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ORIGINAL ARTICLE

Is the first flowering event and corresponding maturity phase in trees related to radial wood density changes?

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Abstract

Key message **Although first flowering in trees causes clear changes in main stem morphological trends, we prove that it does not totally explain changes in wood density trends.**

Abstract The phase change produced by a tree's first flowering event (that marks the limit between juvenile and mature stage) causes changes in the morphological trends of the main stem. As has been documented in a number of tree species, the number of growth units per annual shoot, the annual shoot length, and the growth unit length are some of the variables that exhibit abrupt changes when first flowering occurs. Considering the strong impact of first flowering on tree morphology—and considering also trends that have been observed in wood density and related variables, we tested the relationship between the first flowering event and wood density trend changes in *Pinus radiata* D. Don at three different site conditions with differing climates. The relationship was studied using different criteria to select the proper variables and methodology. Some interesting relationships between first flowering and certain variables were found, particularly the variables earlywood density, latewood density accumulated, latewood proportion, and ring area. However, these relationships were not consistent among the three sites. Overall, there is not enough evidence to conclude that the first flowering causes or contributes to changes in wood density trends. Our findings suggest that studies should be conducted on the relationship between the architectural development of the crown and changes in internal wood characteristics. We corroborate the suggestion as reported by Burdon et al. (For Sci 50:399–415, 2004) of adopting the terms corewood and outerwood instead of juvenile and mature wood.

Keywords 4 to 6 Pinus radiata · Wood density · Phase change · Flowering · Tree architecture · Maturity

Introduction

Strong correlations have been found between wood density and some characteristics such as strength and stiffness; yield and pulp characteristics among others (Bouffier et al. 2009; Gapare et al. 2006; Kumar 2002; Panshin and de Zeeuw 1980; Zobel and Sprague 1998; Zamudio et al. 2005; Li and Wu 2005). But high variability in wood density has been found between species, between individuals of the same species, in the same tree at different heights, and within growth rings. There are a number of reasons for this variation, including genetics, cambial activity, age, seasons, environmental conditions, and silvicultural management (Larson 1969; Koubaa et al. 2002; Harris and Cown 1991).

In softwoods, the wood produced during the tree's first years, called juvenile wood, often differs from the mature wood produced by an older cambial tissue (Zobel and

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Sprague 1998). These differences include a higher proportion of earlywood (Mansfield et al. 2009), lower density, higher microfibrillar angle, greater flexibility [or less stiffness (MoE)], a higher ratio of lignin to cellulose, and more reaction wood (Clark et al. 2006; Gapare et al. 2006; Zamudio et al. 2005). The terms "juvenile wood" and "mature wood" have been discussed by Amarasekara and Denne (2002), Burdon et al. (2004), and Zobel and Sprague (1998). The terms core wood, crown-formed wood, immature wood, inner wood, or pith wood have also been used for juvenile wood (Alteyrac et al. 2006).

Variation in wood density is correlated with the age; the variability decrease is correlated with cambial maturity (we prefer the term "cambial aging") and with the appearance of mature wood (Zobel and Sprague 1998). The boundary between the juvenile and mature wood is diffuse, creating a transition zone (Alteyrac et al. 2006; Clark et al. 2006; Gapare et al. 2006). Trees with high density wood have, in general, sharper gradients in the boundary and less juvenile wood (Harris and Cown 1991).

It has also been documented that wood density increases in the stem base until the tree reaches its final height (Kucêra 1994), while the low density wood is produced in the crown zone (Zobel and Sprague 1998). The gradient and the increments of wood density in the radial axis can vary between trees and sites (Burdon et al. 2004; Harris and Cown 1991; Mansfield et al. 2009; Zobel and Sprague 1998, Zobel and van Buijtenen 1989), indicating that genetic and environmental factors contribute to the variation. Zobel and Sprague (1998) reviewed different variables that could affect the transition of wood characteristics from the pith to the bark (or from juvenile to mature wood).

Biologically, the shift from the juvenile stage to maturity is normally related to the development of reproductive capability. In trees, the appearance of the flowers and their continuation (Zimmerman et al. 1985) are indicators of the phase change—i.e., the transition from a juvenile to a mature phase (Greenwood 1995). The external characteristics of a tree exhibit variations when the phase change occurs, typically resulting in a longitudinal asymmetry in the plant shape (Jones 1999). Vegetative growth slows considerably in maturity (Meilan 1997; Burdon 1994). The phase change also can be evidenced by characteristics such as branching and foliar morphology (Greenwood 1995; Heuret et al. 2006). Some authors have suggested that this change is abrupt (Sweet 1972). This behavior has been documented in *Pinus radiata,* where the first appearance of the female cones is associated with abrupt changes in the annual rates of growth in height, the number of growth units per year, and the length of each growth unit, with shorter and more frequent growth units after flowering begins, with a growth unit defined as a shoot section with a proximal zone of cataphylls and a distal zone bearing lateral buds or axis, that develops during a continuous growing flush (Fernández et al. 2007) (Fig. 1). As a polycyclic species, *P. radiata* can produce one or more growth units per annual shoot. In a growth unit, the stem portion between its base and its upper part is normally called internode.

