# Bordered Pitting Arrangement with Age of Dahurian Larch (*Larix gmelinii*) and Japanese Larch (*Larix kaempferi*)

Do Hoon Kim,<sup>a,1</sup> Byantara Darsan Purusatama,<sup>b,1</sup> and Nam Hun Kim,<sup>a,\*</sup>

The bordered pitting arrangements in the tracheids of Dahurian larch (DL) and Japanese larch (JL) growing in Korea were studied using optical microscopy to gain insights into the wood identification of both species. Differences were noted in the tracheid pitting patterns between the species. In juvenile wood, DL dominantly exhibited uniseriate pitting along the earlywood within a growth ring, whereas JL displayed biseriate pitting at the start of the earlywood, transitioning to uniseriate pitting. In the transition wood, DL predominantly showed biseriate pitting at the beginning, with uniseriate pitting at the end, while JL exhibited uniseriate pitting initially and at the end, with biseriate pitting in the middle. The mature wood of both species predominantly exhibited a biseriate pitting arrangement with a few uniseriate pitting at the end of the earlywood. JL exhibited a higher proportion of biseriate pitting than DL. The proportion of biseriate pitting and radial tracheid width in both species increased with increasing growth ring number, showing a positive correlation. While some differences were found between DL and JL, further investigation is needed to evaluate the consistency and practical applicability of pit patterns for wood identification in diverse field conditions.

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Keywords: Bordered pitting; Dahurian larch; Japanese larch; Juvenile wood; Mature wood; Wood identification

Contact information: a: Department of Forest Biomaterials Engineering, Kangwon National University, Chuncheon 24341, Republic of Korea; b: Institute of Forest Science, Kangwon National University, Chuncheon 24341, Republic of Korea;

\* Corresponding author: kimnh@kangwon.ac.kr; <sup>1</sup>Both authors equally contributed in the manuscript

### INTRODUCTION

Understanding the anatomical characteristics is important for identifying and utilizing wood species. Wood identification is essential in various contexts, including commercial, forensic, archaeological, and paleontological (Wheeler and Baas 1998). Several studies have been performed on the anatomical properties of softwood to identify wood species, including cross-field pitting (Gerards *et al.* 2007; Tarmian *et al.* 2011; Purusatama and Kim 2020), spiral thickening (Patel 1963; Jutte and Levy 1973; Timell 1978; Anagnost *et al.* 1994), epithelial cells (Kim and Kwon 2006; Denne and Gasson 2008), and resin canals (Chauhan *et al.* 2022). Additionally, many studies have examined the radial variation of wood anatomical characteristics for evaluating wood quality, such as latewood proportion (Clark *et al.* 2007; Adamopoulos *et al.* 2009; Guller *et al.* 2012), tracheid properties (Herman *et al.* 1998; Sarén *et al.* 2001; Chong and Park 2008; Fonti *et al.* 2015; Gogoi *et al.* 2019), ray properties (Rahman *et al.* 2005; Kim *et al.* 2009; Kim *et al.* 2021), crystalline properties (Eun *et al.* 2008; Purusatama *et al.* 2019, 2023; Kim *et al.* 

2022), and microfibril angle (Eun and Kim 2008; Purusatama et al. 2019, 2023).

In addition to the aforementioned indices, the tracheid pitting arrangement is an important factor for wood identification and quality indices of various softwood species. Anagnost *et al.* (1994) investigated pit seriation to distinguish *Larix* and *Picea* spp. They reported that *Larix gmelinii* and *Larix kaempferi* had biseriate bordered pitting, and *Picea* spp. had both uniseriate and biseriate arrangements. Jagels *et al.* (2001) reported the existence of uniseriate and partially biseriate bordered pitting in *Larix altoborealis*. Blokhina *et al.* (2011) studied the age-related variation of wood anatomical characteristics to determine a quality index, such as bordered pitting in *Larix cajanderi*. They reported that tracheid pitting was only uniseriate near the pith and that biseriate tracheid pitting appeared from the 5<sup>th</sup> growth ring and became abundant from the 15<sup>th</sup> growth ring.

