Preformation, neoformation, and shoot structure in *Nothofagus dombeyi* (Nothofagaceae)

Javier G. Puntieri, María S. Souza, Daniel Barthélémy, Cecilia Brion, Martín Nuñez, and Camilo Mazzini

Abstract: Buds in different positions along trunk, main branch, secondary branch, and short branch parent shoots of young *Nothofagus dombeyi* (Mirb.) Blume trees were dissected, and the number of organs of their rudimentary shoots was counted. Bud contents were compared with the number of organs of sibling shoots developed in positions equivalent to those of the dissected buds. Cataphyll number was relatively constant for all buds. The number of green leaf primordia differentiated in each bud depended both on the position of the bud on the parent shoot and on the size of the parent shoot. Sibling shoots derived distally from large parent shoots had more nodes than the rudimentary shoots of buds in a similar position. Proximal sibling shoots derived from large parent shoots and all sibling shoots derived from small parent shoots are entirely preformed. In *N. dombeyi*, the size gradient of the sibling shoots derived from a particular parent shoot relates mostly to variation in organ preformation, organ neoformation, and internode extension. The expression of each of these sources of variation is related to the position of the sibling shoot on the parent shoot and on the position of the parent shoot on the tree. Consideration is given to the role of environmental conditions on tree development, in view of the species' morphogenetic gradients

Key words: branching, bud content, sylleptic branching, leaf primordia, *Nothofagus*, preformation.

Résumé : Les auteurs ont disséqué des bourgeons occupant différentes positions sur le tronc, les branches principales, les branches secondaires et les tiges mères des branches courtes de jeunes *Nothofagus dombeyi* (Mirb.) Blume et ils ont compté le nombre d'organes de leurs tiges rudimentaires. Ils ont comparé les contenus des bourgeons avec le nombre d'organes de tiges sœurs développées en positions équivalentes à celles des bourgeons disséqués. Le nombre de cataphylles est relativement constant dans tous les bourgeons. Le nombre de primordiums de feuilles vertes différenciés dans chaque bourgeon dépend à la fois de la position du bourgeon sur la tige mère et de la dimension de la tige mère. Les tiges sœurs dérivées distalement de grosses tiges mères ont plus de nœuds que les tiges rudimentaire des bourgeons occupant une position similaire. Les tiges sœurs proximales dérivées de grosses tiges mères et les tiges sœurs dérivées de petites tiges mères sont toutes préformées. Chez le *N. dombeyi*, le gradient de dimension des tiges sœurs dérivées d'une tige mère donnée est relié surtout à la variation de la préformation des organes, à la néoformation d'organes et à l'extension des entrenœuds. L'expression de chacune de ces sources de variation est reliée à la position de la tige sœur sur la tige mère et à la position de la tige mère sur l'arbre. On discute le rôle des conditions environnementales sur le développement de l'arbre, dans la perspective des gradients morphogénétiques des espèces.

Mots clés : ramification, contenu des bourgeons, ramification sylleptique, primordium foliaire, *Nothofagus*, préformation.

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Introduction

The aboveground architecture of a tree is the way in which the structural units of the stem are arranged at a given time. In the architectural analysis of trees, the organization of different levels of structural units at different stages of tree growth are studied to unveil the endogenous developmental rules characteristic of the species concerned (Hallé and Oldeman 1970; Hallé et al. 1978). The axis, which may be defined as an approximately straight-growing stem portion, is one of these structural units. Axis length growth in vascular plants consists of two processes: organogenesis and

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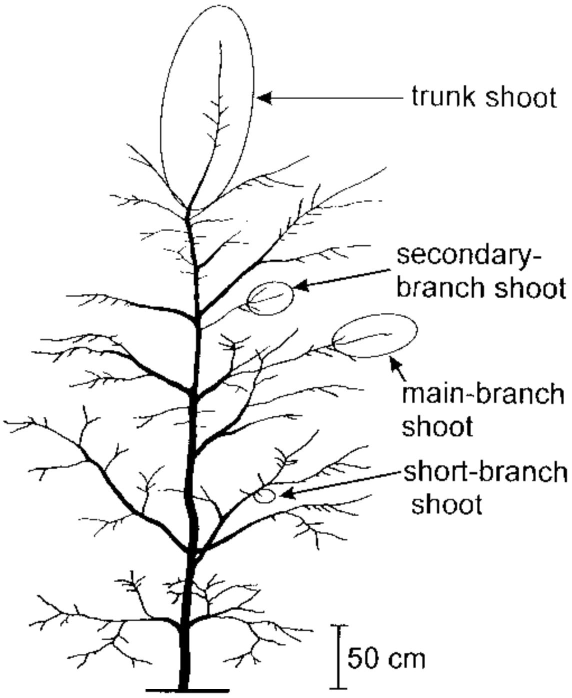
extension. During organogenesis, each of the cells that will contribute to a specific organ differentiates from the meristem located at or close to the axis' distal end. In the more directly visible extension process, all differentiated cells increase in size irreversibly (Steeves and Sussex 1989). Between organogenesis and extension, differentiated rudimentary organs may be enclosed in a bud, whose formation may be recognised a posteriori by the scars left by cataphylls and associated short internodes. Organ primordia that were differentiated in a bud before their extension are referred to as "preformed organs." Organs that are never included in a bud as primordia and extend as they are differentiated by the meristem are named "neoformed organs" (Hallé et al. 1978; Caraglio and Barthélémy 1997).

In many woody species, each axis may be viewed as consisting of segments or shoots reflecting the alternation of periods of extension and periods of rest. A number of studies support the notion of "morphogenetic gradient" or "differentiation sequence," by which each plant species has a characteristic sequence of variation in shoot morphology and physiology that is expressed along each axis. Such a sequence would be endogenously determined; the environment would vary the rate of passage from one stage of that sequence to the next, but it would not affect the sequence itself. Among the morphological features of shoots usually linked to particular morphogenetic stages of a species are length, number of internodes, number of preformed and neoformed organs, and the probabilities of apex persistence, branching, and flowering (Barthélémy et al. 1997).

