



## Structural differentiation among annual shoots as related to growth dynamics in *Luma apiculata* trees (Myrtaceae)

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### ARTICLE INFO

Edited by Favio Gonzalez

#### Keywords:

Allometric relationships  
Axis differentiation  
Bud structure  
Growth dynamics  
Preformation  
Reproductive effort

### ABSTRACT

A close examination of axis differentiation is key to the understanding of plant architecture and its ecological consequences. The present study was aimed at evaluating the relationship between shoot size/structure and shoot growth dynamics in *Luma apiculata* (Myrtaceae), a widely distributed and cultivated but little investigated Andean-Patagonian woody species. We assessed (a) organ preformation in apical buds, (b) shoot growth through repeated observations of labeled axes, (c) axillary production, leaf area, stem volume, dry mass of leaves, stem and reproductive structures, and allometric relationships for annual shoots of different sizes. The number of preformed organs in apical buds of *L. apiculata* averages seven nodes and exhibits little variability, in contrast with the highly variable growth period (from few weeks up to more than six months) and the size reached by annual shoots. The leaf area/stem volume ratio increased more sharply during shoot extension in short than in long shoots. Stem thickness was proportionally higher than other shoot-size measures in short shoots and long shoots as compared to intermediate-size shoots. Both the presence and the position of branches along shoots varied notably with shoot size. Proportional mass and meristem allocation to fruit production decreased with shoot size. Unlike many other broadleaved temperate and temperate-cold tree species so far studied, the size reached by *L. apiculata* shoots developed in the same year varies notably among axes in the same topological position, and seems to depend largely on the expansion of non-preformed organs. A low dependency of shoot growth on organ preformation may increase plasticity in axis structure, at the cost of a less hierarchical (more shrubby) architecture. Simultaneous organ differentiation and expansion may allow plants to fine-tune shoot structure to the prevailing conditions during the growing season, and represent an advantage whenever conditions favourable for growth exhibit high between-year variations.

### 1. Introduction

Axis differentiation is one of the ruling concepts of plant architecture and may be considered among those traits that define the structure of all plants of a particular species at a precise ontogenetic stage (Hallé et al., 1978). The study of intraspecific axis differentiation provides useful information about plant ecology (e.g. Godin et al., 1999; Valladares and Niinemets, 2008; Costes et al., 2014; Carvalho and Ribeiro, 2018), and allows a better understanding of ontogenetic changes in plant form, and of the evolution of plant architecture (e.g. Puntieri et al., 1998, 2003; Sabatier and Barthélémy, 1999, 2001; Seleznyova et al., 2002; Suzuki, 2002; Costes, 2003; Grosfeld and

Barthélémy, 2004; Costes et al., 2014; Chomicki et al., 2017). Good examples of axis differentiation are provided by *Pinus* spp. and *Betula* spp., in which long axes and short axes are clearly distinguished. These two axis categories have been related, respectively, to exploratory and exploitative functions, and to peripheral and non-peripheral axis positions in the tree crown (Macdonald and Mothersill, 1983; Heuret et al., 2006). For many other species, differences among axis categories and the links between axis growth, structure and function are virtually unknown. The extent to which a plant axis is concerned with specific functions depends strongly on the anatomy, morphology and mass allocation of the structural units making up that axis. This is why the understanding of axis differentiation in a plant species usually begins

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<https://doi.org/10.1016/j.flora.2018.10.005>

Received 9 October 2018; Accepted 19 October 2018

Available online 23 October 2018

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**Fig. 1.** (a) General view of an adult *Luma apiculata* tree such as those included in the present study. (b) Detail of the distal end of a shoot with an apical bud (AB) like those dissected in this study (photograph taken in May 2017). A disal leaf (DL) and a distal axillary bud (AxB) are indicated. Scale bars = 0,5 m (a) and 1 mm (b).

with studies centered on shoot structure (Costes et al., 2013; Torres et al., 2016).

In woody plants from temperate and cold regions, each leafy axis is made up by the succession of structural units, each of them developed from an apical meristem during the favourable period of the year. In many of these plants, each such units, known as “annual shoot” or simply “shoot” (Barthélémy and Caraglio, 2007; Stecconi et al., 2017), consists exclusively of internodes, nodes and leaves extended after an event of meristematic activity followed by a period of stockage of the embryonic organs in a bud (e.g. Remphrey, 1989; Remphrey and Davidson, 1994). In this modality of axis length growth, the size reached by a shoot depends on the extent of apical meristematic activity at the end of the preceding growing season, i.e. organ preformation. In contrast, other shoots include not only preformed organs, but also additional organs derived from events of meristematic activity and organ extension during the season of shoot extension (e.g. Sabatier and

Barthélémy, 1999, 2001; Souza et al., 2000; Costes, 2003; Mezghani et al., 2008; Costes et al., 2014). It has been suggested that these additional organs may redefine the structure and function of a shoot (Takenaka, 1994; Yang et al., 2010; Miyata et al., 2011; Morales et al., 2014; Anfodillo et al., 2016) and fine-tune the phenotype of a plant to current conditions (Guédon et al., 2006).

The study of shoot structure provides useful information concerning resource allocation to different functions, such as water uptake, carbon fixation and mechanical stability (Puntieri et al., 2007a). In this regard, an informative basis for comparing shoots and axes of the same species is obtained by analyzing paired variables through exponential equations of the general form  $Y = X^\beta$ , often referred to as allometric equations (e.g. Suzuki and Hiura, 2000; Dahle and Grabosky, 2009). Among the variables most frequently used as descriptors of shoot structure are: stem cross-sectional area (SCA), stem length (SL), leaf area (LA), stem dry mass (SM), leaf dry mass (LM) and the dry masses or

numbers of reproductive structures and branches (e.g. Preston and Ackerly, 2003; Sun et al., 2005; Valladares and Niinemets, 2007; Osada et al., 2002; Osada, 2011; Osada et al., 2015; Yang et al., 2015). Under the assumption that resource allocation to leaves and stems should be sustained along the whole range of shoot sizes within a species, it has been proposed that *LM* and *SM* would vary isometrically (i.e.  $\beta = 1$  in the exponential equation; Sun et al., 2005). In studies concerning both intraspecific and interspecific comparisons (e.g. Niklas and Enquist, 2002; Sun et al., 2005), *LA* has been shown to increase proportionally more than *SCA* (a truly allometric relationship, with  $\beta = 1.5$ , when  $SCA = X$  and  $LA = Y$ ). Relationships between morphological descriptors of shoots or axes may vary depending on environmental condition, shoot type (Alla et al., 2012; Jarčuška and Milla, 2012) and cultivar (Normand and Laurie, 2012). Several hypotheses have been proposed to validate both isometric and allometric relationships among stem size and leaf size descriptors (Niklas and Enquist, 2002; Preston and Ackerly, 2003; Sun et al., 2005). For instance, developmental compromises have been proposed to justify conservative relationships between variables describing leaf and stem size (as discussed by Sun et al., 2005). Nevertheless, no reports of the connection between shoot growth-dynamics and shoot structure are, to our knowledge, available. This is a relevant issue as the relative amounts of preformed organs and additional organs produced during one growing season could greatly affect the balance between leaf mass and/or area and stem structure, and, as a result, plant fitness following environmental changes. The present study was aimed at testing the hypothesis that intraspecific variations in shoot growth dynamics may have sharp consequences on shoot structure and function.