In South Korea, the two major larch species, Dahurian larch (DL) (*Larix gmelinii* var. *olgensis* (A. Henry) Ostenf. and Syrach) and Japanese larch (JL) (*Larix kaempferi* (Lamb.) Carriere), are valuable commercial wood resources for building construction and wood-based panels. DL is found only in a small area of Gangwon Province, whereas JL is widely distributed in the mountains throughout the country. The two species are often referred to as "larch" because they are difficult to distinguish in the characteristics of the trees and the woods (Hwang and Park 2007; Chong and Park 2008).

Only a few studies have been conducted on the wood characteristics of DL and JL. Eom (2015) described the general anatomical characteristics of DL and JL growing in Korea. Han *et al.* (2017) reported the anatomical characteristics of the radial sections of DL and JL wood, such as tracheid pitting, ray tracheids, and ray parenchyma cells, including their physical and mechanical properties.

Previous studies reported the radial variation in the ray, tracheid, and crystalline properties of DL and JL for wood identification keys and quality indices (Kim *et al.* 2021, 2022). More basic studies on wood quality and the identification of DL and JL woods growing in Korea are needed to identify and evaluate the quality of both species efficiently. As mentioned above, the bordered pitting arrangement in the tracheid wall can be used for wood identification and quality evaluation (Anagnost *et al.* 1994; Jagels *et al.* 2001; Blokhina *et al.* 2011). However, no study has compared the arrangement of bordered pitting in the tracheids of DL and JL, including the radial variation. Therefore, to provide valuable information for the effective utilization of both species, this study aimed to investigate the bordered pitting arrangement in tracheids with age and within a growth ring and the relationship between the biseriate pitting proportion in tracheids and tracheid width. Despite these efforts, the similarity in bordered pitting arrangement between DL and JL could be a significant challenge for reliable wood identification. The potential for overlap and variability underscores the need for further research to validate the consistency and practical applicability of these findings for field identification.

### **EXPERIMENTAL**

### Materials

Details of the sampled trees (Table 1 and 2) were obtained from previous studies (Kim *et al.* 2021, 2022). Three trees of each species were age 71 to 72 years for DL and 37 to 41 years for JL. They were harvested from a plantation site in the research forest of Kangwon National University, South Korea (N 37°77', E 127°81'). Three wood discs were obtained at breast height from three trees of each species. The oven-dried density was

randomly measured from the whole part of the wood discs, which ranged from 0.68 to 0.81 g/cm<sup>3</sup> for DL and 0.45 to 0.65 g/cm<sup>3</sup> for JL. In the DL, the specimens were prepared from every five growth rings from the 5<sup>th</sup> growth ring until the 50<sup>th</sup> growth ring and every 10th growth ring after the 50<sup>th</sup> growth ring. Japanese larch specimens were prepared from every five growth rings from the pith to the bark until the 35<sup>th</sup> growth ring. All specimens were obtained from four directions of each wood disc (Fig. 1).



Fig. 1. Dahurian larch (left) and Japanese larch (right) wood discs at breast height. Scale bars: 10 cm

| Table 1. | Basic | Information        | of the | Sample | Trees |
|----------|-------|--------------------|--------|--------|-------|
| 10010 11 | Dadio | in in or in lation | 01 010 | Campio | 11000 |

| Common Name    | Scientific Name   | Tree Age<br>(year) | DBH*<br>(mm) | Oven-dry<br>Density<br>(g/cm <sup>3</sup> ) | Heartwood<br>Rate<br>(%) |
|----------------|---|--------------------|--------------|---|--------------------------|
| Dahurian larch | <i>Larix gmelinii</i> var.<br><i>olgensis</i> (A. Henry)<br>Ostenf. and<br>Syrach | 71 to 72           | 338 to 354   | 0.68 to 0.81                                | 79 to 84                 |
| Japanese larch | <i>Larix kaempferi</i> (Lamb.) Carriere   | 37 to 41           | 351 to 365   | 0.45 to 0.65                                | 72 to 74                 |

