

Article

Linking Dendrometry and Dendrochronology in the Dominant Azorean Tree *Laurus azorica* (Seub.) Franco

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Abstract: As in many archipelagos, the Azorean primary forest was largely cleared and replaced by secondary forest and grassland, the Azorean tree *Laurus azorica* (Seub.) Franco being one of the dominant trees in the remaining natural forests. Dendrochronological and dendrometric studies in the Azores mainly focused on non-indigenous trees, either used for timber (e.g., *Cryptomeria japonica* D. Don) or considered as invasive (*Pittosporum undulatum* Vent.). Therefore, this study aims to describe the growth ring anatomy of *L. azorica*, and to understand the relationship between dendrometric traits (e.g., trunk diameter at breast height; tree height), and the number of growth rings. Growth ring anatomy was accessed by wood anatomical preparation of microcore samples while tree age estimation was based on growth ring counts in wood cores taken at breast height and at base. A total of 145 trees were sampled, resulting in 262 increment cores, at six representative stands of laurel forest in São Miguel Island (Azores). The wood anatomical analysis confirmed the presence of clear annual ring boundaries, and a high structural similarity towards *Laurus novocanariensis* Rivas Mart., Lousã, Fern.Prieto, E.Díaz, J.C.Costa & C.Aguiar. Age at tree base averaged 33 years, with 60% of the trees between 25 and 50 years old, and only about 15% above 50 years old. This suggests the existence of a secondary forest that is more recent than expected, probably due to human disturbance. The allometric models showed best fit when calculated by stand, suggesting the effect of local environmental conditions on growth rate. Radial growth rate was estimated at 0.68 cm·year^{−1}. Given the known dominance of this species and the threats affecting natural forests, this baseline study will allow a better understanding of forest distribution and dynamics, and support a more effective forest management approach.

Keywords: tree age; tree growth rings; wood anatomy; laurel forest; primary forest; forest management; Azores

1. Introduction

Knowledge on tree growth rate and age–size relationships is the basis for understanding tree population dynamics and evaluating changes in both natural and managed forests, being crucial for sustainable forest restoration [1–4]. Dendrochronology techniques are commonly used in forest management to determine woody plant growth rates in seasonal climates [2,5,6]. Accurate tree age estimates are critical to a range of forest and ecological studies [7], for which a standard identification of the earlywood–latewood boundary is necessary [8].

The distribution of natural forests in the Azores Archipelago evolved in unique conditions, due to a pronounced isolation, relatively homogeneous climatic conditions and a limited number of native woody species [9–11]. According to Elias et al. [12], the best preserved and largest remnants of dominant woody natural vegetation in the Azores are located on São Miguel, Pico, Terceira, and Flores islands. Four altitudinal belts have been recognized [12]: Coastal (*Erica-Morella* coastal woodlands); lowland (*Picconia-Morella* lowland forests); submontane/montane (*Laurus* submontane forests, *Juniperus-Ilex* montane forests and *Juniperus* montane woodlands); and altimontane/alpine (*Calluna-Juniperus* altimontane scrublands, *Calluna-Erica* subalpine scrubland and *Calluna* alpine scrubland). Changes in land use have drastically reduced the area occupied by natural forest, and new threats include climate change, which may affect the distribution of dominant species of bryophytes and vascular plants, leading to changes in the structure and distribution of plant communities [13,14]. Moreover, non-indigenous invasive species such as *Pittosporum undulatum* Vent., *Hedychium gardnerianum* Sheppard ex Ker-Gawl., *Leycesteria formosa* Wall., *Clethra arborea* Aiton and tree ferns (*Sphaeropteris cooperi* (F.Muell.) R.M.Tryon; *Sphaeropteris medullaris* Bernh and *Dicksonia antarctica* Labill.), are currently threatening the conservation of endemic Azorean species and natural forests [2–4,15–19].

Dendrochronological studies performed in the Azores mainly addressed exotic species, such as *Pittosporum undulatum* following a possible strategy of biomass valorization [2,4], and *Pinus pinaster* Aiton in order to determine the limiting factors for tree growth in the Azores [20]. Therefore, a lack of research dedicated to other native or endemic trees is evident. Among Azorean endemic trees, *Laurus azorica* (Seub.) Franco is considered as a relic of the Tertiary Mediterranean flora [21], being a characteristic and dominant element in submontane forests and woodlands which are reduced to only 5% of their original cover [9,22]. There are different visions regarding *Laurus* taxonomy. If for some authors *L. azorica* is distinguishable from *Laurus Nobilis* L. and *L. novocanariensis* Rivas Mart., Lousã, Fern.Prieto, E.Días, J.C.Costa & C.Aguiar [23], for others phylogeographic evidence supports the existence of only one *Laurus* taxon [23,24]. A potential threat to *L. azorica* is the possible hybridisation with *Laurus nobilis* as intermediate forms are sometimes found. However, species delimitation within the genus *Laurus* is highly controversial and hybrid occurrence has not been confirmed [25,26].

A few studies have focused on the Lauraceae family in some of the Macaronesian islands. Câmara [27] analyzed forest structure and estimated tree age at breast height at two stands of laurel forest located in a protected area, and Rego et al. [28], with a focus on the ecological characterization of unprotected areas in São Miguel Island, also analyzed growth rings at a laurel forest invaded by *Clethra arborea*. Other studies focused on *Laurus novocanariensis* growth rings, namely in Tenerife laurel forest, where wood structure was characterized, and it was shown that the whole cross section of its diffuse porous stems can be functional, even when many individual vessels are embolized [21,29]. Recently, the potential of the neotropical lauraceae in dendrochronology was revised by Reis-Avila and Oliveira [30], showing that growth rings generally include a boundary marked by an abrupt transition between thick-walled and radially flattened fibers in the latewood of a ring, and thin-walled earlywood fibers of the consecutive ring; or, otherwise, a marginal parenchyma band delimiting tree-rings. The same authors concluded that the Lauraceae have distinct, synchronic and climate-sensitive tree-rings, and recommended their use in dendrochronological studies.

This work focuses on tree ring analysis of *Laurus azorica*, an endemic and dominant tree in the Laurel forests on the Azores Islands. *Laurus azorica* growth rings have been reported to be easily identified, due to the existence of a sharp transition between latewood and earlywood, that is evident macroscopically [27].

In this context, the main goals for this work were a characterization of *L. azorica* growth ring anatomy, in order to confirm the descriptions given for other Lauraceae, and the establishment of age–size relationships and growth rate estimates, using models that relate dendrometric traits with tree age.

2. Materials and Methods

2.1. Study Area

The Azores archipelago, situated in the north Atlantic Ocean ($36^{\circ}55'–39^{\circ}43' \text{ N}$ and $25^{\circ}00'–31^{\circ}15' \text{ W}$), is composed of three island groups: Eastern (Santa Maria and São Miguel islands), Central (Terceira, Pico, Faial, São Jorge and Graciosa islands) and Western (Flores and Corvo islands). This study was carried out in São Miguel (Figure 1), the largest island, with 745 km^2 , a coastline length with 213 km and the highest peak at 1105 m [31].

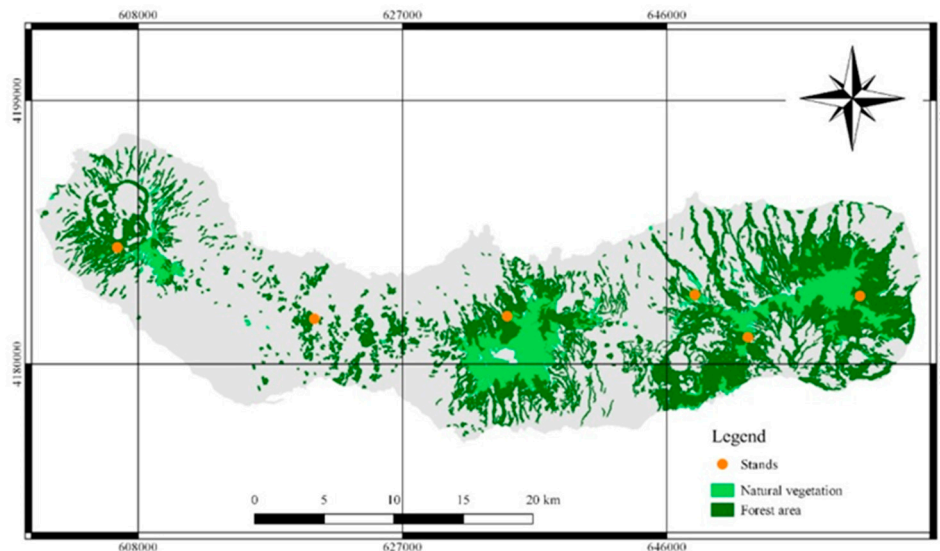


Figure 1. Distribution of the six selected stands of natural vegetation in São Miguel Island. From left to right: Sete Cidades; Pinhal da Paz; Lombadas; Achada das Furnas; Povoação; Tronqueira. In the map, natural vegetation is not entirely forest.