The morphological and physical changes produced with the first flowering are in some cases so distinct that we could hypothesize that first flowering also could be related to changes in wood characteristics. As far as we know, this hypothesis has not yet been tested. If there is not a relationship, then the use of the terms juvenile and mature wood should be revisited, in order to ensure consistency in the scientific literature. In this study, we examine trees from stands in three areas of Chile to assess the possible correlation between phase change and changes in wood characteristics.

Materials and methods

Three unmanaged stands of *P. radiata* D. Don were sampled. The stands are located in three different sites under different climate regimes in a latitudinal gradient between central and southern Chile: La Trupa, in a relatively dry and temperate environment, and Villucura and Lemunantú, areas with more rainfall but colder temperatures (Table 1). We sampled 17, 7, and 10 trees, respectively, at these sites. The year 1998 was an extremely dry year in La Trupa (302 mm precipitation in comparison with the average of 785 mm), distorting some behavioral variables; therefore, data from 1998 were excluded from the analysis.

In each stand, healthy and well developed trees with diameters close to the average value for the stand were felled. In each tree, the first flowering position was determined by the presence of female cones or cone scars. Measurements included total tree height and diameter at breast height (DBH). Applying the methodology used by Fernández et al. (2007), a growth unit is a shoot section that develops during a continuous growing season showing a proximal zone of cataphylls and a distal zone bearing lateral buds or axis. In each growth unit the total length, basal diameter, number of lateral branches, number of female strobili (cones), state of the cones (alive or aborted), and presence of lateral buds were determined.

A disk at the base of the tree and at the base of the growth unit presenting the first evidence of flowering was taken (Fig. 1a). Each disk was oven dried (30–50 °C), polished, and scanned at a 300 dpi resolution. Ring boundaries were determined and measured with Adobe ® Photoshop ® CS 4 Extended; the results were corroborated with visual analysis of the disks. Total tree age and first flowering age were assessed.

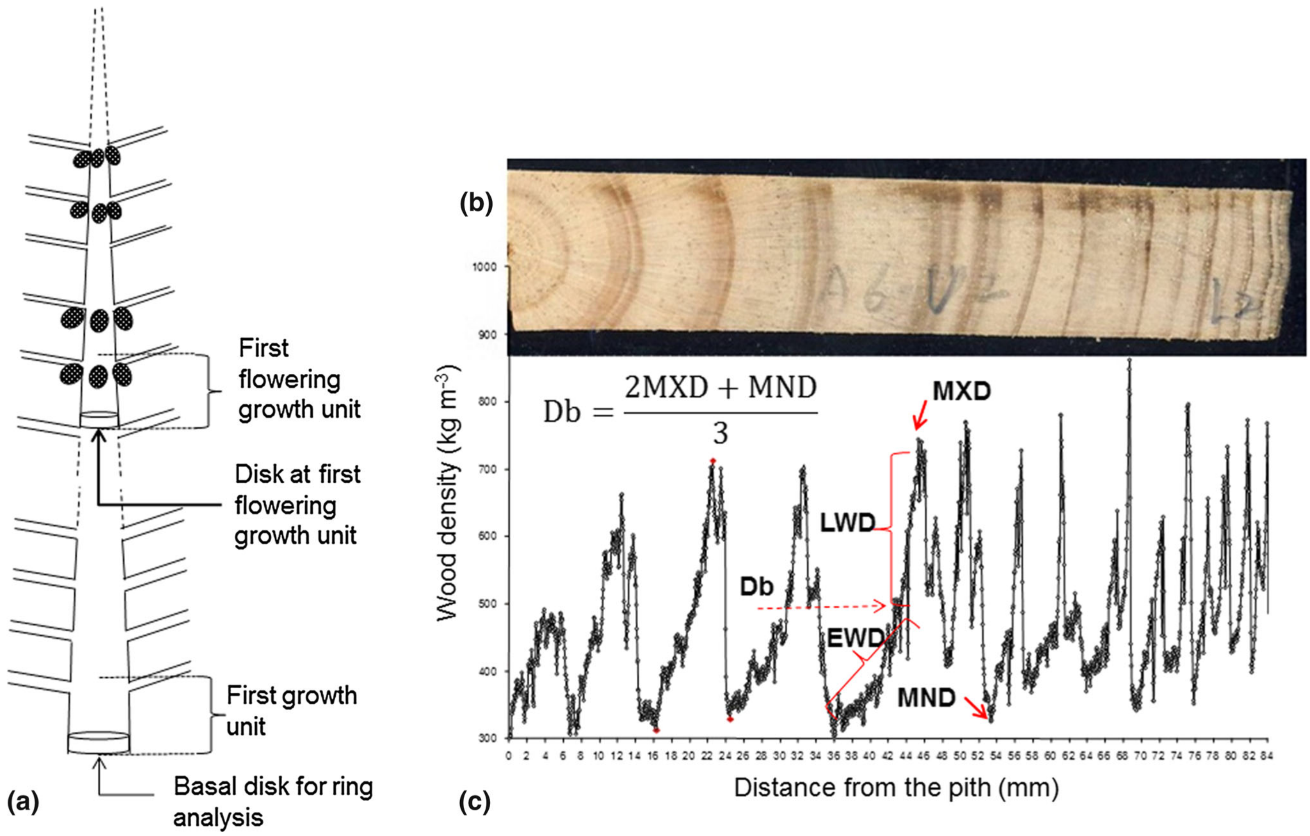


Fig. 1 **a** Disk positions at the base of the tree and at the base of the first flowering growth unit; **b** a view of variations in the wood and their positions in the ring; **c** the X-ray density profiles corresponding to the growth ring sections, which indicate a good boundary determination. *EWD* earlywood density; *LWD* latewood density; *MXD* maximum ring density; *MND* minimum ring density; *Db* boundary between early and latewood inside a ring

