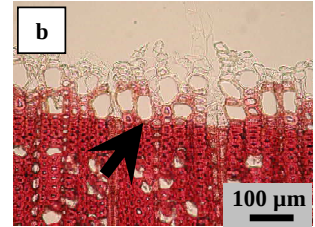
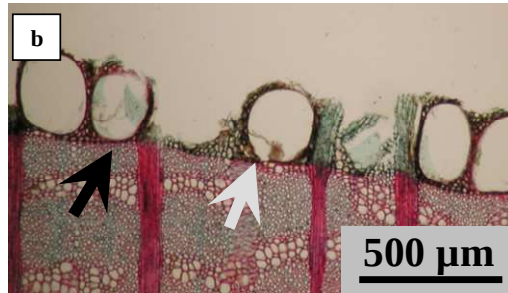


Fig. 2-5

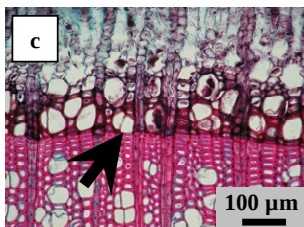


Fig. 2-3

Fig. 2-4

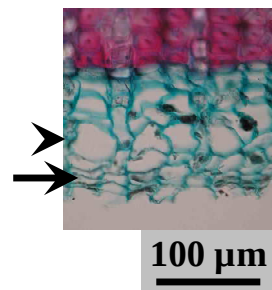


Fig. 2-6

2.2.2 Assessment of vessel formation

Vessel development begins with cambial cell division, followed by enlargement of vessel elements, deposition and lignification of the secondary cell wall, and disintegration of the end walls (Imagawa and Ishida 1972; Wakuta et al. 1973; Fukushima et al. 2003; Fromm 2013). I assessed lignification of the first-formed vessels, which formed first in the current growth ring, often adjacent to the annual ring border, by red staining (Sass 1951; Imagawa and Ishida 1972).

Wood core samples were cut into 5-10 mm long segments in the radial direction. Transverse sections (15-30 μm in thickness) were cut using a sliding microtome (Yamato TU-213; Saitama, Japan) equipped with a freezing unit, double-stained with 1% safranin (in 50% ethanol, about 20 min) and 1% fast green (in 95% ethanol, about 1 min), dehydrated with an ethanol series (50, 70, 80, 90, 95, and 100%), ethanol replaced with xylene, and then mounted in Canada balsam on glass slides (Sass 1951) for light microscopy (Olympus BX-50-32; Tokyo, Japan). Staining with safranin served as an approximate method to judge the lignification of the vessels. In addition, the result of double staining with safranin and fast green was compared to that of the phloroglucinol-hydrochloric acid reaction.

2.3 Results and Discussion

2.3.1 Observation of the time course of vessel formation

In *Cercidiphyllum japonicum* (Cj-243), a diffuse-porous species, formation of vessels was not observed on 23 April (Fig. 2-3a); enlargement of the first-formed vessels was observed next to the annual ring border on 22 May (the first-formed vessels; Fig. 2-3b). All first-formed vessels developed with progression of their lignification on 3 June (Fig. 2-3c). From these observations, it was evident that lignification of all first-formed vessels started after 22 May but before 3 June. The process of vessel formation in Cj-242, -244 and -245, but not -246, was basically the same as that in Cj-243 (Fig. 2-3, Table 2-1). All first-formed vessels started secondary cell wall lignification between 22 May and 17 June in *Cercidiphyllum japonicum* (Table 2-1). In Cj-246, no vessels were formed in the stem between 23 April and 17 June (Table 2-1), although this tree flushed many new leaves. The reason for this is not clear; however, I consider that this tree might have transported enough water to evaporate from the leaves without

the need for new vessels, or that some new vessels might have been formed in the stem facing directions in which wood core samples were not collected. This tree had a large diameter at breast height and it is known that water is transported through vessels of several outer growth rings in the stem of diffuse-porous trees (Kozłowski et al. 1963), such as *Cercidiphyllum japonicum*.

In *Z. serrata* (Zs-203), a ring-porous species, the enlargement of the first-formed wide vessels was initially observed on 22 April (Fig. 2-4a), while in another tree (Zs-204), lignification of some first-formed wide vessels was observed, but not of others, on 22 April (Fig. 2-4b); all first-formed wide vessels took shape with progression of their lignification on 6 May (Fig. 2-4c). Enlargement of the first-formed narrow vessels in the non-pore zone was observed, but without lignification, on 3 June (Fig. 2-4d). Then, lignification of all first-formed narrow vessels in the non-pore zone was observed on 17 June (Fig. 2-4e). From these observations, it was evident that lignification of all first-formed wide vessels started after 22 April but before 6 May, while lignification of narrow vessels in the non-pore zone began after 3 June but before 17 June. The process of vessel formation in Zs-202, -204, -205, and -206 was basically the same as that in Zs-203 (Fig. 4, Table 2-1), although the first-formed wide vessels were all unlignified in some trees and occasionally unlignified in the others two weeks before all first-formed wide vessels took shape with progression of their lignification, and unlignified first-formed narrow vessels in the non-pore zone were observed when all first-formed wide vessels were lignified in Zs-205. All first-formed wide vessels started secondary cell wall lignification between 6 May and 22 May in *Z. serrata* (Table 2-1).

Comparing the development of the first-formed vessels between *Cercidiphyllum japonicum* and *Z. serrata*, the former, a diffuse-porous species, tends to produce the first-formed vessels later and with more variation in timing of development among individuals than does the latter, which is ring-porous. This suggests that the difference in timing and variation in vessel formation between the two species is related to differences in their life strategies.

Therefore, vessel formation of the stems in diffuse- and ring-porous trees could be traced using samples collected with an increment borer. The increment borer method was later applied to five diffuse-porous and five ring-porous species, the results of which will be described in separate chapters.

2.3.2 Quality of judging vessel lignification

The result of double staining with safranine and fast green was compared to that of the phloroglucinol-hydrochloric acid reaction. In practice, the same results were found for the two methods when they were applied to transverse sections of the same sample. For example, when the first-formed vessels were not stained with safranine (*Cercidiphyllum japonicum* Fig. 2-3b, *Z. serrata* Fig. 2-4a), they were also not stained with phloroglucinol-hydrochloric acid (*Cercidiphyllum japonicum* Fig. 2-5a, *Z. serrata* Fig. 2-5c), and when they were stained with safranine (*Cercidiphyllum japonicum* Fig. 2-3c, *Z. serrata* Fig. 2-4c), they were also stained with phloroglucinol-hydrochloric acid (*Cercidiphyllum japonicum* Fig. 2-5b, *Z. serrata* Fig. 2-5d). Based on a comparison of the results, the double-staining method is considered to be applicable to investigations of vessel formation, including the timing of vessel lignification.

2.3.3 Examination of sample quality

The outermost part of a core sample including the bark and cambial zone was often detached from the sample during extraction of the wood core samples from the increment borer, or while cutting transverse sections using a sliding microtome, especially during the growing season, although sometimes it was not detached (Figs. 2-3b, c). Therefore, it is not usually suitable to observe periodic changes in cambial activity. Thus, the increment borer method has not been used to trace wood formation. Main purpose of this study was to determine the timing of vessel formation, especially its lignification. For this reason, wood core samples collected with an increment borer even without a cambial zone can be used.

Observation of transverse sections revealed that naturally lignified vessels, and even the unlignified vessels, remained in the outermost part of the section, as shown in Figs. 2-3b and 2-4a, b, d (gray arrow). This result concurred with the observations reported by Priestley et al. (1933). In addition, transverse sections cut from the samples of the bark and cambial zone detached from the wood showed no vessels in this side (Fig. 2-6). However, care should be taken in judging whether a vessel element can conduct water based on the safranine-stained secondary wall,

because vessel elements with thick secondary walls sometimes retain cytoplasm. Observation of the radial section is helpful when it is uncertain whether a vessel element functions.

The negative effect of taking samples with an increment borer is small, and sample trees were still viable 2 years after boring. Therefore, vessel formation could be traced in such viable sample trees.

Chapter 3 Relationship between the timing of vessel formation and leaf phenology in ten ring-porous and diffuse-porous deciduous tree species

3.1 Introduction

In broad-leaved deciduous species, there is a difference in the temporal relationships of leaf phenology and vessel formation in ring-porous and diffuse-porous species. Stem diameter begins to increase before or at the time of leaf appearance in ring-porous species, whereas it begins after leaf appearance in diffuse-porous species (Friesner 1942; Komiyama et al. 1987; Komiyama 1991; Maruyama et al. 1992).

At the microstructural level, the onset of cambial activity in early spring is controlled by growth hormones (Wilcox 1962; Fahn and Werker 1990). Cell division in bud bases begins just before or simultaneously with bud break in both ring-porous and diffuse-porous species (Ladefoged 1952). However, several studies have shown that cambial activity (e.g., Wareing 1950; Ladefoged 1952) and radial growth (Priestley & Scott 1936; Wareing 1950) in diffuse-porous species starts at the base of winter buds and then spreads slowly downward and that cell division occurs more quickly in the basipetal direction in ring-porous species than in diffuse-porous species. The cambium in ring-porous trees became highly sensitive to extremely low indole-3-acetic acid stimulation during their evolution from diffuse-porous species (Aloni 2013). Therefore, cell division at the base of stems begins earlier in ring-porous species, a few days before or simultaneously with bud break, whereas diffuse-porous species do not show division at the stem base until foliage has partly or completely expanded (Priestley and Scott 1936; Ladefoged 1952). Other studies have reported that ring-porous species have overwintering cells (Tepper and Hollis 1967; Zasada and Zahner 1969; Imagawa and Ishida 1972), and that both ring- and diffuse-porous species initiate cambial cell division simultaneously after bud break, but overwintering cells in the cambial zone are enlarged prior to cell division in ring-porous species (Frankenstein et al. 2005). Thus, it is important to specify the stage of vessel formation that is being compared between ring- and diffuse-porous species.

After cell division and the subsequent enlargement of vessel elements in ring-porous species, the first vessel elements formed in the new growth ring, which are adjacent to the annual

ring border, mature 1 week before or up to 3 weeks after leaf expansion (Suzuki et al. 1996). Vessel maturation also occurs around the time of leaf appearance (Zasada & Zahner 1969; Suzuki et al. 1996, 2000; Sass-Klaassen et al. 2011). In contrast, vessel elements in diffuse-porous species mature more than a month after leaf expansion (Suzuki et al. 1996), or around the time of full leaf expansion (Suzuki et al. 2000). Thus, the timing of vessel maturation clearly differs between ring-porous and diffuse-porous species.

Many previous studies of wood anatomy have not considered the stages of leaf development or patterns of leaf appearance. Similarly, many ecological studies have not investigated the process of vessel formation or vessel microstructure. To clarify the relationship between leaf phenology and vessel formation from the ecophysiological viewpoint, both traits need to be examined concurrently to determine how they relate to photosynthesis and water transport. Such studies in natural forests are very limited, as are studies of seasonal changes in vessel formation in individual trees over a year in both the twigs and stems of ring- and diffuse-porous species. Suzuki et al. (1996, 2000) examined seasonal vessel formation in stems only, and Ladefoged (1952) examined cell division, but not lignification, in different trees over the course of a year.

To examine the anatomical relationship between leaf phenology and vessel formation from an ecophysiological viewpoint, relating photosynthesis and water transport, I examined vessel lignification at the time of leaf appearance and full leaf expansion. I used an increment borer to sample stems and to observe the phenology of vessel lignification of trees.

This is a case study of the timing of budburst in relation to xylem vessel development in both twigs and stem wood for species of ring- and diffuse-porous deciduous trees growing together in a temperate forest in central Japan. The objective of this study is to clarify how different aspects of plant functions are coordinated developmentally.

3.2 Materials and methods

3.2.1 Sampling

The study was conducted at the Ashiu Forest Research Station (Chapter 1). Sample trees (Table 1-1 in Chapter 1) were selected from canopy trees of five ring-porous species and five

diffuse-porous species. Cylindrical wood core samples were collected with an increment borer (Chapter 2) from five trees of each species in 2004 and 2005 (except that in 2005, four trees were sampled for *Q. mongolica* var. *grosseserrata* and six trees for *Cercidiphyllum japonicum*).

Twigs with sun leaves were collected from each tree using 3-m-long pruners and a ladder in 2004, or 12-m-long pruners in 2005. One tree of each species was sampled in 2004 and three or more trees of each species were sampled in 2005 (except that only one tree of *H. tomentella* was sampled in 2005). In this study, twigs that were newly extended within the previous year were regarded as 1-year-old twigs. I observed vessel formation in 1-year-old twigs in 2004 and in 1-year-old (or occasionally 2- or 3-year-old) twigs in 2005 at 0.5–1.5 cm from bud bases. Stem cores and twigs were fixed with 3 % aqueous glutaraldehyde soon after sampling.

In 2004, samples were collected biweekly between April 22 and July 1 and monthly between July 22 and November 16, for a total of 11 times. In 2005, samples were collected weekly between April 19 and 28 and biweekly between April 28 and July 21, for a total of eight times.

3.2.2 Assessment of vessel formation

Transverse sections were double-stained in preparation for light microscopy (Chapter 2). When double staining was ambiguous for lignification, a phloroglucinol–hydrochloric acid reaction was used (Chapter 2). The date of lignification was recorded as the first date on which lignification of almost all of the first-formed vessels was observed.

3.2.3 Observation of leaf phenology

Leaves of each sample tree were examined at each sampling conducted. Phenological parameters such as completion of leaf appearance and full leaf expansion were observed (Chapter 1).

3.3 Results

3.3.1 Timing of vessel lignification in twigs relative to leaf appearance

Lignification of the first-formed vessels in twigs was observed 0–2 weeks after leaf

appearance in both ring- and diffuse-porous species in 2004, and 0–2 weeks before leaf appearance in ring-porous species and 2 weeks before to 2 weeks after leaf appearance in diffuse-porous species in 2005 (Fig. 3-1). The timing of vessel lignification was not significantly different between ring and diffuse-porous species in 2004, but was significantly different in 2005 ($p < 0.01$, Table 3-1). Four of the 20 ring-porous trees sampled showed lignification of vessels prior to leaf appearance, although one *Q. mongolica* individual did not show vessel lignification until 2 weeks after leaf appearance. On the other hand, of the 24 diffuse-porous trees, 11 showed vessel lignification after leaf appearance, although two samples (one *Aesculus turbinata* and one *Fagus crenata*) showed vessel lignification prior to leaf appearance. The timing in 2005 was somewhat earlier than that in 2004.