While there is a variation in the climatic conditions from one extreme of the archipelago to the other, and a significant altitudinal variation within each island, the Azorean climate might be classified as wet mesothermic with oceanic traits, low thermal amplitude and a mean annual temperature of 17°C at sea level. Rainfall is generally high and rainfall ranges from 1500 to more than 3000 mm per year, increasing with altitude and from east to west [32]. Wind is also considered as a relevant factor in the distribution of forests in the case of the Azores, being a limiting factor, particularly at very exposed locations, both at low and high elevation [9]. Of the 2350 km^2 that correspond to the total land area in the Azores, about 43% is now dominated by pastures, planted or exotic forests (22%), with natural vegetation occupying about 13% [33,34]. Table 1 includes a climatic characterization and the type of soil at each of the study sites.

Table 1. Characterization of the sampling stands of *Laurus azorica* (Seub.) Franco trees in São Miguel Island. Elevation (E, m), number of trees (N), number of cores at breast or at the base (CB, CA), Annual Mean Temperature (MT, °C), Maximum Temperature of the Warmest Month (TW, °C), Minimum Temperature of the Coldest Month (TC, °C), Annual Temperature Range (TR, °C), Annual Precipitation (AP, mm), Precipitation of the Wettest Quarter (PW, mm), Precipitation of the Driest Quarter (PD, mm). Soil Types: Ins—Insaturated; Ferr—Ferruginous; Shal Aloph—Shallow Alophanic soil; Reg—Regosol. Climatic data from Karger et al. [35] and Soil data from Ricardo et al. [36]. Native and exotic species as mentioned in the text.

Stands	Code	E (m)	Sampling			Physical Description								Main Species		Other Information
			N	CB	CA	MT	TW	TC	TR	AP	PW	PD	Soil Type	Native	Exotic	Protection
Lombadas	LO	569	25	25	0	14	22	7	15	1684	641	167	Ins And	<i>L. azorica</i> , <i>I. azorica</i> , <i>M. faya</i>	<i>P. undulatum</i> , <i>H. gardnerianum</i>	Habitat/Species Management Area
Achada das Furnas	AF	600	20	20	0	13	22	7	15	2108	789	221	Shal Aloph Ins/Ferr And	<i>L. azorica</i> , <i>I. azorica</i>	<i>C. arborea</i> , <i>P. undulatum</i> , <i>H. gardnerianum</i>	Unprotected
Pinhal da Paz	PP	322	25	31	29	15	23	8	15	1294	494	133	Shal Aloph Reg	<i>L. azorica</i> , <i>M. faya</i>	<i>P. undulatum</i> , <i>H. gardnerianum</i>	Recreational Forest Reserve
Sete Cidades	SC	599	25	29	28	13	22	7	15	1850	698	195	Ins And	<i>L. azorica</i> , <i>I. azorica</i> , <i>M. faya</i>	<i>C. japonica</i> , <i>P. undulatum</i> , <i>H. gardnerianum</i>	Protected Landscape
Tonqueira	TR	629	25	25	25	13	22	6	15	2092	816	199	Shal Aloph Ins/Ferr And	<i>L. azorica</i> , <i>I. azorica</i> , <i>M. faya</i> , <i>J. brevifolia</i>	<i>C. arborea</i> , <i>H. gardnerianum</i>	Habitat/Species Management Area
Povoação	PO	541	25	25	25	14	23	7	15	1557	596	152	Shal Aloph Ins/Ferr And	<i>L. azorica</i> <i>I. azorica</i>	<i>H. gardnerianum</i>	Unprotected
Total			145	155	107											

2.2. Target Species

The Lauraceae family is classified within the order Laurales, being widely distributed in tropical and subtropical climates, including approximately 45 genera and 2850 species [37,38]. Molecular phylogenetic studies clearly show the monophyly of the family [39] and while intergeneric relationships in Lauraceae are becoming established [40–42], generic circumscriptions are plagued by polyphyly [41]. The family has an important ecological role, namely in the Atlantic Forest with many representatives contributing to the species richness of this biome [43]. In Macaronesia it is also one of the plant families structuring the laurel forest in the Azores, Madeira, and in the Canary Islands [44].

The Laureacea family presents a high number of species with a diversity of uses, from cooking, to paper factoring, timber works, constructions, chemical industries and folk medicine. This family has a considerable economic value with a wide range of applications, which has led to an increasing exploitation over the years, making several species vulnerable or endangered [45]. A variety of wood types from this family are used for carpentry and paper factoring while aromatic and oil producing species are mostly used as raw-materials in industry, the range of applications being very wide, and using several plant parts (i.e., timber, leaves, fruits and seeds) [46].

The conservation status of *L. azorica* (Seub.) Franco is currently Least Concern according to the IUCN (International Union for Conservation of Nature) criteria, the population numbers are large and the trend is now stable, and although the species has a restricted area of occupancy, it is not declining [19,47]. *Laurus azorica* is one of the major components of the laurel forest but it is also present in other types of forest, namely in four zonal vegetation types [12]: *Picconia-Morella* lowland forests (100–300 m), *Laurus* submontane forests (300–600 m), *Juniperus-Ilex* montane forests (600–900 m), and less frequently in *Juniperus* montane woodlands (700–1000 m). It is also found in lava flows, margins of cultivated land, coastal scrubland, mountain scrubland and forested peat bogs. It is a dioecious tree that can reach heights from 5 to 20 m, although with an average height around 10 m [18]. It is distinguishable by an upright trunk branching at a short distance from the base, alternate, hairy young leaves and stems, the old leaves becoming glabrous.

2.3. Stand Characterization

The studied stands (Figure 1) include *Laurus azorica* (Seub.) Franco trees of different size classes, growing at remaining laurel forests, in São Miguel Island. In order to maximize the representativeness of the study we distributed all six sampling plots through the typical laurel forests of the island (Figure 1, Table 1), mostly inside protected areas, except Achada das Furnas and Povoação.

2.4. Field Sampling

The study was carried out between March and May 2018 and includes a total of trees of different size classes, growing at remaining laurel forests, in São Miguel Island (Figure 1). A total of 145 *L. azorica* (Seub.) Franco trees were sampled (Table 1). At each site, trees were selected following a stratified random sampling scheme, according to the following diameter classes in cm: (<5, 5–10, 10–15, 15–20, >20 cm). However, the final sample was dependent on the individuals that were available at each stand.

For each tree, the following dendrometric parameters were taken [3,5,8]: Diameter at Breast Height (at 130 cm), Diameter at Base (20 cm) and Maximum Tree Height. Diameters were measured with a diameter measuring tape, Friedrich Richter Messwerkzeuge GmbH & Co. KG, Speichersdorf, Germany, and total tree height using Vertex IV 360 and Transponder T3, Haglöf Sweden AB, Långsele, Sweden.

Wood samples were collected at breast height [48] for tree age determination. Whenever possible, samples were also taken at the base (about 20 cm above soil), but this was dependent on tree and terrain features. In those cases, trunk diameter was also registered at that level. Increment cores (5 mm) were collected using a Pressler borer Haglöf Sweden AB, Långsele, Sweden while microcores (2 mm) were sampled using a Trephor University of Padua, Costruzioni Meccaniche Carabin C., Valle di Cadore, Belluno, Padova, Italy [49]. For the latter, a total of 14 individual trees were sampled to

allow for possible wood anatomical variation. Wood microcores were immediately immersed in a fixative solution of Formalin, Acetic acid and Alcohol (FAA) CHEM-LAB, Zedelgem, Belgium and transported in Eppendorf®, Deltalab S.L., Barcelona, Spain, tubes to the lab.