\* D.B.H.: Diameter at Breast Height

| Table 2. | Physical | Properties | of Dahurian | Larch ar | nd Japanese | Larch (Kim | et al. |
|----------|----------|------------|-------------|----------|-------------|------------|--------|
| 2022)    |          |            |             |          |             |            |        |

|                            | Species        | Heartwood  | Sapwood    | Average    |
|----------------------------|----------------|------------|------------|------------|
| Growth ring width<br>(mm)  | Deburier Lorob | 2.0±0.4    | 1.8±0.4    | 1.9±0.4    |
|                            | Danunan Laich  | *1.5-3.0   | *1.0-2.8   | *1.0-3.0   |
|                            | Japanese Larch | 4.6±1.1    | 2.6±0.4    | 3.7±1.3    |
|                            |                | *2.8-7.0   | *2.0-3.4   | *2.0-7.0   |
| Latewood<br>percentage (%) | Deburier Lorob | 42.6±5.0   | 41.7±4.4   | 42.2±4.7   |
|                            | Danunan Larch  | *29.3-51.7 | *34.1-50.2 | *29.3-51.7 |
|                            | lananaga Larah | 31.3±5.1   | 36.5±4.9   | 33.7±5.8   |
|                            | Japanese Larch | *21.5-42.6 | *24.6-46.4 | *21.5-46.4 |

\*: the range values

#### Optical microscopy

The prepared specimens with dimensions of 10 mm (L)  $\times$  10 mm (R)  $\times$  10 mm (T) were softened in a 1:1 boiling mixture of glycerin and distilled water for 30 min. The 15–20-µm-thick radial sections were obtained using a sliding microtome (Nippon Optical Works Co, Ltd., Tokyo, Japan). The radial sections were stained with 1% safranin and light-green solutions and dehydrated using grade series of alcohol (50%, 70%, 90%, 95%, and 99%) and xylene. Permanent slides were prepared using Canada balsam. Tracheid pitting from the beginning to the end of the earlywood in both species was observed using an optical microscope (Nikon eclipse Si, Tokyo, Japan) and analyzed using image analysis software (IMT I-Solution Lite; British Columbia, Canada).

The radial tracheid diameter was measured in 50 tracheids in each growth ring. The proportion of tracheids with biseriate-bordered pitting in the growth ring was measured in the radial section using Eq. 1,

$$X(\%) = \frac{A}{B} \times 100 \tag{1}$$

where "X" indicates the proportion of the tracheids with dominantly biseriate bordered pitting, "A" shows the number of tracheids dominantly with biseriate bordered pitting, and "B" shows the total number of tracheids in earlywood.

#### Statistical analysis

The significant difference between species in the average proportion of tracheids with biseriate pitting and radial tracheid width was analyzed with a one-way ANOVA. The relationship between the proportion of tracheids with biseriate pitting and the radial tracheid width was analyzed using linear regression and Pearson's coefficient through multivariate analysis. All the statistical analyses were performed using SPSS version 26 (IBM Corporation, NY, USA).

### **RESULTS AND DISCUSSION**

### Tracheid Pitting Characteristics within a Growth Ring and With Age

Optical micrographs of the radial section of each growth ring of the DL are shown in Figs. 2 through 4. The earlywood of the 5<sup>th</sup> and 10<sup>th</sup> growth ring universally exhibited tracheids with uniseriate pitting (Fig. 2). The beginning part of the earlywood at the 20<sup>th</sup> and 30<sup>th</sup> growth rings predominantly showed biseriate-pitting tracheids, whereas the end part of the earlywood mostly showed uniseriate pitting tracheids (Fig. 3). The earlywood from the 40<sup>th</sup> to 70<sup>th</sup> growth rings universally exhibited a biseriate pitting arrangement, with a few uniseriate pitting tracheids at the end of the earlywood (Fig. 4). Biseriate pitting in each growth ring showed the opposite pattern.