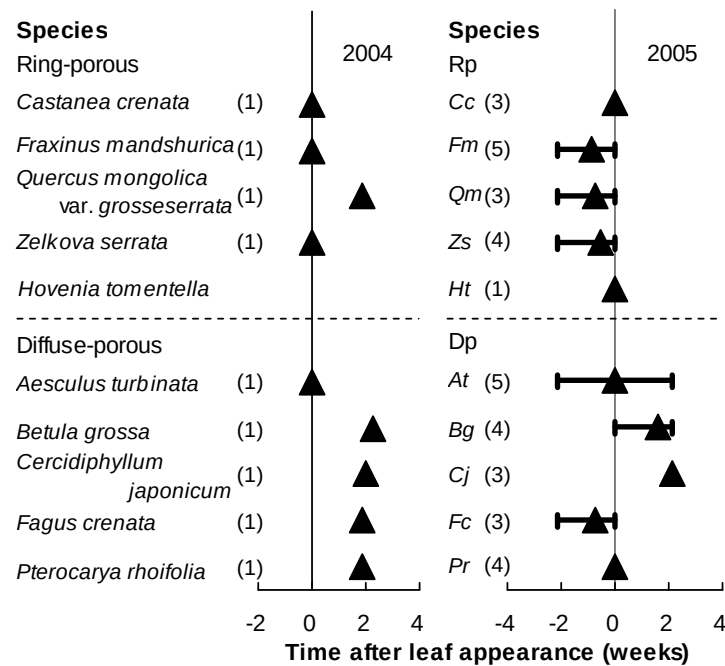


Fig. 3-1 Timing of lignification of the first-formed vessels in twigs relative to the time of leaf appearance of the whole tree in 2004 (left) and 2005 (right). Numbers in parentheses indicate the numbers of trees; filled triangles average, bar range

The timing of lignification of the first-formed vessels in twigs was around the time of leaf appearance (± 2 weeks) in both ring- and diffuse-porous species. Lignification of the

first-formed vessels in twigs relative to leaf appearance tended to occur 1.1 weeks earlier in ring-porous species than in diffuse-porous species (Table 3-1).

Table 3-1 Difference of 5 temporal traits of vessel formation between ring- and diffuse-porous species

Lignification of the first-formed vessels		Ring-porous species mean $\pm$ se (weeks)	Diffuse-porous species mean $\pm$ se (weeks)	difference in t-value (weeks)
2004	in twigs relative to leaf appearance	0.5 $\pm$ 1.1	1.6 $\pm$ 1.0	1.1 ^{ns}
	in stems relative to leaf appearance	0.2 $\pm$ 1.0	5.5 $\pm$ 0.7	5.4 ^{**}
	in stems compared to twigs	-0.6 $\pm$ 1.4	4.8 $\pm$ 1.9	5.3 ^{**}
	in twigs relative to full leaf expansion	-2.6 $\pm$ 1.2	-1.4 $\pm$ 1.3	1.2 ^{ns}
	in stems relative to full leaf expansion	-2.5 $\pm$ 0.7	3.0 $\pm$ 0.6	5.5 ^{**}
2005	in twigs relative to leaf appearance	-0.5 $\pm$ 0.5	0.6 $\pm$ 0.7	1.1 ^{**}
	in stems relative to leaf appearance	0.6 $\pm$ 0.7	5.9 $\pm$ 0.6	5.3 ^{**}
	in stems compared to twigs	0.5 $\pm$ 0.7	4.8 $\pm$ 0.7	4.3 ^{**}
	in twigs relative to full leaf expansion	-3.1 $\pm$ 0.5	-2.0 $\pm$ 0.7	1.1 [*]
	in stems relative to full leaf expansion	-1.9 $\pm$ 0.7	3.4 $\pm$ 0.8	5.3 ^{**}

ns is non-significant, * 0.01 < P < 0.05, ** P < 0.01

3.3.2 Timing of vessel lignification in stems relative to leaf appearance

Lignification of the first-formed vessels in stems was observed 2 weeks before to 4 weeks after leaf appearance in ring-porous species, and 2–8 weeks (2004) or 4–8 weeks (2005) after leaf appearance in diffuse-porous species (Fig. 3-2). Ring-porous species showed significantly earlier vessel lignification than diffuse-porous species in both 2004 and 2005 ($p < 0.01$, Table 3-1). Of the ring-porous species, three individuals (two *Q. mongolica* in 2004 and one *H. tomentella* in 2005) showed vessel lignification 4 weeks after leaf appearance. Across all ring-porous species, 16 of 44 samples examined showed vessel lignification after leaf appearance. Nine of ten *Fraxinus mandshurica* samples and one *Castanea crenata* (2004) had vessel lignification 2 weeks before leaf appearance. In contrast, among the diffuse-porous species, two *B. grossa* (2004) had vessel lignification 2 weeks after leaf appearance, and no new vessels were observed in one *Cercidiphyllum japonicum* and one *Fagus crenata* in 2004 and one *Cercidiphyllum japonicum* in 2005 (Table 1-1) (Chapter 2, describe some possible reasons for the latter occurrence).

The time of the lignification of the first-formed vessels in stems relative to the time of

leaf appearance was 5.4 weeks (2004) or 5.3 weeks (2005) earlier in ring-porous species than in diffuse-porous species (Table 3-1).

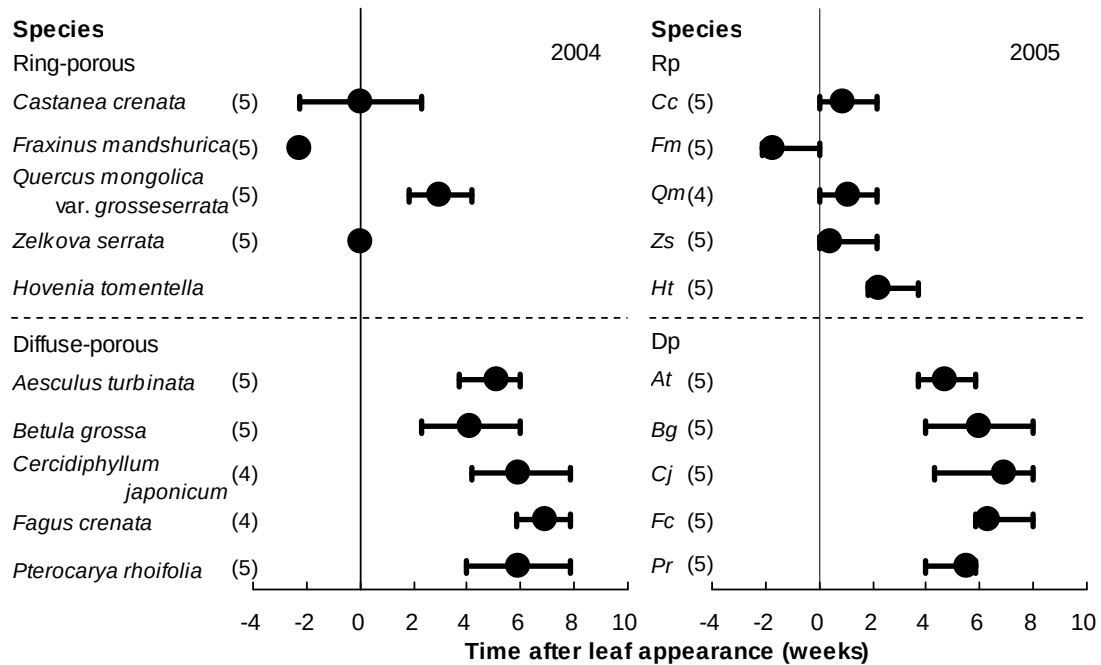


Fig. 3-2 Timing of lignification of the first-formed vessels in stems relative to the time of leaf appearance of the whole tree in 2004 (*left*) and 2005 (*right*). Numbers in parentheses indicate the numbers of trees; filled circles average, bar range

3.3.3 Timing of vessel lignification in stems and twigs

Compared with the timing of lignification of vessels in twigs, vessel lignification in stems occurred 0–2 weeks earlier in 2004 or within 2 weeks in 2005 in ring-porous species and 2–6 weeks (2004) or 2–8 weeks (2005) later in diffuse-porous species (Fig. 3-3). Vessel lignification was 5.3 weeks (2004) or 4.3 weeks (2005) earlier in ring-porous species ($p < 0.01$, Table 3-1). Three of six *Fraxinus mandshurica* samples had vessel lignification in stems 2 weeks before in twigs. Lignification of vessels in stems was later than that in twigs in six of 20 ring-porous trees, a pattern similar to that of all diffuse-porous trees.

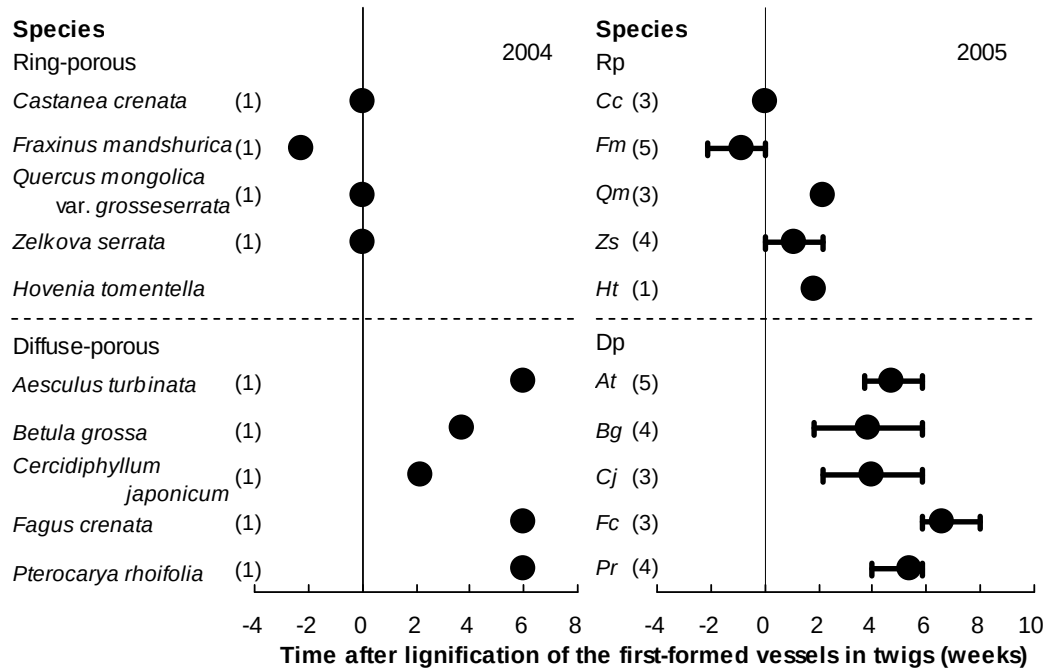


Fig. 3-3 Timing of lignification of the first-formed vessels in stems compared to twigs in 2004 (left) and 2005 (right). Numbers in parentheses indicate the numbers of trees; filled circles average, bar range

3.3.4 Timing of vessel lignification in twigs and stems relative to full leaf expansion

The first-formed vessels in twigs were lignified 2–4 weeks before full leaf expansion in ring-porous species and 0–2 weeks (2004) or 0–4 weeks (2005) before full leaf expansion in diffuse-porous species (Fig. 3-4). Differences in the timing of vessel lignification between ring- and diffuse-porous species were significant in 2005 ($0.01 < p < 0.05$) but not in 2004 (Table 3-1). Ring-porous species showed vessel lignification 1.1 weeks earlier than diffuse-porous species in 2005 (Table 3-1). Several diffuse-porous trees had vessel lignification concurrent with full leaf expansion.

Lignification of vessels in stems occurred 4 weeks before to 0 weeks after (2004) or 4 weeks before to 2 weeks after (2005) full leaf expansion in ring-porous species, 5.5 weeks (2004) or 5.3 weeks (2005) earlier than in diffuse-porous species ($p < 0.01$), which showed lignification of vessels 0–6 weeks (2004) or 0–8 weeks (2005) after full leaf expansion (Fig. 3-5; Table 3-1). Across all of the ring-porous species, one *H. tomentella* showed vessel lignification 2 weeks after full leaf expansion.

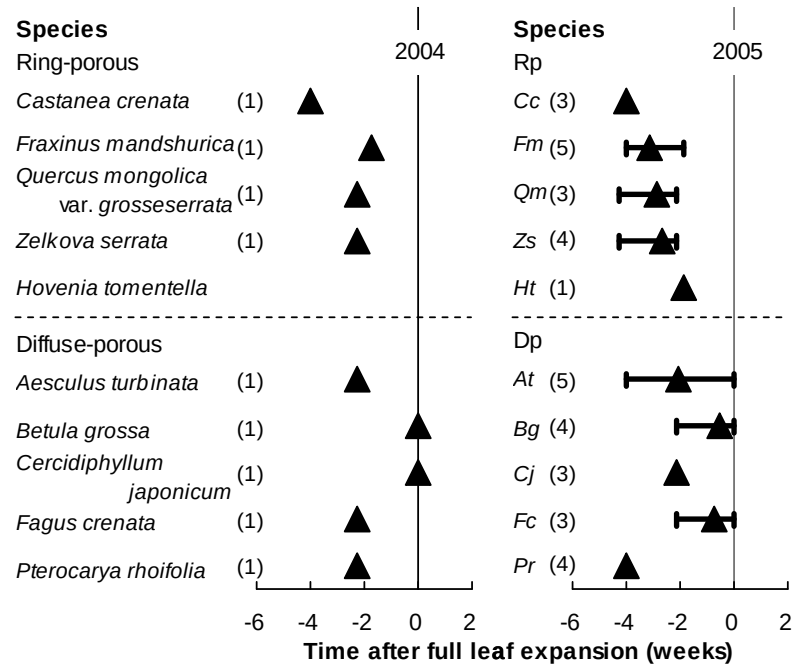


Fig. 3-4 Timing of lignification of the first-formed vessels in twigs compared to the time of full leaf expansion of the whole tree in 2004 (left) and 2005 (right). Numbers in parentheses indicate the numbers of trees; filled triangles average, bar range

