Timing of vessel formation in twigs and trunks in relation to porosity and leaf flushing

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Abstract

In order to understand the coordination of leaf phenology and functional xylem anatomy, the timing of vessel wall lignification in twigs and stems in relation to leaf appearance was studied in nine species with different porosity patterns. Cylindrical stem cores and twigs were collected from early spring through late summer from deciduous (*Quercus serrata*, *Liquidambar styraciflua*, and *Acanthopanax sciadophylloides*), and evergreen (*Castanopsis cuspidata*; *Cinnamomum camphora*, *Ilex pedunculosa*, *Symplocos prunifolia*, *Quercus glauca* and *Quercus myrsinifolia*) species in a temperate forest. The first-formed twig vessels lignified at the time of leaf appearance or before in all species. The timing of stem vessel lignification in relation to leaf appearance in semi-ring-porous deciduous species was overlapping with that of ring-porous deciduous species and diffuse-porous deciduous species. Evergreen species showed a great variation in the timing of stem vessel lignification, relative to leaf flushing. The main conclusions are that 1) Vessel lignification occurs much earlier in twigs than in trunks of the same trees, with hardly any overlap between the two; 2) Deciduous trees do not differ much from evergreen species, but there is a weak tendency for evergreen species to have later vessel differentiation than deciduous species; 3) The timing of vessel formation shows little relation with porosity patterns and overlaps between diffuse-porous and ring-porous species. This suggests a much greater intergradation of timing of vessel formation in species of different porosity pattern in evergreen and deciduous species than recognized in the literature.

Keywords: Vessel arrangement, lignification, leaf phenology, functional ecology, evergreen, deciduous.

Introduction

This study attempts to clarify how variation in vessel porosity affects the timing of vessel formation in relation to leaf appearance. Temperate broad-leaved trees are evergreen or deciduous and may show different wood porosities (Wheeler *et al.* 1989; Hayashi 1991; Itoh 1995). The differences in the size and distribution of water-conducting cells are related to variations in the phenology of organ development and the seasonal efficiency of water conduction. Porosity patterns themselves show gradations and overlap. Many species range from diffuse-porous to semi-ring-porous, or from ring-porous to semi-ring-porous (Wheeler *et al.* 1989) and show a considerable plasticity in their xylem (Scholz *et al.* 2014).

Previous studies revealed that the timing of vessel formation in relation to leaf appearance in typical ring-porous deciduous species differs from that in typical diffuse-porous species (Ladefoged 1952; Suzuki *et al.* 1996, 2000; Takahashi *et al.* 2013, 2015). The first-formed vessels, which develop new rings adjacent to the annual ring border, mature around the time of leaf appearance in stems of ring-porous species (Zasada & Zahner 1969; Suzuki *et al.* 1996, 2000; Sass-Klaassen *et al.* 2011; González-González *et al.* 2013; Takahashi *et al.* 2013; Kudo *et al.* 2015). In contrast, stem vessels of diffuse-porous species mature more than two weeks after leaf appearance (Suzuki *et al.* 1996, 2000; Čufar *et al.* 2008; Takahashi *et al.* 2013).

The wide vessels of the pore-zone in ring-porous deciduous species are known to transport water just in the one growing season (Greenidge 1955; Chaney & Kozlowski 1977; Ellmore & Ewers 1986; Utsumi *et al.* 1999; Umebayashi *et al.* 2008), while in diffuse-porous deciduous species vessels in several rings adjacent to the cambium retain their water transport ability for a number of years (Greenidge 1955; Chaney & Kozlowski 1977; Utsumi *et al.* 1998; Umebayashi *et al.* 2008). The time of stem vessel formation relative to leaf appearance is closely related to water distribution pattern within annual sapwood rings in ring-porous and diffuse-porous deciduous species (Ladefoged 1952; Lechowicz 1984; Suzuki *et al.* 1996; Takahashi *et al.* 2013).