In each basal disk sampled, a radial section strip was analyzed by X-ray in the FP INNOVATION Laboratory (FPINNOVATIONS, Canada). Radial Sects. 18 mm wide and 1.67 mm in height were chosen so as to avoid compression wood, knots, wounds, or cracks. They were extracted using a Soxhlet with a solution of cyclohexane/ethanol (2:1) for 24 h, an ethanol solution for 8 h, and then a hot water bath for 8 h (Koubaa et al. 2002; Savva et al. 2010). After the extraction, the samples were air dried to prevent warping and stabilized at 12 % humidity. The samples were X-rayed to obtain wood density measurements using a QTRS-01X Tree-Ring Scanner (QMC, Knoxville, Tennessee), with a linear resolution of 0.04 mm for each measurement (Savva et al. 2010).

Analysis of external architectural features and flowering age

A comparison was conducted between sites for several variables: first flowering age, total height at the first flowering, number of cones in the first flowering growth unit, accumulated number of growth units, and accumulated number of branches at the first flowering. Data normality was assessed with Kolmogorov–Smirnov test; the Student's t test using the statistical package Minitab 15® was applied to compare pairs of sites. When necessary, the Wilcoxon rank-sum test was applied (to non-normal or small data sets).

Comparison of wood characteristics between sites

A description and comparison of wood characteristics was conducted between sites. The different sites were sampled at different ages; thus, the comparison was made based on the cumulative values of the different variables during the first 13 rings.

Analysis of wood density profiles and selection of variables

From the X-ray profiles, total ring boundary (RB) and earlywood (EW) and latewood (LW) boundaries were determined. The following variables were calculated for the inside of each ring: average ring density (RD), earlywood density (EWD), latewood density (LWD), maximum density (MXD), minimum density (MND) (Fig. 1b), ring area (RA), earlywood area (EWA), latewood area (LWA), and, as the

Table 1 Description of sites and stands

Stand name	La Trupa	Villucura	Lemunantú
Location	VI Region "del Libertador Bernardo O'Higgins", "Provincia Cardenal Caro"	VIII Region "del Bío Bío", "Provincia de Bío Bío"	IX Region "de la Araucanía", "Provincia de Cautín"
Latitude	34°43′40″S	37°33′27″S	39°29′30.26″S
Longitude	71°53′8″O	71°53′46″O	72°20′58.63″O
Altitude (meters over sea level)	130	499	408
Climate classification[a]	Mediterranean with oceanic influence	Temperate Mediterranean	Oceanic fresh
Average annual temperature (°C)[b]			
Minimum	7.1 (2.1)	6 (2.6)	7.3 (2.3)
Average	12.7 (3.2)	13 (9.7)	12.4 (7)
Maximum	18.4 (4.4)	19.8 (5.8)	17.3 (4.6)
Average annual precipitation (mm)[b]	785 (301)	1432 (426.7)	2073 (325)
Soil series[c]	San Pedro de Alcántara	Santa Bárbara	Lumaya
Soil Classification	Alfisol	Andisol	Andisol
Stand density (trees per ha)	1600	1333	1111
Plantation year	1993	1991	1992
Sampling date	July-2009	January-2010	February-2010
Plantation age (years)	16	19	18
Number of sampled trees	17	7	10
Silvicultural management	None	Pruning in the first meter	Pruning in the first meter
Average tree height (m)[1]	19 (1.4)	26 (2.8)	20 (1.6)
Average Diameter at 1.3 m (cm)[1]	18 (1.6)	23 (9.5)	26 (3.8)

Precipitation and temperatures correspond to the life period of each stand

[a] Climate type according to the Santibáñez and Uribe (1993) classification

[b] For temperature, rainfall and tree parameters average values are given, followed by the standard deviation in brackets (SD)

[c] Instituto Nacional de Investigación Agropecuaria (1985) and Centro de Información de Recursos Naturales (1996)

ratio between latewood area and total ring area (LWA/RA), latewood proportion (LWP).

Following Alteyrac et al. (2006) the RA was selected as a more representative variable of tree growth and ring size than ring width. RA is related to new foliage formed at the time the ring was formed (Larson 1969; Mäkelä 2002) and is thereby a better expression of the whole growth process. RA, EWA, and LWA were computed assuming a circular profile of the rings. The average RD was calculated as weighted average EWD and LWD, considering the respective areas.

The advantages and drawbacks of different methods for the demarcation of early and latewood limit in a ring were analysed, such as the threshold method (Gaspar et al. 2008; Kumar 2002; Koubaa et al. 2002), the Mork index (Denne 1988; Koubaa et al. 2002; Kumar et al. 2009; Park et al. 2006), and some mathematical models (Pernestal et al. 1995; Koubaa et al. 2002). The method proposed by Hylen (1999) was chosen because it is simple and produces good results. Using this method, the demarcation between earlywood and latewood in each ring was determined to be the point where density equals the minimum value plus two-thirds of the difference between the maximum (MXD) and the minimum (MND). Equation 1 shows how the boundary (Db) between early and latewood is calculated:

$$\mathrm{Db} = \mathrm{MND} + \frac{2}{3} \cdot (\mathrm{MXD} - \mathrm{MND})$$

$$\mathrm{Db} = \frac{(2 \cdot \mathrm{MXD} + \mathrm{MND})}{3} \quad (1)$$

Wood densities higher than this limit were classified as latewood densities and lower ones as earlywood densities.