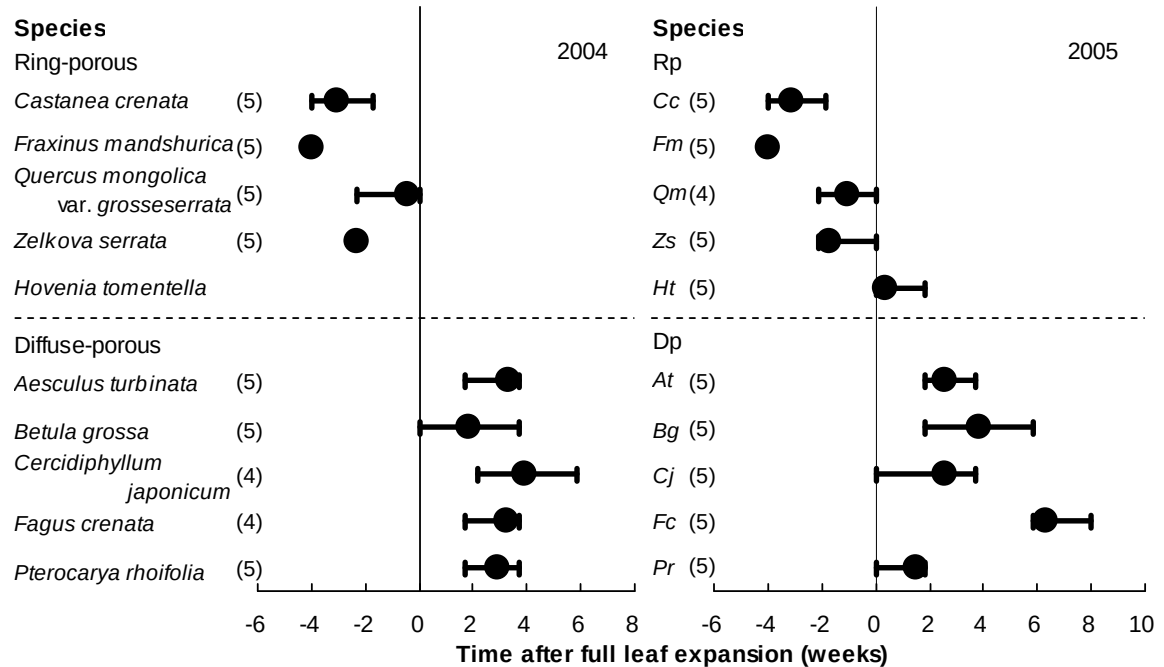


Fig. 3-5 Timing of lignification in the first-formed vessels in stems compared to the time of full leaf expansion of the whole tree in 2004 (left) and 2005 (right). Numbers in parentheses indicate the numbers of trees; filled circles average, bar range

3.4 Discussion

3.4.1 Order and time-lag of leaf and vessel formation

It has generally been reported that the timing of vessel formation in relation to leaf phenology differs between ring-porous and diffuse-porous species (Suzuki et al. 1996). Studies on ring-porous species have shown completion of secondary wall deposition in stems between 1 week before and 3 weeks after leaf expansion (Suzuki et al. 1996), or maturation between 1 week before and 1 week after leaf budding (Suzuki et al. 2000), coinciding with the unfolding of the first leaves (Lodewick 1928; Priestley et al. 1933; Zasada & Zahner 1969; Atkinson & Denne 1988; Fonti et al. 2007; Sass-Klaassen et al. 2011). In diffuse-porous species, secondary wall deposition occurred 4–9 weeks after leaf expansion (Suzuki et al. 1996). I found that lignification of the first-formed vessels in stems occurred between 2 weeks before and 4 weeks after leaf appearance in the case of flush-leaves in ring-porous species, and 2–8 weeks after leaf appearance in diffuse-porous species (Fig. 3-2). Thus, I confirm previous findings that vessel formation tends to occur earlier in ring-porous species than in diffuse-porous species.

Vessel elements in twigs begin to lignify before buds open (Zasada and Zahner 1969), and cell division at bud bases begins before or simultaneously with budbreak in both ring- and diffuse-porous species (Ladefoged 1952), indicating no difference in timing between ring- and diffuse-porous species. However, in this study, ring-porous species showed significantly earlier vessel formation in twigs than diffuse-porous species in 2005 (Table 3-1). Lignification of the first-formed vessels in twigs occurred somewhat earlier in ring-porous species than in diffuse-porous species relative to the timing of leaf appearance of flush-leaves (Fig. 3-1). In relation to the timing of full leaf expansion, I indicated that lignification of vessels in twigs occurred before full leaf expansion in ring-porous species and before or concurrently with full leaf expansion in diffuse-porous species (Fig. 3-4). Thus, in 1-year-old twigs 0.5–1.5 cm from bud bases, the same as in stems, there was a difference in the timing of vessel lignification in relation to the timing of leaf phenology between ring-porous and diffuse-porous species.

Ladefoged (1952) showed that cell division occurs more rapidly in the basipetal direction in ring-porous species than in diffuse-porous species, and so there is a relatively long period between the onset of cell division at the bud and stem bases in diffuse-porous species. I

also found that the lignification of vessels occurred later in stems than in twigs in both ring-porous and diffuse-porous species, but the time-lag was longer in diffuse-porous species (Fig. 3-3). Other studies have shown that the lignification of vessels in twigs and stems lower in the tree occurs prior to bud opening in ring-porous species (Zasada and Zahner 1969). In this study, the first-formed vessels in twigs were lignified by the time of leaf appearance in nearly all of the ring-porous trees examined (Fig. 3-1), while only some trees showed vessel lignification in stems at this time (Fig. 3-2). This suggests that not all of the first-formed vessels are needed for water transport in stems at the time of leaf appearance in ring-porous species with flush-leaves, though they are probably needed in twigs.

The development stages of ring-porous species can be summarized as follows: lignification of the first-formed vessels in twigs occurs first, followed by the appearance of flush-leaves, lignification of stem vessels, and full expansion of leaves (occasionally lignification of stem vessels occurs before leaf appearance). The development stages of diffuse-porous species can be summarized as follows: the appearance of leaves occurs first, followed by lignification of twig vessels, full expansion of leaves, and lignification of stem vessels. The time-lag between the lignification of the first-formed vessels in twigs and leaf appearance is short or absent in both ring- and diffuse-porous species. In contrast, the time-lag between the lignification of stem vessels and nearly simultaneous occurrences of leaf appearance and lignification of vessels in twigs is short or absent in ring-porous species and long in diffuse-porous species. The time-lag between vessel lignification in twigs or stems and full leaf expansion is variable in both ring- and diffuse-porous species.

3.4.2 Differences in physiological traits in ring- and diffuse- porous species

In early spring, transpiration begins in leaves as soon as they open, and transpiration and photosynthetic capacities increase rapidly as leaves expand and their areas increase (Šesták 1985; Koike 1990; Miyazawa and Terashima 2001; Muraoka and Koizumi 2005). This creates a temporal relationship between leaf phenology and vessel formation.

Almost all ring-porous species tend to lignify the first-formed vessels in twigs before or simultaneous with the appearance of flush-leaves and before full expansion of the leaves (Figs.

3-1, 3-4). This suggests that the first-formed vessels in twigs are ready for water transport by the time transpiration begin in flush-leaves. Further, in the case of the tree that did not show lignification of vessels until 2 weeks after leaf appearance, it was still prepared for water transport before leaves reached maximal transpiration.

In contrast to vessels in twigs, vessels in the stems of ring-porous species were lignified 0–4 weeks after (or somewhat before) the appearance of flush-leaves and before or concurrently with full expansion of these leaves (Figs. 3-2, 3-5). This suggests that the first-formed vessels in stems are prepared to transport water while the transpiration and photosynthetic capacities of the leaves are rapidly increasing during leaf expansion. It is vital that trees complete the first-formed vessels in twigs and stems before near maximal transpiration is reached at the time of full leaf expansion, because wide vessels transport water only in the year they develop (Greenidge 1955; Chaney and Kozlowski 1977; Ellmore and Ewers 1986; Utsumi et al. 1999; Umebayashi et al. 2008). At temperatures that occur at the present study site, the wide vessels of ring-porous species may become embolized by the freezing of xylem (Zimmermann 1964; Sperry et al. 1994; Utsumi et al. 1996). Thus, all of the first-formed vessels in twigs and stems prepare to transport water by the time when the flush-leaves reach near-maximal rates of transpiration, and allow photosynthesis to proceed smoothly. In *H. tomentella*, the first stem vessels are not prepared for transporting water until 0–2 weeks after the leaves have fully expanded. This may be related to the phenology of successive-leaves produced after flush-leaves open (Chapter 4).

In diffuse-porous species, lignification of twig vessels occurred within 2 weeks of leaf appearance and at or before full leaf expansion (Figs. 3-1, 3-4). This suggests that the first-formed vessels in twigs prepare to transport water while transpiration and photosynthesis are rapidly increasing, and are functional by the time the near-maximum capacity of the leaves is reached. This is similar to the stems of ring-porous species.

Diffuse-porous species did not show lignification of the first stem vessels until 2–8 weeks after the appearance of flush-leaves (Fig. 3-2). Other studies have shown that the lignification of stem vessels occurred around the time of full leaf expansion in diffuse-porous species (Suzuki et al. 2000). However, the present study found that lignification of stem vessels did not occur until several weeks (up to 8) after full expansion of these leaves (Fig. 3-5). This

suggests that the first stem vessels in these species prepare to transport water after the transpiration and photosynthetic capacities of these leaves have increased to near-maximal levels. Diffuse-porous species can do this because vessels in several outer rings, not just the current year's ring, can transport water (Greenidge 1955; Chaney and Kozlowski 1977; Utsumi et al. 1998; Umebayashi et al. 2008). Thus, the first-formed vessels in twigs are prepared to transport water by the time transpiration needs are near-maximum. Stem vessels are prepared after the rapid increase in transpiration and allow photosynthesis to proceed at a steady rate.

In summary, the ring-porous species produce single-year leaves and most functional vessels in a short period; whereas the diffuse-porous species produce single-year leaves and multiple-year vessels in a long period.

Chapter 4 Relationship between vessel porosity and leaf emergence pattern in ring- and diffuse-porous deciduous trees in a temperate hardwood forest

4.1 Introduction

In temperate trees, woody tissue is characterized by one of three types of porosity (Chapter 1). Leaf emergence pattern has been systematically investigated (Kikuzawa 1983). I attempted to elucidate the relationships between intra-annual changes in vessel diameter and leaf emergence pattern in ring-porous and diffuse-porous species by determining the relationships between leaf phenology and vessel formation.

Previous studies have revealed that the timing of vessel formation relative to leaf expansion of ring-porous species is different from that of diffuse-porous species in the early stage of annual ring formation (Chapter 3). Wide vessels of ring-porous species are known to transport water mostly during the year in which they are formed (Greenidge 1955; Chaney and Kozlowski 1977; Ellmore and Ewers 1986; Utsumi et al. 1999; Umebayashi et al. 2008). In contrast, vessels in several rings adjacent to the cambium retain their water transportation ability in diffuse-porous species (Greenidge 1955; Chaney and Kozlowski 1977; Utsumi et al. 1998; Umebayashi et al. 2008). Thus, ring-porous species produce single-year leaves and most functional vessels in a short period, whereas diffuse-porous species produce single-year leaves and multiple-year vessels in a long period (Chapter 3). There are prominent differences in the wood-formation process between ring- and diffuse-porous species, but such comparisons have generally been restricted to the early stage of annual ring formation.

In the later stage of annual ring formation, small vessels of the first-formed latewood in ring-porous species are completed by full leaf expansion (Suzuki et al. 2000), whereas later-formed earlywood vessels are completed several weeks after full leaf expansion (Zasada and Zahner 1969). The relationship between leaf phenology and vessel formation in the later stage of wood formation remains unclear. I tested the hypothesis that vessel porosity and vessel density within an annual ring are associated with leaf emergence pattern in deciduous broad-leaved trees.

To elucidate the functional linkage between intra-annual changes in vessel diameter and leaf emergence pattern, I determined the temporal relationships between vessel formation and leaf

phenology throughout the early stage to the later stage of annual ring formation in ring-porous trees and compared them with those in diffuse-porous trees in a same temperate forest. According to the theoretical relative conductance predicted by the Hagen–Poiseuille law, flow rate is proportional to the fourth power of the capillary radius (Ellmore and Ewers 1986; Tyree and Zimmermann 2002). In this study, vessels were categorized as the widest vessels, which had the highest water transport capacity, medium-sized vessels, which had 16- to 256-fold less conductivity than the widest vessels, and narrow vessels, which had ≥ 256 -fold less conductivity than the widest vessels.

4.2 Materials and methods

4.2.1 Sampling

The study was conducted at the Ashiu Forest Research Station (Chapter 1). Sample trees (Table 1-1 in Chapter 1) were selected from canopy trees of five ring-porous species and five diffuse-porous species. Cylindrical wood core samples were collected with an increment borer (Chapter 2) from five trees of each species in 2004 and 2005 (except that in 2005, four trees were sampled for *Q. mongolica* var. *grosseserrata* and six trees for *Cercidiphyllum japonicum*).

In 2004, samples were collected biweekly between April 22 and July 1 and monthly between July 22 and November 16, resulting in 11 sampling events in total. In 2005, samples were collected on April 19 and biweekly between April 28 and July 21, resulting in eight sampling events.

4.2.2 Assessment of vessel formation

Transverse sections were double-stained in preparation for light microscopy (Chapter 2). When double staining was ambiguous for lignification, a phloroglucinol–hydrochloric acid reaction was used (Chapter 2). The date of lignification was recorded as the first date on which lignification of at least one enlarged vessel was observed.

In ring-porous species, three size classes of vessels (Ewers and Fisher 1989) were measured on the basis of the maximum radial diameter (rd_{max}) of the first-formed vessels, which formed first in the current growth ring, often adjacent to the annual ring border (Fig. 4-1). Wide

vessels are defined as $>0.5 rd_{max}$, medium-sized vessels as $\leq 0.5 rd_{max}$ and $>0.25 rd_{max}$, and narrow vessels as $\leq 0.25 rd_{max}$. The current research focused on observation of large diameter vessels, given their functional significance for hydraulic conductance. Sparse wide vessels are those that are present in lower density than first-formed vessels or equal half the number of first-formed vessels. Vessel size and density were measured tangentially using 2-3-mm-long transverse sections.