The above-mentioned studies on seasonal changes in vessel formation in relation to leaf phenology and water distribution patterns in the sapwood rings lead to the hypothesis that different porosities affect the timing of vessel formation in relation to leaf appearance. In this study, we tested this hypothesis in a number of species representing a wide range of porosities, aiming to clarify the coordination between leaf phenology and functional xylem anatomy. In particular, the seasonal relationship between leaf appearance and twig and stem vessel formation was studied in nine tree species with different wood porosity and leaf habits growing together in the same temperate forest stand.

Materials and methods

Study site and sampled trees

The study was conducted in a secondary forest of deciduous and evergreen trees at the Kamigamo Experimental Forest Station (35° 04′ N, 135° 46′ E, 109–225 m above sea level) of Kyoto University in Japan. The mean annual temperature over a 30-year period 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 (data from 1976 to 2005, Forest Research Station of Graduate School of Agriculture, Kyoto University 2007).

This study included observations performed on nine tree species with differing leaf habits (deciduous and evergreen) and different types of vessel porosities, from ring-porous to diffuse-porous (Table 1; Fig. 1 & 2). Porosity patterns themselves show gradations and overlap. Types of vessel porosities in this study were classified according to Wheeler *et al.* (1989), the FFPRI website, and the InsideWood website. The type of radial-porous was defined as suggested by Gasson (1985) and Noshiro & Sasaki (2011). *Castanopsis cuspidata* is characterized as a (semi-)ring-porous to radial-porous species. Evergreen diffuse-porous species are categorized as diffuse-porous with very weak to more pronounced semi-ring-porous tendencies. The sampled trees were selected from individuals with a diameter at breast height ranging from 12 to 56 cm, and a height between 7 and 29 m, with relatively straight stems. *Liquidambar styraciflua* and *Quercus myrsinifolia* individuals were planted trees.

Sampling

Sampling for vessel formation was performed repeatedly during the entire growing season through wood cores and twig sampling from three to six trees of each examined species. 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 monthly between July 4 and August 29, in 2006.

Cylindrical wood core samples with a diameter of 7 mm and a length of 20 mm were collected at breast height (1.3 ± 0.3 m above the ground) using an increment borer (Mattson, Mora, Sweden) from each tree (Takahashi *et al.* 2008).

Twigs with sun-exposed leaves were collected from each tree using a 12-m-long pruner or by climbing up the trunk and using a 3-m-long pruner. Twigs that had grown within the previous year were regarded as 1-year-old twigs. In *Acanthopanax sciadophylloides*, we observed vessel formation in 1-year-old twigs and occasionally in 2- to 4-year-old twigs at 0.5–1.5 cm below the bud base. Stem cores and twigs were fixed in 3% aqueous glutaraldehyde soon after sampling.

Assessment of vessel formation in cores and twigs

Transverse sections with a thickness of 15–30 μm were cut from each twig or stem sample using a sliding microtome (Yamato TU-213; Saitama, Japan). The sections were double-stained with 1% safranin and 1% fast green (Sass 1951) for light microscopic study.

Vessel lignification occurs between cell wall deposition and the disintegration of the end walls (Imagawa & Ishida 1972; Wakuta *et al.* 1973; Fukushima *et al.* 2003; Fromm 2013). Lignification of the first-formed vessels in the current growth ring, often adjacent to the annual ring border, was determined by the presence of red color from safranin staining (Sass 1951; Imagawa & Ishida 1972; Takahashi *et al.* 2008). If lignification was not clear after double staining, a phloroglucinol-hydrochloric acid reaction was used (Takahashi *et al.* 2008). The lignification date was defined as the time when the lignification of almost all (more than 80%) of the first-formed vessels was observed, tangentially using 2–3 mm long transverse sections.

When enlargement of the first-formed vessels but no lignification of the walls in stems was found by June 20 or July 4, and lignification of the vessels was observed in August 1 or 29, the date of the lignification was judged to be two weeks later than June 20 or July 4, respectively. In two trees of *Quercus myrsinifolia*, enlargement of the first-formed vessels in the stem was found by August 1, and the vessel lignification date was judged to be two weeks later than August 1.

Observation of leaf phenology

The leaves of each sampled tree were examined weekly using binoculars (Nikon 8 × 30, 8.8° WF; Tokyo, Japan) and photographed *in situ* on the selected trees and sampled twigs. Leaf appearance was defined at the shoot level, as the date when the lamina separated from the shoot axis (Kikuzawa 1983). We observed the first flush of leaves in spring weekly between 29 March and 20 June, 2006. The leaf appearance date was defined as the time when almost all (more than 80% by visual estimate) of flush leaves appeared.