We investigated intraspecific variations in the structure and growth dynamics of main-branch shoots of *Luma apiculata* trees (Myrtaceae). It has been shown that the number of preformed organs in apical winter buds of this species is low (Sosa and Puntieri, 2016), and that shoot size and phenology are highly variable both between and within *L. apiculata* trees (Donoso et al., 2006). Thus, recurrent meristematic activity during the growing season and a significant influence of shoot growth dynamics and shoot structure may be hypothesized for this species. By examining the link between shoot growth and shoot structure, this study contributes to better understand the morphogenetic basis of axis differentiation in plants.

## 2. Materials and methods

### 2.1. Study species

*Luma apiculata* (Myrtaceae) is a widely distributed and one of the most emblematic woody species in the Patagonian forests of South America (Fig. 1; Demaio et al., 2017). It inhabits temperate and temperate-cold forests of Chile and Argentina, preferably close to lakes and rivers and under the canopy of larger trees, where extreme temperatures become moderate (Weinberger, 1978); many of these areas are nowadays affected by man-generated disturbances (Hauenstein et al., 2014). According to molecular studies, *L. apiculata* is a southern species related to a tropical-subtropical lineage (Thornhill et al., 2015). This evergreen species grows as a large shrub or tree up to 25 m high, often developing intricate systems of flexuose branches (Fig. 1a; Movia and Rotman, 1988; Mosbach, 1992; Donoso et al., 2006; Demaio et al., 2017). The growth rate of *L. apiculata* is highly dependent upon the availabilities of water and minerals (Riedemann and Aldunate, 2004). A recent study dealt with shoot growth and bud structure in *L. apiculata* (Sosa and Puntieri, 2016), but the architecture and axis differentiation of this species have not been investigated up to now.

### 2.2. Preformation in winter buds

In order to examine the extent of organ preformation in apical buds of main branches of *Luma apiculata*, we dissected, between May and

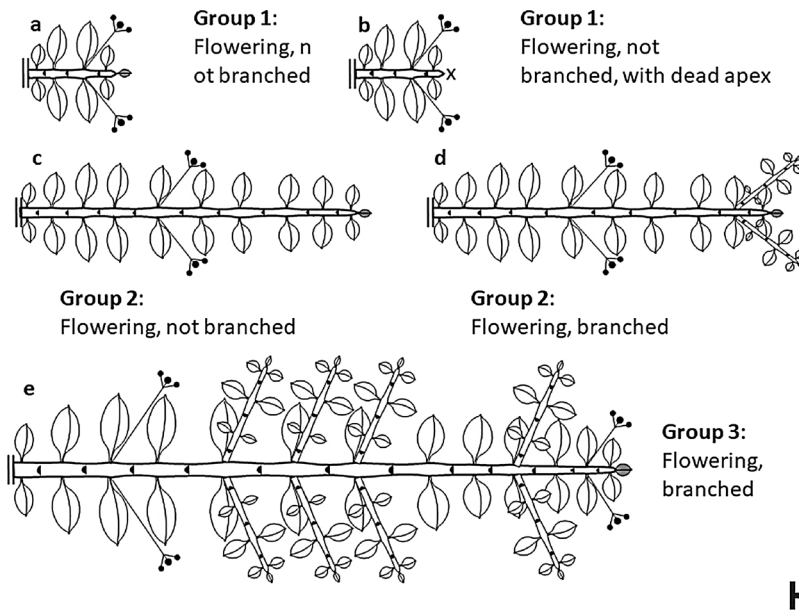
June 2017, ninety buds corresponding to 11 individual trees growing within the native range of this species in Argentina (Fig. 1b). Five of these trees were planted in public and private parks at the locality of El Bolsón (41°58' S, 71°32' W, 313 m a.s.l.), three were planted in public parks at the locality of Bariloche (41°08' S, 71°18' W, 780–840 m a.s.l.), and three developed naturally along the coast of the Nahuel Huapi Lake, in the Nahuel Huapi National Park, Argentina (41°03' S, 71°33' W, 780 m a.s.l.). Between 4 and 12 shoots per tree, ranging from 2.1 cm to 41.0 cm long, were sampled from the distal end of main branches. All of these branches were formed from the trunk between 1 and 3 m above the ground and reached the tree crown's periphery. The apical bud of each shoot was dissected under a stereo-microscope (Leica EZ4D, 40X), and its number of preformed nodes was counted. The smallest, most distal leaf primordia were distinguished as 10  $\mu$ m long domes (Sosa and Puntieri, 2016). This primordia size was congruent with that recorded for other plant species for which bud dissections have been analyzed (Puntieri et al., 2000, 2002; Souza et al., 2000; Magnin et al., 2012).

### 2.3. Intra- and inter-individual variations in shoot structure

In order to encompass as much shoot-size variation as possible, in March 2017, seventeen *Luma apiculata* trees (including those indicated in Section 2.2) were selected at different localities within the native range of this species in Argentina. Thirteen of these trees were planted in public or private green spaces, eight at Bariloche, and five at El Bolsón. The remaining four trees were growing at three public nature reserves: one tree at Nahuel Huapi National Park (41°03' S, 71°33' W, 780 m a.s.l.), two at Lago Puelo National Park (42°06' S, 71°36' W, 202 m a.s.l.), and one at Los Alerces National Park (42°43' S, 71°43' W, 523 m a.s.l.). All of them had developed reproductive structures in the 2016–2017 growth season, and were between 3 and 6 m in height, with several codominant basal trunks between 10 and 30 cm in diameter at ground level (Fig. 1a). To our knowledge, none of these trees had been subject to severe defoliation or pruning in recent years. On the basis of consultations to local authorities and neighbours, those trees that had been planted were between 10 and 30 years old. It was not possible to estimate the age of those trees in natural populations, but their size and structure suggested that they resembled those at Bariloche and El Bolsón in age.

Distal ends of three to 10 main branches were cut for each selected tree from March to May 2017; the severed branches derived from the tree trunk between 1–3 m above ground, at intermediate positions. Observations made on the labeled shoots of one of the selected trees as well as previous results (Sosa and Puntieri, 2016) indicate that the co-occurrence of a relatively short internode and small leaves is a reliable morphological marker of the limit between two successive shoots in this species. This marker allowed us to include entire shoots in the severed main-branch segments. Between 10 and 20 shoots were cut per tree, except for one of the trees at El Bolsón, which was more intensively sampled. The numbers of shoots per population were 106 in Bariloche, 124 in El Bolsón, 20 in the Nahuel Huapi National Park, 41 in the Lago Puelo National Park, and 21 in Los Alerces National Park.