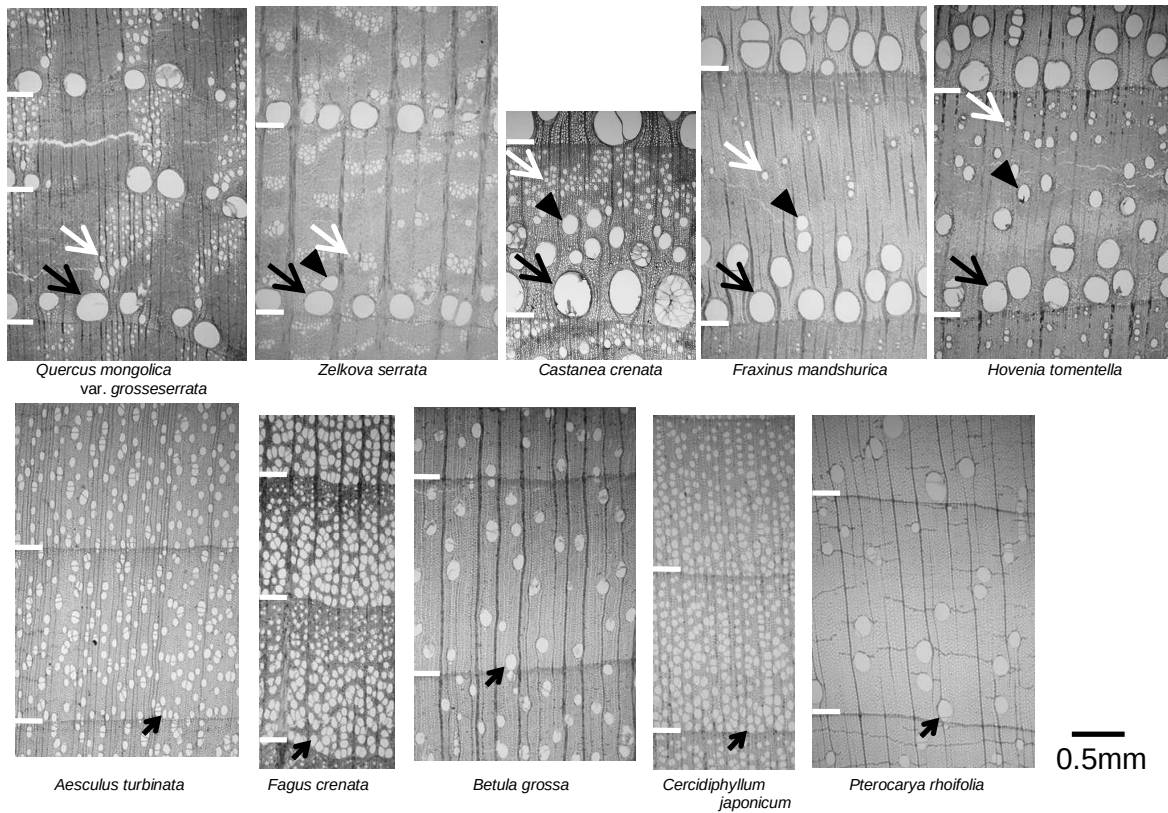


Fig. 4-1 Photomicrographs of ring-porous species (*upper*) and diffuse-porous species (*lower*). Ring-porous species with flush-type leaf emergence pattern: *Quercus mongolica* var. *grosseserrata* and *Zelkova serrata*. Ring-porous species with intermediate-type leaf emergence pattern: *Castanea crenata*, *Fraxinus mandshurica*, and *Hovenia tomentella*. In ring-porous species, *large black arrows* point to wide vessels; *large black arrowheads* indicate medium-sized vessels; *large white arrows* point to narrow vessels. Diffuse-porous species with flush-type leaf emergence pattern: *Aesculus turbinata* and *Fagus crenata*. Diffuse-porous species with intermediate-type leaf emergence pattern: *Betula grossa*, *Cercidiphyllum japonicum*, and *Pterocarya rhoifolia*. In diffuse-porous species, *small black arrows* point to the first-formed vessels. *White lines* indicate ring borders

4.2.3 Observation of leaf phenology

Leaves of each sample tree were examined at each sampling conducted. The following phenological parameters such as the beginning of leaf appearance, completion of leaf appearance, full leaf expansion, and the end of shoot elongation were observed (Chapter 1).

4.3 Results

4.3.1 Temporal relationships between leaf phenology and vessel formation

in ring-porous species

4.3.1.1 Timing of wide vessel lignification

At least one wide vessel had lignified between 4 weeks before and 2 weeks after the completion of leaf appearance in ring-porous species (Fig. 4-2B). In species with flush-type leaf emergence patterns, at least one wide vessel had lignified between 0 and 2 weeks after the beginning of leaf appearance (Fig. 4-2A), and most wide vessels were lignified 0–2 weeks before full leaf expansion (Fig. 4-2D). In species with intermediate-type leaf emergence patterns, at least one wide vessel had lignified between 0 and 2 weeks before the beginning of leaf appearance (Fig. 4-2A), and lignification of most wide vessels was observed between 4 weeks before and 2 weeks after full leaf expansion (Fig. 4-2D). Lignification of sparse wide vessels was observed 2 weeks before full leaf expansion in the trees that showed lignification of most wide vessels 4 weeks before full leaf expansion (Fig. 4-2E). Therefore, independent of wide vessel density, wide vessels lignified between 2 weeks before and 2 weeks after full leaf expansion. Vessel lignification occurred later in *H. tomentella*; specimens showed lignification of at least one wide vessel 0–2 weeks before full leaf expansion, although vessel lignification was observed in other species 2–6 weeks before full leaf expansion (Fig. 4-2C). Sparse wide vessels had become lignified close to the time of full leaf expansion (± 2 weeks), although these vessels formed 2–6 weeks after full leaf expansion when second leaves appeared in *Z. serrata* (Fig. 4-2E).

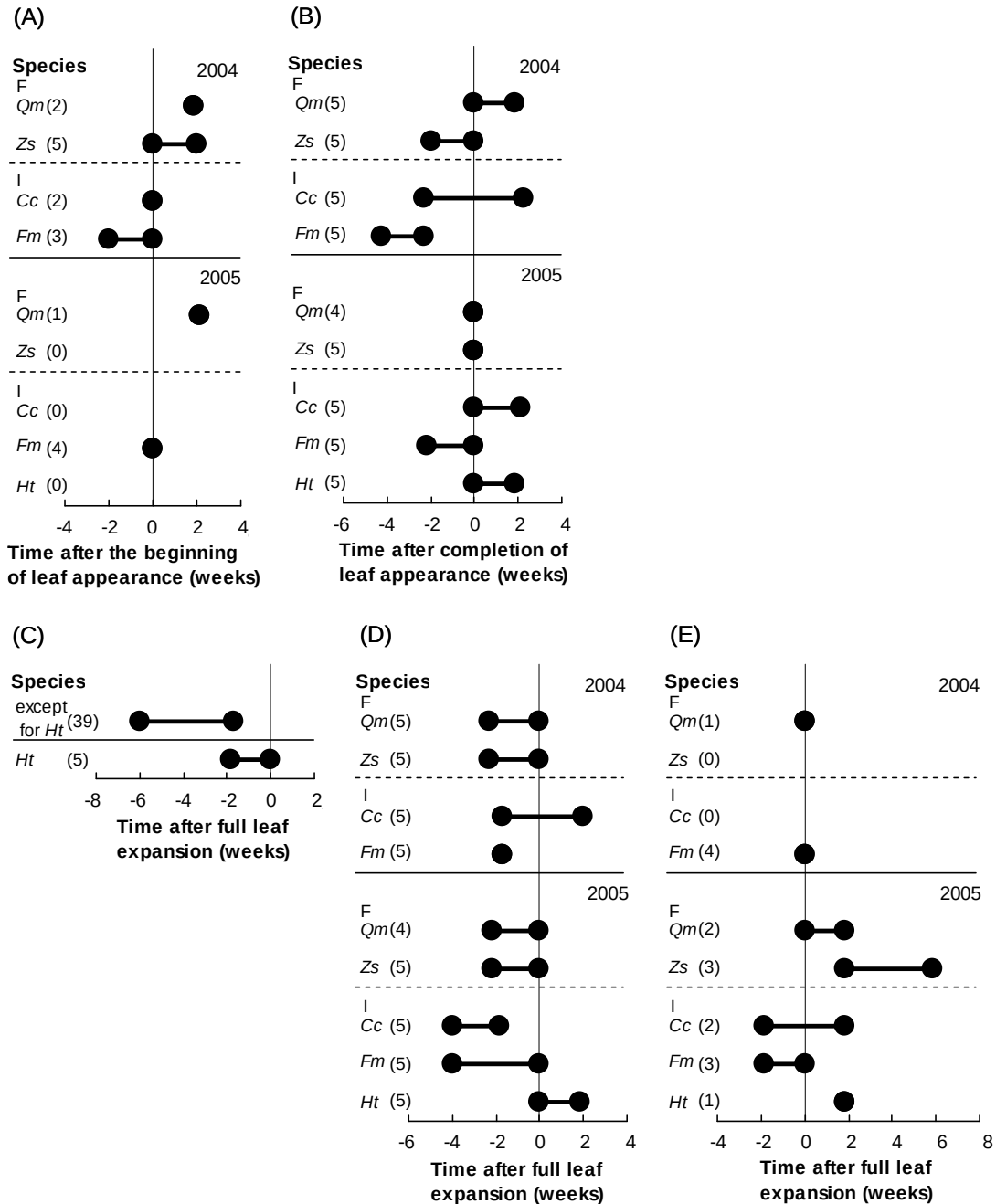


Fig. 4-2 Timing of vessel lignification relative to leaf phenology in ring-porous species in 2004 (upper) and 2005 (lower). (A) Timing of lignification of at least one wide vessel relative to the beginning of leaf appearance. (B) Timing of lignification of at least one wide vessel relative to completion of leaf appearance. (C) Timing of lignification of at least one wide vessel relative to full leaf expansion. (D) Timing of lignification of most wide vessels relative to full leaf expansion. (E) Timing of lignification of sparse wide vessels relative to full leaf expansion. (F) Timing of lignification of at least one medium-sized vessel relative to full leaf expansion. (G) Timing of lignification of most medium-sized vessels relative to the end of shoot elongation. (H) Timing of lignification of most medium-sized vessels relative to full leaf expansion of successive leaves. (I) Timing of lignification of narrow vessels relative to full leaf expansion. (J) Timing of lignification of narrow vessels relative to the end of shoot elongation. Numbers in parentheses indicate the numbers of trees; circles indicate range; F, flush-type leaf emergence pattern; I, intermediate-type leaf emergence pattern; Qm, *Quercus mongolica* var. *grosseserrata*; Zs, *Zelkova serrata*; Cc, *Castanea crenata*; Fm, *Fraxinus mandshurica*; Ht, *Hovenia tomentella*.

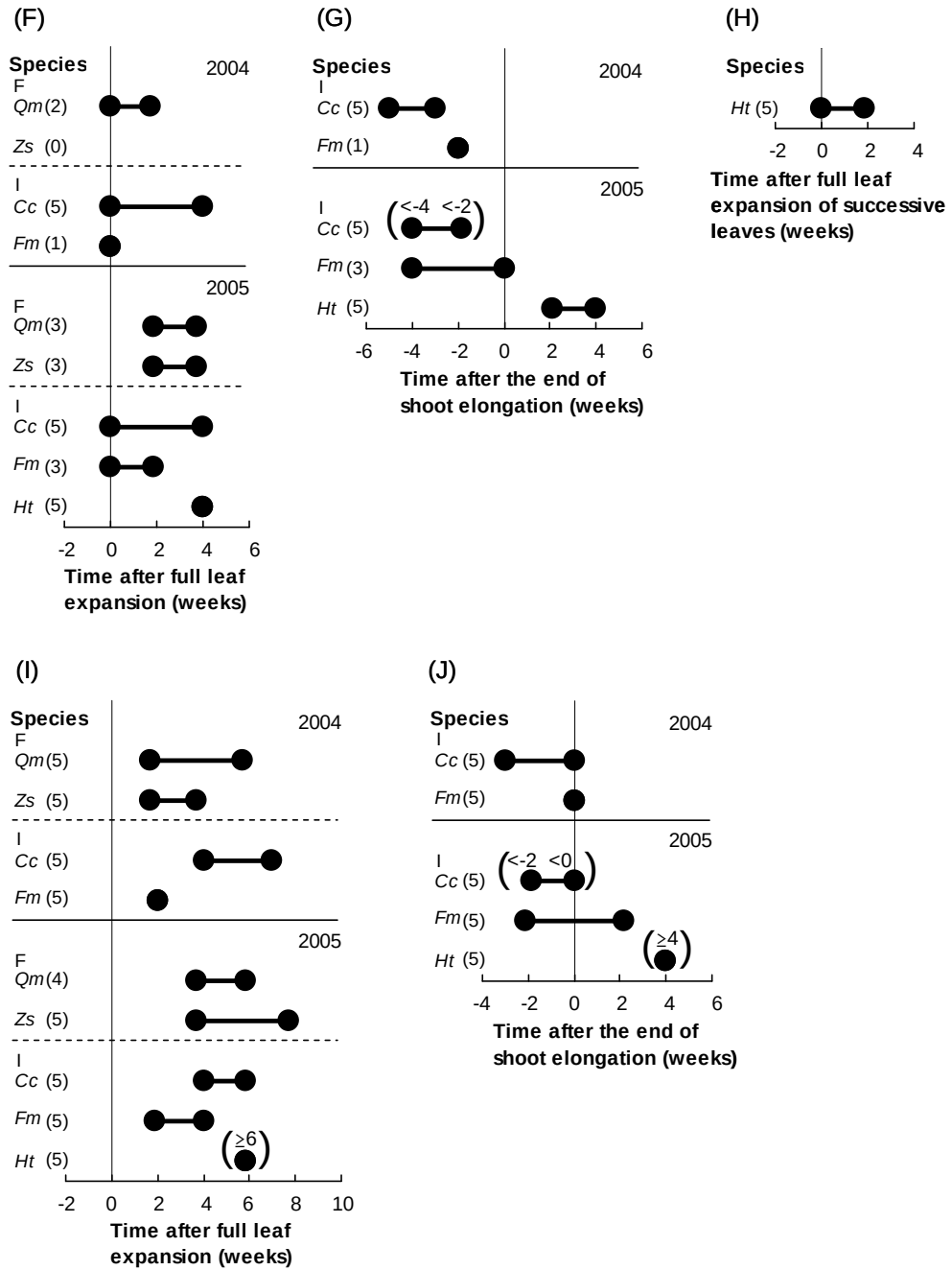


Fig. 2 (concluded)

4.3.1.2 Timing of medium-sized vessel lignification

Among species with flush-type leaf emergence patterns, medium-sized vessels formed in five of nine *Q. mongolica* var. *grosseserrata* specimens and did not form in *Z. serrata* in 2004 (Fig. 4-2F). In contrast, among species with intermediate-type leaf emergence patterns, all *Castanea crenata* and *H. tomentella*, and four of ten *Fraxinus mandshurica* specimens formed medium-sized vessels (Fig. 4-2F). Four other *Fraxinus mandshurica* specimens formed sparse wide vessels rather than medium-sized vessels.

Lignification of at least one medium-sized vessel occurred 0–4 weeks after full leaf expansion in *Q. mongolica* var. *grosseserrata*, and 0–4, 0–2, and 4 weeks after full leaf expansion in *Castanea crenata*, *Fraxinus mandshurica*, and *H. tomentella*, respectively (Fig. 4-2F). Lignification of most medium-sized vessels occurred >2–4 weeks before, 0–4 weeks before, and 2–4 weeks after the end of shoot elongation or 0–2 weeks after full leaf expansion of successive leaves in *Castanea crenata*, *Fraxinus mandshurica*, and *H. tomentella*, respectively (Figs. 4-2G, 4-2H). Thus, intermediate-type species had a stronger tendency to form medium-sized vessels or sparse wide vessels than flush-type species, and vessel lignification occurred the latest in *H. tomentella* (Figs. 4-2E, 4-2F, 4-2G).

