Bud Content and its Relation to Shoot Size and Structure in *Nothofagus pumilio* (Poepp. et Endl.) Krasser (Nothofagaceae)

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Buds of shoots from the trunk, main branches, secondary branches and short branches of 10–21 year-old *Nothofagus pumilio* trees were dissected and their contents recorded. The number of differentiated nodes in buds was compared with the number of nodes of sibling shoots developed at equivalent positions during the following growing season. Axillary buds generally had four cataphylls, irrespective of bud position in the tree, whereas terminal buds had up to two cataphylls. There were more nodes in terminal buds, and the most distal axillary buds, of trunk shoots than in more proximal buds of trunk shoots, and in all buds of shoots at all other positions. The highest number of nodes in the embryonic shoot of a bud varied between 15 and 20. All shoots had proximal lateral buds containing an embryonic shoot with seven nodes, four with cataphylls and three with green leaf primordia. The largest trunk, and main branch, shoots were made up of a preformed portion and a neoformed portion; all other shoots were entirely preformed. In *N. pumilio*, the acropetally-increasing size of the sibling shoots derived from a particular parent shoot resulted from differences in: (1) the number of differentiated organs in the buds; (2) the probability of differentiation of additional organs during sibling shoot extension; (3) sibling shoot length; (4) sibling shoot diameter; and (5) the death of the apex and the most distal leaves of each sibling shoot.   © 2000 Annals of Botany Company

Key words: Axis differentiation, branching, bud structure, leaf primordia, neoformation, *Nothofagus pumilio*, preformation, size gradient.

INTRODUCTION

The above-ground portion of a plant can be viewed as consisting of an hierarchical system of foliated axes. In regions where a period favourable for plant growth, and a period unfavourable for plant growth, can be clearly distinguished each year, it may be possible to recognize, in each axis of woody plants, segments known as annual shoots. A shoot may result from one or more events of organogenesis and extension, sometimes called growth units (Caraglio and Barthélémy, 1997). Shoot morphology depends not only on the plant species concerned but also on the position of the shoot on the plant (Hallé, Oldeman and Tomlinson, 1978). A predictable sequence of variation in shoot morphology, the morphogenetic gradient, may be recognized for each species; such a sequence is expressed along each of the axes of a plant (Reffye, Dinouard and Barthélémy, 1991; Reffye *et al.*, 1997). Therefore, shoot morphology is related to the ontogenetic stage of the axis from which the shoot is derived. For some species, changes in leaf morphology, phyllotaxis, branching pattern, number of growth units per shoot, and shoot orientation have been interpreted as externally-visible expressions of

morphogenetic gradients (Barthélémy, Caraglio and Costes, 1997; Nicolini, 1998; Sabatier and Barthélémy, 1999).

In this context, within-plant variation in the number of organs differentiated during each event of shoot growth and its relation to final shoot size are particularly relevant for the understanding of the organization of a plant's architecture. Organogenesis and extension may take place at different times and be separated by a resting period when the embryonic organs are enclosed within a bud. In such cases, the organs developed from these primordia are said to be preformed (Caraglio and Barthélémy, 1997). On the other hand, growth may result from simultaneous organogenesis and extension, without a resting period or bud formation; in this case, the resulting organs are referred to as neoformed organs. A shoot may thus consist of: (1) preformed organs only; (2) preformed organs and neoformed organs; or (3) neoformed organs only. In some species, the relative numbers of preformed and neoformed organs developed by a particular shoot depend on the ontogenetic stage of the axis from which the shoot is derived (Reffye, Dinouard and Barthélémy, 1991; Costes, 1993). This information provides useful raw data for modelling and simulating plant growth in a biologically meaningful way (see Guédon and Costes, 1997), as well as for the diagnosis of tree health. However, studies of the number of leaf primordia in buds and its variation with bud position on the tree have only been carried out for a few

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species (e.g. Rivals, 1965; Caesar and Macdonald, 1983; Remphrey and Davidson, 1994; Thorp, Aspinall and Sedgey, 1994; Sabatier *et al.*, 1995). The present study aimed to analyse: (1) variation in the content of buds of *Nothofagus pumilio* (Poepp. et Endl.) Krasser (Nothofagaceae) according to bud position on a shoot, and shoot position on the tree; and (2) the relationship between bud content and the size of the shoots which subsequently develop.