The study of the morphogenetic gradients of plants and the morphological features associated with them contributes to the understanding of tree architecture and the effects of environmental conditions on it (Reffye et al. 1997). Recent advances in the mathematical modelling and simulation of plant growth have rendered this information relevant for detailed plant growth simulations with applications in forestry, horticulture and landscape design (Reffye et al. 1993, 1995, 1997). The basic developmental information feeding these models is limited. In particular, studies concerning organ preformation and neoformation are scarce (Hallé et al. 1978; but see Caesar and Macdonald 1983; Remphrey and Powell 1984).

Nothofagus dombeyi (Mirb.) Blume (Nothofagaceae) is one of the largest and most widespread tree species in temperate-cold regions of southern South America. It forms self-sown pure or mixed stands in the wettest areas of the Subantarctic Forest Region (Roig 1998). The ecological aspects of *N. dombeyi* forests have been the subject of a number of studies (Veblen et al. 1977; Veblen 1985; Dezzotti 1996; Veblen et al. 1996*a*, 1996*b*; among others). Far less attention has been paid to the vegetative morphology of this and other species of *Nothofagus*, although this could significantly contribute to our understanding of their architectural and ecological features and their evolutionary links (but see Thiébaut et al. 1997; Puntieri et al. 1998; Raffaele et al. 1998; Barthélémy et al. 1999).

Fig. 1. Diagrammatic illustration of the branching system of a 10-year-old *Nothofagus dombeyi* tree, representative of those selected for the present study, indicating the four positions from which shoots were sampled.



In *Nothofagus* spp., axis extension is seasonal, as in many other tree species (Hallé et al. 1978; Barthélémy et al. 1999). By the end of its spring–summer extension period, each shoot has developed a set of axillary buds and, sometimes, a terminal bud, all of which remain dormant until the following spring bud break. In the present study, we analysed the content of buds in different positions on young *N. dombeyi* trees, and the relationship between bud content and the morphology of the shoots derived, the following year, from buds in equivalent positions.

Materials and methods

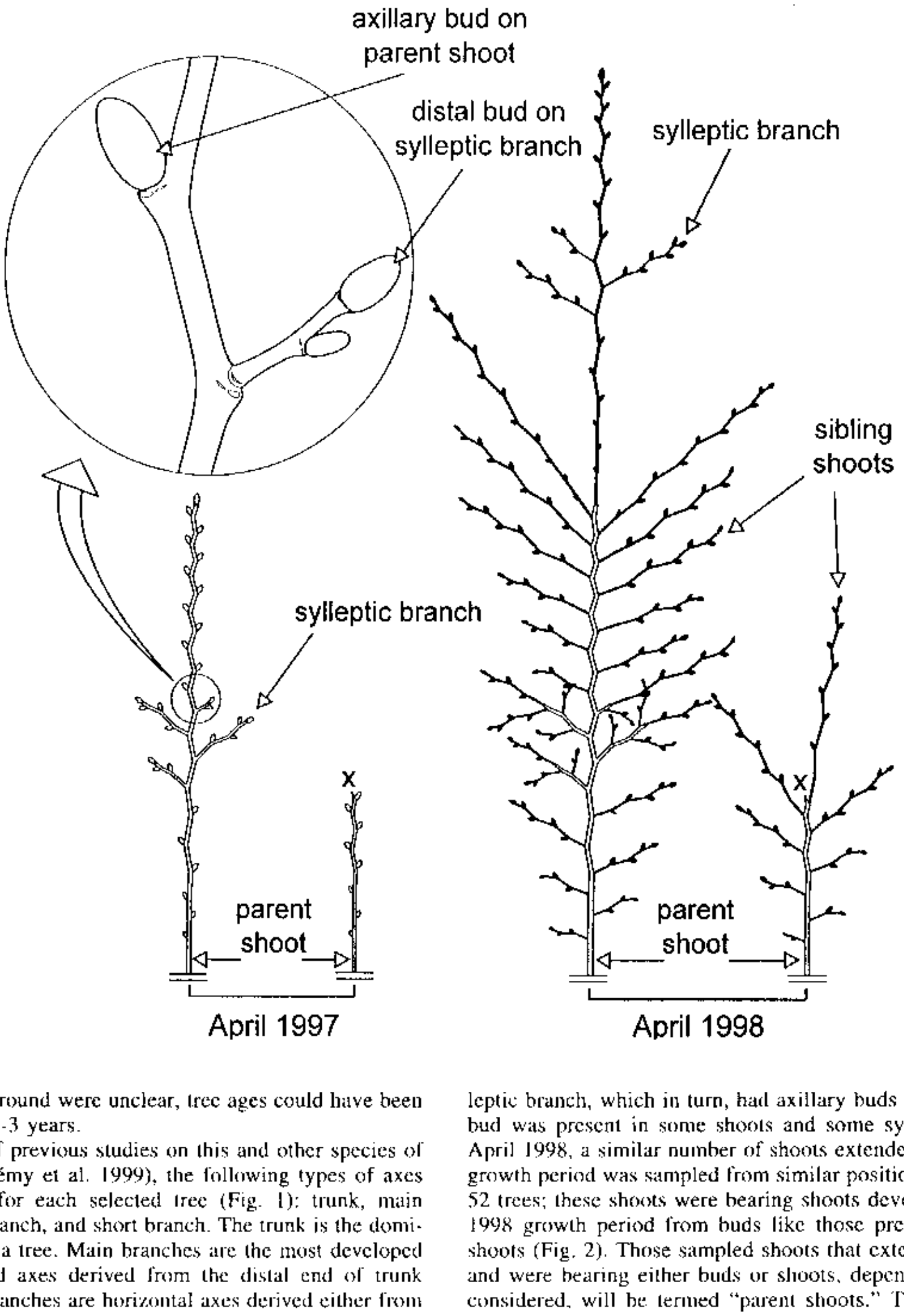
Sampling site

Samples were taken from a stand along a roadside between the localities of San Carlos de Bariloche and Villa Mascardi, Argentina (41°10′S, 71°10′W, 850 m altitude). This population was selected because of the high number of healthy, young *N. dombeyi* trees with a well-differentiated vertical trunk and vigorous main branches not shaded by neighbouring individuals. Mean annual precipitation in this area reaches about 1000 mm and concentrates in autumn and winter; mean temperatures are 14.0°C for the warmest month and 2.4°C for the coldest (Conti 1998). The soil is derived mostly from volcanic ash (Scoppa 1998). The population sampled represents a naturally regenerated mixed *Austrocedrus chilensis* (D. Don) Pic. Serm. & Bizzarri (Cupressaceae) – *N. dombeyi* forest, which occupied the area before road construction.