Results

Timing of twig vessel lignification in relation to leaf appearance

The lignification of the first-formed twig vessels was observed concurrently with leaf appearance in a ring-porous deciduous *Quercus serrata*, and 0–3 weeks before leaf appearance in a diffuse-porous deciduous *Liquidambar styraciflua* (Fig. 3). Twig vessel lignification occurred 0–1 week before leaf appearance in *Acanthopanax sciadophylloides*; 2 weeks before to 1 week after leaf appearance in evergreen species (Fig. 3). In twigs, overall, the time of the first-formed vessel lignification was close to the time of leaf appearance and ranged between 3 weeks before and 1 week after leaf appearance (Fig. 3).

Timing of stem vessel lignification in relation to leaf appearance

The timing of lignification in the first-formed stem vessels is summarized in Figure 4, and ranges from nearly simultaneous with leaf flush up to 15 weeks after leaf appearance. There appears to be a strong overlap between deciduous species, although the total range in timing relative to leaf flush is wider in evergreen species than in deciduous ones. There is hardly any relationship between type of porosity and timing of vessel wall lignification of the earlywood and there is a big variation in the timing within some of the species.

The time of lignification of the first-formed stem vessels in relation to the time of leaf appearance was earlier in a ring-porous deciduous *Quercus* serrata than in diffuse-porous deciduous *Liquidambar* *styraciflua*, and evergreen *Cinnamomum camphora*, *Symplocos prunifolia*, *Quercus glauca* and *Q. myrsinifolia* (Fig. 4). *Acanthopanax sciadophylloides*, *Castanopsis cuspidata* and *Ilex pedunculosa* followed a time pattern of vessel lignification overlapping with that of ring-porous and diffuse-porous deciduous species (Fig. 4).

Timing of stem and twig vessel lignification

The timing of lignification in the first-formed stem vessels relative to twig vessels is summarized in Figure 5, and ranges between nearly simultaneous with and 14 weeks after lignification in the first-formed twig vessels. Vessel lignification in twigs occurs earlier than in stems, with hardly any overlap with timing in stems.

Discussion

Comparison among species with different and intergrading porosities

In this study, we found that the lignification of the first-formed vessels in twigs occurred concurrently with leaf appearance in both deciduous and evergreen species and in the various types of porosity (Fig. 3). This suggests that twig vessel lignification follows a similar time pattern as in the typical ring-porous deciduous *Quercus serrata* and the typical diffuse-porous deciduous *Liquidambar styraciflua* (Fig. 3). These results are consistent with previous findings. Twig vessel elements begin to lignify before bud break (Zasada & Zahner 1969), and cambial cell division at bud bases begins before or simultaneously with bud break in both ring-porous and diffuse-porous deciduous species (Ladefoged 1952).

Lignification of the first-formed stem vessels occurred shortly after leaf appearance in the typical ring-porous deciduous *Q. serrata* and long after leaf appearance in the typical diffuse-porous deciduous *L. styraciflua* (Fig. 4). These results are similar to previous findings (Ladefoged 1952; Suzuki *et al.* 1996, 2000; Takahashi *et al.* 2013). However, if one considers the timing in other deciduous and evergreen diffuse-porous, semi-ring-porous, ring-porous or radial-porous species (Fig. 4) the pattern becomes more complex or even blurred.

Acanthopanax sciadophylloides is categorized as semi-ring-porous (FFPRI website). In this study, *A. sciadophylloides* has narrower earlywood vessels compared to ring-porous species, and sometimes its rings are diffuse-porous (Fig. 2e). Some of *A. sciadophylloides* individuals produced leaves and stem vessels for a short period, similarly to ring-porous deciduous species, while other individuals produced leaves and stem vessels for a long period, similarly to diffuse-porous deciduous species (Fig. 3–5). Tyloses are present in old sapwood rings of *A. sciadophylloides* (Saitoh *et al.* 1993), so it is possible that *A. sciadophylloides* transports water in several annual rings. However, Umebayashi *et al.* (2008) categorized *A. sciadophylloides* as a ring-porous species, given that its wide vessels are known to transport water mostly during the year in which they are formed, similarly to other ring-porous species. Thus, it seems that changes in water distribution pattern within annual rings affect the timing of vessel formation in relation to leaf appearance in the semi-ring-porous deciduous *A. sciadophylloides*.