4.3.1.3 Timing of narrow vessel lignification

Narrow vessels formed after the appearance of wide or medium-sized vessels. At least one narrow vessel had lignified between 2 and 8 weeks after full leaf expansion in all specimens (Fig. 4-2I). Relative to the end of shoot elongation, lignification of narrow vessels occurred at or before this stage in *Castanea crenata*, within ± 2 weeks of this stage in *Fraxinus mandshurica*, and ≥ 4 weeks after this stage in *H. tomentella* (Fig. 4-2J).

4.3.2 Temporal relationships between leaf phenology and vessel formation

in diffuse-porous species

4.3.2.1 Timing of lignification of the first-formed vessel

At least one of the first-formed vessels lignified 0–8 and 0–6 weeks after full leaf expansion in species with flush- and intermediate-type leaf emergence patterns, respectively, or 9

weeks before to 2 weeks after the end of shoot elongation (Figs. 4-3A, 4-3B). No new vessels were observed in one *Cercidiphyllum japonicum* tree in 2004 and 2005 or in one *Fagus crenata* tree in 2004 (Table 1-1).

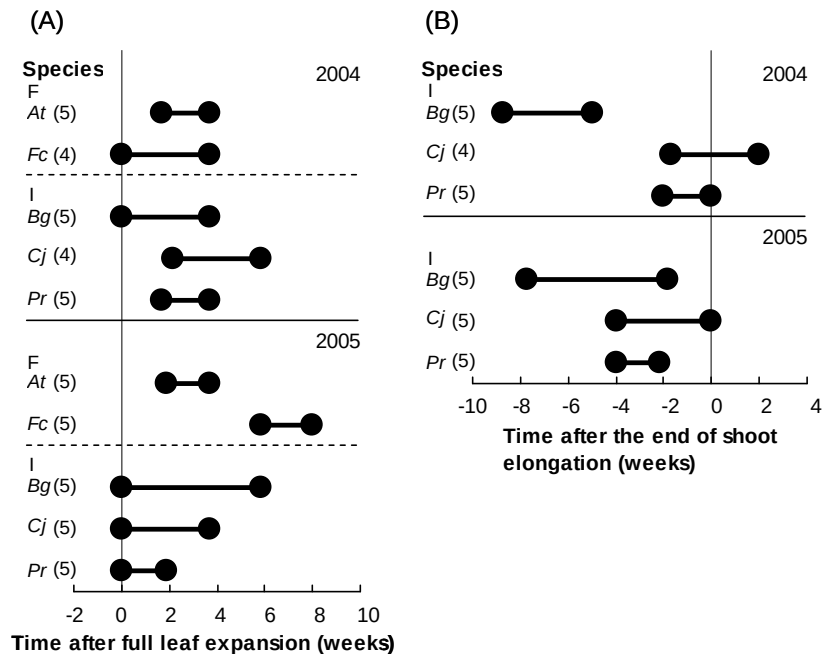


Fig. 4-3 Timing of vessel lignification relative to leaf phenology in diffuse-porous species in 2004 (upper) and 2005 (lower). (A) Timing of lignification of the first-formed vessel relative to full leaf expansion. (B) Timing of lignification of the first-formed vessel relative to the end of shoot elongation. Numbers in parentheses indicate the numbers of trees; circles indicate range; F, flush-type leaf emergence pattern; I, intermediate-type leaf emergence pattern; At, *Aesculus turbinata*; Fc, *Fagus crenata*; Bg, *Betula grossa*; Cj, *Cercidiphyllum japonicum*; Pr, *Pterocarya rhoifolia*.

4.3.3 Relationship between leaf emergence pattern and vessel arrangement

Ring- and diffuse-porous species have both flush- and intermediate-type leaf emergence patterns (Table 4-1). The type of porosity and leaf emergence pattern of each species investigated in this study are shown in Table 1-1; microphotographs of vessel arrangement in each species are shown in Fig. 4-1. There were two groups of ring-porous species. *Q. mongolica* var. *grosseserrata* and *Z. serrata* formed wide vessels first in the current growth ring and then formed narrow vessels and few medium-sized vessels, whereas *Castanea crenata*, *Fraxinus mandshurica*, and *H. tomentella* formed wide vessels first and then formed one or more rows of medium-sized vessels (or sparse wide vessels) and narrow vessels (Figs. 4-1, 4-2E, 4-2F). The transition

between the pore- and non-pore zones in the former group was abrupt and that in the latter group was gradual. There was no apparent relationship between leaf emergence pattern and vessel arrangement in diffuse-porous species (Table 1-1, Fig. 4-1).

Table 4-1 Porosity and leaf emergence patterns

Porosity ^a	Leaf emergence pattern ^b	
	Flush-type	Intermediate-type
Ring-porous	<i>Quercus mongolica</i> var. <i>grosseserrata</i> *	<i>Picrasma quossoides</i>
	<i>Maackia amurensis</i> var. <i>buergeri</i>	<i>Kalopanax pictus</i>
		<i>Fraxinus mandshurica</i> var. <i>japonica</i> *
		<i>Ulmus davidiana</i> var. <i>japonica</i>
		<i>Ulmus laciniata</i>
		<i>Phellodendron amurense</i>
		<i>Castanea crenata</i> *
Diffuse-porous	<i>Fagus crenata</i> *	<i>Betula platyphylla</i> var. <i>japonica</i>
	<i>Prunus sargentii</i>	<i>Betula davurica</i>
	<i>Prunus ssiori</i>	<i>Betula ermanii</i>
	<i>Sorbus alnifolia</i>	<i>Betula maximowicziana</i>
	<i>Acer mono</i>	<i>Cercidiphyllum japonicum</i> *
	<i>Aesculus turbinata</i> *	<i>Cornus controversa</i>
	<i>Carpinus cordata</i>	<i>Populus sieboldii</i>
	<i>Acer palmatum</i> var. <i>matsumurae</i>	<i>Populus maximowiczii</i>
	<i>Acer japonicum</i>	<i>Juglans ailanthifolia</i>
	<i>Tilia japonica</i>	<i>Alnus japonica</i>
	<i>Tilia maximowicziana</i>	<i>Magnolia obovata</i>
	<i>Syringa reticulata</i>	<i>Sorbus commixta</i>
	<i>Styrax obassia</i>	<i>Salix sachalinensis</i>
		<i>Salix hultenii</i> var. <i>angustifolia</i>
		<i>Salix subfragilis</i>
		<i>Ostrya japonica</i>

Note: Scientific names are based on those reported by Kikuzawa (1983).

^aPorosity classes for each species were assigned as defined in Hayashi (1991), Itoh (1995, 1997), and Inoue et al. (2002).

^bThe type of leaf emergence pattern was reported for each species as defined by Kikuzawa (1983); flush and intermediate types were examined.

*Species were examined.

4.4 Discussion

4.4.1 Relationship between leaf phenology and vessel formation

4.4.1.1 Comparison of ring-porous and diffuse-porous species

In ring-porous species, secondary wall deposition of the first vessel elements is completed from 1 week before to 3 weeks after leaf expansion (Suzuki et al. 1996), and maturation occurs within 1 week of leaf budding (Suzuki et al. 2000). This timing coincides with or occurs just before bud opening (Lodewick 1928; Zasada and Zahner 1969) and after the onset of leaf unfolding (Atkinson and Denne 1988; Fonti et al. 2007). The timing of first vessel maturation relative to bud break is species-specific (Sass-Klaassen et al. 2011). In the present study, at least one wide vessel was lignified between 2 weeks before the beginning of leaf appearance and 2 weeks after the completion of leaf appearance in ring-porous species (Figs. 4-2A, 4-2B). Relative to the beginning of leaf appearance, at least one wide vessel was lignified between 0 and 2 weeks after in flush-type species and between 0 and 2 weeks before in intermediate-type species (Fig. 4-2A). Thus, at least one wide vessel would be formed within a short time of leaf appearance, and the timing, which is before or after leaf appearance, is considered to be species-specific. Moreover, lignification of most wide vessels in ring-porous species was observed 4 weeks before to 2 weeks after full leaf expansion (Fig. 4-2D). Lignification of sparse wide vessels was observed 2 weeks before full leaf expansion in the trees that showed lignification of most wide vessels 4 weeks before full leaf expansion (Fig. 4-2E). Therefore, independent of wide vessel density, wide vessels were lignified between 2 weeks before and 2 weeks after full leaf expansion. Most wide vessels would be formed within a short time of full leaf expansion.

A rapid decline in vessel diameter at the end of pore-zone formation coincided with the cessation of shoot elongation (Denne, 1976); therefore, the pore–non-pore transition in ring-porous species occurred when shoot extension ceased (Digby and Wareing 1966). Lignification of medium-sized vessels occurred 0–2 weeks after full leaf expansion in flush-type species, except for *Z. serrata*, which formed wider vessels when second leaves appeared, and one *Q. mongolica* var. *grosseserrata* tree, which had Japanese oak wilt disease (Fig. 4-2F). In intermediate-type species, medium-sized vessels were lignified between 0 and 4 weeks after full leaf expansion (Fig. 4-2F) and before the end of shoot elongation (Fig. 4-2G), or around full leaf expansion of successive leaves (Fig. 4-2H). Thus, the decline in vessel diameter is considered to

coincide with the cessation of shoot extension – from full leaf expansion to the end of shoot elongation or full leaf expansion of successive leaves.

These findings suggest that ring-porous species produce leaves and most hydraulically functional vessels over a short time in the early stage of annual ring formation; after early growth, these species synchronously produce subsequent leaves and vessels. This suggests that ring-porous species change vessel diameters according to the progress of leaf formation.

In contrast, in diffuse-porous species, at least one of the first-formed vessels lignified 0–8 weeks after full leaf expansion in flush-type species and slightly sooner in intermediate-type species (Fig. 4-3A), which was the same as or later than that reported by Lodewick (1928) and Suzuki et al. (2000). Lignification of the first-formed vessels occurred before the end of shoot elongation in most intermediate-type species (Fig. 4-3B). Thus, diffuse-porous species are considered to begin forming the first vessels after full leaf expansion or before the end of shoot elongation in intermediate-type species. Further, diffuse-porous species might form no new vessels in at least one direction; a few of samples showed no new vessels.

These findings suggest that diffuse-porous species produce leaves and vessels over an extended period in the early stage of annual-ring formation and produce subsequent leaves and vessels asynchronously after the early growth. This suggests that the time of formation of vessels is not closely related to that of leaves in diffuse-porous species.

A phylogenetic tree (Fig. 4-4) was constructed for all measured species from the APG III (2009) with a program, Phylomatic (<http://phylodiversity.net/phylomatic>). Phylogenetic analysis revealed that ring-porous *Castanea crenata* and *Q. mongolica* var. *grosseserrata* showed synchronous formation of leaves and vessels, whereas diffuse-porous *Fagus crenata* showed asynchronous formation of these elements among Fagaceae (Fig. 4-4). Although phylogenetically distant, ring-porous species showed the same tendency of leaf and vessel formation (e.g., *Castanea crenata* and *Fraxinus mandshurica*; Fig. 4-4) and, although phylogenetically distant, diffuse-porous species showed the same tendency of leaf and vessel formation (e.g., *Fagus crenata* and *Cercidiphyllum japonicum*; Fig. 4-4).

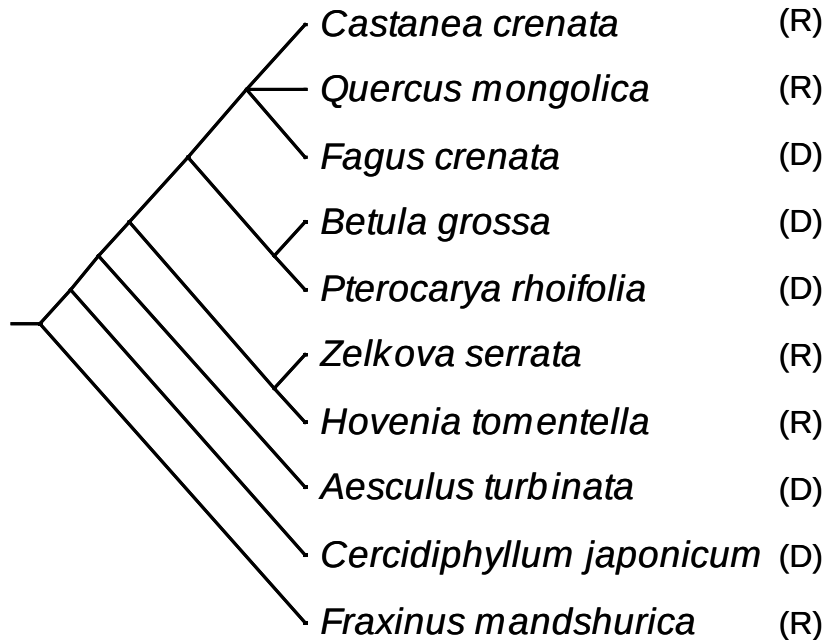


Fig. 4-4 Phylogenetic tree of sample species (from the APG III 2009 with a program, Phylomatic <http://phylodiversity.net/phylomatic>, last accessed 14 December 2014). R, ring-porous species; D, diffuse-porous species.

4.4.1.2 Timing of narrow vessel formation in ring-porous species

The first-formed narrow vessels in ring-porous species are thought to be completed around the time of full leaf expansion (Suzuki et al. 2000), coinciding with final leaf expansion (Lodewick 1928). However, the non-pore zone was reported to form several weeks after maturation of foliage in *Quercus rubra* (Zasada and Zahner 1969). In the present study, narrow vessels were lignified 2–8 weeks after full leaf expansion in flush-type species (Fig. 4-2I), as described by Zasada and Zahner (1969). In contrast, narrow vessels were also lignified between full leaf expansion and the end of shoot elongation (Figs. 4-2I, 4-2J) or immediately after full expansion of successive leaves in intermediate-type species, consistent with the findings of Lodewick (1928) and Suzuki et al. (2000). Thus, lignification of narrow vessels might occur after

full leaf expansion in flush-type species and around final leaf expansion in intermediate-type species.