Shoot sampling and data records

Trees were randomly selected from those 10–15 years old and 2.8–5.3 m high, with a basal diameter of 37–95 mm. Individuals with signs of damage caused by exogenous factors were avoided. Tree age was estimated by counting the number of annual shoots of the trunk, which are limited by cataphyll scars. Since cataphyll

Fig. 2. Diagrammatic illustrations of two representative *Nothofagus dombeyi* parent shoots sampled by the end of the 1996–1997 growth period (April 1997) and by the end of the 1997–1998 growth period (April 1998). Open lines correspond to shoots extended in the 1996–1997 growth period (= parent shoots), including their sylleptic branches. Shoots extended in the 1997–1998 growth period (= sibling shoots), including their sylleptic branches, are shown as solid lines. A detail of a parent shoot portion showing an axillary bud on the parent shoot and the distal bud of a sylleptic branch are indicated. The double-thickness lines indicate the proximal limit of parent shoots. X, dead apex of the parent shoot.

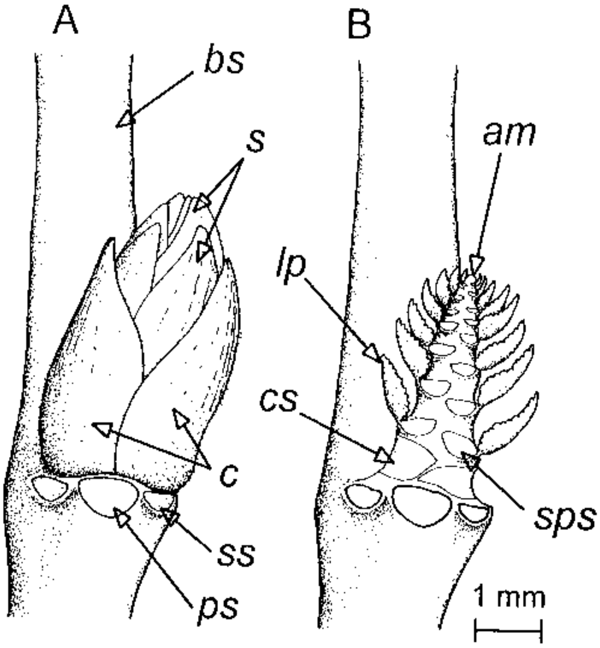


scars closest to the ground were unclear, tree ages could have been underestimated by 1–3 years.

On the grounds of previous studies on this and other species of *Nothofagus* (Barthélémy et al. 1999), the following types of axes were distinguished for each selected tree (Fig. 1): trunk, main branch, secondary branch, and short branch. The trunk is the dominant vertical axis of a tree. Main branches are the most developed horizontal or slanted axes derived from the distal end of trunk shoots. Secondary branches are horizontal axes derived either from intermediate nodes of trunk shoots or from main branch shoots. Short branches are horizontal axes derived from nodes close to the proximal end of trunk, main branch, and secondary branch shoots.

In April 1997 (early autumn), shoots extended in the 1996–1997 growth period were sampled from each of 52 trees (Fig. 2). Four shoots, corresponding to each of the types of axes mentioned above, were sampled from each tree. In each axillary position, these shoots had an axillary bud or, in some trunk shoots, a sylleptic branch, which in turn, had axillary buds (Fig. 2). A terminal bud was present in some shoots and some sylleptic branches. In April 1998, a similar number of shoots extended in the 1996–1997 growth period was sampled from similar positions of another set of 52 trees; these shoots were bearing shoots developed in the 1997–1998 growth period from buds like those present on April 1997 shoots (Fig. 2). Those sampled shoots that extended in 1996–1997 and were bearing either buds or shoots, depending on the sample considered, will be termed "parent shoots." The expression "sibling shoots" was considered appropriate for those shoots borne by the parent shoots and extended in the 1997–1998 growth period. The extent of organ differentiation in buds in the early autumn to winter period was assessed by means of a sample of parent shoots collected in September 1997, about 2 weeks before bud break. This sample included 20 trunk parent shoots. Hereafter, April 1997, September 1997, and April 1998 samples will be referred to as sample I, sample II, and sample III, respectively. All shoots except

Fig. 3. Illustrations of a closed bud (A) and a dissected bud (B) of *Nothofagus dombeyi*. Both cataphylls (c) and the stipules of green leaf primordia (s) are shown in Fig. 3A. In Fig. 3B, the scars left by the second cataphyll (cs) and by the stipule of the first green leaf primordium (sps), the blade primordium of the second green leaf (lp) and the apical meristem (am) of the rudimentary shoot are indicated. bs, portion of the bud's parent shoot; ps, scar left by the petiole of the subtending leaf; ss, scar of one of the stipules of the subtending leaf.



trunk shoots were selected from intermediate positions (1.5–2.0 m high) within each tree.