Nothofagus pumilio is a widespread deciduous tree species from temperate-cold regions of South America. It forms pure (extending over approx. 1 200 000 ha) as well as mixed stands (approx. 330 000 ha; Bava, 1998). Throughout its range, it is the principal species of the high altitude treeline forests of the Andes. The population ecology of *N. pumilio* forests has been relatively well studied (Mutarelli and Orfila, 1969, 1971; Veblen *et al.*, 1977; Veblen, 1985; Carabelli, 1991; Richter and Frangi, 1992; Rusch, 1993; Donoso, 1994; Veblen, Hill and Read, 1996; Martínez-Pastur *et al.*, 1997). In recent years, emphasis has been on the study of the morphological and architectural features of this species (Thiébaut *et al.*, 1997; Puntieri *et al.*, 1998; Barthélémy *et al.*, 1999).

MATERIALS AND METHODS

Study site and sampling

Nothofagus pumilio trees growing within a 10 ha area at Cerro Otto, San Carlos de Bariloche, Argentina (41°09′S, 71°10′W, 1350 m altitude) were studied. Annual precipitation in this area reaches 800 mm and is mainly concentrated in autumn and winter (Conti, 1998). The soil is derived principally from volcanic ash (Scoppa, 1998). The sampling area has a patchy cover of *N. pumilio* trees and regeneration of this species is relatively abundant. A few individuals of the introduced tree species *Pinus monticola* (Pinaceae) and *Pseudotsuga menziesii* (Pinaceae) also occur in this area. Other plant species include low shrubs and herbs. This site was selected because of the large number of healthy young individuals of *N. pumilio* with a well-differentiated vertical trunk and well-developed branches not shaded by neighbouring individuals.

For the present study, 104 trees were selected randomly from those between 10 and 21 years old and 2·5 and 7·5 m in height, with a basal diameter of between 30 and 100 mm. Trees with trunks damaged by exogenous factors were avoided. The small diameter of the trunks prevented the use of cores to count annual growth rings. Tree age was determined by counting the number of annual shoots on the trunk. Because the scars marking the between-shoot limits closest to the ground become unclear as trunk diameter increases, it is likely that tree ages were underestimated by 1 to 3 years.

The following axis types (described as homogeneous axis categories in a previous study; Barthélémy *et al.*, 1999), were identified for each selected tree: trunk, main branches, secondary branches and short branches (Fig. 1). For each sample, shoots developed in the 1996–97 growth period

were collected from one of each of the axis types of each selected tree. These shoots are termed 'parent shoots'.

To study the relationship between the number of organs in buds and the number of organs in subsequently-developed shoots, parent shoots were collected in April 1997 (at the end of the 1996–97 growing season) and in April 1998 (Fig. 2). For each of these samples, 52 trees were labelled and one parent shoot of each axis type was collected from each tree. None of the trees selected in April 1997 were selected in April 1998. Axillary buds and, sometimes, a terminal bud were present on each parent shoot collected in April 1997. Each parent shoot collected in April 1998 carried a set of shoots developed in the 1997–98 growth period. The shoots developed by each parent shoot are referred to as 'sibling shoots' (Fig. 2). To determine if organogenesis occurred in the buds during late autumn to winter, a set of parent shoots was collected in September 1997, about 2 weeks before budbreak. Due to time constraints and the fact that the highest organogenesis level was expected for the most vigorous shoots, this sample included only 20 trunk parent shoots. The April 1997, September 1997 and April 1998 samples are referred to as samples I, II and III, respectively.

Data record

At the end of their extension, most shoots of *N. pumilio* consist of an unbranched sequence of nodes and internodes; each node bears a single leaf. The most proximal nodes of each shoot are separated by very short internodes and bear bladeless leaves, i.e. cataphylls, consisting of two stipules (Barthélémy *et al.*, 1999). All other nodes usually bear green leaves. At the time of each sampling the following information was recorded for each parent shoot: origin (from a terminal or axillary bud), length, basal diameter, number of nodes, number of cataphylls, number of green leaves and condition of the apex (dead or surviving). Cataphyll-bearing nodes were distinguished from green leaf-bearing nodes by the abscission scars of leaves on the stem. In cases of unclear scars, both cataphyll and green leaf numbers were considered missing from the respective shoot. Shoot length was measured to the nearest mm with a tape measure, whereas shoot basal diameter was measured to the nearest 0·1 mm with digital callipers. Shoots showing evident damage due to exogenous factors (chiefly insects) were excluded from the study.