2.5. Wood Sample Preparation for Macroscopic Analysis

Samples were allowed to air-dry in wood supports for at least one week [2], and were mounted on wood supports with glue. Sample surface preparation was carried out by polishing with sandpaper of increasing gradation [50]—100, 180, 220, 320, 400 and 600.

The number of tree rings was estimated through counting under a stereomicroscope (Leica-Zoom 2000, Model NO.Z30 V, 240 vac, maximum magnification of 100×, Leica Microsystems Inc., Buffalo Grove, IL, USA). Images were taken using Stemi 2000-C with built-in 6 V, 10 W, 230 vac, and Zeiss ZEN software. Fisher Bioblock Scientific, Cedex, France; Carl-Zeiss-Straße, Oberkochen, Germany.

2.6. Wood Sample Preparation for Microscopic Analysis

Wood microcores were processed following a standard protocol [51]. Progressive dehydration with alcohol, clearing with D-limonene Across Organic™ Thermo Fisher Scientific and paraffin infiltration were performed in an automatic tissue processor. After that, microcore samples were paraffin blocked, and trimmed for section cutting. In a rotary microtome, sections with a thickness of 8 µm were prepared, transferred to Glycerin-Albumin Sigma-Aldrich Corporation, St. Louis, MO, USA coated slides, and stored at 37 °C overnight. Two sets of histological slides were prepared for two distinct staining methods. Paraffin was removed using D-limonene and rehydration was performed with alcohol solutions CHEM-LAB, Zedelgem, Belgium with decreasing concentration. The first set of slides was stained with Toluidine Blue Sigma-Aldrich Corporation, St. Louis, MO, USA and the second set was stained with a water solution of 100 mL demineralized water, Astrablue (150 mg), Santa Cruz Biotechnology, Dallas, TX, USA safranin (40 mg) CHEM-LAB, Zedelgem, Belgium and 2 mL acetic acid CHEM-LAB, Zedelgem, Belgium (adapted from [52]). Sections were then dehydrated and mounted in DPX (Distrene Plasticizer Xylendene The samples were observed under a light microscope LEICA DM1000 Leica Microsystems Inc., Buffalo Grove, IL, USA, associated to CoolSnap-Pro, Media Cybernetics and images taken using Image Pro-Plus 5.0. software.

2.7. Statistical Analyses

2.7.1. Dendrometric Traits

Descriptive statistics and graphical comparisons were produced for tree diameter, height and age (based on the number of rings). A preliminary analysis revealed the existence of outliers when analyzing data per site. However, when grouping the trees by size class (<10, 10–20, >20 cm), irrespective of sampling site, no outliers were detected. Boxplot and bar chart representations were obtained for the above listed parameters, according to the site, the type of sample (tree base or breast height) or the diameter class. We used Kruskal-Wallis (*K-W*) test, followed by a multiple comparison test, (i) to compare sites for tree height, trunk diameter, and tree age, and (ii) to compare diameter classes for tree height and tree age. We used Mann-Whitney (*M-W*) test to compare samples at base of the tree or at breast height, both for trunk diameter and tree age. Data analysis was made using IBM SPSS version 24.

2.7.2. Annual Increment

From a total of 101 base/breast sample pairs, the average number of years to attain breast height and radial growth rate were calculated. To estimate radial growth, Periodic Annual Increment (PAI) was used:

$$PAI = \frac{D_1 - D_2}{Age_1 - Age_2}$$

where D_1 is the diameter at base (20 cm above ground), D_2 is the diameter at breast height (130 cm above ground), Age_1 is the number of years at base, and Age_2 is the number of years at breast height for each tree [2,53].

2.7.3. Relationship between Tree Age and Dendrometric Traits

Models relating tree age with other dendrometric variables (e.g., diameter at breast height, tree height, crown diameter, crown volume, leaf area) are available for different species and different geographic areas [54,55], including the Azores [2]. In our study, allometric equations were used to relate tree age, as derived by tree ring counts, with diameter at breast height, tree height and basal area.

We followed the general procedures for the calculation of allometric equations that have been previously described [2–4], including data logarithmization in order to ensure homocedasticity. We evaluated candidate models using corrected Akaike's Information Criterion (AIC), square root mean error and mean relative error [2–4,56]. Statistical analyses were performed using R software, version 3.2.3 [57].

3. Results

3.1. Tree Ring Structure

We found clear growth ring patterns in *L. azorica* (Seub.) Franco increment cores and wood samples taken in São Miguel Island (Figure 2). Some of the samples showed unclear rings transitions, presenting some different coloration or other anomaly, detected in field or laboratory work, which made tree ring counting difficult or impossible (Figure 2B). Therefore, those samples were not considered in the study. The diffuse-porous structure of the wood was clearly visible in the macroscopic preparations (Figure 2A,D).

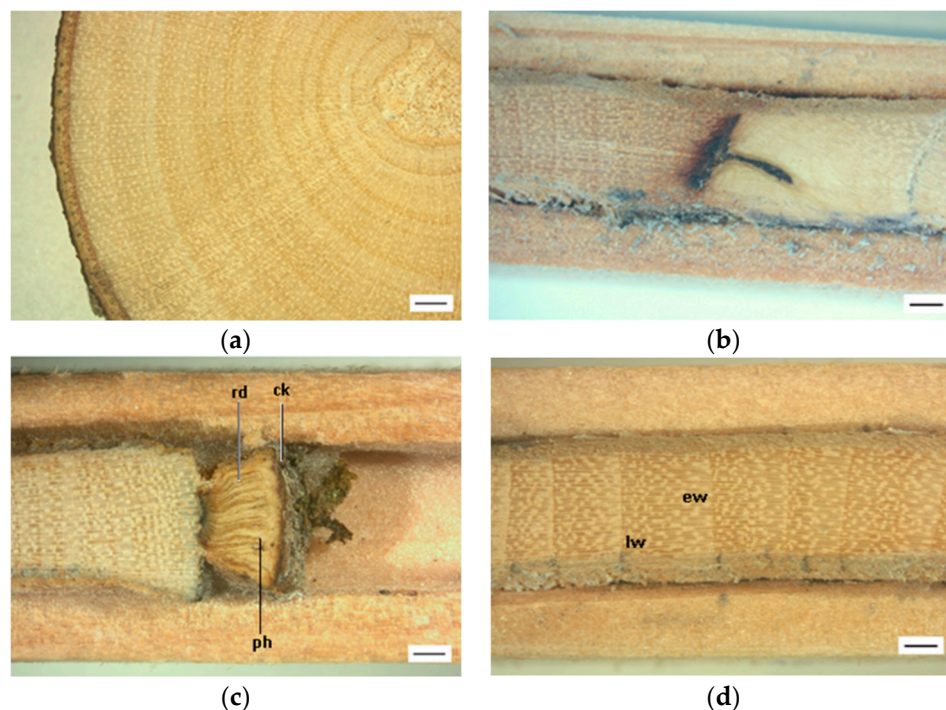


Figure 2. Macroscopic view of *L. azorica* (Seub.) Francostem disc (a) and of increment cores (b,c,d) taken in São Miguel Island. (a) Pith, distinct tree rings with latewood (lw) and earlywood (ew), and bark. (b) Example of some of the anomalies found in increment cores. (c) Younger tree rings close to cambium, phloem (lighter, ph), ray dispersion (darker, rd) and cork (ck). (d) Tree rings with distinct ring boundaries, and different coloration of early (ew) and latewood (lw), with vessels equally distributed along the ring. Scale bar 1 mm.