For each parent shoot, the following information was recorded at about the time of each sampling: origin within the plant (terminal bud or axillary bud), length, basal diameter, number of nodes, number of basal cataphylls, number of green leaves, and apex condition (dead or persistent). Shoot length was measured to the nearest millimetre with a measuring tape. Shoot basal diameter was measured to the nearest 0.1 mm with digital calipers. Cataphylls and green leaves were distinguished by the presence in the latter of a lamina between the stipules. All nodes of each parent shoot were correlatively ranked starting with the distal node (terminal bud: rank = 0, distal axillary bud: rank = 1). The presence of a bud (samples I and II), a sibling shoot (sample III), or a sylleptic branch (all samples) were recorded for each node (Fig. 2). The nodes of each sylleptic branch developing on each parent shoot were counted. In some nodes, no evident axillary structure was present. The terminal structure of each parent shoot with a persistent apex was always a bud (samples I and II) or a sibling shoot (sample III). Parent shoots with an intra-annual relay branch developed after the death of the apex (a usual response to damage due to exogenous factors in this species; Puntieri et al. 1998) as well as parent shoots, buds, sylleptic branches, and sibling shoots with signs of damage caused by herbivores were all excluded from the study.

Sample I and sample II parent shoots were preserved in 96% ethanol for 2 weeks after sampling, to facilitate bud dissection. The terminal bud (whenever present) and the axillary bud on each parent shoot node were dissected under a stereomicroscope (Olympus SZ30, 40×; Fig. 3). For the rudimentary shoot present in each bud, nodes, cataphylls, and green leaf primordia were counted. The number of nodes differentiated in the distal bud of each sylleptic branch (Fig. 2, detail) was registered. Number of nodes, length and basal diameter were recorded for the terminal (if present) and the axillary sibling shoots derived from sample III parent shoots. The nodes of the distal sibling shoot derived from a parent shoot's sylleptic branch were counted.

Data analysis

A previous study had shown that bud origin (terminal or axillary), number of nodes, and presence–absence of sylleptic branches are related to the branching pattern of a *N. dombeyi* shoot (Puntieri et al. 1998). To enable comparisons among samples, parent shoots within each sample were regrouped according to these features. The resulting groups are defined in Table 1. Additional groups could have been formed, but they contained too few parent shoots to be included in the analyses.

For groups 1 and 2, the mean number of nodes of the rudimentary shoots present in terminal buds was compared with the mean number of nodes in sibling shoots derived from terminal buds (one-way ANOVA; Sokal and Rohlf 1981). For all other groups, terminal buds and sibling shoots derived from terminal buds were insufficient for statistical analyses.

For each sample I and sample II parent shoot, an average number of nodes per axillary bud was obtained for the following ranges of ranked nodes: positions 1–5, all groups; positions 6–10, groups 1–4; positions 11–15, groups 1–3; positions 16–20, groups 1 and 2; and positions 21–25, group 1. Nodes devoid of an axillary bud or bearing a sylleptic branch were considered missing values. A mean number of nodes within each group was, in turn, obtained for each range of positions. Similar procedures were followed for the number of nodes of sample III sibling shoots of each group and for the number of nodes in buds and sibling shoots developed distally on sylleptic branches of group 1 shoots. For each group, comparisons among nodes in axillary buds and nodes in sibling shoots were carried out (one-way ANOVA) for each range of ranked positions.

For parent shoots in group 1, the number of nodes in axillary buds and in sibling shoots directly derived from parent shoots were compared with those in buds and sibling shoots on sylleptic branches (two-way ANOVA). Factors in these comparisons were (*i*) the bearing structure of the bud or sibling shoot (i.e., parent shoot or sylleptic branch) and (*ii*) the sample (I, II, and III).

Results

Parent shoot size

Trunk parent shoots corresponded mostly to groups 1 and 2 (Table 1). Parent shoots of main branches were included in groups 1–4; those of secondary branches corresponded mostly to group 4, and those of short branches to group 5 (Table 1).

For each sample, both length and basal diameter of the parent shoot decreased from group 1 to group 5 (Table 2). On average, parent shoots in group 1 were 20 times longer than those in group 5. Differences among groups in the basal diameter of parent shoots depended on the sample considered (Table 2). Basal diameter increased between samples I and III (because of cambial activity) for parent shoots corresponding to groups 1, 2, and 3 but not for those of groups 4 and 5. The basal diameter of parent shoots in group 1 did not vary notably between samples I and II. Cataphylls were restricted to the proximal end of each parent shoot. Most parent shoots in group 3 had zero to two cataphylls, whereas most parent shoots in groups 1, 2, 4, and 5 had two or three

Table 1. Morphological features of parent shoots corresponding to groups 1–5: position of the bud of origin (axillary or terminal), number of nodes, and presence or absence of sylleptic branches.

	Morphological features			No. of shoots corresponding to each position			
Shoot group	Bud position	No. of nodes	Sylleptic branches	Trunk	Main branch	Secondary branch	Short branch
1	Axillary	21–41	Present	52	13	0	0
2	Axillary	21–41	Absent	19	32	0	0
3	Terminal	11–20	Absent	2	23	2	0
4	Axillary	11–20	Absent	0	22	79	10
5	Axillary	<11	Absent	0	0	21	91

Table 2. Length, basal diameter, number of basal cataphylls, and number of green leaves of parent shoots of *Nothofagus dombeyi* for samples I (April 1997), II (September 1997), and III (April 1998) and groups 1–5.