For each sample I and sample II parent shoot, the origin (terminal or axillary) and rank number or position number of each bud on its parent shoot were recorded. Rank numbers started at the distal end of the parent shoot (terminal bud: rank = 0, most distal axillary bud: rank = 1). After preservation in 96% ethanol for 2 weeks, the buds were dissected under a stereo-microscope (Olympus SZ30, 40X; Fig. 3). The numbers of nodes, cataphylls and green leaf primordia of the embryonic shoot in each bud were counted. In all groups, the parent shoot nodes corresponding to the cataphylls did not subtend any externally-visible axillary structure. The nodes of the two or three most proximal green leaves of each parent shoot usually subtended a relatively small axillary bud (less than
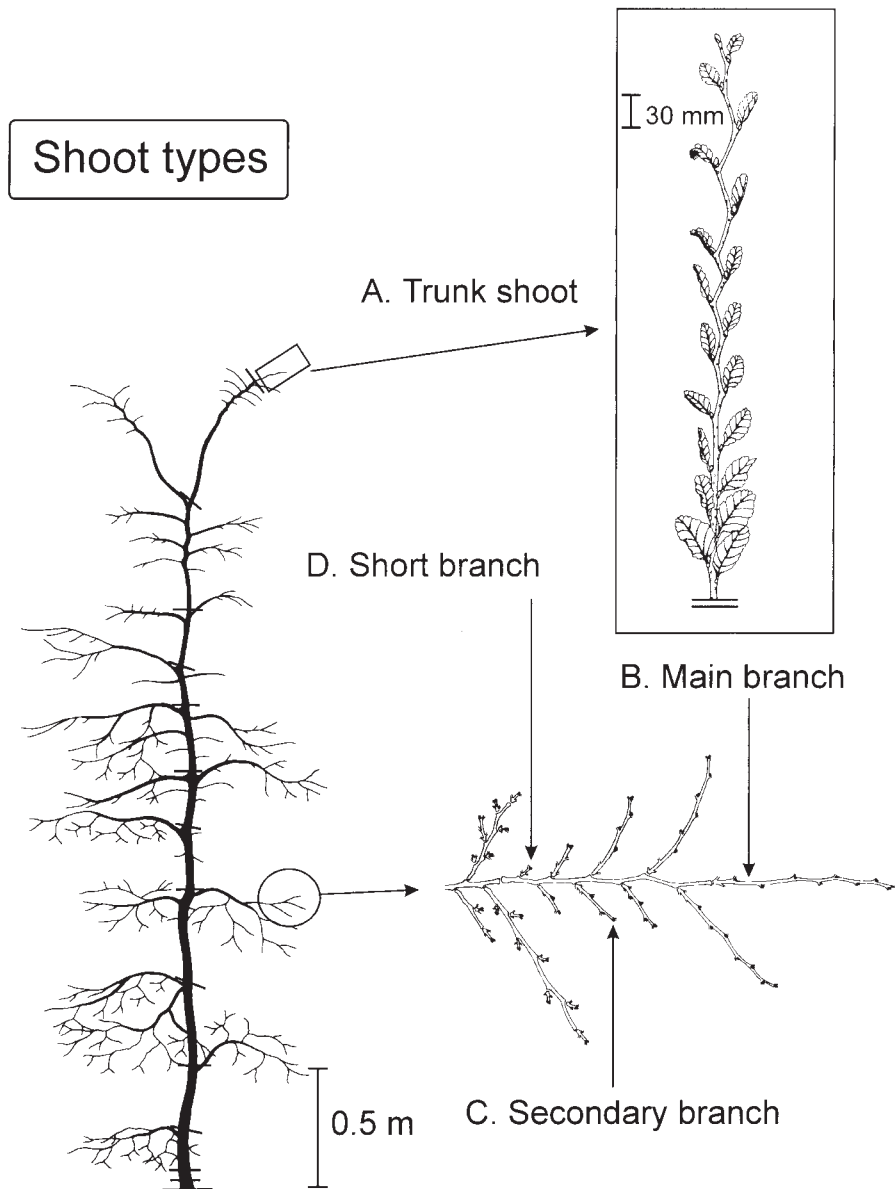
F IG. 1. Diagram of a young *N. pumilio* tree similar to those selected for the present study showing the positions from which shoots were sampled. A typical main trunk shoot appears in the upper right frame. A secondary branch and a short branch are indicated in an expanded view of one of the tree's main branches (bottom right). Horizontal lines on the trunk indicate the limits between annual shoots.