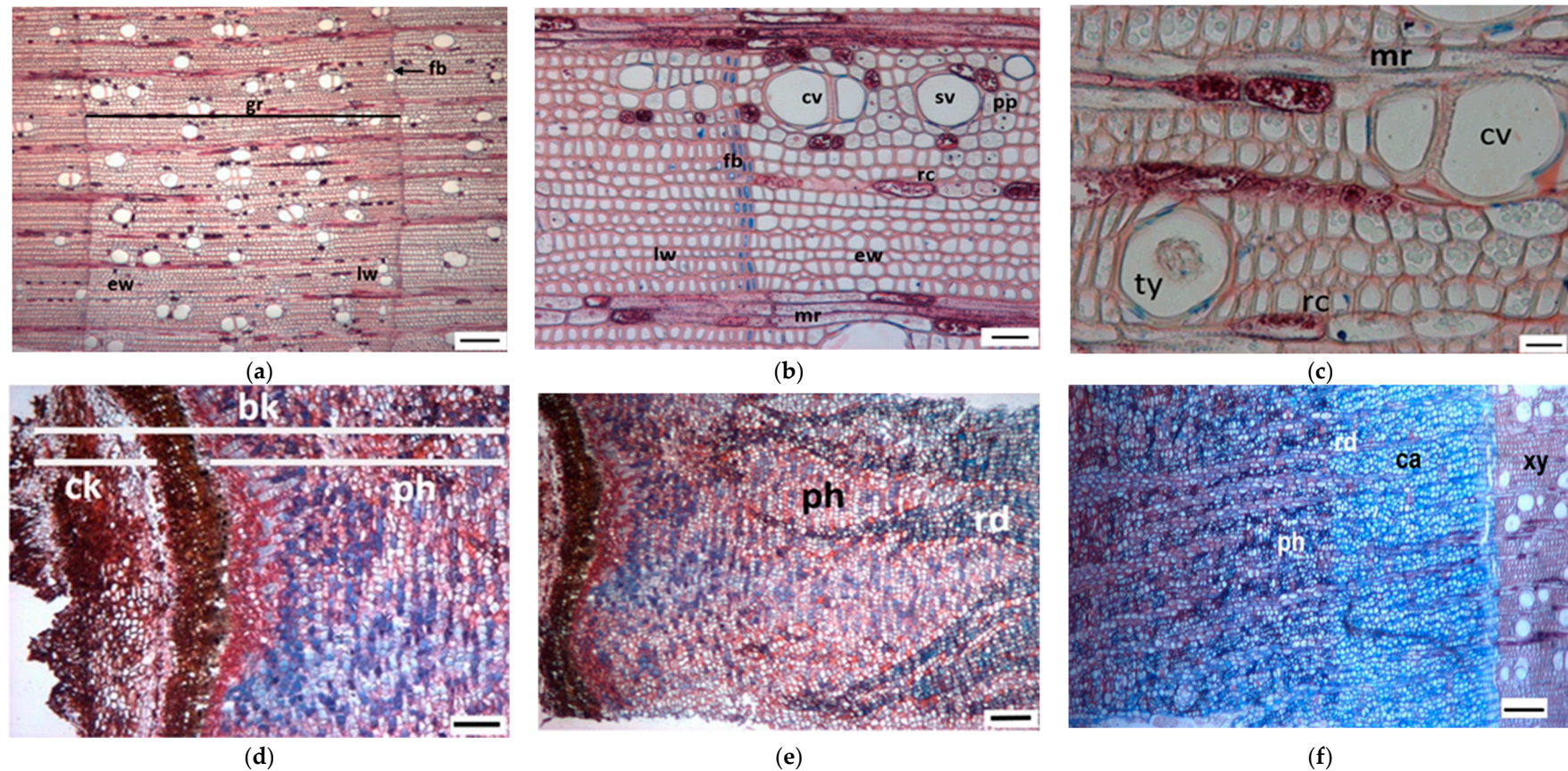


Figure 3. Cross-section view of *L. azorica* (Seub.) Franco tree rings under light microscope: First row: (a) General view of growth rings (gr) with thick-wall fibrous boundaries (fb), earlywood (ew) and latewood (lw), xylem rays and vessels with equal size and distribution along annual ring (scale bar 200 μ m); (b) Growth ring boundary (fb), paratracheal parenchyma cells around vessels (pp), multiseriate rays (mr), solitary vessels (sv), clustered vessels (cv) and reddish cells (rc) (scale bar 50 μ m); and (c) Unlignified tylosis (ty) (scale bar 25 μ m). Second row: (d,e), cross section view of bark with phloem wood: (d) Simple construction of phloem, with phloem elements difficult to distinguish but differentiated from ray dilatation (scale bar 150 μ m); (e) Bark layers. rd—ray dilatation; ph—phloem; ck—cork layers; bk—bark (scale bar 200 μ m); (f) Cross-section of cambial zone from *L. azorica*, cambium is crossed by xylem rays that dilate in phloem to the bark. xy—xylem; ca—cambium; ph—phloem; rd—ray dilatation (scale bar 200 μ m).

Laurus azorica xylem showed a diffuse-porous structure, with solitary or clustered vessels, in radial oriented groups, randomly distributed and with no relationship with the ring boundaries (Figure 3A). Ring boundaries, the transition from latewood to earlywood, are marked by thick-walled, radially flattened fibers (Figure 3A,B). Reddish cells appear randomly distributed across tree rings due to dye retention (Figure 3A,B). Xylem vessels appear solitary or in clusters. Series of radial rays produced in cambium are arranged in parallel and elongated, connecting the medullar and cortical areas (Figure 3B). Xylem fibers in latewood tend to show a relatively smaller cellular lumen and thicker cell wall, as compared with earlywood fibers (Figure 3B). Small, thin-walled, unlignified tylosis were also observed (Figure 3C).

The transition between xylem, vascular cambium and phloem is evident in cross-section (Figure 3, second row). Cambium is crossed by xylem rays that dilate into phloem and to the bark. An outermost cork layer is also evident (Figure 3, second row).

In longitudinal view, plates were observed between consecutive xylem vessels, intervessel pits were visible along the xylem vessels, and multiseriate rays were common (Figure 4).

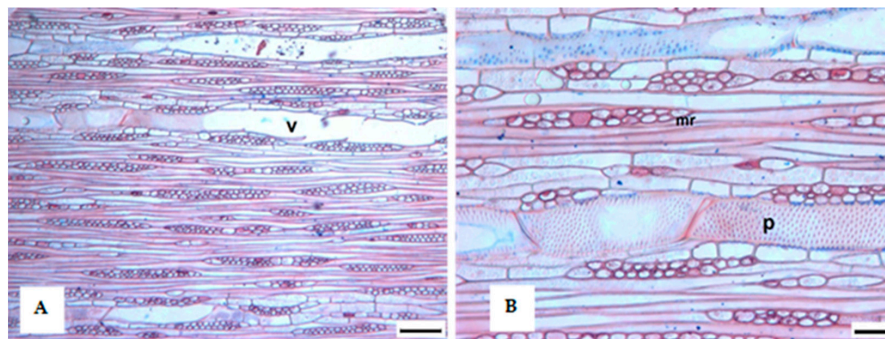


Figure 4. Tangential section from *L. azorica* (Seub.) Franco secondary xylem. (A) General view including xylem vessels (v) and multiseriate rays (scale bar 150 μ m). (B) A more detailed view showing a closer perspective of multiseriate rays (mr) and the presence of intervessel pits (p) (scale bar 50 μ m).

3.2. Dendrometric Traits

From the total of 256 samples collected at base and breast height a high amplitude of values was found for all the analyzed parameters (Figure 5). Trunk analysis has shown that the diameter of the mostly sampled trees ranges between 5 and 25 cm (Figure 5A).

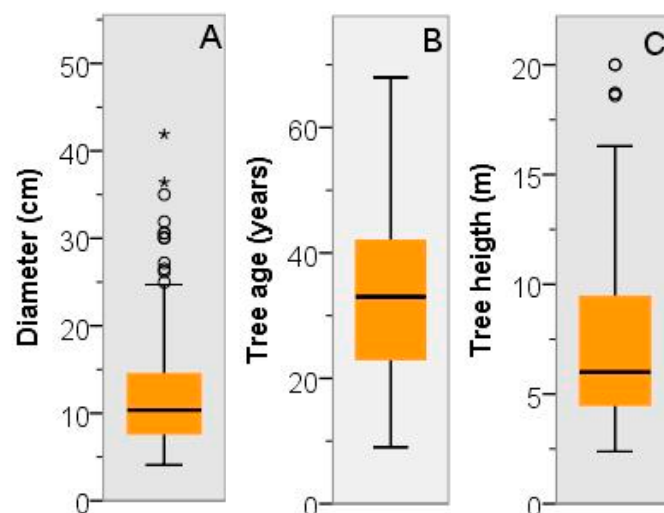


Figure 5. The estimated distribution of trunk diameter (A), tree age (B), and maximum height (C), of the 256 *L. azorica* (Seub.) Franco samples measured in São Miguel Island.