Sample and group	Length (mm)	Diameter (mm)	No. of cataphylls	No. of green leaves	Persistent apex (%)	*N*
I						
1	578 (43)	6.4 (0.4)	2 (0.3)	26 (1.1)	41	27
2	335 (31)	3.3 (0.1)	3 (0.3)	23 (1.1)	54	25
3	203 (27)	2.8 (0.2)	1 (0.3)	15 (0.8)	25	14
4	95 (8)	2.4 (0.1)	2 (0.1)	12 (0.3)	0	46
5	25 (2)	1.6 (0.1)	2 (0.1)	6 (0.2)	0	63
II						
1	526 (55)	6.2 (0.5)	2 (0.3)	25 (1.5)	25	12
III						
1	569 (46)	9.9 (0.7)	2 (0.2)	28 (1.2)	46	26
2	415 (34)	8.2 (0.7)	2 (0.1)	25 (1.0)	39	24
3	197 (16)	3.7 (0.3)	1 (0.2)	15 (0.7)	31	13
4	111 (6)	2.8 (0.1)	2 (0.0)	13 (0.3)	12	65
5	28 (2)	1.5 (0.1)	2 (0.1)	6 (0.2)	0	49

Note: Values are means with SE given in parentheses. The percentage of parent shoots with persistent apices is also indicated. *N*, total number of shoots of each group in each sample.

cataphylls (Table 2). Differences among groups in leaf number per parent shoot were largely due to differences in the number of green leaves. The percentage of parent shoots with persistent apices was higher for groups 1, 2, and 3 than for groups 4 and 5 (Table 2). Within each group, parent shoots sampled at different times were similar in mean length and number of leaves.

In parent shoots of all groups, neither the cataphylls nor the one to eight green leaves closest to the shoot's proximal end subtended any externally visible organ. A tiny (<1 mm long) bud, not dissectable with the available means, was seen in some of these axillary positions. All other nodes had either a dissectable bud (samples I and II) or a sibling shoot (sample III), or had developed a sylleptic branch as the parent shoot extended (group 1). Sylleptic branches were found mostly among ranked positions 6 to 25 of parent shoots in group 1. Each of these shoots had between 1 and 16 sylleptic branches. The number of nodes of the sylleptic branches in each range of ranked positions was as follows: positions 6–10, 8 ± 0.5 (mean ± SE); positions 11–15, 10 ± 0.5; positions 16–20, 12 ± 0.8; and positions 21–25, 12 ± 1.0.

Number of nodes in buds and sibling shoots

Each bud had a rudimentary shoot with a cataphyll or a green leaf primordium at each of its nodes. None of the buds contained flower primordia.

The number of nodes in terminal buds of sample I was 11 ± 0.9 ($N = 18$) for group 1 and 10 ± 1.0 ($N = 11$) for group 2. The mean number of nodes of sibling shoots derived from terminal buds was 23 ± 2.0 ($N = 15$) for group 1 and 19 ± 1.3 ($N = 14$) for group 2; these numbers were significantly higher than the corresponding mean number of nodes in terminal buds (group 1: $F = 30.7$, group 2: $F = 26.8$, $p < 0.001$).

The number of nodes in axillary buds was clearly higher in distal than in proximal positions for groups 1, 2, and 3 and varied only slightly with bud position for group 4 and was constant for group 5 (Fig. 4). The distal axillary buds of groups 1, 2, and 3 had a higher number of nodes than those of group 4, which in turn, had a higher number of nodes than those of group 5 (Fig. 4). For groups 1 and 2, the number of nodes in the distal axillary buds (positions 1–5; Tables 3 and 4) was significantly higher ($p < 0.05$) than the number of nodes in terminal buds of the same groups ($F = 4.5$ and $F = 6.5$, respectively). The mean number of nodes of the distal buds of sample I shoots was similar among groups 1, 2 and 3, and higher for these than for the most distal buds of groups 4 and 5 (Fig. 4, Tables 3 and 4).

For group 1, a significant difference ($p < 0.001$) among samples was found for the range of ranked nodes (1–5): sample III sibling shoots had, on average, more nodes than sample I buds, but sample II buds were not different from sample I buds or from sample III sibling shoots (Fig. 4A.

Fig. 4. Mean number of nodes of the rudimentary shoot in sample I buds (filled circles) and in sample II buds (shaded triangles) and the mean number of nodes in sibling shoots of sample III (open squares) for positions ranked from the distal end of the parent shoot, for groups 1 (A), 2 (B), 3 (C), 4 (D), and 5 (E). Error bars are SE.