For each sampled shoot we measured the maximum and minimum stem diameters at proximal and distal ends (with digital calipers, to the nearest 0.1 mm), the length of each internode, the total stem length, and the length of one of the leaves at each node. It should be indicated that *Luma apiculata* leaves are arranged in opposite-decussate phyllotaxis, and that both leaves at a node resemble one another in size and shape (Sosa and Puntieri, 2016). The formation of axillary structures per node was assigned to one of the following types: branch, reproductive structure and bud. The “branch” type included all axillary productions that bore green leaves in at least some of their nodes, irrespective of the axillary or terminal development of reproductive structures. Reproductive structures included inflorescences and infructescences that lacked green leaves. All axillary structures consisting of leaf primordia and devoid of green leaves and reproductive



**Fig. 2.** Schematic representation of *Luma apiculata* shoots. (a) Flowering, unbranched short shoot, (b) Flowering, unbranched short shoot with dead apex (X), (c) Flowering, unbranched intermediate-size shoot, (d) Flowering, branched intermediate-size shoot, (e) Flowering, branched long shoot. Only leaves corresponding to one plane were drawn. For simplicity, all inflorescences are trimerous (flowers: black circles). Inflorescences and branches were located at the most likely positions for shoots of each size. Scale bar = 20 mm.

structures were termed axillary buds. In some cases, one or, more frequently, two branches developed from one of the most distal nodes of a shoot after the end of its length growth; this relay branching process was, sometimes, related to the death of the bearing shoot’s apex (Fig. 2). In these cases, the renewal branch or the longest one of these branches (in case two branches developed) was considered part of the shoot, so that its internodes and leaves were included in the evaluation of the length and number of leaves per shoot. Most generally, both axillary structures at a node belonged to the same type. Whenever a reproductive structure and a bud were present in the same node, that node was assigned to the “reproductive structure” type, and every time a branch and a bud shared the same node, the node was assigned to the “branch” type. The combination of a reproductive structure and a branch on the same node was not found. The number of nodes per branch produced by a shoot was recorded. The total number of nodes (TN) of each shoot was obtained by adding the number of nodes of its main stem and the number of nodes of all branches derived from it. The apical or axillary position of each shoot as well as the persistence or not of its apex were recorded.

After shoot measuring, stem, leaves and reproductive structures were manually removed and oven dried at 70 °C for 48 hs in paper bags. The dry masses of stem (SM), leaves (LM) and reproductive structures (RM) were measured separately to the nearest 0.001 g (ACCULAB ALC-210.4). Vegetative mass (VM) was computed as SM + LM, and total mass (TM) as VM + RM.

**2.4. Intra-individual variations in shoot growth**

Shoot growth dynamics was assessed to evaluate the growth rates of shoots and verify previously established morphological markers of shoot limits. The relationship between shoot growth rate and size at the end of the growing season was also evaluated. In November 2016, fifty shoots at the beginning of their extension period were labeled on main branches of a single *Luma apiculata* tree developed in a private garden at El Bolsón. At that time, this tree was about 18 years old and 5 m in height, and had four trunks about 25 cm in diameter diverging as co-dominant axes from the ground. The labeled shoots were all distally located on main branches derived from the trunk between 1–3 m above ground. The labeled shoots were randomly oriented with respect to the magnetic North. For each labeled shoot, one of the leaves of the most distal node was tagged with a small dot using a permanent marker at each of the following dates: 26 November 2016 (t<sub>1</sub>), 17 December 2016

(t<sub>2</sub>), 28 December 2016 (t<sub>3</sub>), 13 January 2017 (t<sub>4</sub>), 29 January 2017 (t<sub>5</sub>), 11 February 2017 (t<sub>6</sub>), and 11 March 2017 (t<sub>7</sub>). At t<sub>7</sub>, all shoots had reached the autumn-winter resting phase. In each case, the tagged leaf belonged to the most distal fully expanded pair of leaves of the 2016–2017 shoot. The number of nodes developed between those leaves labeled at each time was registered upon completion of shoot growth.

**2.5. Data analyses**

For each of the 312 shoots that were cut, the stem proximal cross-sectional area, SCA, was computed under the assumption of an ellipsoidal outline. Using the maximum, d1, and minimum, d2, stem diameters, Eq. (1) was applied.

$$SCA = d1 * d2 * \pi \tag{1}$$

For each shoot, the mean stem diameter was computed for the proximal and the distal ends, Dp and Dd, respectively. From these values and total stem length, stem volume was calculated with Eq. (2), assuming that the stem was a truncated cone.

$$stem\ volume = 1/3 * \pi * total\ stem\ length * [(Dp/2)^2 + (Dd/2)^2 + (Dp/2).(Dd/2)] \tag{2}$$

The stem volume was measured from the proximal to the distal end of the shoot.

The area of each measured leaf was estimated from its length using Eq. (3).

$$Leaf\ area = 0.4505 * leaf\ length^{1.903} \tag{3}$$

This equation was obtained after measuring the length and area of a sample of 199 leaves that were randomly sampled from 20 *Luma apiculata* trees at different localities (r<sup>2</sup> = 0.93). The unifacial area of each leaf was determined by means of the J-image free-access software after leaf scanning with a flatbed scanner. Leaf area at each node and total leaf area of each shoot were computed. For each node of each shoot, the ratio between the added leaf area and stem volume up to that node were calculated.

The sampled shoots were classified in three size groups: G1, short shoots, with less than 8 nodes (N = 125); G2, intermediate-size shoots, with 8–20 nodes (N = 146); G3, long shoots, with more than 20 nodes (N = 41). The following relationships were analysed separately for each group: TN vs SCA, SM vs SCA, LM vs SCA, VM vs SCA, TM vs SCA, SM vs

TN, and LM vs SM, by means of type II regressions (geometric mean regressions; Sokal and Rohlf, 1981). The slopes of the regressions on log-transformed variables ( $\beta$ ) were compared with the predicted values 1.0 and 1.5, and between groups by using the 95% confidence intervals of  $\beta$ , assuming that the standard error of  $\beta$  resembles that of the slope of a type I least-squares regression (Sokal and Rohlf, 1981).

For those shoots that bore mature fruits at the time of sampling, the reproductive effort (RE) was computed as the RM/TM ratio. Differences in RE between shoot groups were evaluated through analysis of covariance (generalized linear model for unbalanced designs), including VM (log-transformed) as a covariable, account taken of the relationship between this variable and RE. For this analysis, RE was arcsin-transformed so as to normalize its distribution.

For each of the shoots that were repeatedly observed over the growing season, relative growth rate (RGR) was computed for the period between  $t_1$  and  $t_4$  by means of equation [4]:

$$[4] RGR = \log_{10}(\text{number of nodes at } t_4) - \log_{10}(\text{number of nodes at } t_1)$$

The selection of this period was based on the fact that the majority of the labeled shoots were extending in that period whereas many of them did not extent later on. RGR was correlated (Pearson’s correlation) with each of the following variables: number of nodes, total stem length, total leaf area, SM, LM, VM and TM at the end of the growing season (all variables log-transformed).

In all statistical comparisons, a 0.05 error probability was adopted. These analyses were performed with the R 2.12.0 package (R Development Core Team, 2012).

### 3. Results

#### 3.1. Preformation

An average of 6.6 preformed nodes was found in the dissected buds. Variations in this regard were low, as expressed in variance (var = 1.55) and standard error (SE = 0.13) around the mean (Fig. 3). Neither reproductive structures nor branches could be identified at the primordial axillary buds in any of the dissected buds.