Estimated tree ages showed high variability, ranging from about 10 to almost 70 years old. For all the collected samples the central quartiles of tree age distribution concentrated between 23 and 42 years (Figure 5B). Tree height ranged from about 3 up to 20 m (Figure 5C).

Differences between stands were found in the distribution and variability of dendrometric data (i.e., tree age, maximum tree height, diameter at breast height, diameter at base). Tree height ranged from about 7 m in Achada das Furnas up to 20 m in Povoação. For most stands, tree height was concentrated between 4 and 9 m, except for Povoação, where the central quartiles ranged from about 9 to 14 m (Figure 6A). In general, diameter at the base was slightly larger than diameter at breast height, with the former ranging from 5.2 to 41.9 cm and the latter from 4.1 to 36.4 cm (Figure 6B). Povoação showed the largest trunk diameters, differing from the remaining sites with values concentrated below 15 cm (Figure 6B). We found a remarkable variability in tree age, varying between 12 and 68 years at base, and between 9 and 62 years at breast height (Figure 6C). Minimum age was found at Pinhal da Paz and maximum age at Povoação. In addition, a large variation was found at each stand. Povoação, Sete Cidades and Tronqueira stands were those with tree rings more concentrated at older ages (Figure 6C).

Three diameter classes were created (<10, 10–20, >20 cm), to better understand the relationship between trunk diameter, tree height and tree age. Only 24 individuals represented the 3rd diameter class, while 121 and 111 represented the 1st class and 2nd classes, respectively. As diameter class increased also tree height increased (Figure 6D). In the 1st, 2nd and 3rd classes, tree height was concentrated around median values of 5, below 7.5 and between 12.5 and 15 m, respectively. As expected, an increase in tree age with diameter was found (Figure 6E). Considerable overlap was found between tree age estimates obtained at the base and at breast height for the 1st and 2nd diameter classes, but not so much for the 3rd diameter class (Figure 6E).

Differences in age structure between stands were clear when representing tree age distribution for the three diameter classes (Figure 7). Most populations were concentrated in the 1st and 2nd diameter classes, while Povoação was concentrated in the 2nd and 3rd classes. Again, a considerable overlap of the distribution of the estimated ages in the 1st and 2nd diameter classes was found, namely for Lombadas and Sete Cidades, and also in the 2nd and 3rd classes for Povoação (Figure 7).

There were significant differences between sites for tree height (K -W test, $\chi^2 = 109.027$, $df = 5$, $p < 0.001$) trunk diameter (K -W test, $\chi^2 = 88.102$, $df = 5$, $p < 0.001$), and tree age (K -W test, $\chi^2 = 95.774$, $df = 5$, $p < 0.001$), forming three homogeneous groups (results of a multiple comparison test, with $p > 0.05$ within each group): (i) Povoação; (ii) Sete Cidades, Lombadas and Pinhal da Paz; and (iii) Achada das Furnas and Tronqueira. As expected, there were significant differences between samples at the base of the tree or at breast height, both for trunk diameter (M -W test, $U = 5574.0$, $p < 0.001$, mean rank base = 150.90, mean rank breast = 113.17) and tree age (M -W test, $U = 5579.5$, $p < 0.001$, mean rank base = 148.93, mean rank breast = 114.52). As expected, there were significant differences between the three defined diameter classes both for tree height (K -W test, $\chi^2 = 78.147$, $df = 2$, $p < 0.001$) and tree age (K -W test, $\chi^2 = 64.779$, $df = 2$, $p < 0.001$), with significant differences among the three classes for both parameters (all p values from the multiple comparison tests < 0.05).

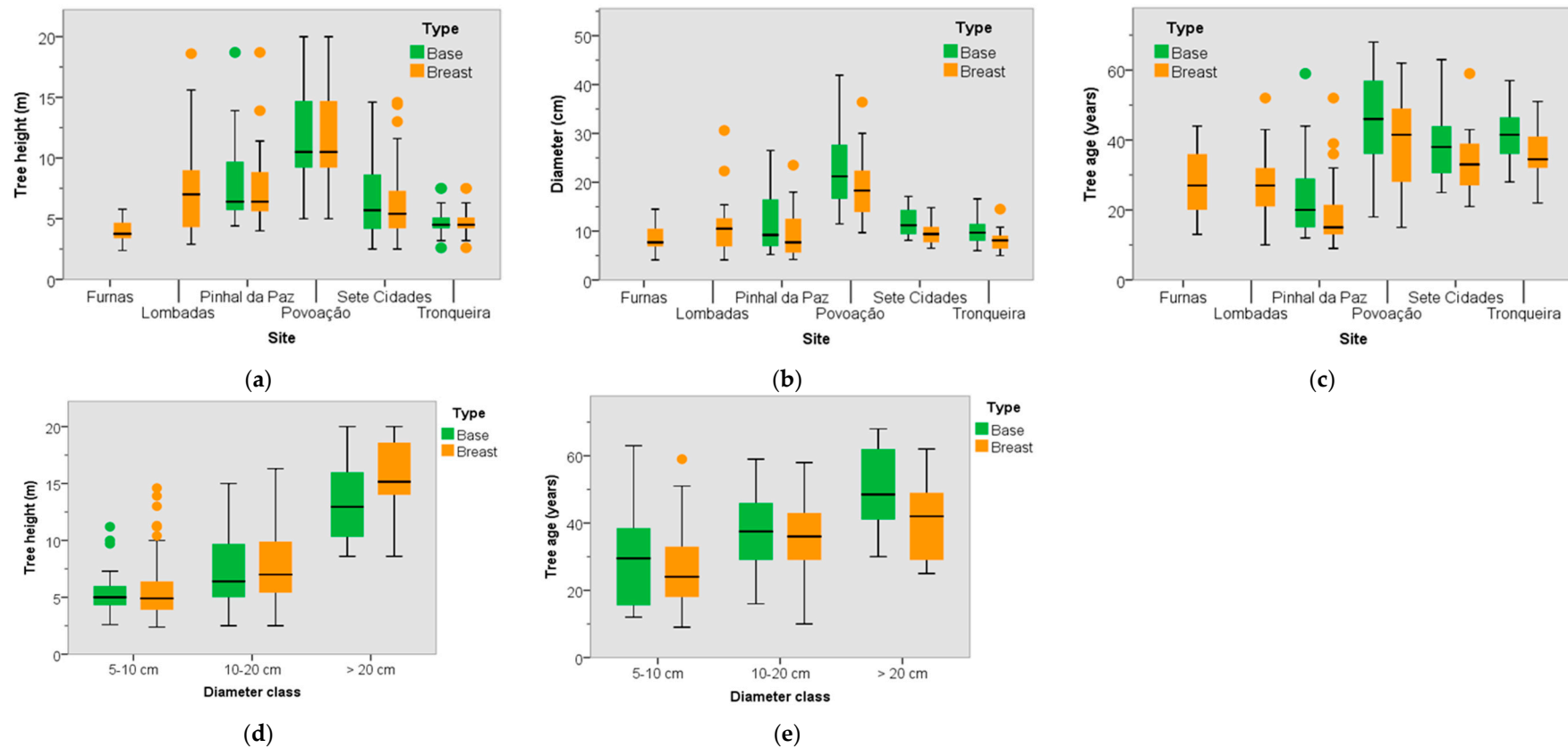


Figure 6. First row: Distribution of tree height (a), trunk diameter (b) and tree age (c) for each sampled stand in São Miguel Island. Second Row: Distribution of tree height (d) and estimated tree ages (e) by diameter class (<10, 10–20, >20 cm). From a total of 256 samples of *L. azorica* (Seub.) Franco collected in São Miguel Island, collected at breast height (130 cm above substrate) or at tree base (20 cm above substrate). In the boxplots, circles represent outliers.