Inside of a ring, the average of EWD and LWD was calculated as the averages of all the values below or above the Db, respectively (Fig. 1a).

In addition to the previous variables, the cumulative averages of RD, EWD, LWD, and LWP were calculated (RD_a, EWD_a, LWD_a, LWP_a respectively), weighting each value according to the respective ring areas. The average accumulated values obtained along the rings' profiles represent the average value that could be obtained if an average wood density had been measured considering a profile from the pith to the on-going ring.

As first flowering occurs at different ages between locations and between trees at the same site, the standardization procedure proposed by Fernández et al. (2007) was applied. It consists of establishing the first onset of female flowering as the zero biological reference date on the main axis, allowing the expression in common ontogenetical ages the years before the first flowering as negative values and the years after the first flowering as positive values.

Each variable was plotted against ontogenetical age for a graphic elucidation of the best and clearest trends. Also the OLSCUSUM procedure with bootstrap for time series change points analysis after Taylor (2000) and Edopka and Ogbeide (2013) was performed on all the variables. The null hypothesis indicates that non-significant changes are detected. The results are expressed as the confidence level that a change occurred. Typically, 90–95 % is required before one states that a significant change has beed detected. After completing both analyses, the best variables were chosen.

Estimation of transition age from juvenile to mature wood using segmented linear regression

For each tree and each selected wood variable, the transition age between juvenile and mature wood was estimated using segmented linear regressions with the program SegReg, as suggested by Abdel-Gadir and Krahmer (1993); Alteyrac et al. (2006); Gapare et al. (2006); Mansfield et al. (2009), and Sauter et al. (1999). This procedure determines the change of slope in a data series. The model consists of finding two linear regressions to characterize the variation in wood characteristics (variable Y) with respect to cambial age (variable X). With a, b, c, and d parameters, and the transition point (X_o) estimated by an optimization procedure to obtain the best coefficient of determination.

$$Y = aX + b + \varepsilon \text{ for } \quad X \leq X_0 \tag{2}$$

$$Y = cX + d + \varepsilon \text{ for } \quad X \geq X_0 \tag{3}$$

$$X_0 = \frac{d-b}{a-c} \tag{4}$$

Once the segmented regression and the change of slope in the data were determined, a correlation between the first flowering date and the slope change date for each variable in each site was established.

Results and discussion

External architectural features and flowering age results

Table 2 presents the first flowering age and the conditions of its occurrence, including the average stem height at first flowering, the number of cones involved in the first flowering, the number of growth units generated before the first flowering, and the associated number of branches. The Table 2 also shows the comparison of those parameters between the different stands.

At the La Trupa site, the onset of flowering began at an average age of 4 years, significantly earlier than in Villucura and Lemunantú, which flowered with 8 and 10 years, respectively (Table 2). Among Villucura and Lemunantú, there are no significant differences for the first flowering

Table 2 First flowering age, height, and architectural variables of the stem for the three sites. Mean comparison (t Student test) used to compare the different sites

Variable	La Trupa ($n = 17$) Average (sdev)	Villu-cura ($n = 7$) Average (sdev)	Lemun-antú ($n = 10$) Average (sdev)	La Trupa/ Villucura p value	La Trupa/ Lemun-antú p value	Villucura/ Lemunantú p value
First flowering age (years)	4 (1.1)	9 (4.2)	10 (1.9)	0.030*	0.000*	0.256
Stem height at first flowering (m)	5 (1.5)	12 (6.1)	10 (2.7)	0.026*	0.000*	0.538
Number of cones at first flowering	2 (1)	3 (2)	2 (1)	0.562	0.684	0.392
Number of growth units accumulated before first flowering	12 (3)	24 (12)	20 (6)	0.036*	0.003*	0.421
Number of branches accumulated before first flowering	59 (24)	128 (59)	118 (38)	0.024*	0.001*	0.700
Basal area of base disk at 13 years (cm^2)	215 (50)	669 (132)	446 (107)			

n = sample size; average values with standard deviation in brackets; * significant differences with p value <0.05

age or for the other variables evaluated (stem height at the first flowering occurrence, number of growth units generated before the first flowering, number of branches generated before the first flowering). Compared to the other two sites, La Trupa is warmer and drier (with an average water balance that supplies half of the species' annual requirements). The trees flowered earlier, with a shorter number of accumulated growth units, fewer accumulated branches, and less total height at the first flowering. This means that the flowering not only occurred earlier chronologically, but also earlier in terms of the number of structures and the size of the trees. Thus, in a first glance we could also expect some internal differences between La Trupa and Villucura and Lemunantú.

Comparison of general wood characteristics between sites

Table 3 shows the results of the comparison of accumulated wood characteristics of the first 13 rings between sites.

Analyzing the general wood characteristics for the three sites (Table 3), there is a gradient of average wood density, La Trupa being the site with higher average density and Villucura the site with lower average density. Villucura has a higher MXD and a higher total LWD than Lemunantú, but nevertheless a lower total density. It seems clear that the low contribution of LWA to the total ring area (around 20 % for the three sites) implies that the earlywood density values are the more significant contributors to total wood density values in the three sites.