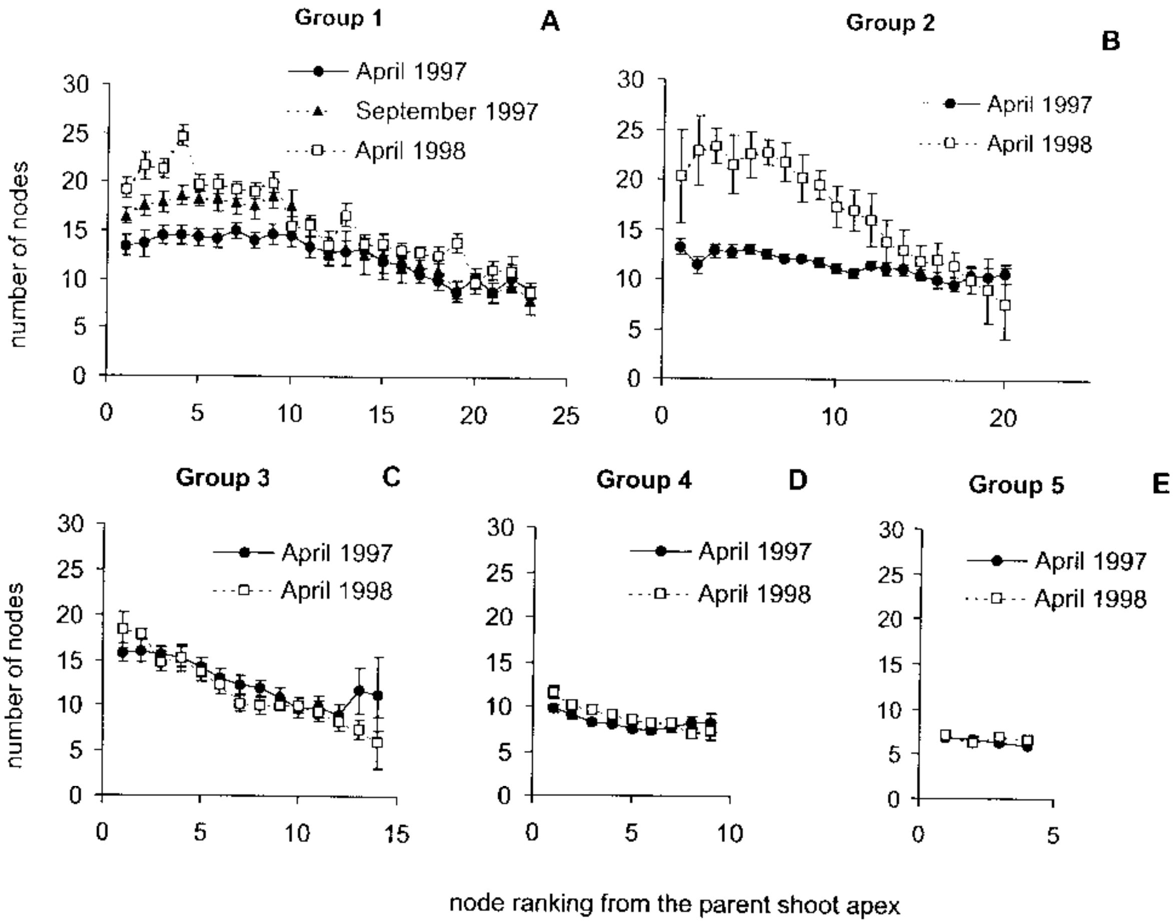


Table 3). For the range of ranked nodes 6–10, a less significant ($p < 0.05$) difference in node number was found among samples (Fig. 4A, Table 3). For the same range of ranked nodes, buds and sibling shoots of sylleptic branches had a very significantly lower number of nodes than buds and sibling shoots directly developed on the parent shoot (Table 3). For positions closer to the proximal end of group 1 shoots, buds and sibling shoots derived from parent shoots had a similar number of nodes than those derived from sylleptic branches. For position range 16–20, sample I buds had a significantly lower number of nodes than sample II buds and sample III sibling shoots.

For group 2, sibling shoots in positions 1–5 and 6–10 had a very significantly higher number of nodes than axillary buds in the same positions; buds and sibling shoots in other positions had a similar number of nodes (Fig. 4B, Table 4). For shoots in group 3, the number of nodes of sibling shoots in each range of ranked positions was similar to that of the corresponding buds. Distal sibling shoots of group 4 had a significantly higher number of nodes than distal buds of the same group. For group 5, the number of nodes of sibling shoots was constant and similar to the number of buds in similar positions.

Length and diameter of sibling shoots

The mean length of sibling shoots derived from terminal buds was 405 ± 60 mm for group 1 and 212 ± 27 mm for group 2. The length of axillary sibling shoots decreased exponentially from distal to proximal positions in groups 1, 2, and 3 (Fig. 5A). A less notable trend in the length of sibling shoots according to position was observed for group 4. For group 5, the length of sibling shoots did not vary with their position on the parent shoot.

The mean basal diameters of sibling shoots derived from terminal buds were 4.1 ± 0.4 and 2.2 ± 0.1 mm for group 1 and group 2, respectively. The basal diameter of axillary sibling shoots tended to increase linearly from proximal to distal positions of parent shoots corresponding to groups 1, 2, 3, and 4 (Fig. 5B). For group 5, the basal diameter of sibling shoots did not vary with their position.

Discussion

Preformation and neoformation in *N. dombeyi*

By the end of its extension period, each *N. dombeyi* shoot has well-developed buds that include cataphylls and green leaf primordia. Like *Nothofagus pumilio* (Poepp. & Endl.)

Table 3. (A) Number of nodes in axillary buds (samples I and II) and sibling shoots (sample III) in different ranges of positions ranked from the apex of parent shoots in group 1. (B) Number of nodes of the most distal bud (samples I and II) and the most distal sibling shoot derived from a sylleptic branch (sample III), according to the position of the sylleptic branch on the parent shoot. (C) Summary of *F* tests comparing the number of nodes among samples (positions 1–5) or both between samples and between organs derived from parent shoots and organs derived from sylleptic branches, for each range of ranked positions.

(A) No. of nodes in axillary buds and sibling shoots.

	Positions 1–5		Positions 6–10		Positions 11–15		Positions 16–20		Positions 21–25	
Sample	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*
I	14 (1.0)	20	14 (0.8)	20	13 (0.8)	14	10 (0.7)	14	9 (0.5)	7
II	18 (0.7)	12	18 (1.0)	10	17 (0.9)	10	13 (1.4)	8	10 (1.0)	7
III	22 (1.2)	20	18 (1.2)	22	15 (1.7)	17	12 (1.4)	12	8 (1.5)	8

(B) No. of nodes of the most distal bud and the most distal sibling shoot derived from a sylleptic branch.

	Positions 1–5		Positions 6–10		Positions 11–15		Positions 16–20		Positions 21–25	
Sample	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*
I	—		11 (1.5)	7	12 (0.8)	14	10 (0.8)	11	9 (0.8)	8
II	—		14 (0.5)	8	14 (1.1)	6	12 (0.6)	3	8 (1.6)	2
III			12 (1.5)	8	16 (1.5)	13	12 (1.8)	12	13 (2.6)	4

(C) ANOVA results.

	Positions 1–5	Positions 6–10	Positions 11–15	Positions 16–20	Positions 21–25
Bearing structure	—	14.7***	0.2ns	0.7ns	0.0ns
Sample	14.9***	3.3*	2.9ns	4.5*	0.8ns
Bearing structure × sample	—	0.4ns	2.1ns	1.1ns	0.2ns

Note: Statistical significance is as follows: ***, $p < 0.001$; *, $p < 0.05$; ns, $p > 0.05$.

Table 4. Number of nodes of rudimentary shoots in axillary buds (sample I) and number of nodes in sibling shoots (sample III) in different ranges of positions counted from the apex of parent shoots of groups 2, 3, 4, and 5.