2 mm in length) which was impossible to dissect with the available instruments. All other nodes had either a dissect-able bud (samples I and II parent shoots) or a sibling shoot (sample III parent shoots). Buds with partial or total damage due to exogenous factors were excluded from the analysis. A total of 1950 buds corresponding to all sample shoots were dissected for this study; 131 buds had been damaged by insect larvae.

For each sample III parent shoot, sibling shoots were rank-numbered as described for buds, and their origin, length, basal diameter, node number, cataphyll number, green leaf number and apex condition were recorded.

Data analysis

A previous study of a related species of *Nothofagus* indicated that both the number of nodes of a shoot and its origin may affect the size of its branches, as well as the variation in branch size according to branch position on the parent shoot (Puntieri *et al.*, 1998). Parent shoots of each sample were, therefore, re-grouped on the basis of their number of nodes, origin and type of axis on which they were formed. Six groups were defined as detailed in Table 1. Additional groups could have been formed, but they contained too few parent shoots to allow statistical comparisons.
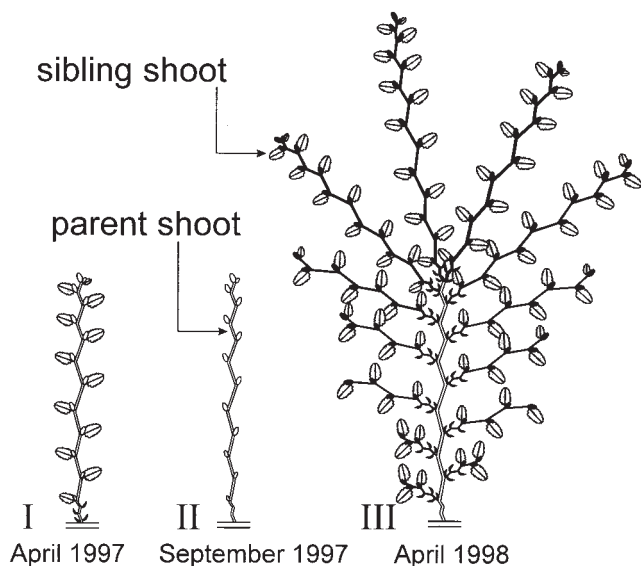
FIG. 2. Diagrammatic representation of parent shoots (unshaded) of *N. pumilio* sampled at the end of the 1996–97 growth period (April 1997, sample I), before the beginning of the 1997–98 growth period (September 1997, sample II) and at the end of the 1997–98 growth season (April 1998, sample III). Leaves and buds are included for April 1997 shoots, and buds for September 1997 shoots. Shoots developed from parent shoots during the 1997–98 growth season (sibling shoots, black) are shown for April 1998 parent shoots. The horizontal double lines indicate the proximal end of the parent shoot.
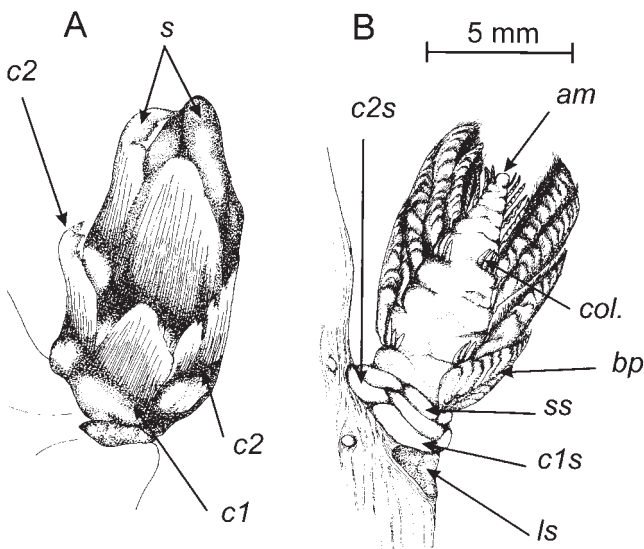

FIG. 3. A, Bud of *N. pumilio*: the first cataphyll (*c1*), consisting of two fused stipules, both stipules of the second cataphyll (*c2*) and green-leaf primordia stipules (*s*) are indicated. B, dissected bud. *c1s* and *c2s*, Scars left by the first and the second cataphylls respectively; *ss*, scar left by one of the stipules of the first green-leaf primordia; *bp*, blade primordium of the first green leaf; *ls*, scar of the subtending leaf; *col*; colleters (stipule resinous glands); *am*, apical meristem.