Castanopsis cuspidata has (semi-)ring-porous to radial-porous wood (Wheeler *et al.* 1989; InsideWood 2004-onwards; FFPRI website). In this study, the first-formed stem vessels lignified not only long after but also simultaneous with leaf appearance in this species (Fig. 4). Hirano (1998) reported that secondary wall deposition in stems was completed concurrently with leaf appearance in *Castanopsis sieboldii*, a member of the same group. On the other hand, tyloses of *C. sieboldii* are present in old sapwood rings (Saitoh *et al.* 1993), so it is possible that *C. sieboldii* transport water in several annual rings. These results suggest that the species categorized as semi-ring-porous, ring-porous or radial-porous, such as *Castanopsis* spp., have a broad range of timing of vessel lignification in the stems, overlapping with the timing in ring-porous and diffuse-porous deciduous species.

Cinnamomum camphora is categorized as semi-ring-porous to diffuse-porous (Wheeler *et al.* 1989; FFPRI website), whose earlywood vessels are wider than in other diffuse-porous species (Fig. 1b, 2g–i). In this study, evergreen *Cinnamomum camphora* produced leaves and stem vessels for a long period, similar to diffuse-porous deciduous species (Fig. 4). *Pterocarya rhoifolia*, which is also categorized as semi-ring-porous to diffuse-porous (Wheeler *et al.* 1989; InsideWood 2004-onwards; FFPRI website), also produces leaves and stem vessels for a long period (Takahashi *et al.* 2013). These results suggest that semi-ring-porous to diffuse-porous evergreen species have a time pattern of stem vessel lignification similar to diffuse-porous deciduous species. *Ilex pedunculosa* and *Symplocos prunifolia* are diffuse-porous (Fig. 2h, i) (Wheeler *et al.* 1989; FFPRI website). Stem vessel lignification in relation to leaf appearance in *I. pedunculosa* occurred earlier than in *Cinnamomum camphora* and *S. prunifolia*

(Fig. 4). Especially, stem vessel lignification of a few *I. pedunculosa* individuals was not different from ring-porous deciduous species (Fig. 4). Diffuse-porous *Fagus sylvatica* has been shown to complete vessel wall deposition in stems 2 weeks after leaf expansion (Čufar *et al.* 2008). These results suggest that a few diffuse-porous trees show stem vessel lignification and leaf appearance occurring within a short time, while the majority take a long time.

Quercus glauca and *Q. myrsinifolia* are categorized as diffuse-porous to radial-porous (Gasson 1985; Wheeler *et al.* 1989; FFPRI website; Noshiro & Sasaki 2011). Stem vessel lignification occurred long after leaf appearance in *Q. glauca* and *Q. myrsinifolia* (Fig. 4). Additionally, vessels in several rings adjacent to the cambium retain their water transport ability for multiple years in radial-porous evergreen species (Hirose *et al.* 2005; Umebayashi *et al.* 2010). This suggests that the time of stem vessel lignification in radial-porous evergreen plants may be similar to that in diffuse-porous deciduous species. However, Hirano (1998) also showed that secondary wall deposition in stems was completed concurrent with leaf appearance in the radial-porous evergreen species *Lithocarpus edulis*. It is suggested that some species or trees might need current year's stem vessels at the time of leaf appearance.

Our study shows that the timing of stem vessel differentiation in semi-ring-porous deciduous species overlaps with that of ring-porous deciduous species and diffuse-porous deciduous species. Evergreen species showed a great variation in the timing of stem vessel lignification, relative to leaf flushing. The various types of porosity of deciduous and evergreen species clearly intergrade and overlap in their timing of lignification of the first earlywood vessels. This contradicts the hypothesis that different porosities affect the timing of vessel formation in relation to leaf appearance, especially in evergreen species and semi-ring-porous species.