#### 3.2. Shoot structure

The shoots sampled from 17 trees varied from 0.7 to 63.6 cm in length, from 0.9 to 5.9 mm in basal stem diameter, and from 2 to 46 in number of nodes of the main stem. Out of 312 sampled shoots, 125 had < 8 nodes (G1), 146 had 8 to 20 nodes (G2) and 41 had > 20 nodes (G3). In the majority of the selected trees, shoots in at least two of these groups were sampled (Table 1). On average, those shoots with more nodes had longer internodes than those with fewer nodes (Fig. 4a).

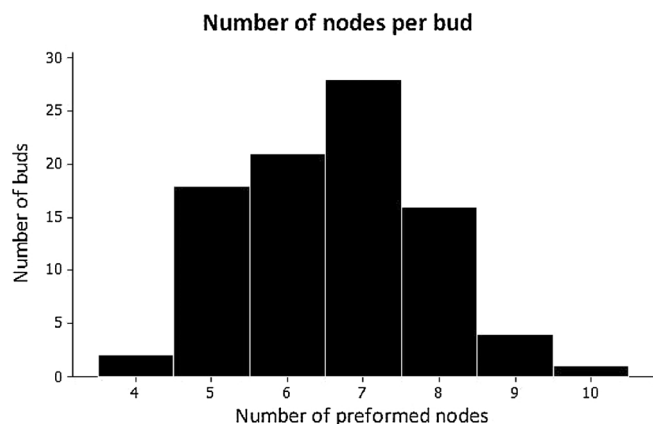


Fig. 3. Frequency distribution of the number of preformed nodes in dissected apical buds of *Luma apiculata*.

Table 1

Number of sampled *Luma apiculata* shoots for each size group defined by number of nodes (Group 1: < 8 nodes, Group 2: 8–20 nodes, and Group 3: > 20 nodes) for trees selected at Bariloche, El Bolsón, Parque Nacional Nahuel Huapi (PNNH), Parque Nacional Lago Puelo (PNLP) and Parque Nacional Los Alerces (PNLA).

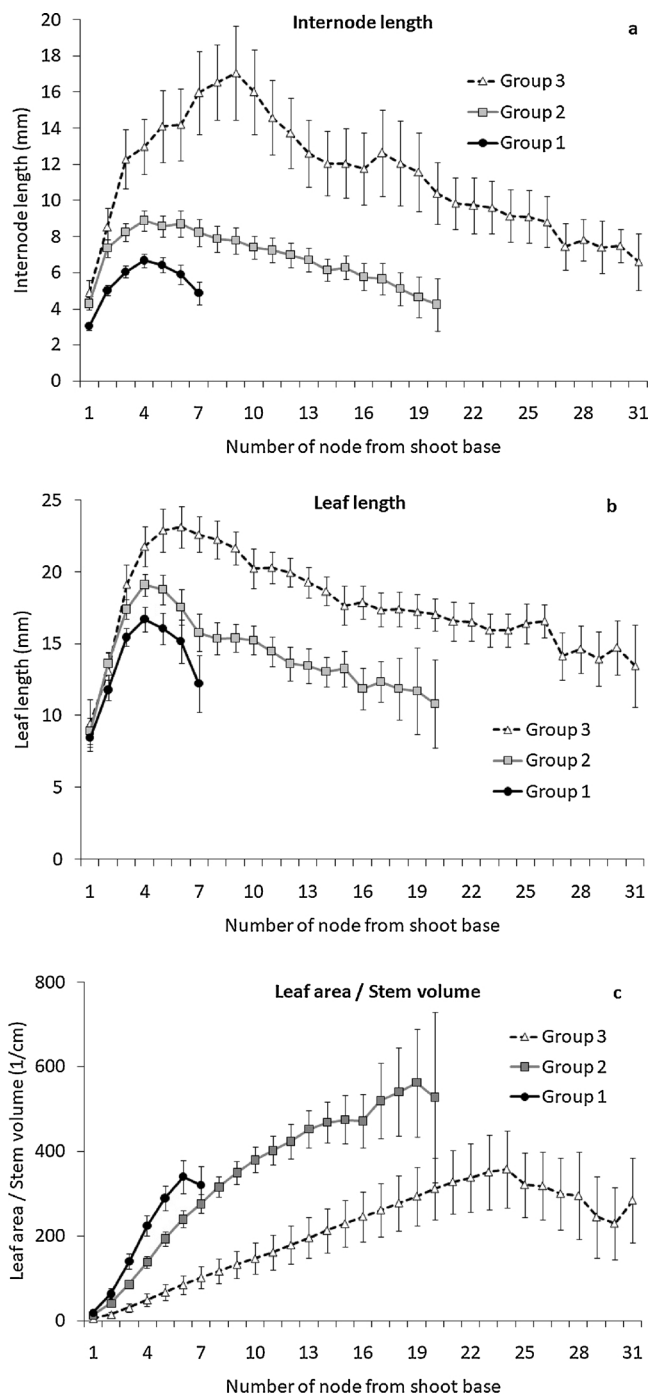
Tree locality	N° of trees	Number of shoots per size group		
		Group 1	Group 2	Group 3
Bariloche	8	0	8	4
		0	0	10
		12	8	0
		2	18	0
		2	12	1
		10	2	0
		0	4	2
0	11	0		
El Bolsón	5	21	40	12
		14	6	0
		3	4	3
		0	3	5
		0	9	4
PNNH	1	4	16	0
PNLP	2	19	2	0
		20	0	0
PNLA	1	18	3	0

Mean internode length increased between the four proximal nodes and diminished gradually toward the distal end in G1 and G2 shoots (Fig. 4a). In G3 shoots, mean internode length increased steadily up to the ninth node and decreased distally. Average leaf length also increased as shoots increased in number of nodes (Fig. 4b). Maximum values of leaf length were recorded for nodes 4 to 5 in G1 and G2 shoots, and for nodes 4 to 9 in G3 shoots. In all three groups, leaf length tended to decrease towards the distal end of the shoot.

Increments in the leaf area/stem volume ratio from the proximal end to intermediate-distal nodes were observed in all three shoot groups; this increment was high for G1 shoots, intermediate for G2 shoots and low for G3 shoots (Fig. 4c). In the latter group, a reduction in the leaf area/stem volume ratio was evident from node 24 onwards.

Axillary structures varied in frequency depending on size and node position along the shoot. The development of reproductive structures was more frequent in smaller than in larger shoots, viz 100%, 84.9% and 29.3% in G1, G2 and G3 shoots, respectively. In G1 shoots, the proportion of nodes with reproductive structures was high for all except for the two most proximal nodes (Fig. 5a). In G2 shoots, the proportion of nodes with reproductive structures was even between nodes 4 and 12, and diminished in distal nodes (Fig. 5b). The proportion of nodes of G3 shoots with reproductive structures was low (< 10%), but peaks could be identified between nodes 3 and 5, and between nodes 21 and 28 (Fig. 5c). The percentage of shoots that developed branches during their elongation increased with shoot size, viz 3.2%, 63.0% and 95.1% in G1, G2 and G3 shoots, respectively. In some cases, reproductive structures derived from these branches, but the extent of flowering was not quantified (data not shown). In G2 shoots, the probability of branch development, although low for all nodes, increased gradually between nodes 3 and 15. In G3, the frequency of branches was highest between nodes 9 and 11; a lower peak occurred between nodes 18 and 21. Axillary bud was the most frequent axillary production in almost all nodes of G2 and G3 shoots.