La Trupa presents significant differences in almost all the wood characteristics when compared with the other two sites. There are no significant differences between Villucura and Lemunantú in total ring density, total EWD, total LWD, or LWP.

Results of the analysis of wood density profiles and selection of variables

The null hypothesis of the OLSCUSUM procedure with bootstrap for time series change points indicates that non-significant changes are detected. The results are expressed as the confidence level that a change occurred. Typically, 90–95 % is required before determining that a significant change has been detected. Those confidence levels are presented in Table 4 by variable and site.

From this analysis, the variables RD, LWD, EWD, and LWP were chosen because at least in one site per variable there was evidence of significant changes (confidence level larger or equal to 90 %). In the case of RD, LWD, and EWD, the site Lemunantú presented values above 90 %. In the case of LWP, the variable presented significant changes in all the three sites. The variable MND was dismissed because of its confidence levels, indicating that non-significant changes in the series tendencies were detected. Although MXD larger confidence level was 79.6 %, the variable was chosen after visual inspection. Finally and regarding RA (and its related EWA and LWA), they present consistent changes in the three site conditions after the analysis and after visual inspection. Therefore the variable RA is maintained and shown in Fig. 2, although not used in further analysis because of its high relationship with stand density conditions.

In addition, a visual inspection resulted in the selection of the variables LWD_a and LWP_a.

Figure 2 shows the selected variables normalized after the first flowering year (equal to ontogenetical age 0).

When presented graphically (Fig. 2), some variables exhibit a clear change at the point of the first flowering (the "0" ontogenetical age in the graphs). The La Trupa site shows more marked trend changes, particularly in LWD, RA, MXD, and LWP. LWD increases until the first

Table 3 Means and standard deviations of wood density and ring area for the first 13 rings in the three sites. Mean comparison significance (*t* Student) used to compare sites

Variables	La Trupa ($n = 17$)	Villucura ($n = 7$)	Lemunantú ($n = 10$)	La Trupa/ Villucura	La Trupa/ Lemunantú	Villucura/ Lemunantú
Total ring density (kg m^{-3}) (RD)	512 (40)	477 (28)	488 (36)	0.027*	0.120	0.487
Total earlywood density (kg m^{-3}) (EWD)	475 (35)	435 (24)	462 (34)	0.006*	0.368	0.073
Total latewood density (kg m^{-3}) (LWD)	666 (46)	610 (37)	589 (44)	0.008*	0.000*	0.309
Total minimum density (kg m^{-3}) (MND)	386 (33)	358 (15)	386 (23)	0.012*	0.943	0.009*
Total maximum density (kg m^{-3}) (MXD)	716 (54)	643 (33)	607 (28)	0.001*	0.000*	0.040*
Total ring area (cm^2) (RA)	215 (50)	669 (132)	446 (107)	0.000*	0.000*	0.004*
Total earlywood area (cm^2) (EWA)	172 (36)	506 (88)	353 (83)	0.000*	0.000*	0.004*
Total latewood area (cm^2) (LWA)	43 (16)	163 (49)	94 (33)	0.001*	0.001*	0.009*
Total latewood proportion (%) (LWP)	20 (3)	24 (3)	21 (5)	0.011*	0.525	0.118

n = sample size; standard deviation in brackets; critical p value = 0.05

Table 4 Confidence level (expressed as a percentage) of significant changes detected in the time series of each variable

	La Trupa (%)	Villucura (%)	Lemunantú (%)
Ring density (RD) (kg m^{-3})	81.5	71	94.2
Earlywood density (EWD) (kg m^{-3})	32.1	86.6	99.5
Latewood density (LWD) (kg m^{-3})	65.4	77.8	98.6
Minimum density (MND) (kg m^{-3})	37.8	43.1	27.4
Maximum density (MXD) (kg m^{-3})	60.9	79.6	68.5
Ring area (RA) (cm^2)	100	99.5	99.9
Earlywood area (EWA) (cm^2)	99.8	91.7	99.5
Latewood area (LWA) (cm^2)	96.9	70.6	98.8
Latewood proportion (LWP) (%)	99.6	93.5	98.9

flowering year, drops for 2 years, then culminates in value the third year after first flowering. Then it stabilizes for several years, with a particular peak at the end of the time series. LWD_a presents even a clearer trend, increasing until the onset of flowering and then stabilizing. Comparing LWP and LWP_a, the latter exhibits more marked stabilization, diminishing until one year after first flowering and then stabilizing. MXD and RA also culminate at the flowering year and one year later, respectively. Crown architecture data from La Trupa (data not shown) shows the same changes after the first flowering as those reported in Fernández et al. (2007). The data indicate a steady increase in the number of growth units per annual shoot until the onset of flowering, then stabilization in the value. The data also show that the annual shoot increases in length until first flowering and then stabilizes, resulting in a shorter growth unit after first flowering. Thus, in La Trupa, flowering seems to affect both external and internal characteristics of the trees.