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Table captions

Table 1. Description of the tree species sampled.

[a]Classes of porosity were defined based on the FFPRI website, InsideWood (2004-onwards), Gasson (1985), Wheeler et al. (1989) and Noshiro & Sasaki (2011). *Castanopsis cuspidata* is characterized as (semi-)ring-porous to radial-porous species. Evergreen diffuse-porous species are categorized as diffuse-porous with very weak to more pronounced semi-ring-porous tendencies.

[b]Scientific names are based on Hayashi *et al.* (1987).

[c]Diameter at breast height.

[d]O: twigs were sampled of this tree.

Figure captions

Figure 1. Light micrographs of cross sections in deciduous (a,b) and evergreen (c,d) sample trees. – a: *Quercus serrata*; black arrow, a wide vessel in the pore zone; white arrow, a narrow vessel in the non-pore zone. – b: *Liquidambar styraciflua*. – c: *Quercus glauca*. – d: *Quercus myrsinifolia*. – Ring borders are marked. – Scale bar is 300 μm.

Figure 2. Light microphotographs of cross sections in deciduous (e) and evergreen (f–i) sample trees. – e: *Acanthopanax sciadophylloides*; arrowhead, a relatively wide vessel. – f: *Castanopsis cuspidata*; black arrow, a relatively wide vessel; white arrow, relatively narrow vessels. – g: *Cinnamomum camphora*. – h: *Ilex pedunculosa*. – i: *Symplocos prunifolia*. – Ring borders are marked. – Scale bar is 300 μm.

Figure 3. Timing of lignification of the first-formed vessels in twigs relative to the time of leaf appearance in 2006. Numbers in parentheses indicate the number of trees; bars indicate the time ranges.

Figure 4. Timing of lignification of the first-formed vessels in stems relative to the time of leaf appearance in 2006. Numbers in parentheses indicate the numbers of trees; - - represents a single tree that had not yet completed lignification; bars indicate the time ranges.

Figure 5. Timing of lignification of the first-formed vessels in stems compared to twigs in 2006. Numbers in parentheses indicate the numbers of trees; - - represents a single tree that had not yet completed lignification; bars indicate the time ranges.

Table 1 Description of the tree species sampled.

Leaf habit	Porosity[a]	Species[b]	Family	Tree No.	DBH[c] (cm)	Tree height (m)	Twig[d]
Deciduous	Ring-porous	*Quercus serrata* Thunb.	Fagaceae	149	32	14	
				156	23	8	O
				157	36	12	O
				158	33	14	O
				159	22	13	
	Diffuse-porous	*Liquidambar styraciflua* L.	Hamamelidaceae	182	30	25	O
				183	22	22	O
				184	33	25	
				185	40	29	
				186	44	24	O
				187	56	29	O
	Semi-ring-porous	*Acanthopanax sciadophylloides* Fr. et Sav.	Araliaceae	164	21	7	O
				165	16	7	O
				170	22	13	
				173	21	13	O
				177	27	14	O
				178	29	17	O
Evergreen	ring- (or radial-) porous	*Castanopsis cuspidata* (Thunb.) Schottky	Fagaceae	167	56	26	O
				168	39	23	O
				169	12	7	O
	Diffuse-porous	*Cinnamomum camphora* Sieb.	Lauraceae	171	45	19	O
				172	29	16	O
				176	35	15	O
		Ilex pedunculosa Miq.	Aquifoliaceae	153	22	9	O
				161	24	8	O
				162	19	10	O
				163	21	11	O
				166	24	9	
		Symplocos prunifolia Sieb. et Zucc.	Symplocaceae	150	24	7	O
				151	19	9	O
				152	21	9	O
				154	22	9	O
				155	22	8	O
	Radial-porous	*Quercus glauca* Thunb.	Fagaceae	160	24	11	O
				174	29	13	O
				175	20	12	
				180	27	13	O
				181	44	17	O
		Quercus myrsinifolia Blume	Fagaceae	144	22	17	O
				145	22	17	
				146	28	15	O
				147	53	20	O
				148	30	20	O

[a]Classes of porosity were defined based on the FFPRI website, InsideWood (2004-onwards), Gasson (1985), Wheeler et al. (1989) and Noshiro & Sasaki (2011). *Castanopsis cuspidata* is characterized as (semi-)ring-porous to radial-porous species. Evergreen diffuse-porous species are categorized as diffuse-porous with very weak to more pronounced semi-ring-porous tendencies.