4.4.2 Leaf emergence pattern and vessel arrangement

There is no apparent association of wood structure and leaf development synchronicity, because within both ring- and diffuse-porous groups, species with flush- and intermediate-type leaf emergence patterns were observed (Tables 1-1, Fig. 4-1). Ring-porous species can have abrupt or gradual transitions between pore- and non-pore zones (Woodcock 1989; Carlquist 2001). In the ring-porous species investigated in this study, I noted abrupt transitions in flush-type species and gradual transitions in intermediate-type species (Table 1-1, Fig. 4-1), although there was no relationship between leaf emergence pattern and vessel arrangement in diffuse-porous species (Table 1-1, Fig. 4-1).

Ladefoged (1952) and Denne (1976) described the relationships between vessel diameter and shoot elongation. Findings of this study suggest that these relationships apply to ring-porous species only, which form leaves and vessels synchronously. Thus the leaf emergence pattern might be predicted from the observations of vessel arrangement, and *vice versa*.

Chapter 5 Phenological relationship between leaf appearance and vessel formation in twigs and stems of species with contrasting vessel arrangement

5.1 Introduction

Temperate broad-leaved trees have evergreen or deciduous leaf habits, and their woody tissue is classified by porosity to ring-porous, diffuse-porous, and semi-ring-porous (Wheeler et al. 1989). Radial-porous wood is characterized by vessels in radial pattern (Wheeler et al. 1989; Hayashi 1991; Itoh 1995). I attempt to clarify how different leaf habits and variation in porosity and vessel arrangement affect species adaptation to their environment by examining the temporal association of vessel formation in relation to leaf appearance.

In Chapter 3, the ring-porous species were shown to produce single-year leaves and most functional vessels in a short period; whereas the diffuse-porous species produced single-year leaves and multiple-year vessels in a long period. In ring-porous and diffuse-porous species, twig vessel lignification occurs around leaf appearance (Chapter 3). However, studies of leaf and vessel production in deciduous species are not many, as are studies of seasonal changes in vessel formation in evergreen species. The ring-porous evergreen species, *Castanopsis sieboldii*, and the radial-porous evergreen species, *Lithocarpus edulis*, have shown completion of secondary wall deposition in the stem at the time of leaf appearance (Hirano 1998). Phenological studies on vessel formation lead to the following hypotheses that ring- and radial-porous evergreen species show leaf and vessel production in short duration.

Regarding water distribution, vessels in several rings adjacent to the cambium retain their water transport ability through the years in diffuse-porous (Umebayashi et al. 2010) and radial-porous evergreen species (Hirose et al. 2005; Umebayashi et al. 2010). The semi-ring-porous deciduous species, *Acanthopanax sciadophylloides*, has narrower vessels in the pore-zone compared to those in ring-porous species and sometimes has porosity similar to that of diffuse-porous species. Umebayashi et al. (2008) categorized *Acanthopanax sciadophylloides* as ring-porous species, because water is transported mostly through its wide vessels during the year and these vessels are formed in the same manner as in other ring-porous species. Studies on water distribution lead to the following hypotheses that diffuse- and radial-porous evergreen species

show leaf and vessel production in long duration compared to the semi-ring-porous deciduous *Acanthopanax sciadophylloides* with leaf and vessel production in short duration.

I tested above-mentioned hypotheses and aimed to clarify the developmental coordination among different aspects of plant function. In particular, phenological relationship between leaf appearance and twig and stem vessel formation was studied in nine tree species with different porosity and vessel arrangement in a temperate forest, where all species grew in the same forest stand.

5.2 Materials and methods

5.2.1 Sampling

The study was conducted at the Kamigamo Experimental Forest Station (Chapter 1). Sample trees (Table 1-2 in Chapter 1) with six different types of porosity and vessel arrangement from nine species were selected. Cylindrical wood core samples were collected with an increment borer (Chapter 2) from three to six trees of each species.

Twigs with sun leaves were collected from each tree using 12-m-long pruners or by climbing using 3-m-long pruners. Three to five trees of each species were sampled. In this study, twigs that were newly extended within the previous year were regarded as 1-year-old twigs. In *Acanthopanax sciadophylloides*, I observed vessel formation in 1-year-old twigs and occasionally in 2- to 4-year-old twigs at 0.5–1.5 cm above the bud base.

Twig samples were collected biweekly between March 14 and June 6 in 2006. Stem samples were collected biweekly, between March 14 and June 20, and additionally monthly, between July 4 and August 29, in 2006.

5.2.2 Assessment of vessel formation

Lignification of the first-formed vessels was determined by the same procedure used in Chapter 3. Lignification date was defined as the time on which lignification of almost all the first-formed vessels was observed.

5.2.3 Observation of leaf phenology

Leaf observation began on March 14 and was conducted weekly, between March 29 and June 20, 2006. Completion of leaf appearance was observed (Chapter 1).

5.3 Results

5.3.1 Time of twig vessel lignification in relation to leaf appearance

Lignification of the first-formed twig vessels was observed concurrently with leaf appearance in the ring-porous deciduous *Q. serrata*, and 0–3 weeks before leaf appearance in the diffuse-porous deciduous *L. styraciflua* (Fig. 5-1). Differences in the time of vessel lignification between ring-porous and diffuse-porous species were not significant (Table 5-1). Twig vessel lignification occurred 0–1 week before leaf appearance in the semi-ring-porous deciduous *Acanthopanax sciadophylloides*; 0–2 weeks before leaf appearance in the ring-porous evergreen *Castanopsis cuspidata*; 2 weeks before to 1 week after leaf appearance in the diffuse-porous *Castanopsis cuspidata*; 2 weeks before to 1 week after leaf appearance in the diffuse-porous

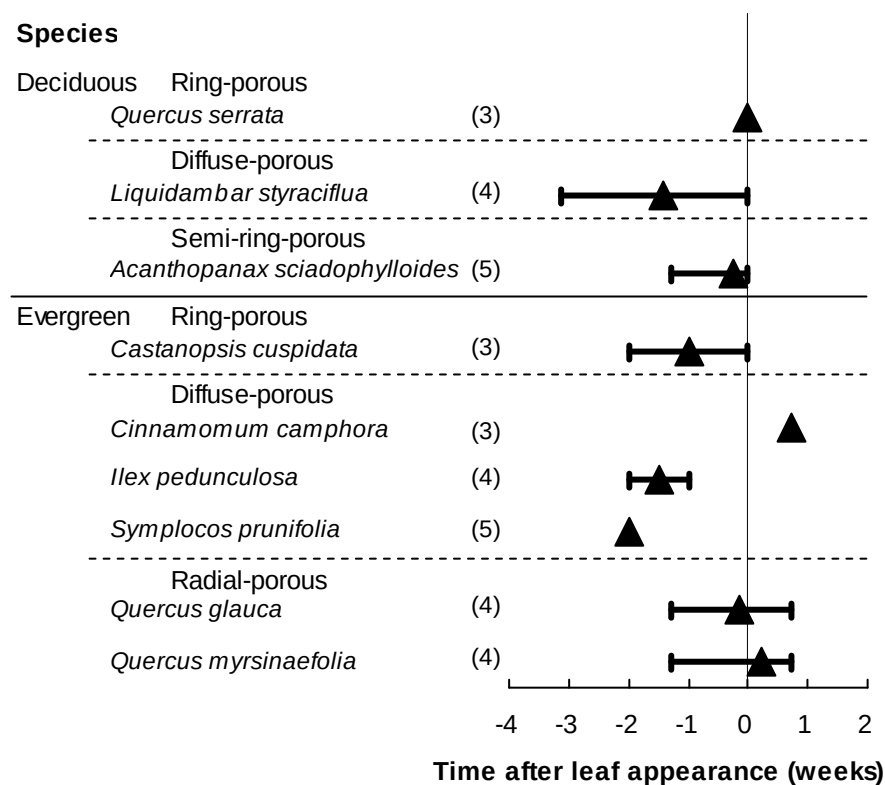


Fig. 5-1 Lignification time of the first-formed twig vessels in relation to the time of leaf appearance at the whole-tree level. Numbers in parentheses indicate the number of trees; filled triangles average, bar range

evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia*; and within 1 week after leaf appearance in the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia* (Fig. 5-1). The time of twig vessel lignification was different within diffuse-porous evergreen species; twig vessel lignification was observed just after leaf appearance in *Cinnamomum camphora*, while in *I. pedunculosa* and *S. prunifolia*, it was observed just before leaf appearance (Fig. 5-1). The Dunnett's test results showed that the time of twig vessel lignification was not significantly different between the semi-ring-porous deciduous, ring-porous evergreen, diffuse-porous evergreen, or radial-porous evergreen species and the ring-porous deciduous or diffuse-porous deciduous species (Table 5-1). The time of the first-formed twig vessel lignification was close to the time of leaf appearance and ranged between 3 weeks before and 1 week after leaf appearance (Fig. 5-1).

Table 5-1 Differences of 3 temporal traits of vessel formation in ring-porous and diffuse-porous species compared to other species

Lignification of the first-formed vessels	Leaf habit	Deciduous			Evergreen			total (trees)			
		Vessel arrangement	Ring-porous	Diffuse-porous	Semi-ring-porous	Ring-porous	Diffuse-porous		Radial-porous		
in twigs compared	count (trees)	3		4	5	3	12	8	35		
to leaf appearance	mean (weeks)	0.0	a*	-1.4	a	-0.3	a	-1.0	a	0.0	a
	var	0.00		1.67		0.33		1.00		1.41	0.76
in stems compared	count (trees)	5		6	6	3	13	10		43	
to leaf appearance	mean (weeks)	2.4	a	9.5	b*	4.6	ab	5.0	ab	8.2	b
	var	0.80		18.18		3.81		19.00		17.86	17.07
in stems compared	count (trees)	3		4	5	3	12	8		35	
to twigs	mean (weeks)	2.0	a	12.0	b	5.2	ab	6.0	ab	9.7	b
	var	0.00		18.32		5.20		28.00		18.79	16.86

*Different letters within each column indicate differences at 5% significance level by Dunnett's test.

5.3.2 Time of stem vessel lignification in relation to leaf appearance

Lignification of the first-formed stem vessels was observed 2–4 weeks after leaf appearance in the ring-porous deciduous *Q. serrata*, and 7–18 weeks after leaf appearance in the diffuse-porous deciduous *L. styraciflua* (Fig. 5-2). Ring-porous deciduous trees showed significantly earlier vessel lignification than diffuse-porous deciduous trees ($P < 0.05$, Table 5-1). Stem vessels lignified 2–7 weeks and 0–8 weeks after leaf appearance in the semi-ring-porous deciduous *Acanthopanax sciadophylloides* and the ring-porous evergreen *Castanopsis cuspidata*,

respectively (Fig. 5-2). On the basis of results of the Dunnett's test, the time of stem vessel lignification was not significantly different between the semi-ring-porous deciduous or ring-porous evergreen species and the ring-porous deciduous or diffuse-porous deciduous species (Table 5-1). On the other hand, stem vessels of the diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia* lignified 1–13 weeks after leaf appearance, while those of the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia* lignified 6–17 weeks after leaf appearance (Fig. 5-2). Stem vessel lignification in these two species occurred significantly later than that in ring-porous deciduous species ($P < 0.05$, Table 5-1), but it was not significantly different from that in diffuse-porous deciduous species (Table 5-1). The time of stem vessel lignification was different within diffuse-porous evergreen species. Stem vessel lignification in *I. pedunculosa* occurred 1–6 weeks after leaf appearance, which was earlier than that in *Cinnamomum camphora* (7–13 weeks after leaf appearance) and in *S. prunifolia* (8–12 weeks after leaf appearance) (Fig. 5-2).

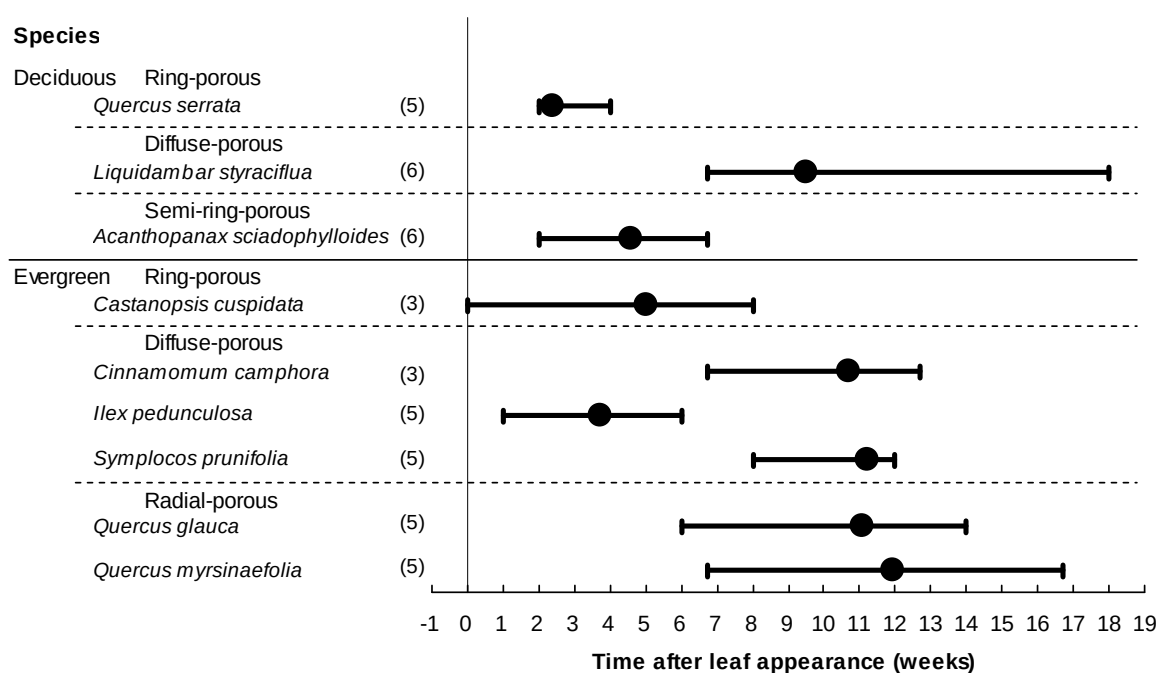


Fig. 5-2 Lignification time of the first-formed stem vessels in relation to the time of leaf appearance at the whole-tree level. Numbers in parentheses indicate the number of trees; filled circles average, bar range

The time of lignification of the first-formed stem vessels in relation to the time of leaf appearance was earlier in ring-porous deciduous species than in diffuse-porous deciduous, diffuse-porous evergreen, and radial-porous evergreen species (Fig. 5-2). Semi-ring-porous deciduous and ring-porous evergreen species followed a time pattern of vessel lignification similar to ring-porous and diffuse-porous deciduous species (Fig. 5-2).