At Villucura the average RD exhibits a rather steady increase, although there are some local variations. At the onset of flowering the trend changes, with an abrupt drop caused by lower values in both earlywood and latewood. There is a strong correlation between the drop in latewood density (LWD) and a drop in máximum density (MXD). In both cases, the drop begins the third year after the first flowering (again, using the flowering year as the reference year). Nevertheless, pine cones mature over a span of three years (Cremer 1992), and the ongoing production of new flowers in the following years could be producing a delayed effect on wood properties. Thus, there is evidence for a transition age between no flowering and full flowering capacity. LWD does not show such an evident trend as LWP_a (Fig. 2f). After the first flowering event, Villucura LWP_a data stabilizes near 20 %.

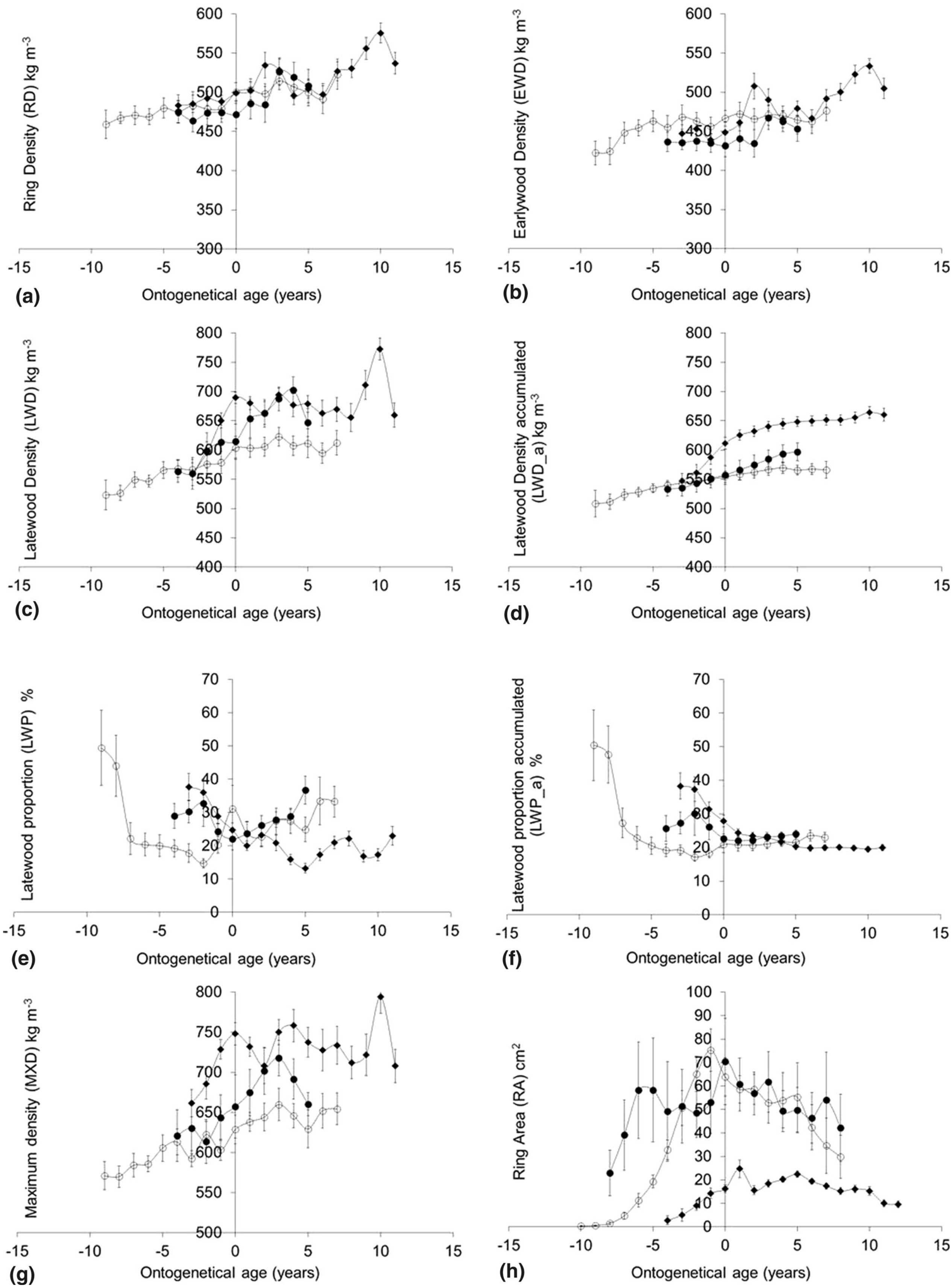
Reviewing RA data, Villucura presents variable behavior before the first flowering, reaches a peak at the flowering year, then begins to drop steadily.

All the variables related to wood density (RA, EWD, LWD, and LWD_a) exhibit less evident trend changes in Lemunantú than in Villucura. Nevertheless, MXD increases steadily until 3 years after the first flowering, with the LWD showing the same trend. Again, changes appear after a delay of three years after the first flowering, a phenomenon that could be related to a transition period between first flowering and full flowering phases. LWP_a stabilizes after the first flowering. Finally, RA steady increases, culminating 1 year before the zero ontogenetical age and dropping steadily after that. Thus, MXD, LWP_a, and RA values are apparently related to the first flowering event.