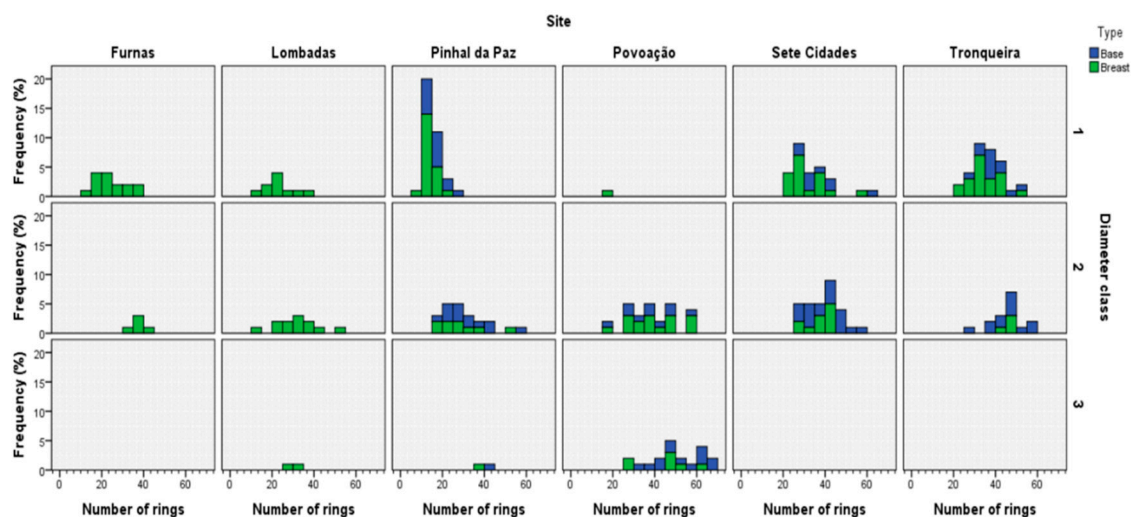


Figure 7. Distribution of the cambial age of *L. azorica* (Seub.) Franco trees obtained at six sites in São Miguel Island. The chart allows to appreciate the age and size structure of the different populations. Diameter classes (cm): (1, <10; 2, 10–20; 3, >20 cm).

3.3. Tree Age and Dendrometric Traits

Scatter plots show a correlation between tree age and trunk diameter, although with some variation between sites (Figure 8). The multiple linear regression models showed a reduced fit when we tested for the entire sample, both for diameter at breast height and at the base (a selection of models is shown in Table 2 and Figure 9), both with or without log transformation, with a significant number of observations far from the regression predicted value, and a high residual error (see Table S1 for the full list of tested models). Not all tested models showed significant regression coefficients ($p < 0.05$).

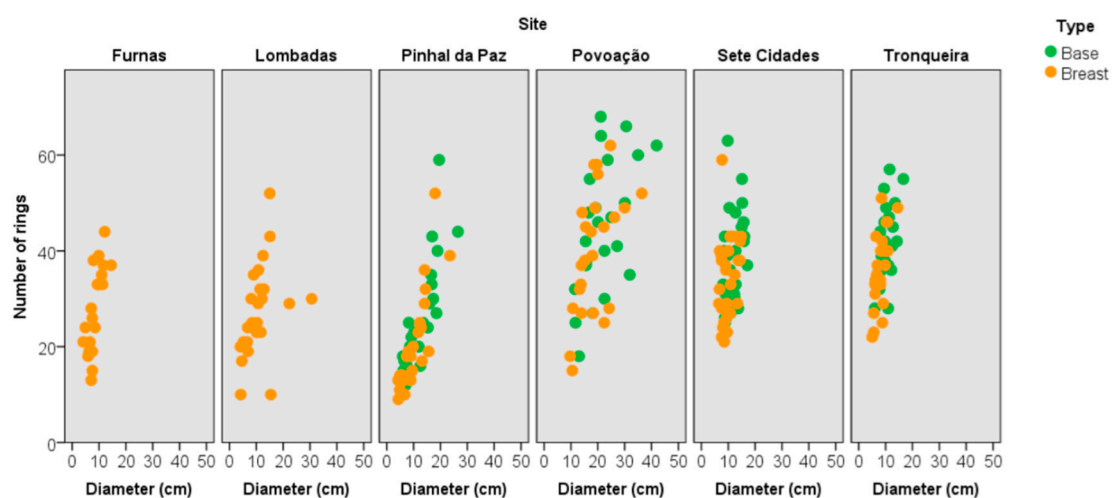


Figure 8. Scatter plots representing the relationship between estimated tree age and trunk diameter at base and at breast height, for six stands of *L. azorica* (Seub.) Franco in São Miguel Island.

Separate regressions were calculated for each stand (a selection of models is shown in Table 2 and Figure 9), showing an increase in the R^2 value, particularly for Pinhal da Paz. Dendrometric parameters also showed a better predictive ability to estimate tree age for several stands when outliers were removed from the data set, namely for Lombadas, Achada das Furnas and Tronqueira (a selection of models is shown in Table 2 and Figure 9). The full set of tested models for each stand, and with or without outliers are shown in Tables S2–S7.

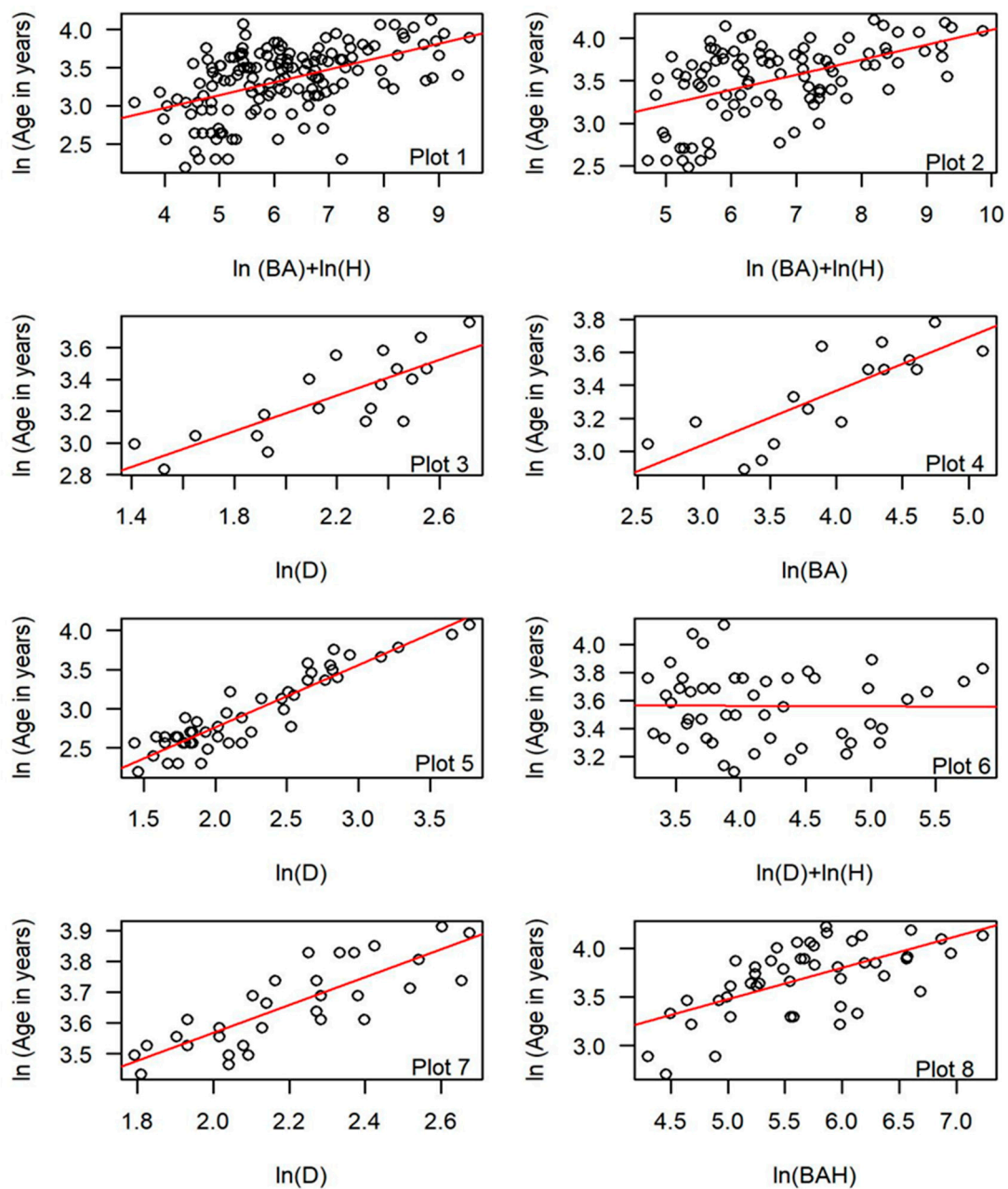


Figure 9. Scatter plots of the allometric equations used to predict *L. azorica* (Seub.) Franco age from dendrometric traits (BA, Basal Area; D, Diameter at Breast Height; H, Maximum Tree Height) Comparison of models for all the available samples at breast height, (**plot 1**, as defined in Table 2), at tree base (**plot 2**, as defined in Table 2) and for each stand (**plots 3–8**, as defined in Table 2). This is a selection of the best models. See Table 2 and Tables S1–S7 for the full set of tested models.