5.3.3 Time of stem and twig vessel lignification

Lignification of stem vessels occurred 2 weeks after the lignification of twig vessels in the ring-porous deciduous *Q. serrata*, while that in the diffuse-porous deciduous *L. styraciflua* occurred 8–18 weeks after the lignification of twig vessels (Fig. 5-3). Vessel lignification occurred significantly earlier in ring-porous than in diffuse-porous deciduous species ($P < 0.05$, Table 5-1). Stem vessel lignification occurred 2–8 weeks and 0–10 weeks after twig vessel lignification in the semi-ring-porous deciduous *Acanthopanax sciadophylloides* and the ring-porous evergreen *Castanopsis cuspidata*, respectively (Fig. 5-3), and the time was not significantly different from that for ring-porous or diffuse-porous deciduous species (Table 5-1). On the other hand, stem vessel lignification of the diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia* and the radial-porous *Q. glauca* and *Q. myrsinaefolia* occurred 2–14 weeks and 6–16 weeks after twig vessel lignification, respectively (Fig. 5-3), which was significantly later than that for ring-porous deciduous species ($P < 0.05$, Table 5-1), but not significantly different from that for diffuse-porous deciduous species (Table 5-1). The time of stem vessel lignification was different within diffuse-porous evergreen species. Stem vessel lignification in *I. pedunculosa* occurred 2–8 weeks after twig vessel lignification, time that was comparatively earlier than this in *Cinnamomum camphora* and *S. prunifolia* that occurred 6–12 weeks and 10–14 weeks after twig vessel lignification, respectively (Fig. 5-3).

The time of the first-formed stem vessel lignification in relation to twig vessel lignification was earlier in ring-porous deciduous species compared to diffuse-porous deciduous, diffuse-porous evergreen, and radial-porous evergreen species (Fig. 5-3). Some individuals of semi-ring-porous deciduous and ring-porous evergreen species followed a time pattern of stem

vessel lignification in relation to twig vessel lignification similar to that by ring-porous or diffuse-porous deciduous species (Fig. 5-3).

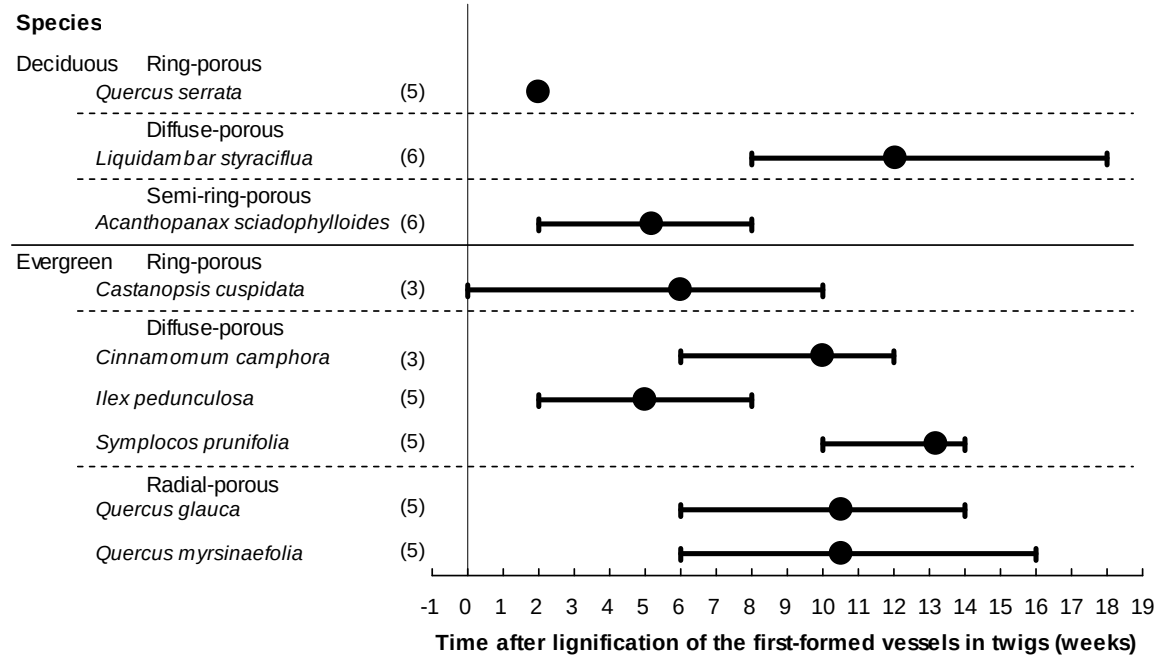


Fig. 5-3 Lignification time of the first-formed stem vessels in relation to lignification time of twig vessels. Numbers in parentheses indicate the number of trees; filled circles average, bar range

5.4 Discussion

5.4.1 hypothesis test

In the ring-porous deciduous *Q. serrata*, stem vessels were lignified shortly after leaf appearance (Fig. 5-2). Lignification of stem vessels in the diffuse-porous deciduous *L. styraciflua* occurred long after leaf appearance (Fig. 5-2). The diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia*, and the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia* showed a time pattern of stem vessel lignification similar to that for diffuse-porous deciduous species (Fig. 5-2). The semi-ring-porous deciduous *Acanthopanax sciadophylloides* and the ring-porous evergreen *Castanopsis cuspidata* showed a time pattern of stem vessel lignification either similar to ring-porous deciduous species or to diffuse-porous deciduous species (Fig. 5-2). The time of stem vessel lignification in relation to leaf appearance in *I.*

pedunculosa was earlier than that in other evergreen diffuse-porous species (Fig. 5-2). The results for the diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa* and *S. prunifolia* and the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia* were consistent with the hypothesis of long duration in leaf and vessel production, although a few individuals of diffuse-porous evergreen *I. pedunculosa* showed leaf and vessel production different from this hypothesis. Some individuals of the semi-ring-porous deciduous *Acanthopanax sciadophylloides* and the ring-porous evergreen *Castanopsis cuspidata* differed from the hypothesis of short duration in leaf and vessel production.

5.4.2 Relationship between leaf phenology and vessel formation

5.4.2.1 Comparison of ring-porous and diffuse-porous deciduous species

I found that the first-formed twig vessel lignification occurred concurrently with leaf appearance in the ring-porous deciduous *Q. serrata* and 0–3 weeks before leaf appearance in the diffuse-porous deciduous *L. styraciflua* (Fig. 5-1), indicating that time difference between these two species is not significant, similarly as in Chapter 3.

Lignification of the first-formed stem vessels occurred 2–4 weeks after leaf appearance in the ring-porous deciduous *Q. serrata* and 7–18 weeks after leaf appearance in the diffuse-porous deciduous *L. styraciflua* (Fig. 5-2). Hence, a ring-porous deciduous species showed significantly earlier stem vessel formation than a diffuse-porous deciduous species (Table 5-1). Stem vessel formation in relation to leaf appearance in ring-porous species tends to occur earlier than that in diffuse-porous species, similarly as in Chapter 3.

I also found that the lignification of stem vessels occurred 2 weeks after the twig vessel lignification in the ring-porous deciduous *Q. serrata* and 8–18 weeks after the twig vessel lignification in the diffuse-porous deciduous *L. styraciflua* (Fig. 5-3). Thus a ring-porous deciduous species showed significantly earlier stem vessel formation than a diffuse-porous deciduous species (Table 5-1). Lignification of stem vessels occurs later than that of twig vessel in both ring-porous and diffuse-porous species, and the time-lag between these two events is longer in diffuse-porous than in ring-porous species, similarly as in Chapter 3.

5.4.2.2 Comparison between semi-ring-porous deciduous, ring-porous evergreen, diffuse-porous evergreen, or radial-porous evergreen species, and ring-porous or diffuse-porous deciduous species

I found that lignification of the first-formed twig vessels occurred between 2 weeks before and 1 week after leaf appearance in the semi-ring-porous deciduous *Acanthopanax sciadophylloides*; the ring-porous evergreen *Castanopsis cuspidata*; the diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia*; and the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia* (Fig. 5-1). Same results were also obtained from the ring-porous deciduous *Q. serrata* and diffuse-porous deciduous *L. styraciflua* (Table 5-1; Fig. 5-1). This suggests that twig vessel lignification follows a similar time pattern in these species. On the other hand, ring-porous deciduous species tend to show a significantly earlier twig vessel formation than diffuse-porous deciduous species (Chapter 3). Further studies are required to elucidate these findings.

The first-formed stem vessels lignified 0–8 weeks after leaf appearance in the ring-porous evergreen *Castanopsis cuspidata* (Fig. 5-2). Hirano (1998) reported that secondary wall deposition in stems completed concurrently with leaf appearance in the ring-porous evergreen *Castanopsis sieboldii*. I suggested that ring-porous evergreen species have a time pattern of stem vessel lignification similar to ring-porous and diffuse-porous deciduous species. Stem vessel lignification occurred 6–14 weeks and 7–17 weeks after leaf appearance in the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia*, respectively (Fig. 5-2). Hirano (1998) also showed that secondary wall deposition in stems completed concurrently with leaf expansion in the radial-porous evergreen *Lithocarpus edulis*. I showed that radial-porous evergreen species had a time pattern of stem vessel formation similar to diffuse-porous deciduous species (Table 5-1), and the results were not consistent with those in Hirano (1998), suggesting that the time of stem vessel formation in radial-porous evergreen plants may be species-specific.

In the semi-ring-porous deciduous *Acanthopanax sciadophylloides*, stem vessel lignification occurred 2–7 weeks after leaf appearance (Fig. 5-2). This result indicates that semi-ring-porous deciduous species have a time pattern of stem vessel lignification similar to other deciduous species. Diffuse-porous evergreen species showed stem vessel lignification later

than ring-porous deciduous species but not different from diffuse-porous deciduous species (Table 5-1). Stem vessel lignification in the diffuse-porous evergreen *I. pedunculosa* occurred 1–6 weeks after leaf appearance, which was 7–13 weeks earlier and 8–12 weeks later than that in *Cinnamomum camphora* and *S. prunifolia*, respectively (Fig. 5-2). Stem vessel lignification of some *I. pedunculosa* individuals was earlier than other diffuse-porous deciduous trees, but not different from ring-porous deciduous species.

Stem vessel lignification in relation to twig vessel lignification occurred relatively earlier in the ring-porous deciduous *Q. serrata* than in the diffuse-porous deciduous *L. styraciflua*; diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia*; and radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia*. Variation in the time of stem vessel lignification in relation to twig vessel lignification observed within species of the semi-ring-porous deciduous *Acanthopanax sciadophylloides* and ring-porous evergreen *Castanopsis cuspidata* (Fig. 5-3). These time patterns of stem vessel lignification in relation to twig vessel lignification were similar to those of stem vessel lignification in relation to leaf appearance (Figs. 5-2, 5-3). These results suggest that the time lag between stem vessel lignification and leaf appearance is caused by the transition of vessel formation from twigs to stems. It is known that the time lag between the onset of cell division (Ladefoged 1952) and radial growth (Priestley & Scott 1936; Wareing 1950) at the bud and stem base is different among species.

5.4.3 Differences in phenological traits of species

The ring-porous deciduous *Q. serrata* produces leaves and vessels in shorter duration compared to the diffuse-porous deciduous *L. styraciflua*; diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia*; and radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia*. The rate of leaf and vessel production varies within the semi-ring-porous deciduous *Acanthopanax sciadophylloides* and ring-porous evergreen *Castanopsis cuspidata*. Chapter 3 also showed that ring-porous deciduous species produces single-year leaves and functional vessels in short duration compared to diffuse-porous species, which produces single-year leaves and multiple-year vessels in long duration.

Wide vessels of ring-porous deciduous species are known to transport water mostly during the year in which they are formed (Greenidge 1955; Chaney & Kozlowski 1977; Ellmore & Ewers 1986; Utsumi et al. 1999; Umebayashi et al. 2008), and these species produce leaves and vessels in a short period. These results suggest that ring-porous deciduous species produces leaves and wide vessels in a short period of time, to enable effective photosynthesis and water transport as the temperature rises in the spring following winter dormancy. Furthermore, diffuse-porous deciduous species begin to form leaves and twig vessels in spring, but stem vessel formation begins later. This suggests that diffuse-porous deciduous species, similar to ring-porous deciduous species, produces leaves and twig vessels in short duration, to enable photosynthesis and water transport in twigs as the temperature rises, but these species do not need to form stem vessels shortly after leaf appearance. This is possible because vessels in several rings adjacent to the cambium retain their water transport ability through the years (Greenidge 1955; Chaney & Kozlowski 1977; Utsumi et al. 1998; Umebayashi et al. 2008).

In diffuse-porous and radial-porous evergreen species, the time of leaf appearance, twig and stem vessel formation were similar to that of diffuse-porous deciduous species. Additionally, vessels in several rings adjacent to the cambium retain their water transport ability through the years in diffuse-porous (Umebayashi et al. 2010) and radial-porous evergreen species (Hirose et al. 2005; Umebayashi et al. 2010). This suggests that diffuse-porous and radial-porous evergreen species do not need to produce leaves and stem vessels in short duration, because they have leaves and usable vessels all year round and can start photosynthesis and water transport even before new leaf appearance (Miyazawa & Kikuzawa 2005). Some individuals of evergreen species with diffuse-porous wood in the current study, such as *I. pedunculosa*, were observed to develop leaves and vessels synchronously, suggesting cases in which development of diffuse-porous wood coincide with leaf development.

The semi-ring-porous deciduous *Acanthopanax sciadophylloides* has narrower first-formed vessels compared to ring-porous species, that are similar to diffuse-porous species in porosity. Umebayashi et al. (2008) categorized *A. sciadophylloides* as ring-porous species, based on the observation that its wide vessels transport water only in the outer-most ring, as in typical ring-porous species. It is also known that tyloses of *Acanthopanax sciadophylloides* are present in

several inner rings under the bark (Saitoh et al. 1993). In this study, some *Acanthopanax sciadophylloides* individuals produced leaves and stem vessels in short duration similarly to ring-porous deciduous species, while other individuals produced leaves and stem vessels in long duration similarly to diffuse-porous deciduous species. It seems that *Acanthopanax sciadophylloides* does not need to form stem vessels shortly after leaf appearance, probably because small vessels in several rings adjacent to the cambium of the non-pore zone contribute to effective water transport.

In ring-porous evergreen species, some individuals produce leaves and stem vessels in short duration similarly to ring-porous deciduous species, while other individuals produce leaves and stem vessels in long duration similarly to diffuse-porous deciduous species. The absence of tyloses of *Castanopsis sieboldii* in several outermost rings (Saitoh et al. 1993) suggest that the vessels in these multiple outer rings of ring-porous evergreen species function for water transport. It is also reasonable that hydraulic conductance is maintained in previous years' wood before the formation of current year vessels in evergreen species with ring-porous wood, given that they maintain functional leaves all year.