[b]Scientific names are based on Hayashi *et al.* (1987).

[c]Diameter at breast height.

[d]O: twigs were sampled of this tree.

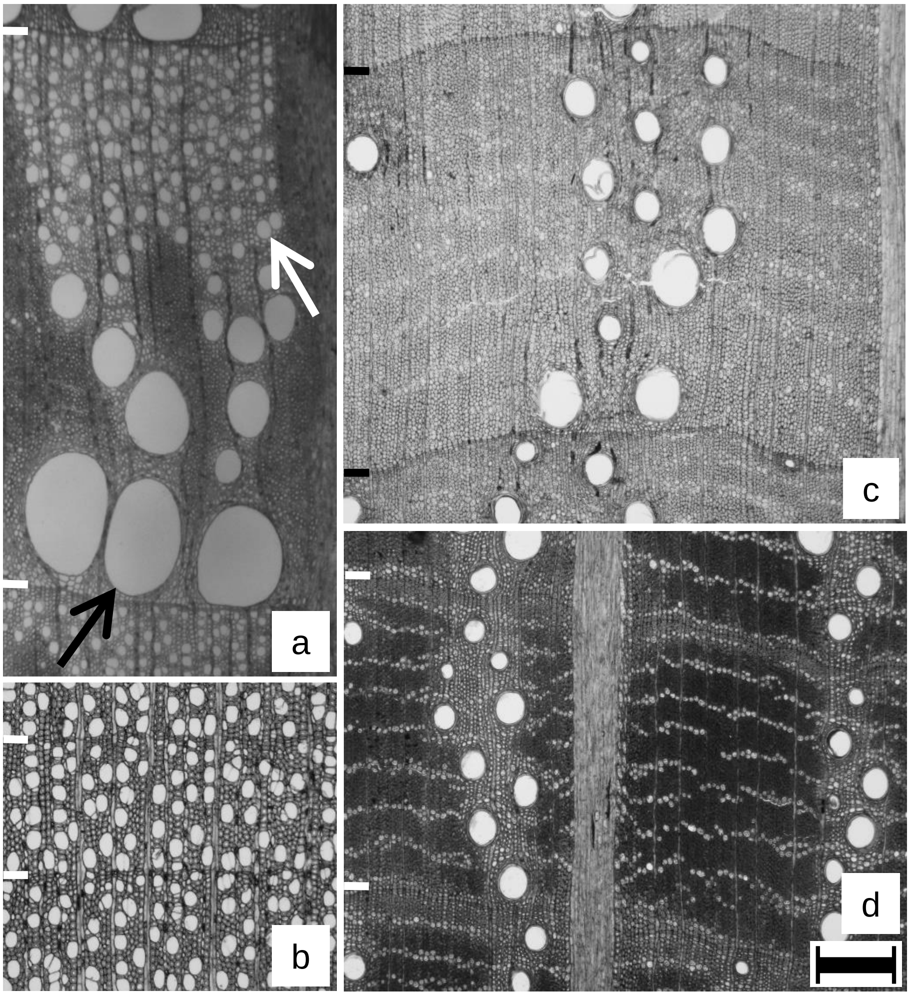

Figure 1. Light micrographs of cross sections in deciduous (a,b) and evergreen (c,d) sample trees. – a: *Quercus serrata*; black arrow, a wide vessel in the pore zone; white arrow, a narrow vessel in the non-pore zone. – b: *Liquidambar styraciflua*. – c: *Quercus glauca*. – d: *Quercus myrsinifolia*. – Ring borders are marked. – Scale bar is 300 µm.