RA for Lemunantú shows a marked trend change, strongly coincident with the first flowering. The same occurs in La Trupa. Villucura also shows a marked trend change in ring area, but in this case the values peak 5 years before the first flowering and then peak again right after flowering. There seems to be a relationship between ring area and first flowering. Nevertheless, ring area has been considered a variable strongly related to stand density. The site index, defined as the height the forest attains at a certain key age (Prodan et al. 1997), has been based on trees' height and not their diameter, because the diameter (related to RA) depends strongly on stand density and competition conditions inside the stand. Foliage biomass and leaf area are related to the cross-section area of the tree (Mäkelä 2002), and vice versa, as the growth (and next ring area) in the following season will depend on the already existing foliage.

Based on stand density, the average DBH of each stand, and a model that relates maximum branch length with DBH (another database, data not shown), we estimated stand closure occurred when La Trupa reached an age of 6–7 years, when Villucura reached an age of 6–7 years, and when Lemunantú reached an age of 5–6 years. If observing ring area culmination based on raw data (based on calendar years and not on ontogenetical age), culmination of RA also occurs in La Trupa between years 6 and 7; in Villucura in year 6 and in Lemunantú in year 8. In other words, in La Trupa and Villucura there is a strong correlation between estimated stand closure and RA culmination values.

From the perspective of stand characteristics, La Trupa is a very dense stand, with 1600 trees per ha but with slow


Ring Density (RD) kg m-3
Ontogenetical age (years)
(a)
Earlywood Density (EWD) kg m-3
Ontogenetical age (years)
(b)
Latewood Density (LWD) kg m-3
Ontogenetical age (years)
(c)
Latewood Density accumulated (LWD_a) kg m-3
Ontogenetical age (years)
(d)
Latewood proportion (LWP) %
Ontogenetical age (years)
(e)
Latewood proportion accumulated (LWP_a) %
Ontogenetical age (years)
(f)
Maximum density (MXD) kg m-3
Ontogenetical age (years)
(g)
Ring Area (RA) cm2
Ontogenetical age (years)
(h)

◀ **Fig. 2** **a** Average ring density (RD), **b** earlywood density (EWD), **c** latewood density (LWD), **d** latewood density accumulated (LWD_a), **e** latewood proportion (LWP), **f** latewood proportion accumulated (LWP_a), **g** maximum density (MXD) and **h** ring area (RA) for La Trupa, Villucura and Lemunantú sites. Horizontal scale expressed in ontogenetical age, with year 0 as the first flowering year. Values correspond to mean values. Density values in kg m^{-3}, ring area in cm^2. Symbols: *La Trupa black square*; *Villucura black circle*; *Lemunantú open circle*. Average values with *error bars*

growth because of a water deficit. Thus, the peak in RA occurs after the first flowering. As a result, the data do not permit us to distinguish between the possible effect of stand closure and the effect of first flowering on RA. Villucura data support the concept of a correlation between stand closure and the RA peak, because this semi-dense stand (1300 trees per ha) with very good growth rates shows an RA peak 5 years before flowering. In Lemunantú, a less dense stand than La Trupa and Villucura, but one with a higher DBH average than those two stands, the RA peak occurs 1 year before flowering; in this case there is no coincidence between stand closure date and the culmination of RA based on calendar year. Alteyrac et al. (2006) concluded that RA was not a proper variable for defining the boundary between juvenile and mature wood, and our data support that conclusion.

Correlation between wood characteristics

Before the segmented regression analysis, a correlation analysis between the different wood variables was conducted. The analysis showed that MXD is highly correlated with LWD ($r = 0.97$, $p < 0.0001$) and that EWD is highly correlated with RD ($r = 0.91$, $p < 0.0001$). Thus, only one variable from each couple was selected for the trend change analysis procedure.

Integrating the graphical analysis of the data, the mean comparison between sitesand the correlation between variables, and the following set of variables were finally selected for the segmented regression procedure: (a) EWD; (b) LWD_a; (c) LWP_a; and (d) RA. This last variable, total ring area, is strongly related to stand density as discussed previously. Thus, it was considered during the analysis to help elucidate the whole formation process, but was not used for the final results and conclusions.

Results of the segmented regression analysis

Table 5 presents the means and standard deviations of the transition age between juvenile and mature wood, as estimated with the selected wood variables.

The transition depends on the variable, varying between 4.27 and 6.36 years in La Trupa, 4.64 and 8.95 years in Villucura and 4.82 and 9.12 years in Lemunantú.

As an average result using the variables EWD, LWD_a, and LWP_a, the average transition age is 5.25 years for La Trupa, 6.68 years for Villucura and 6.55 years for Lemunantú (Table 5).

Relationship between first flowering age and transition age between juvenile and mature wood

A simple regression analysis between the independent variable "first flowering age" and the dependent variable

Table 5 Mean and standard deviation of the transition age between juvenile and mature wood, based on segmented regression analysis for the selected variables