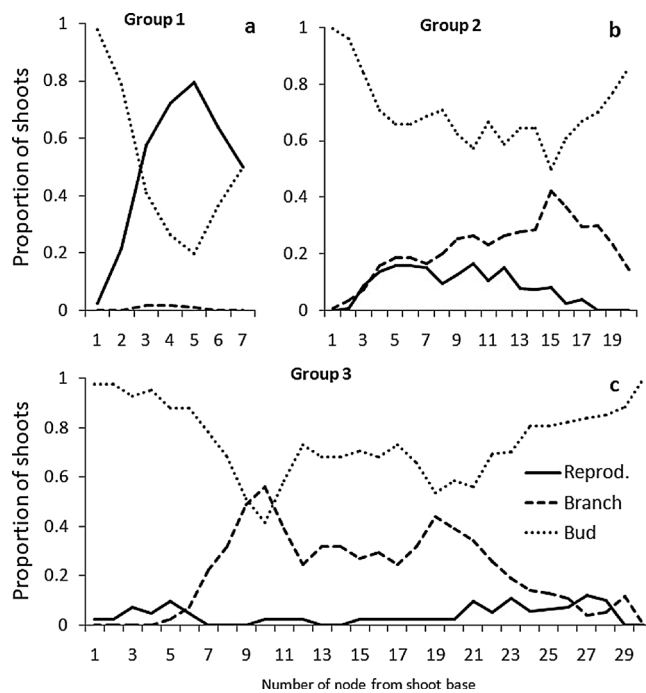
The development of one or two renewal branches was recorded in four G1 shoots (3.2%), 46 G2 shoots (31.5%), and five G3 shoots (12.2%). In approximately 50% of the shoots that developed one or two (co-dominant) renewal branches, the distal end of the shoot was withered; the presence of a fresh but dormant apical bud was verified in the remaining shoots. When considering all shoots (irrespective of the



**Fig. 4.** Mean ( $\pm$  95% confidence interval) internode length (a), leaf length (b) and leaf area / stem volumen (c) for *Luma apiculata* shoots corresponding to three size groups. Group 1 (black circles): shoots with < 8 nodes; Group 2 (gray squares): shoots with 8–20 nodes; Group 3 (white triangles): shoots with > 20 nodes. Internodes and leaves are counted from the proximal to the distal ends of the shoots.

development or not of relay branches), the percentage of shoots with withered apical meristem at the end of the extension period was 20.6% for G1, 7.5% for G2, and nil for G3. No evidence of apical withering caused by herbivory was found.

The correlations between size descriptors were significantly positive for all three shoot groups; the corresponding coefficients of determination were markedly lower for G1 than for G2 and G3 (Fig. 6; Table 2). For G1 shoots, *TN*, *LM*, *VM* and *TM* increased proportionally less ( $\beta < 1$ ) and *SM* increased proportionally more ( $\beta > 1$ ) than *SCA*. This



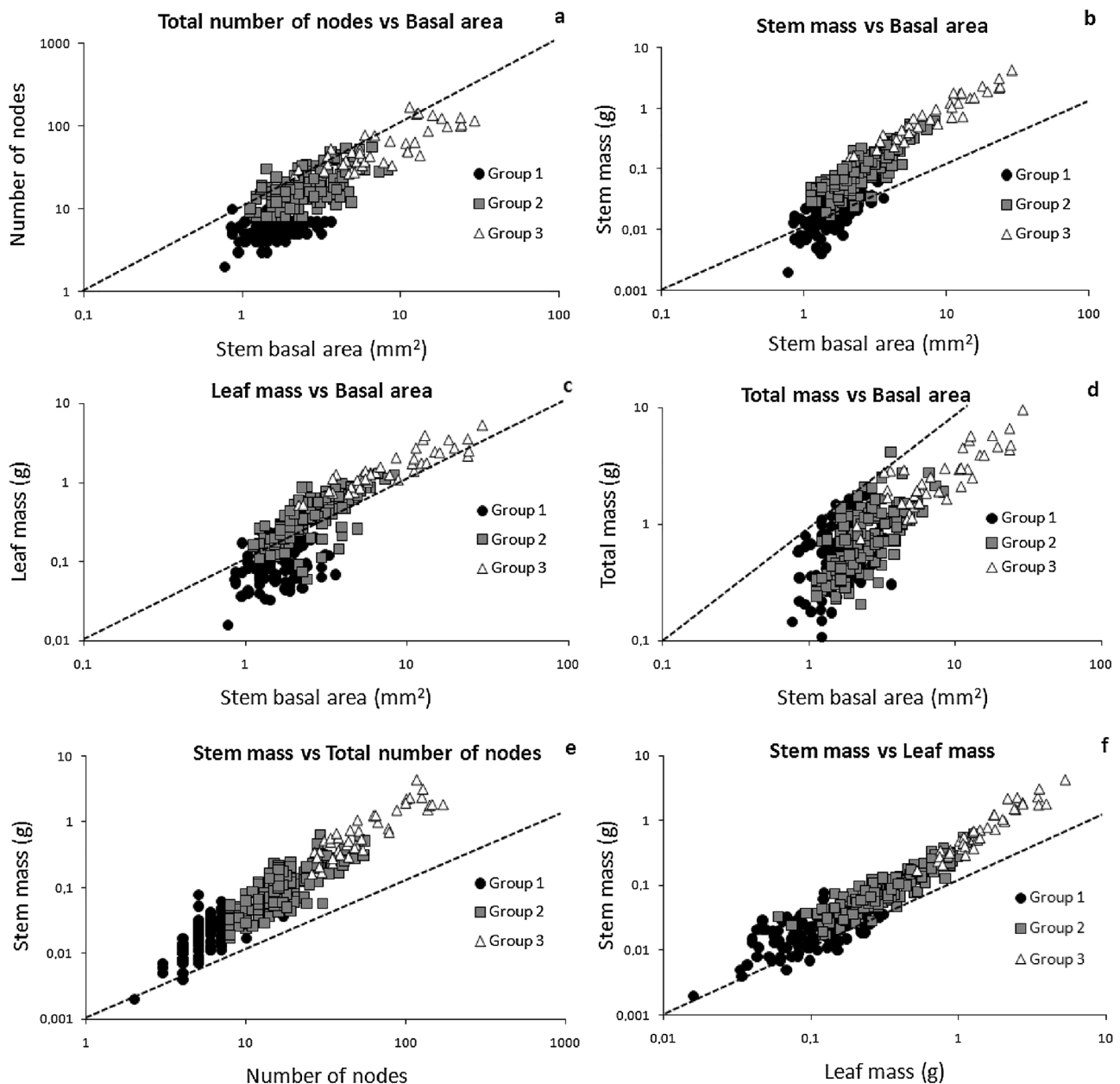
**Fig. 5.** Proportion of *Luma apiculata* shoots corresponding to each size group (Group 1: a; Group 2: b; Group 3: c) with a bud, a branch and a reproductive structure at each of the nodes counted from the shoot's proximal end.