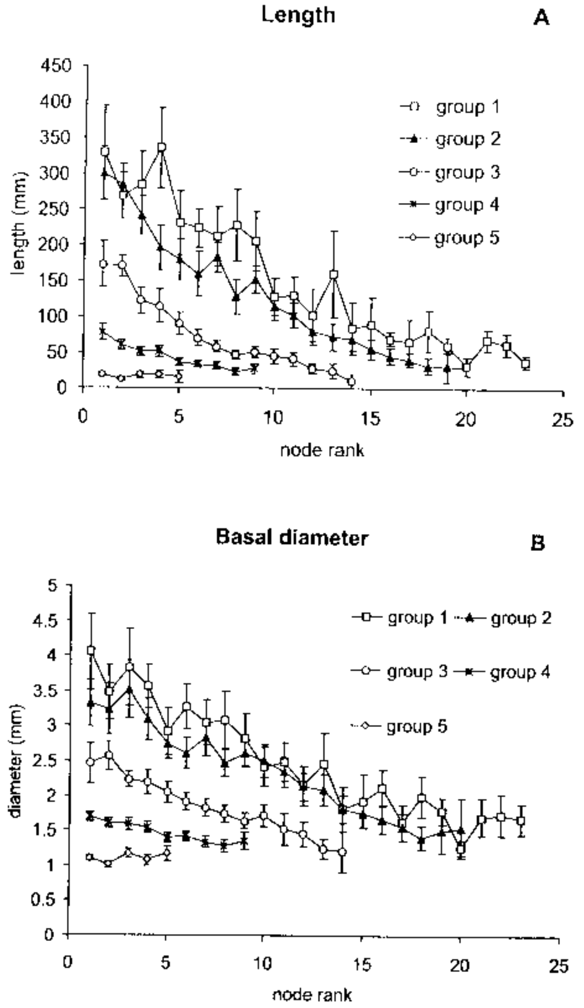
Group and sample	Positions 1–5		Positions 6–10		Positions 11–15		Positions 16–20	
	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*	Mean (SE)	*N*
2								
I	13 (0.6)	24	12 (0.3)	24	11 (0.4)	24	9 (0.5)	20
III	21 (1.0)	18	17 (1.0)	20	12 (1.0)	20	8 (0.8)	13
F	56.3***		35.4***		2.8ns		0.4ns	
3								
I	15 (1.0)	15	12 (0.9)	15	9 (1.1)	10		
III	16 (0.9)	13	11 (0.7)	13	8 (0.6)	7		
F	0.1ns		0.9ns		0.3ns			
4								
I	8 (0.4)	46	7 (0.3)	27				
III	11 (0.5)	65	8 (0.4)	45				
F	10.6**		0.6ns					
5								
I	6 (0.2)	52						
III	7 (0.2)	49						
F	1.2ns							

Note: The results of *F* tests comparing number of nodes of buds (sample I) and sibling shoots (sample III) are summarized for each range of positions and each group. ***, $p < 0.001$; **, $p < 0.01$; ns, $p > 0.05$.

Krasser (Souza et al. 2000), the number of cataphylls in axillary buds of *N. dombeyi* does not depend on bud position, and it is low (between zero and four) compared with that in species of related genera: 10–40 in *Quercus* spp., 5–9 in *Castanopsis sieboldii* (Makino) Hatusima ex Yamazaki et Mashiba, 10–15 in *Lithocarpus edulis* Nakai (Nitta and Ohsawa 1998), and 7–11 in *Fagus sylvatica* L. (Nicolini 1996). In contrast, the number of green leaf primordia in each bud varies notably (from 3 to 26) according to bud position and is high compared with that in other angiosperm tree species studied (Assaf 1965, in Rivals 1965; Macdonald et al. 1984; Remphrey and Davidson 1994).

Each of the proximal axillary buds developed by a *N. dombeyi* shoot after its extension includes a rudimentary shoot with a number of nodes similar to that of the sibling shoot, which will develop from that bud in the following

Fig. 5. Mean length (A) and basal diameter (B) of sibling shoots developed in different axillary positions (ranked from the distal end of the parent shoot) for groups 1, 2, 3, 4, and 5. Error bars are SE.



growing season. These sibling shoots are thus entirely preformed by the end of parent shoot extension. This is the case also for the distal sibling shoots developed by short shoots in this species. On the other hand, the terminal bud and all axillary buds developed distally on larger parent shoots may include, by the end of parent shoot extension, only a proportion of the organs of the sibling shoots that will develop from them. In other tree species, organs additional to those already preformed by the end of parent shoot extension may differentiate later in the autumn (see Rivals 1965), in the following spring, from about the time of bud break (as in long shoots of *Betula papyrifera* Marsh.; Macdonald et al. 1984), and (or) after the extension of preformed organs (as in *Populus trichocarpa* Torr. & Gray; Critchfield 1960). The present results and those of a following study carried out by some of us (J. Puntieri and C. Mazzini, unpublished data) suggest that, in the case of *N. dombeyi*, organs additional to those differentiated in buds by the time parent shoot extension stopped would differentiate from about the time of bud break in spring through to the summer. This would explain the difference in number of nodes among buds sampled in April 1997, buds sampled in September 1997, and sibling shoots sampled in April 1998 for the most distal axillary positions of long shoots (Fig. 4A, Table 3).

A study carried out on another species of *Nothofagus* from South America (*N. pumilio*) yielded similar results resembling the present ones: distal shoots derived from the trunk and the main branches of young trees consisted of preformed organs and neoformed organs (Souza et al. 2000). In contrast, the related species *F. sylvatica* (Fagaceae) seems not to have the possibility of neoformation (Roloff 1987). In *Nothofagus*, the number of organs differentiated in a bud may be influenced by the environmental conditions under which the parent shoot of that bud extended, as in other tree species (Remphrey and Davidson 1994). Environmental conditions in the following growing season might affect the amount of neoformation in extending shoots positioned distally on the trunk and the main branches (Davidson and Remphrey 1994). Neoformation may be seen as an opportunistic way of increasing the exploration of the surrounding environment by the trunk and the main branches of young *Nothofagus* trees. However, extensive neoformation in long shoots of *Nothofagus* may result in the death of their growing distal end late in the growing season, as stressful conditions, not uncommon in northwestern Patagonia during the summer (Conti 1998), take place before shoot growth stops and shoot apices harden.