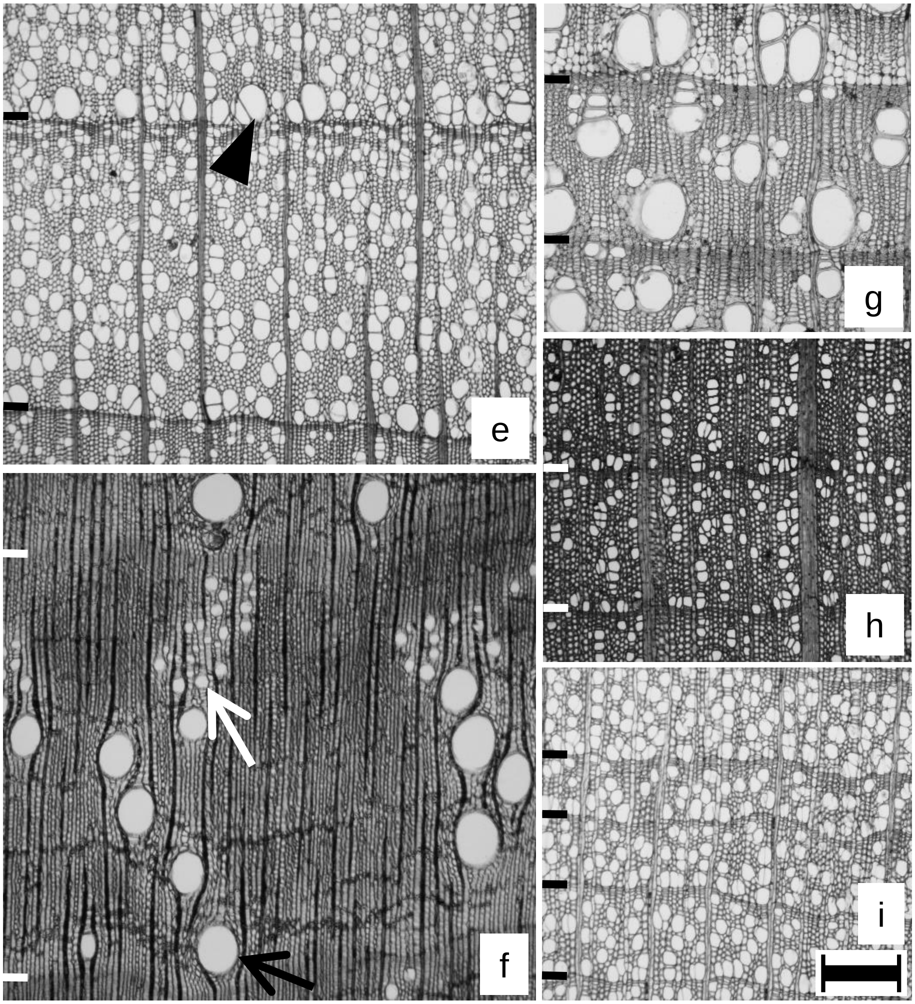

Figure 2. Light microphotographs of cross sections in deciduous (e) and evergreen (f–i) sample trees. – e: *Acanthopanax sciadophylloides*; arrowhead, a relatively wide vessel. – f: *Castanopsis cuspidata*; black arrow, a relatively wide vessel; white arrow, relatively narrow vessels. – g: *Cinnamomum camphora*. – h: *Ilex pedunculosa*. – i: *Symplocos prunifolia*. – Ring borders are marked. – Scale bar is 300 µm.

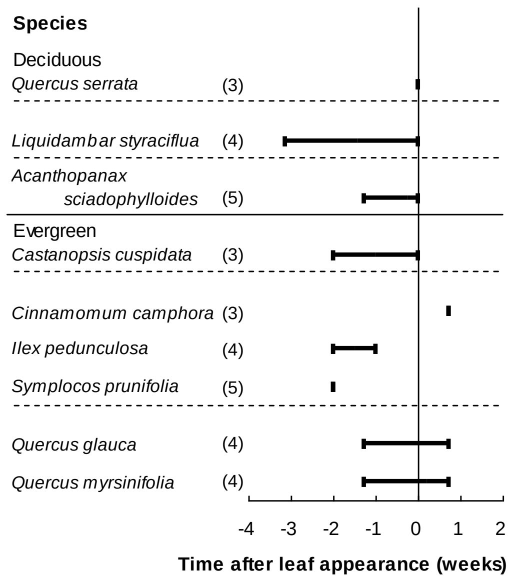

Figure 3. Timing of lignification of the first-formed vessels in twigs relative to the time of leaf appearance in 2006. Numbers in parentheses indicate the number of trees; bars indicate the time ranges.