means that stem thickness of G1 shoots increased disproportionately more with the increments in number of nodes, leaf mass and fruit mass. In G2 shoots, the variables *TN*, *LM*, and *TM* were isometrically related ( $\beta = 1$ ) with *SCA*, and the variables *SM* and *VM* increased proportionally more than *SCA*. In G3 shoots, *VM* and *SCA* were isometrically related, whereas the variables *TN*, *LM* and *TM* increased proportionally less ( $\beta < 1$ ), and *SM* proportionally more than *SCA*. In all three groups, *SM* increased proportionally more than *TN* and *LM* (Fig. 6e, f). Concerning among-group comparisons, the regression slopes indicated proportionally lower increments in *SCA* as compared to those in all other shoot descriptors for G2 shoots than for G1 and G3 shoots. The regression coefficient between *SM* and *TN* was higher for G1 shoots than for G2 shoots and G3 shoots, thus indicating a proportionally higher increase in *SM* as related to that in *TN*. The regression coefficient between *SM* and *LM* was higher for G1 and G3 shoots than for G2 shoots; *SM* tended to increase proportionally more than *LM* in G1 shoots than in larger shoots (Table 2).

These comparisons reveal clear structural differences among shoots assigned to different groups based on their number of nodes. In contrast to shoots of intermediate size (G2), large (G3) shoots and, especially, small shoots (G1) have a proportionally higher stem development (in terms of thickness and mass) as compared to leaf development (in number or mass).

### 3.3. Shoot growth

Ten out of the 50 shoots that were labeled had already ended their elongation by  $t_1$  (26 November), thus no evaluation of their growth rate could be made. Node production in the remaining 40 labeled shoots took place in two phases, viz from October to the first half of January, and from the second half of January up to March (Fig. 7). The majority of these shoots ended the formation of nodes after the first growth phase. Fewer shoots continued node formation up to the end of the second phase. The *RGR* between  $t_1$  and  $t_4$  was not correlated with the number of nodes ( $r = 0.25$ ), total leaf area ( $r = 0.24$ ), total stem length ( $r = 0.32$ ), *LM* ( $r = 0.30$ ), *VM* ( $r = 0.32$ ) or *TM* ( $r = 0.29$ ;  $p > 0.05$  in all cases) of the labeled shoots, and was positively but slightly



**Fig. 6.** Relationships between descriptor variables for shoots of *Luma apiculata* discriminated by size (Group 1: < 8 nodes; Group 2: 8–20 nodes; Group 3: > 20 nodes). (a) Total number of nodes (*TN*) vs stem cross-sectional basal area (*SCA*), (b) stem mass (*SM*) vs *SCA*, (c) leaf mass (*LM*) vs *SCA*, (d) total mass (*TM*) vs *SCA*, (e) *SM* vs *TN* and (f) *SM* vs *LM*. The dashed line indicates the approximate slope for the isometric *X/Y* relationship ( $\beta = 1$ ) crossing through the origin of each graph.

correlated with *SM* ( $r = 0.33, p = 0.042$ ) at the end of their growth.

### 3.4. Reproductive effort

Of the 312 shoots that were sampled, 261 (83.6%) were bearing reproductive structures (flowers and/or fruits); 197 (63.1%) of them had mature fruits at the time of sampling: a total of 121 of them (96.8%) were G1 shoots, 69 were G2 shoots (47.3%) and 7 (17.1%) were G3 shoots. The shoots that bore both flowers and fruits at sampling were: 1 G1 shoot, 17 G2 shoots and 4 G3 shoots.

When considering only those shoots with mature fruits at the time of sampling, *VM* (dry mass of leaves and stem) was directly related to reproductive mass (*RM*), and inversely related to *RE* (Fig. 8). *RM* and *RE* did not differ significantly among shoot groups ( $F = 0.6$  and  $F = 0.5$ , respectively,  $p > 0.1$ ; d.f. between groups = 2; d.f. within groups = 193), when including *VM* as covariable, the effect of which

was significant in both cases ( $F = 5.9, p < 0.05$  for *RM*;  $F = 55.0, p < 0.001$  for *RE*).

## 4. Discussion

### 4.1. Shoot differentiation in *Luma apiculata*

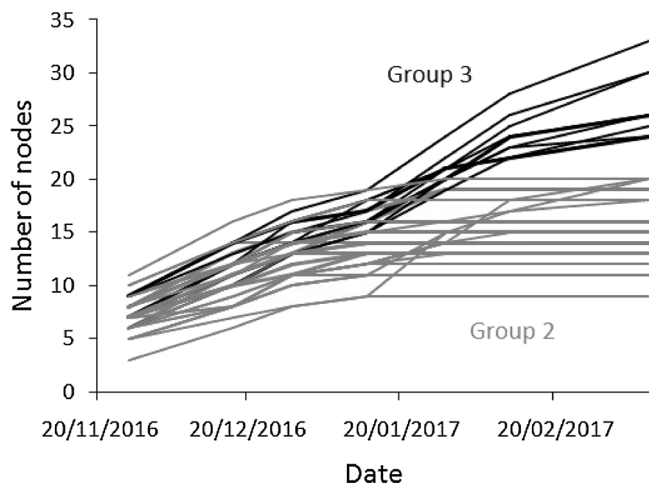
Main-branch shoots of *Luma apiculata* are highly variable in size and structure, which supports the assumption that main branches in this species may play different roles and be included in different functional categories despite their topological similarity. Variability in the structure of *L. apiculata* shoots is linked to the morphogenetic processes involved in shoot development. After comparing the consistently low number of differentiated nodes in apical buds of this species (Sosa and Puntieri, 2016; Fig. 3) and the number of nodes of the smallest shoots sampled here (G1 shoots), it may be concluded that these shoots are

**Table 2**

Type II regression coefficients ( $\beta$ ) and their upper and lower 95% confidence limits ( $\beta 1$  and  $\beta 2$ ) for the relationships between stem cross-sectional area (SCA) and the variables total number of nodes (TN), stem dry mass (SM), dry mass in leaves (LM), vegetative dry mass (VM) and total dry mass (TM), between SM and TN, and between SM and LM for each group of *Luma apiculata* shoots (Groups 1, 2 and 3). In all cases,  $\beta > 1$  indicates a proportionally higher development of the former as compared to the latter descriptor variable; the opposite is true when  $\beta < 1$ .