Variable	Transition age (chronological years)								
	La Trupa (FFA 4 years) (1.1 sd)		Villucura (FFA 9 years) (4.2 sd)		Lemunantú (FFA 10 years) (1.9 sd)		La Trupa/ Villucura	La Trupa/ Lemunantú	Villucura/ Lemunantú
	Mean (sd)	*n*	Mean (sd)	*n*	Mean (sd)	*n*	*p* value	*p* value	*p* value
EWD	6.36 (2.81) pv = 0.008*	11	8.95 (3.05) pv = 0.982	6	8.41 (3.09) pv = 0.191	9	0.128	0.159	0.797
LWD_a	4.27 (1.16) pv = 0.358	16	6.23 (2.98) pv = 0.433	3	6.66 (2.81) pv = 0.049*	5	0.243	0.099	0.99
LWP_a	5.46 (1.84) pv = 0.0008*	17	4.64 (1.11) pv = 0.093	6	4.82 (0.81) pv = 0.0002*	10	0.262	0.353	0.935
RA	5.99 (2.54) pv = 0.005*	17	6.01 (0.42) pv = 0.244	7	9.12 (1.19) pv = 0.1165	8	0.112	0.004*	0.0006*
Average including EWD, LWD_a, LWP_a	5.25 (2.06) pv = 0.01*	44	6.68 (3.01) pv = 0.274	15	6.55 (2.76) pv = 0.002*	24	0.153	0.077	0.862

Comparison shown between the variables transition age and first flowering age and between sites

Table 6 Regression parameters and R^2 between first flowering age (independent variable) and the transition between juvenile and mature wood (dependent variable)

	Interecept	Coefficient	R^2	*p* value
Earlywood density (EWD)				
La Trupa	6.61	−0.055	0	0.944
Villucura	6.396	0.319	0.16	0.427
Lemunantú	3.526	0.482	0.08	0.439
The three sites	5.047	0.362	0.16	0.046
Latewood density accumulated (LWD_a)				
La Trupa	3.772	0.119	0.01	0.674
Villucura	10.66	−0.476	0.68	0.176
Lemunantú	−6.684	1.335	0.68	0.086
The three sites	3.44	0.278	0.18	0.04
Latewood proportion accumulated (LWP_a)				
La Trupa	5.931	−0.111	0	0.804
Villucura	4.997	−0.041	0.03	0.753
Lemunantú	2.393	0.235	0.3	0.1
The three sites	5.612	0.072	0.03	0.336
Ring area				
La Trupa	4.189	0.432	0.03	0.483
Villucura	6.444	0.048	0.23	0.28
Lemunantú	4.34	0.49	0.47	0.062
The three sites	5.091	0.256	0.13	0.038

Mean value and standard deviation in brackets

Pv p value for the mean comparison between first flowering age and transition age, *FFA* first flowering age, for comparison information, *n* the number of valid samples used in the segmented regression analysis

"transition age between juvenile and mature wood" was done for the selected variables (EWD, LWD_a, LWP_a, RA) in order to detect if the first flowering event is correlated to changes in wood characteristics. The results of the regression analysis are shown in Table 6. The individual regression for each site between the first flowering age and the transition age for the variables LWP_a, LWD_a, EWD, and RA showed no significant relationship. When combining the data from all the sites for each variable, LWD_a, EWD, and RA show a more postive relationship with first flowering age. So, although a tendency is evident, the significance of the results do not permit the conclusion that there is a real relationship.

When taking an average of the wood variables with a better performance (LWD_a, EWD, and RA) and relating it with the first flowering age (Table 6), at the individual site level there is not a clear relationship; however, when combining the site data the coefficient of determination increases ($R^2 = 0.369$, $p < 0.0001$).

The different approaches to correlating the first flowering age to the transition age between juvenile and mature wood do not point to a consistent variable at the three different sites. EWD (Table 5) transition age is coincident with flowering age in Villucura and Lemunantú, but not in La Trupa. LWD_a transition age is coincident with first flowering age in La Trupa and Villucura, but not in Lemunantú. And LWP_a transition age is coincident with first flowering age only in Villucura.

We should take in account that all parameter measured by x-ray densitometry are, due to the analyzing methodology, averaged parameters for the tissues, which do not allow magnification. Maybe a higher resolution by using e.g., SilviScan analysis with a resolution of 25 µm (almost a double of the resolution used in this research) could raise the possibility to enhance the significance of the analysis. Also, the use of the proposed analysis in trees growing without lateral competence could help to discard the cross effect of stand closure.

Conclusions

As shown by Fernández et al. (2007) for *Pinus radiata* and by Heuret et al. (2006) for other species such as *Pinus pinaster*, the first flowering implies a phase change from the juvenile to the mature stage in the tree and is related to a marked change in the species' stem architecture. Our results, however, do not provide enough evidence to conclude that the first flowering could help to explain the changes in wood density trends in the radial direction.

To be consistent with the results, we suggest adopting the terms corewood and outerwood proposed by Burdon et al. (2004) when refereeing to the radial progression of wood from the pith to the bark, instead of juvenile and mature wood, because the radial variation would be not clearly related to the biological concept of tree maturation.

Future studies could assess the relationship between the architectural development of the crown and the internal wood characteristics as proposed by Tondjo et al. (2014), because as Larson (1969) has shown, wood properties are related to yearly crown development. Studies with *Pinus radiata* involving longer time series might also help elucidate the relationship between wood density and other developmental factors, although, given the short rotation of this species, studies of extended length are not always possible.

Author contribution statement M. Paulina Fernández as permanent professor and researcher of the Pontificia Universidad Católica de Chile contributed as main researcher, designing the research, organizing all practical work, supervising the work along the project, and guiding Bárbara Cornejo among other students in her particular contribution. M. P. Fernández contribute greatly to data analysis and final manuscript writing, Bárbara Cornejo, as undergraduate student of the Pontificia Universidad Católica, contributed in data collection, data analysis and initial manuscript writing, as part of her graduate final work.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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