For comparison of the number of nodes of the embryonic shoots in buds and the number of nodes of sibling shoots, terminal structures and axillary structures were treated separately. Because of the low number of terminal

structures, groups were pooled: sample I and sample II terminal bud node numbers were compared with sample III sibling shoot node numbers by one-way ANOVA followed by Tukey–Kramer *a posteriori* tests (Sokal and Rohlf, 1981). Comparisons between numbers of nodes in axillary buds and those in axillary sibling shoots were performed for pre-established ranges of rank numbers, so as to reduce the number of comparisons and account for random variation within each parent shoot in bud content or sibling shoot size. For each sample I and sample II parent shoot, the mean number of nodes per axillary bud was calculated for buds of three successive rank numbers. Similarly, for each sample III parent shoot, the mean number of nodes per axillary sibling shoot was obtained for each range of three successive rank numbers. The ranges of rank numbers compared between samples for each group of parent shoots were: [1–3], [4–6], [7–9], [10–12], [13–15], [16–18] and 19–21] for groups 1, 2 and 3, [1–3], [4–6] and [7–9] for groups 4 and 5 and [1–3] for group 6. For each range of rank numbers of each group, a one-way ANOVA was carried out to compare the number of nodes in buds and the number of nodes in sibling shoots (followed by Tukey–Kramer tests for group 2).

RESULTS

Parent shoot size

Parent shoots of groups 2–6, all of which derived from axillary buds, generally had four cataphylls, whereas group 1 parent shoots, which derived from terminal buds, had up to two cataphylls (Table 2). The use of the number of nodes as a criterion for classifying parent shoots derived from axillary buds resulted in low within-group variability in terms of shoot length and basal diameter. In general terms, parent shoots with more nodes tended to be longer and thicker than those with less nodes. However, main branch parent shoots with 16–32 nodes (group 3) were shorter and had a smaller diameter than trunk parent shoots with a similar number of nodes (groups 1 and 2). Similarly, secondary branch parent shoots (group 5) were shorter and thinner than main branch parent shoots with the same number of nodes (group 4; Table 2). The proportion of parent shoots with a surviving apex after shoot extension was highest for group 1, intermediate for group 2, low for groups 3 and 4 and nil for groups 5 and 6 (Table 2).

Number of nodes in buds and sibling shoots

In sample I, terminal buds were found in group 1 (13 buds), group 2 (two buds) and group 3 (two buds) parent shoots. In the last group, both terminal buds were damaged by insects. In the case of sample II, five terminal buds were found. The mean ($\pm$ s.e.) number of nodes in terminal buds was 16.9 ± 0.83 for sample I and 18.2 ± 1.18 for sample II. Sibling shoots derived from terminal buds were found for groups 1 (four shoots), 2 (seven shoots) and 3 (two shoots). The mean number of nodes in group 1 and group 2 sibling shoots derived from

TABLE 1. *Number of parent shoots corresponding to samples I (April 1997), II (September 1997) and III (April 1998) included in groups 1 to 6*

Group	Morphological features	Number of shoots in each sample		
		I	II	III
1	Terminal-bud derived trunk shoots with 16–32 nodes	24	0	11
2	Axillary-bud derived trunk shoots with 16–32 nodes	23	20	36
3	Axillary-bud derived main branch shoots with 16–32 nodes	27	0	22
4	Axillary-bud derived main branch shoots with eight–15 nodes	16	0	25
5	Axillary-bud derived secondary branch shoots with eight–15 nodes	45	0	46
6	Axillary-bud derived short branch shoots with <eight nodes	52	0	51

The morphological features on which each parent shoot group was defined are indicated.