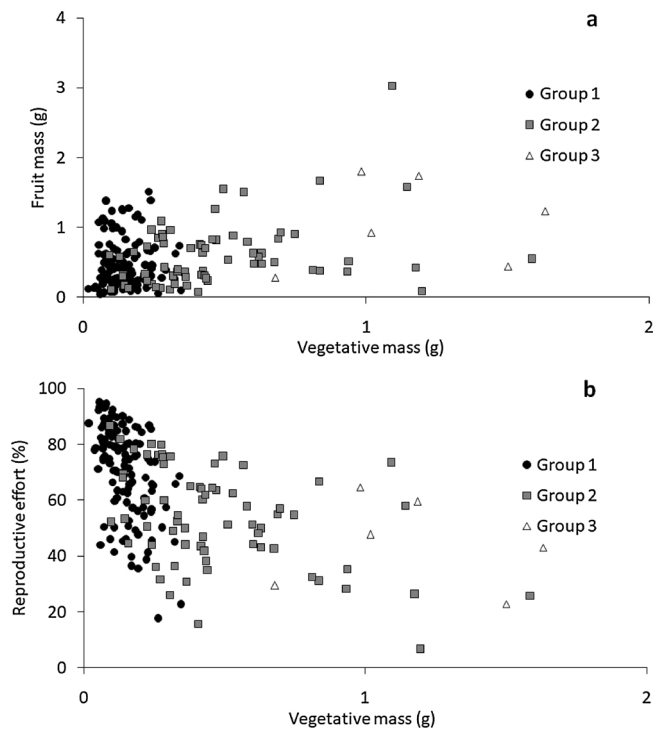
	$\beta$	$\beta 1$	$\beta 2$	$r^2$
<b>Group 1: &lt; 8 nodes</b>				
TN vs SCA <sup>b</sup>	0.848	0.991	0.706	12.9 ***
SM vs SCA <sup>b</sup>	1.285	1.410	1.181	44.1 ***
LM vs SCA <sup>c</sup>	0.503	0.528	0.481	8.0 **
VM vs SCA <sup>c</sup>	0.658	0.701	0.619	14.8 ***
TM vs SCA <sup>b</sup>	0.648	0.686	0.614	11.4 ***
SM vs TN <sup>a</sup>	2.288	2.615	1.961	36.7 ***
SM vs LM <sup>a</sup>	1.717	1.865	1.568	37.8 ***
<b>Group 2: 8–20 nodes</b>				
TN vs SCA <sup>a</sup>	1.066	1.209	0.923	34.4 ***
SM vs SCA <sup>a</sup>	1.511	1.631	1.404	71.6 ***
LM vs SCA <sup>a</sup>	1.052	1.147	0.972	51.8 ***
VM vs SCA <sup>a</sup>	1.151	1.253	1.064	58.8 ***
TM vs SCA <sup>a</sup>	0.956	1.042	0.883	46.9 ***
SM vs TN <sup>b</sup>	1.677	1.841	1.513	65.2 ***
SM vs LM <sup>b</sup>	1.345	1.429	1.260	82.4 ***
<b>Group 3: &gt; 20 nodes</b>				
TN vs SCA <sup>b</sup>	0.833	0.995	0.671	62.4 ***
SM vs SCA <sup>b</sup>	1.217	1.330	1.120	92.0 ***
LM vs SCA <sup>b</sup>	0.748	0.855	0.667	81.0 ***
VM vs SCA <sup>b</sup>	0.908	1.014	0.822	87.5 ***
TM vs SCA <sup>b</sup>	0.711	0.832	0.621	68.9 ***
SM vs TN <sup>b</sup>	1.523	1.745	1.301	78.7 ***
SM vs LM <sup>a</sup>	1.584	1.716	1.452	92.4 ***

$r^2$  coefficients of determination; significance levels: \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ . Low case superscript letters (a, b and c) beside variable names indicate pairwise similarities in  $\beta$  between shoot groups for that variable.



**Fig. 7.** Variation in the number of nodes of 40 shoots of one *Luma apiculata* tree over the 2016–2017 growth season. Black lines: shoots with > 20 nodes at the end of shoot extension. Gray lines and black lines correspond, respectively, to shoots with 8–20 nodes (Group 2) and > 20 nodes (Group 3) at the end of extension.

conformed exclusively by organs that were preformed in the autumn preceding their elongation season. Relatively low levels of preformation in buds, such as those reported for other woody species (Thorp et al., 1994; Souza et al., 2000; Spann et al., 2007), have been related to shoot specialization in light interception (Day et al., 1997; Goulet et al., 2000; Sabatier et al., 2003; Valladares and Niinemets, 2008). This function could also be attributed to short shoots of *L. apiculata*, which combine



**Fig. 8.** Relationships (a) between fruit dry mass and vegetative dry mass (i.e. mass of stem + leaves) and (b) between reproductive effort (% of shoot dry mass in fruits) and vegetative dry mass for *Luma apiculata* shoots discriminated by size (Group 1: < 8 nodes; Group 2: 8–20 nodes; Group 3: > 20 nodes).

exclusively preformed organs with a high leaf area/stem volume relationship (Fig. 4c).

The development of intermediate and large size shoots in main branches of *Luma apiculata* includes the differentiation and expansion of newly formed organs besides those preformed in the preceding autumn. According to previous studies, the development of new organs in addition to preformed organs may take place either immediately before spring budbreak (as in *Fagus sylvatica*; Barthélémy and Caraglio, 2007) or later, as a consequence of neoformation, i.e. simultaneous differentiation and unfolding of organs (as in *Larix*, *Populus*, *Prunus* and *Nothofagus*; Critchfield, 1960; Remphrey and Powell, 1984; Gordon et al., 2006; Guédon et al., 2006). Sharp changes in morphology between preformed leaves and additional leaves have been found for some species (e.g. Critchfield, 1960; Puntieri et al., 2007a), but not for others (Souza et al., 2000; Puntieri et al., 2000). In *L. apiculata*, this transition is gradual, and preformed leaves cannot be distinguished visually from newly formed leaves (Fig. 4a, b); the differentiation of new leaves as shoot expansion takes place may have involved anatomical and/or physiological adjustments to the prevailing conditions during the current growing season (unaccounted for in the present study; e.g. Sims and Percy, 1992).

The length and number of nodes developed by *Luma apiculata* shoots of intermediate and large size were not related to their peak rate of node-internode production, unlike the results observed in other species (Puntieri et al., 1998; Sabatier and Barthélémy, 1999; Stecconi et al., 2000). However, differences in the lengths of proximal internodes and leaves between intermediate and long shoots (Fig. 4a, b) indicate some degree of differentiation between these two shoot groups early in the growing season. A previous study on *Eucalyptus occidentalis* indicated that variations in shoot growth might be related to environmental factors acting on each growth site before budbreak (Jaya et al., 2010). Nonetheless, the fact that seasonal elongation varied notably among shoots in similar positions on the same *L. apiculata* trees suggests that the growth potential of each shoot in this species would be regulated by



endogenous factors, such as the physiological age of the meristem from which the shoot is formed (see Barthélémy and Caraglio, 2007).

In many woody species the number organs of a shoot is highly dependent upon the number of preformed organs in the preceding bud (Remphrey and Powell, 1984; Remphrey and Davidson, 1994; Hover et al., 2017). In these species, axis differentiation is closely related to organ preformation. On the contrary, the present contribution showed that apical buds of *Luma apiculata* include a low and little variable number of preformed organs. Thus, two morphogenetic sources of axis differentiation in woody plants may be distinguished: one that relies strongly on variations in organ preformation, and another one that depends upon meristematic activity in the season of shoot elongation. It could be argued that in plants exhibiting the first pattern, the function of a shoot would be affected by environmental conditions in the growing season preceding that of shoot extension (e.g. Buissart et al., 2018). On the other hand, simultaneous organ differentiation and elongation during the growing season would allow a closer relationship between shoot function and environmental conditions in that particular season. Plants with the latter shoot-growth pattern would be more able to adjust their annual growth to interannual climatic fluctuations, for instance, those related to ENSO (El Niño/Southern Oscillation) and the interaction between this phenomenon and climate change (Latif and Keenleyside, 2009).