Optical micrographs of the radial sections of each JL growth ring are shown in Figs. 5 through 7. In the 5<sup>th</sup> and 10<sup>th</sup> growth rings (Fig. 5), biseriate pitting tracheids were dominant at the beginning of the earlywood. In addition, uniseriate pitting tracheids were the only type of pitting arrangement observed from the middle to the end of the earlywood at the 5<sup>th</sup> growth ring. The tracheids at the 10<sup>th</sup> growth ring predominantly showed uniseriate pitting from the middle to the end of the earlywood, with a few biseriate pittings. The earlywood at the 15<sup>th</sup> growth ring showed a few tracheids with predominantly biseriate

pitting, and the tracheids showed only a uniseriate pitting arrangement toward the end (Fig. 6). The earlywood in the 20<sup>th</sup> growth ring predominantly showed biseriate pitting at the beginning and uniseriate pitting at the end, whereas there was an even distribution of biseriate and uniseriate pitting in the middle (Fig. 6). Tracheids with biseriate pitting at the 25<sup>th</sup> growth ring were universally observed at the beginning, and most tracheids had uniseriate pitting at the middle and end (Fig. 7). In the 30<sup>th</sup> growth ring, the tracheids predominantly showed biseriate pitting at the beginning and uniseriate pitting at the end of the earlywood stage, whereas in the 35<sup>th</sup> growth ring, the tracheids predominantly showed biseriate pitting in Japanese larch universally presents the opposite arrangement.



**Fig. 2.** Optical micrographs of the radial sections in the beginning (B), middle (M), and end (E) parts of earlywood at the 5<sup>th</sup> and  $10^{th}$  growth ring in DL. The white arrows indicate the tracheids with predominantly uniseriate bordered pitting. Scale bars: 200 µm

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**Fig. 3.** Optical micrographs of the radial sections in the beginning (B) and end (E) parts of earlywood at the  $20^{th}$  and  $30^{th}$  growth ring in DL. White arrows indicate the tracheids with predominantly uniseriate bordered pitting, while black arrows indicate the tracheids with predominantly biseriate pitting. Scale bars:  $200 \,\mu m$ 



**Fig. 4.** Optical micrographs of the radial sections in the 40<sup>th</sup>, 50<sup>th</sup>, 60<sup>th</sup>, and 70<sup>th</sup> growth ring in DL. White arrows indicate uniseriate bordered pitting, while black arrows indicate biseriate pitting. Scale bars: 200 µm

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**Fig. 5.** Optical micrographs of the radial sections in the beginning (B), middle (M), and end (E) parts of earlywood of the 5<sup>th</sup> and 10<sup>th</sup> growth ring in JL. White arrows indicate uniseriate bordered pitting, while black arrows indicate biseriate pitting. Scale bars: 200 µm

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**Fig. 6.** Optical micrographs of the radial sections in the beginning (B) and end (E) parts of earlywood of the 15<sup>th</sup> and 20<sup>th</sup> growth ring in JL. White arrows indicate uniseriate bordered pitting, while black arrows indicate biseriate pitting. Scale bars: 200  $\mu$ m

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**Fig. 7.** Optical micrographs of the radial sections in the beginning (B), middle (M), and end (E) parts of earlywood of the 25<sup>th</sup>, 30<sup>th</sup>, and 35<sup>th</sup> growth ring in JL. White arrows indicate uniseriate bordered pitting, while black arrows indicate biseriate pitting. Scale bars: 200 µm

Qualitatively, there was a distinctive difference between the two species in the arrangement of bordered pitting in the tracheid within the growth ring, and with age. According to the IAWA Committee (2004), biseriate tracheid pitting in the radial walls is a typical index for identifying *Larix* spp. Anagnost *et al.* (1994) reported that *Larix lyalli* had uniseriate tracheid pitting, whereas *Larix decidua*, *Larix gmelinii*, and *Larix russica* had biseriate tracheid pitting. Blokhina *et al.* (2011) reported that tracheid pitting in *Larix cajanderi* was only uniseriate from the 1<sup>st</sup> to 4<sup>th</sup> growth ring, but biseriate tracheid pitting was observed after the 5<sup>th</sup> growth ring. Jagels *et al.* (2001) reported that *Larix altoborealis* showed one to two rows of bordered pits on the radial surfaces of the tracheids.