Chapter 6 General discussion

I clarify how plant functions relating photosynthesis and water transport are coordinated developmentally for species with different porosity and vessel arrangement, comparing the timing of leaf phenology and vessel formation in twigs and stems based on an ecophysiological viewpoint.

Fig. 6-1 summarizes the timing of vessel formation in twigs and stems in relation to leaf phenology for species of ring- and diffuse-porous deciduous trees (Chapter 3). The first-formed vessels in twigs were lignified around the time of leaf appearance at the whole-tree level in the case of flush-leaves and at or before full leaf expansion of the tree in both ring- and diffuse-porous species, although the timing tended to be slightly later in diffuse-porous species (also Figs. 3-1, 3-4). In almost all ring-porous species, stem vessels were lignified starting within a short time of leaf appearance and ending by the time of full leaf expansion (also Figs. 3-2, 3-5). *Fraxinus mandshurica* had the earliest vessel lignification, with individuals typically showing vessel lignification prior to leaf appearance, and *H. tomentella* had somewhat later vessel lignification. In contrast, lignification of stem vessels in diffuse-porous species occurred long after leaf appearance and after full leaf expansion (also Figs. 3-2, 3-5). Overall, lignification of vessels in stems occurred concurrently or soon after lignification of vessels in twigs in ring-porous species but relatively later in diffuse-porous species (also Fig. 3-3). *Fraxinus*

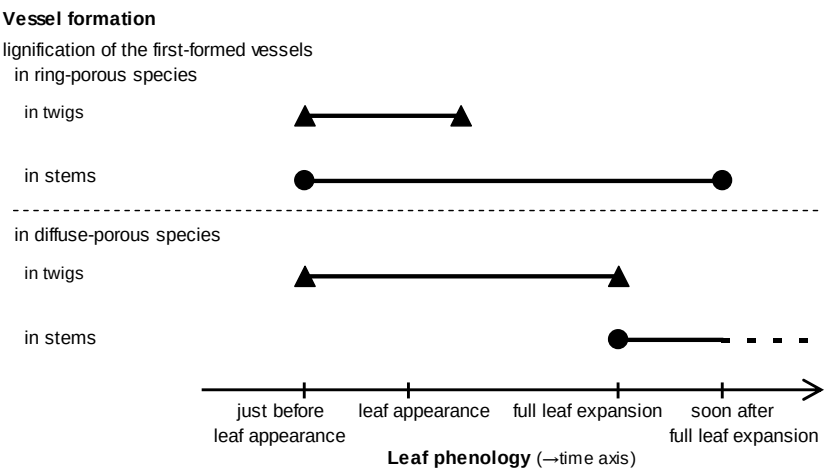


Fig. 6-1 Summary of the relationship between the order and timing of leaf phenology and vessel formation in ring-porous species (upper panel) and diffuse-porous species (lower panel). Filled triangles indicate timing in twigs; filled circles timing in stems; bar range

mandshurica had somewhat earlier vessel lignification in stems in relation to twigs.

Fig. 6-2 summarizes the time of twig and stem vessel formation in relation to leaf appearance for deciduous and evergreen species with different types of porosity and vessel arrangement (Chapter 5). The first-formed twig vessels were lignified around the time of leaf appearance in all species (also Fig. 5-1). In the ring-porous deciduous *Q. serrata*, stem vessels were lignified shortly after leaf appearance and twig vessel lignification (also Figs. 5-2, 5-3). In contrast, lignification of stem vessels in the diffuse-porous deciduous *L. styraciflua* occurred long after leaf appearance and twig vessel lignification (also Figs. 5-2, 5-3). The diffuse-porous evergreen *Cinnamomum camphora*, *I. pedunculosa*, and *S. prunifolia*, and the radial-porous evergreen *Q. glauca* and *Q. myrsinaefolia* showed a time pattern of stem vessel lignification similar to that for diffuse-porous deciduous species (also Figs. 5-2, 5-3). The semi-ring-porous deciduous *Acanthopanax sciadophylloides* and the ring-porous evergreen *Castanopsis cuspidata* showed a time pattern of stem vessel lignification either similar to ring-porous deciduous species or to diffuse-porous deciduous species (also Figs. 5-2, 5-3). The time of stem vessel lignification in relation to leaf appearance and twig vessel lignification in *I. pedunculosa* was earlier than that in other evergreen diffuse-porous species (also Figs. 5-2, 5-3).

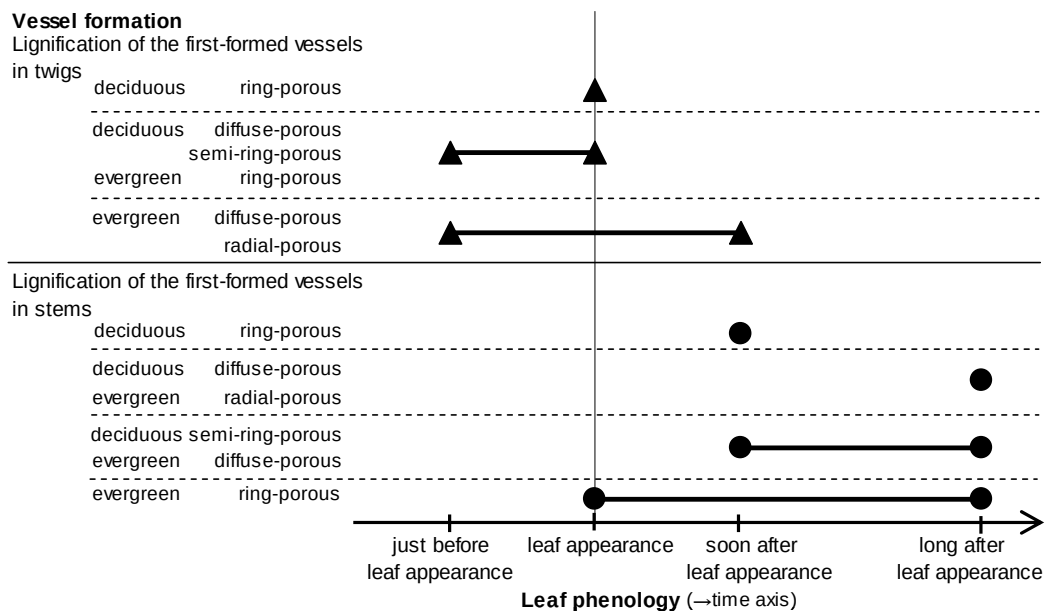


Fig. 6-2 Summary of the order and time of leaf phenology in relation to twig vessel (*upper panel*) and stem vessel formation (*lower panel*). *Filled triangles* indicate timeline of twig vessel formation; *filled circles* timeline of stem vessel formation, *bar* range

From above results, I discuss how porosity and vessel arrangement affects species' adaptation to their environment for species with different types of porosity and vessel arrangement coexist throughout the temperate zone.

In ring-porous deciduous species, all of the first-formed vessels in twigs and stems prepare to transport water by the time when the flush-leaves reach near-maximal rates of transpiration, and allow photosynthesis to proceed smoothly. These species produce leaves and wide vessels within a short period, to enable effective photosynthesis and water transport as the temperature rises in the spring following winter dormancy. It is vital that trees complete the first-formed vessels in twigs and stems before near maximal transpiration is reached at the time of full leaf expansion, because wide vessels transport water only in the year they develop (Greenidge 1955; Chaney and Kozlowski 1977; Ellmore and Ewers 1986; Utsumi et al. 1999; Umebayashi et al. 2008). Ring-porous species produce leaves and most hydraulically functional vessels within a short time in the early stage of annual ring formation; after early growth, these species synchronously produce subsequent leaves and vessels.

Furthermore, in diffuse-porous deciduous species, the first-formed vessels in twigs are prepared to transport water by the time transpiration needs are near-maximum. Stem vessels are prepared after the rapid increase in transpiration and allow photosynthesis to proceed at a steady rate. These species, similar to ring-porous deciduous species, produce leaves and twig vessels in short duration, to enable photosynthesis and water transport in twigs as the temperature rises, but do not need to form stem vessels shortly after leaf appearance. This is possible because vessels in several rings adjacent to the cambium retain their water transport ability through the years (Greenidge 1955; Chaney & Kozlowski 1977; Utsumi et al. 1998; Umebayashi et al. 2008). Diffuse-porous species produce leaves and vessels in long duration in the early stage of annual-ring formation and produce subsequent leaves and vessels asynchronously after the early growth.

The semi-ring-porous deciduous *Acanthopanax sciadophylloides* has narrower vessels in the pore-zone compared to ring-porous species and sometimes its porosity is similar to that of diffuse-porous species. Some *Acanthopanax sciadophylloides* individuals produced leaves and stem vessels in short duration similarly to ring-porous deciduous species, while other individuals

produced leaves and stem vessels in long duration similarly to diffuse-porous deciduous species.

In ring-porous evergreen species, some individuals produce leaves and stem vessels in short duration similarly to ring-porous deciduous species, while other individuals produce leaves and stem vessels in long duration similarly to diffuse-porous deciduous species. Vessels of the pore- or non-pore zone of several outer rings of ring-porous evergreen species may contribute to effective and indispensable water transport in order to keep leaves all year round.

In diffuse-porous evergreen species, the time of leaf appearance, twig and stem vessel formation were similar to that of diffuse-porous deciduous species. These species do not need to produce leaves and stem vessels in short duration, because they have leaves and usable vessels all year round and can start photosynthesis and water transport even before new leaf appearance (Miyazawa & Kikuzawa 2005). Additionally, vessels in several rings adjacent to the cambium retain their water transport ability through the years in diffuse-porous evergreen species (Umebayashi et al. 2010). Some individuals of evergreen species with diffuse-porous wood in the current study, such as *I. pedunculosa*, were observed to develop leaves and vessels synchronously, suggesting cases in which development of diffuse-porous wood coincide with leaf development.

In radial-porous evergreen species, the time of leaf appearance, twig and stem vessel formation were similar to that of diffuse-porous deciduous species. These species do not need to produce leaves and stem vessels in short duration, because they have leaves and usable vessels all year round and can start photosynthesis and water transport even before new leaf appearance (Miyazawa & Kikuzawa 2005). Additionally, vessels in several rings adjacent to the cambium retain their water transport ability through the years in radial-porous evergreen species (Hirose et al. 2005; Umebayashi et al. 2010). Hirano (1998) also showed a short production of leaves and stem vessels in the radial-porous evergreen *Lithocarpus edulis*, so a few species might use stem vessels at the time of leaf appearance.

I clarified the relationship between the timing of vessel formation and leaf phenology in ring-porous and diffuse-porous species. Diffuse-porous and ring-porous species generally followed similar patterns of vessel-leaf phenology within each group. The diffuse-porous *P. rhoifolia* has wider early vessels than other diffuse-porous species, but still followed the same

patterns as other diffuse-porous species. On the other hand, there were some fluctuations by species or by year within ring- or diffuse-porous species. It is unclear why some individuals of *Fraxinus mandshurica* or *H. tomentella* formed vessels in stems earlier or later than other ring-porous trees. Similarly, the timing of vessel formation in twigs fluctuated between the years.

Kudo et al. (2014) experimentally showed that, at partially elevated temperatures of the stem, ring-porous species formed the first vessel elements (as well as a few narrow vessel elements) even in the absence of buds. Therefore, further studies are warranted to elucidate the cause and effect of leaf and vessel formation in connection with environmental factors.

More studies are needed to clarify the relationships between the timing of vessel formation and leaf phenology of species with different wood porosities in different climatic regions. Systematic elucidation of the relationships between leaf phenology and vessel formation requires the analysis of additional species. Furthermore, new methods need to be developed for quantifying phenological characteristics of leaves and vessels at the whole-tree level, to accurately trace the periodic process of vessel formation less destructively but more conveniently and for observing seasonal changes in trunk vessel formation at a given location.

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Summary

I attempt to determine how different aspects of plant function relating photosynthesis and water transport are coordinated developmentally for species with different vessel porosity and arrangements that coexist in the temperate zone. I observed the timing of leaf phenology and vessel formation in twigs and stems from an ecophysiological viewpoint, focusing on how vessel porosity and arrangements affect species' adaptation to their environment.

Chapter 1 reviews the previous studies of the association between leaf production and vessel formation, and describes the study sites and sample trees examined, and the methods for observation of leaf phenology.

Chapter 2 describes the increment borer method to trace the periodic process of vessel formation. The results suggest that the negative effect on trees caused by sampling is small, and vessel formation in sample trees is traced despite occasional separation of cambial zone from core samples. Based on a comparison of dying methods between double staining with safranin and fast green and the phloroglucinol-hydrochloric acid reaction, the former was considered applicable to investigate vessel formation, including the timing of vessel lignification.

In Chapter 3, by using the method of Chapter 2, I examined the temporal relationship between leaf phenology and vessel formation in twigs and stems for species of ring- and diffuse-porous deciduous tree species in a temperate forest. Ring-porous deciduous species form new wide twig and stem vessels concurrently with leaf appearance, because vessels of the pore-zone formed in the previous year have become dysfunctional. In contrast, diffuse-porous deciduous species can transport water in several rings, thus new twig vessels are formed at the time of leaf appearance, long before stem vessel lignification. Although phylogenetically distant, various ring-porous species showed the same tendency of leaf and vessel formation and, although phylogenetically distant, various diffuse-porous species showed the same tendency of leaf and vessel formation.

By using the sample trees of Chapter 3, I determined the temporal relationships between vessel formation and leaf phenology throughout the early stage to the later stage of annual ring formation in Chapter 4. I distinguished between flush leaves and successive leaves and three size

classes of vessels were measured. The results suggest that ring-porous species form leaves and vessels synchronously to accommodate water-transport requirements, whereas diffuse-porous species form these organs asynchronously; thus, unlike the latter, the former species change vessel diameters according to leaf formation.

In Chapter 5, phenological relationship between leaf appearance and twig and stem vessel formation was studied in evergreen and deciduous tree species with different porosity and vessel arrangement in a temperate forest. The difference was tested between the semi-ring-porous deciduous, ring-porous evergreen, diffuse-porous evergreen, or radial-porous evergreen species and the ring-porous deciduous or diffuse-porous deciduous species. These results may indicate a variation in phenology of organ development within each species. Ring-porous deciduous species show leaf and vessel production in shorter duration, compared to diffuse-porous deciduous and radial-porous evergreen species. In diffuse-porous evergreen species, a few individuals showed leaf and vessel production in short duration, while the majority of trees showed a production in long duration. Individuals within semi-ring-porous deciduous and ring-porous evergreen species showed characteristics similar to those of ring-porous or diffuse-porous deciduous species.

Chapter 6 determined how different aspects of plant function are coordinated developmentally for species with different porosity and vessel arrangement coexist throughout the temperate zone and attempted to clarify how porosity and vessel arrangement affects species' adaptation to their environment by determining this.

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