#### 4.2. Variations in shoot structure

Size differences among *Luma apiculata* shoots imply variations in the relative development of several of their structural components. In short shoots the increment in stem cross-sectional area was proportionally higher than those in number of nodes, leaf mass, vegetative mass and total mass. The apparently disproportionate development of conducting tissues in short shoots (see Shinozaki et al., 1964; Niklas and Enquist, 2002; Sun et al., 2005), may be related to their high flower/fruit production. Short shoots may need a high water supply due to the high evapotranspiration of the flowers (Lambrecht et al., 2011), and the strong resource demand from fleshy fruits (Fischer et al., 2012). In shoots of intermediate size, in contrast, number of nodes, leaf mass and total mass related isometrically with stem cross-sectional area. Despite the proportionally higher increase in stem cross-sectional area than in number of nodes in short shoots than in shoots of intermediate size, the former shoots exhibited higher leaf area accumulation as compared to that in stem volume towards the shoot's distal end (Fig. 4c). This difference is related to shorter internodes and a more sharply tapering stem in short shoots than in longer shoots.

Taking into account the dynamics of organ elaboration in *Luma apiculata* shoots (Fig. 7), it may be concluded that the development of organs additional to those that were preformed took place in spring for shoots of intermediate size, and in spring and summer for long shoots. Significant structural differences between these two shoot types are evident from the analyses of the relationships between pairs of descriptive variables. Compared to intermediate-size shoots, long shoots could require more resources to stem development than to leaf development (Fig. 6; Table 2). The development of leafy branches (i.e. excluding those branches that consisted exclusively of flowers) also contributed to the structural differences among shoots of *L. apiculata*. The higher probability of immediate (syllaptic) branching in intermediate than in proximal or distal nodes of long shoots (Fig. 2e; Fig. 5c) complies with the results of previous studies on woody species, in which the development of immediate branches has been positively related to the rate of shoot elongation (Puntieri et al., 1998; Barthélémy et al., 1999; Sabatier and Barthélémy, 1999). Branch development in *L. apiculata* shoots of intermediate size was most likely from distal nodes, and sometimes resulted in the development of codominant distal branches (Fig. 2c; Fig. 5b). Distal branching of a growing shoot is frequently associated with shoot-apex withering (post-traumatic branching; Sabatier and Barthélémy, 1999; Wu and Hinchley, 2001; Barthélémy

and Caraglio, 2007; Puntieri et al., 2007b). The latter was not the case for *L. apiculata*, as we verified that the majority of shoot apices were covered with hydrated leaf primordia after the development of branches from nodes below the apex (Fig. 2b).

The structural differences among *Luma apiculata* shoots of different sizes may be linked with different functions. Short shoots would be involved mostly with light interception early in the growing season, as suggested above, whereas the structure of the largest shoots would indicate more involvement in crown expansion (see Sone et al., 2006). Shoots of intermediate size are not, in structural terms, halfway between short and long shoots; e.g. leaf mass was proportional to stem thickening in intermediate-size shoots but not in smaller or larger shoots (Fig. 6; Table 2). Following the results of previous studies in which the structure of shoots along tree axes were analyzed retrospectively (e.g. Passo et al., 2002; Heuret et al., 2006; Stecconi et al., 2010; Magnin et al., 2017; Buissart et al., 2018), it may be inferred that earlier ontogenetic stages would have been expressed in larger than in smaller shoots of *L. apiculata*. Further studies would be necessary in order to clarify the ontogenetic gradients associated with axis development in *L. apiculata* and the role of climatic factors in the expression of such gradients (e.g. Buissart et al., 2018).

#### 4.3. Flower and fruit development in *L. apiculata*

Shoot differentiation in *Luma apiculata* involves variations in the probability of development of reproductive structures, following an inverse proportion with shoot size. In many woody plants, such as *Pinus* spp., *Juglans* spp. and *Nothofagus* spp., topology and ontogeny are closely related to the flowering capacity of an axis; moreover, the relative position of a particular node in a shoot is also tightly linked to the probability of a flower arising at that node (e.g. Guédon et al., 2001; Puntieri et al., 2009; Charles-Dominique et al., 2010; Torres et al., 2016). Despite the fact that flower production in *L. apiculata* concerned mainly proximal shoot nodes, a high extent of variation in flower position was found, and this variation was related to a long flowering period. Flower development in *L. apiculata* may take place within six months of the year, which is a rather long period for a species living under a temperate-cold climate (Armesto et al., 1987; Donoso et al., 2006). Many of the shoots that were harvested for the present study bore, at the same time, flowers and/or fruits. This indicates that the variations in flowering time recorded for this species in previous studies (Donoso et al., 2006 and references therein) may be partly due to the long period in which each shoot is able to flower. Flower development between November and January (spring to early summer) was registered in shoots of all sizes. Wide within-tree variations in flower position and, therefore, flowering time were observed (data not shown but available upon request).

Fruits are stronger resource sinks than stems and leaves (Fischer et al., 2012; Ayala and Lang, 2015). It has been shown for fruit crops, such as *Malus* spp. and *Mangifera mango*, that leaves close to developing fruits exhibit increased photosynthetic capacity as compared to other leaves of the same trees (Hansen, 1971; Urban et al., 2003). In *Luma apiculata*, a high proportion (up to > 80%) of the biomass of short shoots is included in their fruits. Further studies would be necessary to establish the extent to which the fruit set of a shoot in this species is dependent on resource inputs from other shoots, as found for *Prunus avium* (Ayala and Lang, 2015), or exclusively on the photosynthetic capacity of the fruiting shoot's leaves.

## 5. Conclusions

Shoots developed distally on main branches of adult *Luma apiculata* trees may contrast in size and structure as a consequence of different morphogenetic events involved in shoot growth. Structural differences between entirely preformed shoots and those that included organs additional to preformed ones indicate notable variations in main-branch

growth and function. Compared to other Patagonian tree species so far investigated, *L. apiculata* would exhibit a higher capacity to adjust the yearly extension of main branches following internal and/or external factors operating during the growing season. This would imply more plasticity in axis structure and, therefore, a better fitting to environmental conditions. These results may explain why the architecture of adult but relatively young *L. apiculata* trees often exhibits a low level of differentiation among axes (i.e. a shrubby or less hierarchical development). More investigations on the developmental differences between species from climatically-contrasting regions may help in understanding the connections among plant architecture, plant ecology and climate.

## Acknowledgements

We are indebted to the authorities of Administración de Parques Nacionales de Argentina for allowing the sampling of shoots within nature reserves. We also thank Ezequiel Villacide (Universidad Nacional de Río Negro) for helping in sample processing. This research was supported by CONICET (CCT Patagonia Norte, Argentina). We are much indebted to Claudia Sosa, whose study on preformation in Patagonian trees inspired the development of the present study.

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