Table 2. Selected allometric equations relating tree age and dendrometric parameters tested for 147 *L. azorica* (Seub.) Franco trees sampled at breast height (BH), for 97 *L. azorica* trees sampled at base (Base), and for each stand of *L. azorica* in São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equation (see Tables S1–S7); Stand code (see Table 1). N (number of samples). Plot # (see Figure 9). Adjusted determination coefficient (Adj R²); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE); Correction Factor (CF); Diameter (D); Tree Height (H); Basal Area (BA). This is a selection of the best models. See Tables S1–S7 for the full set of tested models.

Stand	BH/Base	N	Plot #	Regression Model	Adj R ²	AIC	RMSE	MRE	CF ##
All	BH	147		$\ln(\text{Age}) = a + b_1 \ln(D) + \varepsilon$ *	0.34	118.60	10.01	−0.07	1.07
All	BH	147		$\ln(\text{Age}) = a + b_1 \ln(D) + b_2 \ln(H) + \varepsilon$ ***	0.38	110.29	10.05	−0.06	1.06
All	BH	147	1	$\ln(\text{Age}) = a + b_1 \ln(BA) + b_2 \ln(H) + \varepsilon$ **	0.39	106.87	9.75	−0.06	1.06
				Null Model		178.18			
All	Base	97		$\ln(\text{Age}) = a + b_1 \ln(D) + \varepsilon$ *	0.36	74.47	11.60	−0.06	1.06
All	Base	97		$\ln(\text{Age}) = a + b_1 \ln(D) + b_2 \ln(H) + \varepsilon$ ***	0.41	67.46	11.70	−0.06	1.06
All	Base	97	2	$\ln(\text{Age}) = a + b_1 \ln(BA) + b_2 \ln(H) + \varepsilon$ **	0.44	62.31	11.12	−0.05	1.05
				Null Model		116.37			
LO	BH/Base	19	3	$\ln(\text{Age}) = a + b_1 \ln(D) + \varepsilon$ *	0.61	−11.26	4.41	−0.01	1.01
AF	BH/Base	16	4	$\ln(\text{Age}) = a + b_1 \ln(BA) + \varepsilon$ ***	0.62	−6.82	4.63	−0.01	1.01
PP	BH/Base	49	5	$\ln(\text{Age}) = a + b_1 \ln(D) + \varepsilon$ *	0.86	−22.87	3.92	−0.02	1.02
SC	BH/Base	49	6	$\ln(\text{Age}) = a + b_1 \ln(D) + b_2 \ln(H) + \varepsilon$ ***	0.13	1.76	8.60	−0.03	1.03
TR	BH/Base	33	7	$\ln(\text{Age}) = a + b_1 \ln(D) + \varepsilon$ *	0.66	−69.53	3.12	−0.03	1.00
PO	BH/Base	47	8	$\ln(\text{Age}) = a + b_1 \ln(BAH) + \varepsilon$ ***	0.40	17.78	10.77	−0.04	1.04

Note: # All Equations included significant regression coefficients ($p < 0.05$); All equations before application of Correction Factor—See Supplementary Material for BH and base samples (Table S1) and for each stand (Tables S2–S7). Models indicated in bold were tested after removing the outliers. ## Correction factor to remove the bias of regression estimates for logarithmic transformed data.

3.4. Estimated Annual Increment

For 101 pairs of base and breast height cores collected at Pinhal da Paz, Povoação, Sete Cidades and Tronqueira, estimated tree age differences between breast and base height were concentrated between 3 and 7 years, with an estimated average of 5 years to reach breast height. Thus, annual radial growth rate was estimated by periodic annual increment, with an average of 0.68 cm·year^{−1}, and with a concentration of values between 0.300 and 0.833 cm·year^{−1} (Table 3).

Table 3. Descriptive statistics estimated for periodic annual increment and tree age difference, from 101 pairs of *L. azorica* (Seub.) Franco wood cores collected at tree base and at breast height.

Growth Parameters	Mean	Standard Deviation	Standard Error	Minimum	Maximum
PAI (cm·year ^{−1})	0.679	0.659	0.066	0.033	4.200
Age Difference (years)	5.010	3.413	0.340	0.000	18.000

4. Discussion

4.1. Laurel Forest in São Miguel Island

The future of the natural vegetation is seriously threatened by non-indigenous plants already considered as problematic in the Azores [15]. Invasive plants were commonly found at all the sites, the most threatened stands being Sete Cidades and Pinhal da Paz, where *P. undulatum* Vent. and *H. gardnerianum* Sheppard ex Ker-Gawl., two top invasive species [4,58] are very abundant; but also in Lombadas, where the latter is also common. Moreover, the area of natural forest on this island has been reduced [12], presently corresponding to small fragmented remains in the western part, and to larger stands in the central and eastern areas, which is reflected in the collected data (see Figure 1). More than 75% of the tree trunks were more than 20 years old, about 60% were between 25 and 50 years old, and only about 15% were more than 50 years old. This might suggest the existence of a secondary forest that is probably more recent at Achada das Furnas, Lombadas and Pinhal da Paz, due to human disturbance [59] and somewhat older at Povoação, Sete Cidades and Tronqueira. At Tronqueira and

Povoação stands, we found what appears to be older remains of a laurel forest with a general higher density/cover of endemic tree species. The taller, larger and older individuals in this study were found at Povoação, which suggests that a larger forest might have occurred there, in the past, presently restricted to water stream margins, a mesic submontane forest described by Elias et al. [12]. At higher altitudes, such as in Tronqueira, we found as invasive species mostly *H. gardnerianum* and *C. arborea* Aiton, and the dominant native trees often corresponded to *Juniperus brevifolia* (Seub.) Antoine or *Ilex perado* subsp. *azorica* (Loes.) Tutin. Therefore, the vegetation at higher elevation corresponded more to a *Juniperus* Montane Woodland [12]. The dense canopies of old *L. azorica* and *J. brevifolia* trees serve as protection for a wide variety of species, characteristic of the Azores natural forests, providing shelter and creating microhabitats [9,60], those species allow the recovery of other native woody species and the restoration of the natural forests [18].

4.2. Growth Ring Anatomy

The seasonality of the Azorean climate, temperate with mild summers, never too dry, allows the differentiation of earlywood and latewood in secondary xylem, which we think is mostly influenced by precipitation rate variations [61]. As shown in the results, in *L. azorica* (Seub.) Franco increment cores, there was a progressive color differentiation between earlywood, lighter, and latewood, darker, particularly at the ring boundary. There was no visible distinct heartwood in any of the taken increment cores, since no color change or difference in light transmission could be observed along the increment fresh cores, immediately after being taken, confirming earlier *Laurus* observations by [21,62].

In São Miguel Island we found a diffuse-porous wood structure in *L. azorica*, as reported for *L. novocanariensis* Rivas Mart., Lousã, Fern.Prieto, E.Días, J.C.Costa & C.Aguiar from Tenerife by Morales et al. [21]. According to those authors, vessel distribution in the wood of *Laurus* suggests a high efficiency. While relatively small, xylem vessels are present at high density, requiring a much smaller functional xylem cross-section to supply an equivalent leaf area, than either diffuse porous angiosperms or conifers. Cermák et al. [29] also related *L. azorica* to *Quercus* as ring-porous species. The wood structure of *L. azorica* in São Miguel is very close to the description provided for Tenerife, increasing survival in adverse environmental conditions, such as highly exposed locations, and from wet places to almost dry lava flows [9]. Meanwhile, in species with a random distribution of vessels, this often obscures the ring boundaries, making ring identification in diffuse porous genera particularly difficult [5]. However, *L. azorica* showed distinct growth rings as found for other Lauraceae, including the close relative, *Laurus nobilis* L. [21,27,30]. The greater difficulties found in *L. azorica* growth ring analysis corresponded to ring anomalies which can be originated by reaction wood, knots, compartmentalization by fungi or putrefaction, observed in the pith, and mainly in larger individuals.