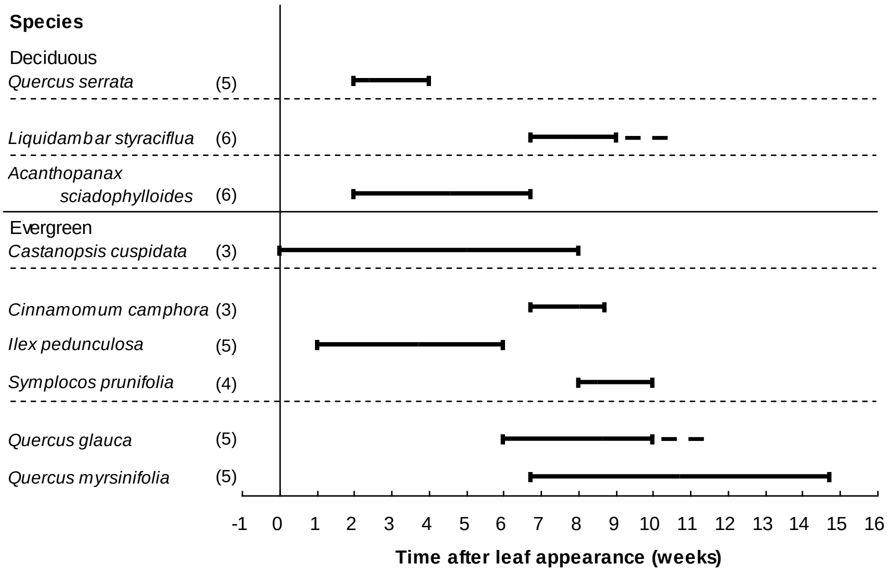

Figure 4. Timing of lignification of the first-formed vessels in stems relative to the time of leaf appearance in 2006. Numbers in parentheses indicate the numbers of trees; - - represents a single tree that had not yet completed lignification; bars indicate the time ranges.

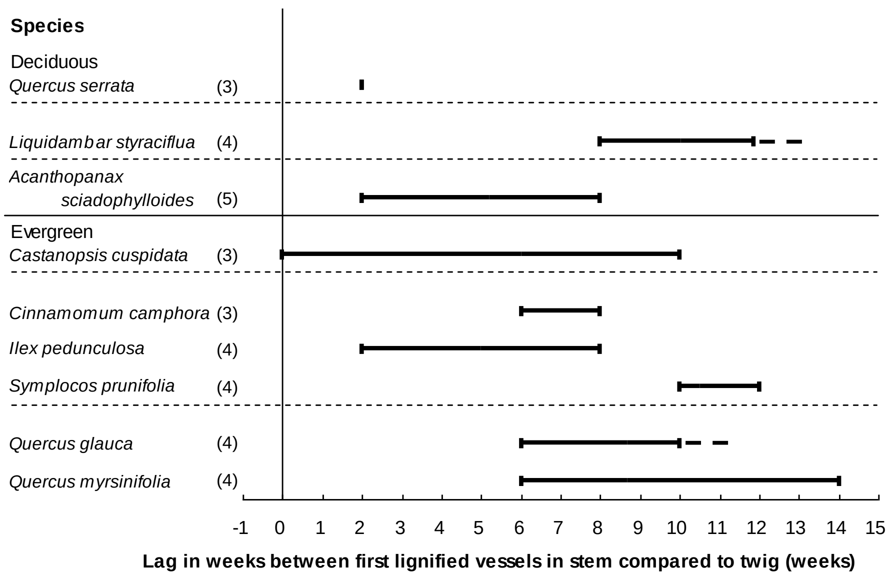

Figure 5. Timing of lignification of the first-formed vessels in stems compared to twigs in 2006. Numbers in parentheses indicate the numbers of trees; - - represents a single tree that had not yet completed lignification; bars indicate the time ranges.