Sibling shoot size gradients and parent shoot size

In general terms, the length, diameter, and number of nodes of sibling shoots increase from the proximal to the distal end of all but the smallest parent shoots of *N. dombeyi*, as in *N. pumilio* (Souza et al. 2000), *Nothofagus antarctica* (G. Forster) Oersted (Stecconi et al. 2000), and other species of the Fagaceae–Nothofagaceae–Betulaceae complex (see Collin et al. 1996; Soumoy et al. 1996; Thiébaut et al. 1997; Nicolini 1998). The extent of preformation in axillary buds is the first observable component of such gradients. For a given parent shoot with more than 10 nodes, the closer to its distal end a bud is formed, the higher the number of rudimentary organs it is likely to have. However, for the largest parent shoots, the number of nodes in buds tends to level off for buds close to the distal position. The lower number of nodes differentiated in the terminal buds of these shoots than in the five distal axillary buds of these shoots may be a consequence of the long extension period of this species; terminal buds are differentiated in early autumn (Puntieri et al. 1998), and the deteriorating environmental conditions at that time would limit organogenesis in these buds (see Rivals 1965). The same explanation would apply to the low amount of preformation found in distal buds of distal sylleptic branches as compared with that of distal buds directly developed on the parent shoot. The higher number of leaf primordia found for other tree species in terminal than in axillary buds (Remphrey and Powell 1984; Remphrey and Davidson 1994) could be due to the shorter extension period of shoots in these species.

Nothofagus dombeyi shoots of very different number of nodes (11–41 nodes) develop sibling shoots that may include neoformed organs in addition to those preformed after parent shoot extension. Since neoformation is more likely in distal than in proximal sibling shoots, it increases the size gradient among them, more notably so for large than for small parent shoots (see Fig. 4).

For parent shoots with less than 11 nodes, diameter and length do not vary among sibling shoots. For larger parent shoots, sibling shoot length tends to increase close to the distal end of each parent shoot, particularly because of differences in internode extension. Stem diameter, on the other hand, varies with sibling shoot position along each type of parent shoot more linearly than stem length. Distal sibling shoots are, therefore, more slender than proximal sibling shoots, especially in the case of large parent shoots. This could explain the tendency to plagiotropy of long shoots in this species (as in other trees fitting Troll's architectural model; Hallé et al. 1978; Oldeman 1989). Differences in stem slenderness among sibling shoots would determine a less efficient flow of resources and photosynthates and a lower tolerance to stressful conditions in longer than in shorter shoots (see Oldeman 1989; Givnish 1995; Rossignol et al. 1998). The more intense cambial activity in larger than in smaller shoots would further increase size differences among sibling shoots as well as increase both the physical strength and the conducting capacities of large shoots (see Table 1, Fig. 5B)

The development of sylleptic branches in the most vigorous shoots of this species alters the pattern of variation in preformation and neoformation along parent shoots only at their distal end, where buds and shoots derived from sylleptic branches have less leaves than the corresponding organs derived directly from the parent shoot.

Differentiation of axes

As shown for other tree species (Hallé and Oldeman 1970; Edelin 1977; Costes 1993; Reffye et al. 1993; Barthélémy et al. 1997), *N. dombeyi* trees exhibit a predictable differentiation of axes according to their position on the tree. Trunk, main branches, secondary branches, and short branches may, with some overlap, be characterized on the basis of the morphological features of their component annual shoots, including number of leaves, length, basal diameter after extension, apex persistence, sylleptic branching probability, and preformation and neoformation capabilities. The morphogenetic gradient along the sibling shoots developed by each parent shoot parallels the gradient found for shoots of different axes. Thus, from the distal to the proximal end of a trunk shoot, sibling shoot morphology resembles that of trunk, main branch, secondary branch, and short branch shoots. At the other extreme of the species' morphogenetic gradient, short shoots develop only short shoots.

The clear differentiation of axes described here applies to young trees of *N. dombeyi* growing in large openings in the forest, considered a favourable environment for the regeneration of this species (Veblen et al. 1996*b*). Under more stressful conditions, each axis of a tree would exhibit a higher rate of shift along the species' morphogenetic gradient (see Froebe and Gleissner 1995; Rossignol et al. 1998). For instance, the trunk would develop, at its distal end, shoots resembling those of main branches or secondary branches of trees growing in a more favourable environment. Such a response to stressful conditions has been observed for *Fagus sylvatica* (Nicolini 1998) and *N. pumilio* (Puntieri et al. 1999). The morphological features of shoots on different types of axes in this species suggest that trees under stressful conditions would have, compared with unstressed trees, more limited possibilities for new space exploration, because of their short shoots with little variation in the size of their branches, and a lower biomass accumulation rate, because of the low number of green leaves in their shoots. However, trees growing in an unfavourable environment would be, compared with unstressed trees, more efficient at light exploitation, because of their plagiotropic shoots with shorter internodes (see Oldeman 1989; Cao 1995; Rossignol et al. 1998), and more tolerant to summer stress caused by early frosts or droughts, because of their shorter period of shoot extension.

On the basis of the present results, each of the shoot types of young *N. dombeyi* trees may be characterized by means of a series of mathematical models describing each of their morphological features (length, diameter, number of preformed leaves, number of neoformed leaves, probability of apex persistence, and probability of sylleptic branching). This information, together with mathematical models describing the probability of development of each particular type of shoot in each position of each tree axis may be used to describe tree architecture (e.g., Godin et al. 1999). Several applications to the resulting descriptive models and the detailed computer simulations derived from them have been proposed (Reffye et al. 1993, 1995).

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