According to Worbes [50] tree ring typology, the growth rings of the Lauraceae show no distinct boundary between latewood and earlywood, but just a thickening of the cell wall and a reduction of the cell lumen from earlywood to latewood. Reis-Avila and Oliveira [30] reported that growth rings in the Lauraceae generally include a boundary marked by an abrupt transition between thick-walled and radially flattened fibers in latewood of a ring, and thin-walled earlywood fibers of the consecutive ring; or otherwise a marginal parenchyma band delimiting tree-rings. Our results indicate a transition from earlywood to latewood, with an increase in cell wall thickness and a decrease in cell lumen, but also of what appears to be a fibrous boundary marking the transition from latewood to earlywood, evident in our micrographs due to the different coloration and extreme flatness of the fibers. The marked boundary between annual growth rings observed in this species is typical of some genera with diffuse porous wood, and could be formed by marginal parenchyma cells [5] or delimited by a fibrous zone [63]. Because of the visible red stained, thick-walled cells, observed at the growth ring boundary, *L. azorica* seems to have ring boundaries delimited by radially flattened latewood fibers, also present in other *Laurus* species [63,64]. However, those fibers show a peculiar affinity for Astrablue, what could indicate the presence of polysaccharides residues from the cell wall, such as cellulose and pectins [65].

We noticed that some modifications occur in *L. azorica* as it gets older, as in other trees, a continuous and progressive decrease in tree ring amplitude tends to happen [63]. The observed width of tree rings showed high variability, between and in the same increment, reflecting the influence of the environmental factors in the growth rate, namely available light, temperature and water [66].

Several xylem rays were visible, perpendicular to the ring boundaries, a trait that is very prominent in hardwoods, providing radial transportation functions [67]. *Laurus azorica* secondary xylem showed strict radial regularities, as found in Tenerife for *L. novocanariensis* [21]. As a hardwood species, *L. azorica* xylem vessels show very evident pits in tangential section, which allows for water transport between individual vessels. Reddish cells, identified in several cross-sections, randomly distributed in the xylem, could correspond to tylosis with simple piths or oil cells associated with rays, since both structures have been observed in *Laurus* [63,64].

4.3. Dendrometry and Dendrochronology

We explored the allometric relationship between tree age (ring counts) and various dendrometric traits in *L. azorica* (Seub.) Franco, since the number of growth rings can be used as a good estimate of stand age [2,68]. Although tree growth is the result of very complex processes [69], allometric models can be a robust alternative to predict the growth and the mean age of tree populations [70]. Furthermore, models relating tree age and other dendrometric variables are available for different species and different geographic areas (e.g., [2–4,54,55]). We analyzed model fit for the entire data set and also separately, according to the sampled stands [2,3,54]. When modeling the global data set, regression models explaining tree age based on trunk diameter, basal area, and/or tree height, were of low quality, in contrast with the results previously obtained by Borges Silva et al. [2,4] for the invasive tree *P. undulatum* Vent. in the Azores. For *L. azorica*, model adjustment clearly increased when modelling separately for each stand, emphasizing the possible importance of local factors (e.g., soil type; microclimatic conditions), leading to a greater predictive ability at a site-specific level (e.g., [55,71]). While the climate in the Azores is somewhat homogeneous, a relevant change in the climatic conditions is to be expected with elevation, corresponding to a decrease in temperature and to an increase in precipitation and relative humidity [32], as shown in the physical characterization of our study sites. In fact, pronounced climatic differences were found between the stands, particularly regarding annual precipitation, with annual values ranging from about 2100 to 1300 mm. Therefore, implementing allometric equations beyond the specific site for which they were developed can affect estimate accuracy [2,72]. Moreover, assuming that the collection of as many samples per tree as possible, in a large number of trees per site, would reduce the level of environmental noise, it might still be possible to improve the fit of the allometric models for this species [73].

The present study suggests that if diameter at breast height is available, a relative simple equation could be used to predict tree age. Trunk diameter is often the best predictor for age in allometric models, because both variables are strongly correlated [74], it is a relatively easy and inexpensive measurement to obtain, and is often available in forest inventory data [75]. In fact, growth estimates of forest trees are typically made by using measurements of diameter at breast height and of total height, which are scaled to the whole tree via allometric equations derived from destructive sampling [4,76]. Age of *L. azorica* trees was also closely correlated with tree height and basal area [77], and our results revealed that, in some cases, models with cubic terms improved age estimates [2,4,53,54], although the performance of simpler models, including only a linear effect, was also acceptable [2,4].

In this research, samples taken at tree base and at breast height allowed to estimate an average radial growth rate of $0.68 \text{ cm} \cdot \text{year}^{-1}$, with an estimated average of 5 years to reach breast height. A higher growth rate than the values reported for the widespread invasive species *P. undulatum*, with a radial growth rate of $0.38 \text{ cm} \cdot \text{year}^{-1}$, and an average of 8 years needed to attain breast height [2,4]. Therefore, other factors might be affecting growth and dispersal of those species, and a local comparison of growth rates would be needed, if the invasion process is to be better understood, since growth rate can provide a general representation of the growth dynamics at certain conditions (climate, hydrology,

soil, and successional stages) [78]. These analyses are essential to support the sustainable management and conservation of natural forests [78].

5. Conclusions

As for other Lauraceae, *L. azorica* (Seub.) Franco has shown a promising potential for dendrochronology studies [30]. We showed that increment cores can be successfully used to estimate tree age, without the need to cut *L. azorica* trees. Moreover, age–diameter relationships can be further validated by increasing sample size and extending the sampling effort to other islands, where laurel forests are still present [12]. In the Azores, dendrochronology can become a helpful tool to evaluate current and future threats to forests, and support forest management, through the comparison of indigenous and non-indigenous trees, and the future integration of climatic parameters. If long chronologies are not available, at least tree age and growth rate can be estimated and related with environmental variables, improving *L. azorica* niche definition. Moreover, older specimens could eventually be found in other islands, such as Terceira and Pico, where larger stands of natural forests are still present.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/7/538/s1>. Table S1: Allometric equations tested for 147 and 97 *Laurus azorica* (Seub.) Franco trees sampled respectively at breast height (BH) and at base in São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree Height (H); Basal Area (BA); Table S2: Allometric equations tested for 25 *Laurus azorica* (Seub.) Franco trees (ALL) and for 19 samples (without outliers, WO) of *Laurus azorica* trees from Lombadas, São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree Height (H); Basal Area (BA); Table S3: Allometric equations tested for 20 *Laurus azorica* (Seub.) Franco trees (ALL) and for 16 samples (without outliers, WO) of *Laurus azorica* trees from Achada das Furnas, São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree Height (H); Basal Area (BA); Table S4: Allometric equations tested for 49 *Laurus azorica* (Seub.) Franco trees from Pinhal da Paz, São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree Height (H); Basal Area (BA); Table S5: Allometric equations tested for 49 *Laurus azorica* (Seub.) Franco trees from Sete Cidades, São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree height (H); Basal area (BA); Table S6: Allometric equations tested for 48 *Laurus azorica* (Seub.) Franco trees (ALL) and for 33 samples (without outliers, WO) of *Laurus azorica* trees from Tronqueira, São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree Height (H); Basal Area (BA); Table S7: Allometric equations tested for 47 *Laurus azorica* (Seub.) Franco trees from Povoação stand, São Miguel Island. Allometric models: * [54]; ** [2]; *** Current study. Regression model equations; Null Model (M_0); Adjusted determination coefficient ($\text{Adj } R^2$); Akaike Information Criterion (AIC); Root Mean Square Error (RMSE); Mean Relative Error (MRE), Correction Factor (CF). Diameter (D); Tree Height (H); Basal Area (BA).

Author Contributions: B.M., L.B.S. and L.S. designed the study, performed the fieldwork, assisted labwork, analyzed the data and prepared a first draft of the manuscript. R.C. and A.S.R. supervised and performed most of the wood anatomical work. R.R. and M.C. assisted with the fieldwork and with data preparation and analysis. All the authors participated in the discussion and revision of the manuscript.

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