# Biseriate tracheid pitting proportion

The proportion of tracheids with biseriate bordered pitting in the earlywood of DL and JL is shown in Table 3 and the radial variation is shown in Fig. 2. At the 5<sup>th</sup> and 10<sup>th</sup> growth rings, JL showed a significantly greater proportion of tracheid with biseriate pitting than DL, whereas both species showed similar properties at the 15<sup>th</sup> and 20<sup>th</sup> growth rings. The average value of DL at the 5<sup>th</sup> to 20<sup>th</sup> growth rings was smaller than that of JL but not significantly different.

| Crowth Dingo | Species                   |                                |  |  |
|--------------|---------------------------|--------------------------------|--|--|
| Growin Rings | DL                        | JL                             |  |  |
| 5            | 1.9±0.1 <sup>aA</sup>     | 4.0±0.2 <sup>bA</sup>          |  |  |
| 10           | 6.6±1.3 <sup>aA</sup>     | 22.7±1.2 <sup>bB</sup>         |  |  |
| 15           | 25.0±10.5 <sup>aB</sup>   | 27.6±5.3 <sup>aB</sup>         |  |  |
| 20           | 23.0±5.1 <sup>aB</sup>    | 29.1±7.4 <sup>aB</sup>         |  |  |
| Average      | 14.1±11.6 <sup>a</sup>    | <b>20.8</b> ±11.6 <sup>a</sup> |  |  |
| 25           | 50.6±11.3 <sup>bCDE</sup> | 32.2±1.6 <sup>aBC</sup>        |  |  |
| 30           | 45.5±6.3 <sup>aC</sup>    | 51.1±15.7 <sup>bD</sup>        |  |  |
| 35           | 47.2±10.2 <sup>aCD</sup>  | 46.5±8.3 <sup>aCD</sup>        |  |  |
| Average      | 47.8±2.6 <sup>a</sup>     | 43.3±9.9 <sup>a</sup>          |  |  |
| 40           | 61.1±9.6 <sup>DEF</sup>   |                                |  |  |
| 45           | 65.0±8.8 <sup>EF</sup>    |                                |  |  |
| 50           | 58.5±4.2C <sup>DEF</sup>  |                                |  |  |
| 60           | 65.4±8.4 <sup>EF</sup>    |                                |  |  |
| 70           | 71.1±10.2 <sup>F</sup>    |                                |  |  |

**Table 3.** Proportion of Biseriate Bordered Pitting Tracheid in the Earlywood of DL and JL

Note: Identical lowercase superscript letters beside the mean values in the same rows indicate insignificant outcomes at the 5% significance level for comparisons among species. Identical uppercase letters beside the mean values in the same columns denote insignificant outcomes at the 5% significance level for comparisons between growth rings.

From the 25<sup>th</sup> to 35<sup>th</sup> growth ring, both species exhibited comparable proportion of biseriate pitting tracheids, showing an average of  $47.8\pm2.6\%$  for DL and  $43.3\pm9.9\%$  for JL. In addition, the values ranged from 45.5% to 50.6% for DL and 32.2% to 51.1% for JL. After the 40<sup>th</sup> growth ring in DL, the proportion of biseriate pitting tracheid increased to above 59%. The proportion of tracheids with biseriate-bordered pitting increased as the growth ring number increased in both species but tended to be constant after the 40<sup>th</sup> and 30<sup>th</sup> growth rings in DL and JL, respectively.

The proportion of tracheids with biseriate-bordered pitting in earlywood can be used to identify juveniles, transitions, and mature wood in both species. The radial variation in the proportion of tracheids with biseriate pitting in the present study was comparable to other identification indices of DL and JL. Kim *et al.* (2021, 2022) reported that the uniseriate and fusiform ray height, relative crystallinity index, tracheid length, and diameter of DL and JL were low near the pith, increased as the number of growth rings increased, and tended to remain constant after the 30<sup>th</sup> growth ring in DL and after the 20<sup>th</sup> growth ring in Japanese larch wood. The authors also mentioned that the transition between juvenile and mature wood in both species was the 30<sup>th</sup> growth ring for DL and the 20<sup>th</sup> growth ring for JL.



Fig. 8. Radial variation of the proportion of the tracheids with biseriate bordered pitting in earlywood of DL and JL

#### Radial tracheid diameter and its correlation with biseriate tracheid pitting proportion

Figure 9 shows the radial variation in the earlywood tracheid width from the pith to the bark in both species. The radial tracheid width of DL and JL was  $52.5\pm13.8 \mu m$  and  $53.1\pm12.4 \mu m$ , respectively, and it increased with increasing the growth ring and tended to be constant after a certain growth ring. Rlee and Kim (2005) observed that the tracheid width of JL exhibited distinct dimensions: measuring 54.2  $\mu m$  (ranging from 47.2 to 60.4  $\mu m$ ) in earlywood and 36.2  $\mu m$  (ranging from 33.4 to 39.4  $\mu m$ ) in latewood. Chong and Park (2008) reported that the tracheid width in the radial direction of JL was 44  $\mu m$  during earlywood formation and 26  $\mu m$  during latewood formation. Fonti *et al.* (2015) provided additional insights, reporting that the tangential tracheid width of DL was 28–30  $\mu m$ .





Regarding radial variation, Kim *et al.* (2022) reported that the tangential tracheid widths of DL and JL were narrow in the pith region and remained constant toward the bark from approximately the 20<sup>th</sup> growth ring. Park *et al.* (1979) reported that the tracheid diameter in the branch and stem wood of *Pinus densiflora* increased from the pith to the bark. Purusatama and Kim (2020) revealed that the radial tracheid diameter of the compression, lateral, and opposite woods in *Ginkgo biloba* increased slightly with an increasing number of growth rings. In addition, the radial tracheid diameter of opposite wood significantly increased with an increasing number of growth rings.

Figure 10 shows the relationship between the earlywood tracheid width and the proportion of tracheids with biseriate bordered pitting. In both species, a positive correlation was observed between these two factors (Pearson's coefficient: 0.651). It can be concluded that biseriate bordered pitting was observed predominantly in wider earlywood tracheids, whereas uniseriate pitting was observed in narrower tracheids. Therefore, the bordered pitting arrangement can be used to determine the boundary between the juvenile and mature wood.



**Fig. 10.** Relationships between the earlywood tracheid width and the tracheid proportion with biseriate pitting in Dahurian larch (DL) and Japanese larch (JL) woods. The dotted line indicates the mean value of both species.

### CONCLUSIONS

- 1. In juvenile wood, Dahurian larch (DL) universally showed tracheids with a uniseriate pitting arrangement along the earlywood, whereas Japanese larch (JL) showed uniseriate pitting tracheids from the middle to the end of the earlywood and biseriate pitting tracheids at the beginning of the earlywood.
- In the transition wood, DL predominantly showed biseriate pitting tracheids at the beginning of the earlywood and uniseriate pitting tracheids at the end of the earlywood. JL showed uniseriate pitting at the beginning and end, whereas the middle part predominantly shows biseriate pitting tracheids.

- 3. The mature wood of both species predominantly showed a biseriate pitting arrangement with a few uniseriate pitting tracheids at the end of the early wood.
- 4. JL tended to exhibit a higher tracheid proportion with biseriate pitting at the 5<sup>th</sup> to 20<sup>th</sup> growth rings compared to DL, and both species showed comparable values at the 25<sup>th</sup> to 35<sup>th</sup> growth rings.
- 5. The tracheid proportion with biseriate pitting and the radial tracheid width of both species increased with increasing growth ring number. Both properties remained constant after the 25<sup>th</sup> growth ring. There was a positive correlation between these two properties.
- 6. In conclusion, the results revealed distinct patterns in bordered pit arrangements between the two species and in radial variation. These findings suggest that analyzing bordered pits could be a promising approach for differentiating DL and JL. However, further research is necessary to ensure the practical application of the method, including validating accuracy across a wider range of larch species and growth conditions and refining the analysis methods.

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