TABLE 2. *Mean (± s.e.) number of cataphylls, number of green leaves, length and basal diameter of the parent shoots corresponding to samples I, II and III for each group (1–6)*

Group	Sample	Cataphylls	Green leaves	Length (mm)	Basal diameter (mm)	Apex survival (%)
1	I	0·6 ± 0·32	22·2 ± 0·81	364 ± 22	5·6 ± 0·30	57
1	III	0·0	22·0 ± 1·59	367 ± 43	9·7 ± 0·74	60
2	I	3·8 ± 0·08	18·9 ± 0·70	358 ± 18	5·3 ± 0·24	26
2	II	4·0 ± 0·07	17·9 ± 0·68	334 ± 23	5·5 ± 0·24	24
2	III	3·9 ± 0·03	19·2 ± 0·83	296 ± 14	9·6 ± 0·32	27
3	I	3·6 ± 0·07	15·6 ± 0·54	214 ± 15	3·7 ± 0·11	6
3	III	4·0 ± 0·04	15·2 ± 0·65	180 ± 10	5·2 ± 0·23	4
4	I	3·7 ± 0·20	9·7 ± 0·41	140 ± 10	3·1 ± 0·10	12
4	III	4·0 ± 0·02	8·1 ± 0·39	106 ± 8	4·0 ± 0·18	0
5	I	3·9 ± 0·06	8·3 ± 0·37	75 ± 3	2·6 ± 0·12	0
5	III	4·0 ± 0·03	6·2 ± 0·33	50 ± 4	2·5 ± 0·10	0
6	I	4·0 ± 0·04	2·9 ± 0·04	5 ± 0·3	1·7 ± 0·01	0
6	III	4·0 ± 0·04	3·0 ± 0·03	3 ± 0·2	1·7 ± 0·02	0

The percentage of parent shoots with a surviving apex after shoot extension is indicated for each sample and each group.

terminal buds was 24·4 ± 1·27 (both groups pooled), which was significantly higher than the mean number of nodes in terminal buds corresponding to sample I and sample II parent shoots ($F = 15·0$, $P < 0·001$); the difference between samples I and II in this respect was not significant.

For the most distal nodes of group 1 and group 2 parent shoots, the mean number of nodes in each axillary bud was 16·8 ± 0·54 (Fig. 4A, B) and was lower towards more proximal rank numbers (9·8 ± 0·57 for the most proximal nodes). Although the mean number of nodes in buds of sample II shoots of group 2 was consistently lower than that of sample I shoots (Fig. 4B), the samples did not differ significantly for any of the ranges of rank numbers compared. The number of nodes of axillary sibling shoots of group 1 and group 2 parent shoots decreased almost linearly from distal to proximal sibling shoots (Fig. 4A, B). For rank numbers 1–3 on the parent shoot, sibling shoots of groups 1 and 2 had more nodes than buds of samples I and II. For rank numbers 13–15 of group 1 parent shoots and rank numbers 10–12 and 13–15 of group 2 parent shoots, sibling shoots had a mean number of nodes which was lower than that of similarly-positioned axillary buds of samples I. For other axillary rank numbers of group 1 and group 2 parent shoots, the number of nodes in the buds was similar to that of the sibling shoots.

In the case of group 3 parent shoots, the mean number of nodes was 14·8 ± 0·51 for the most distal axillary buds (Fig. 4C). For more proximal positions, the number of nodes per bud decreased to a mean of 9·1 ± 0·58. The sibling shoots developed in rank numbers 1–3 on group 3 parent shoots had more nodes than the corresponding axillary buds (Fig. 4C). For rank numbers 7–9 and 10–12 on the parent shoot, the number of nodes of sibling shoots of group 3 was lower than that in buds of similar rank numbers. Similar numbers of nodes were recorded for buds and sibling shoots corresponding to other rank numbers of group 3 parent shoots.

The number of nodes in buds of group 4 parent shoots ranged between a highest value of about 13 nodes in the most distal buds, to a lowest of between seven and ten nodes in the most proximal buds (Fig. 4D). The number of nodes of sibling shoots in rank numbers 1–3 on group 4 parent shoots was similar to that in buds of equivalent positions (Fig. 4D, E). Sibling shoots in rank numbers 4–6 and 7–9 on group 4 parent shoots had fewer nodes than axillary buds of similar rank number.
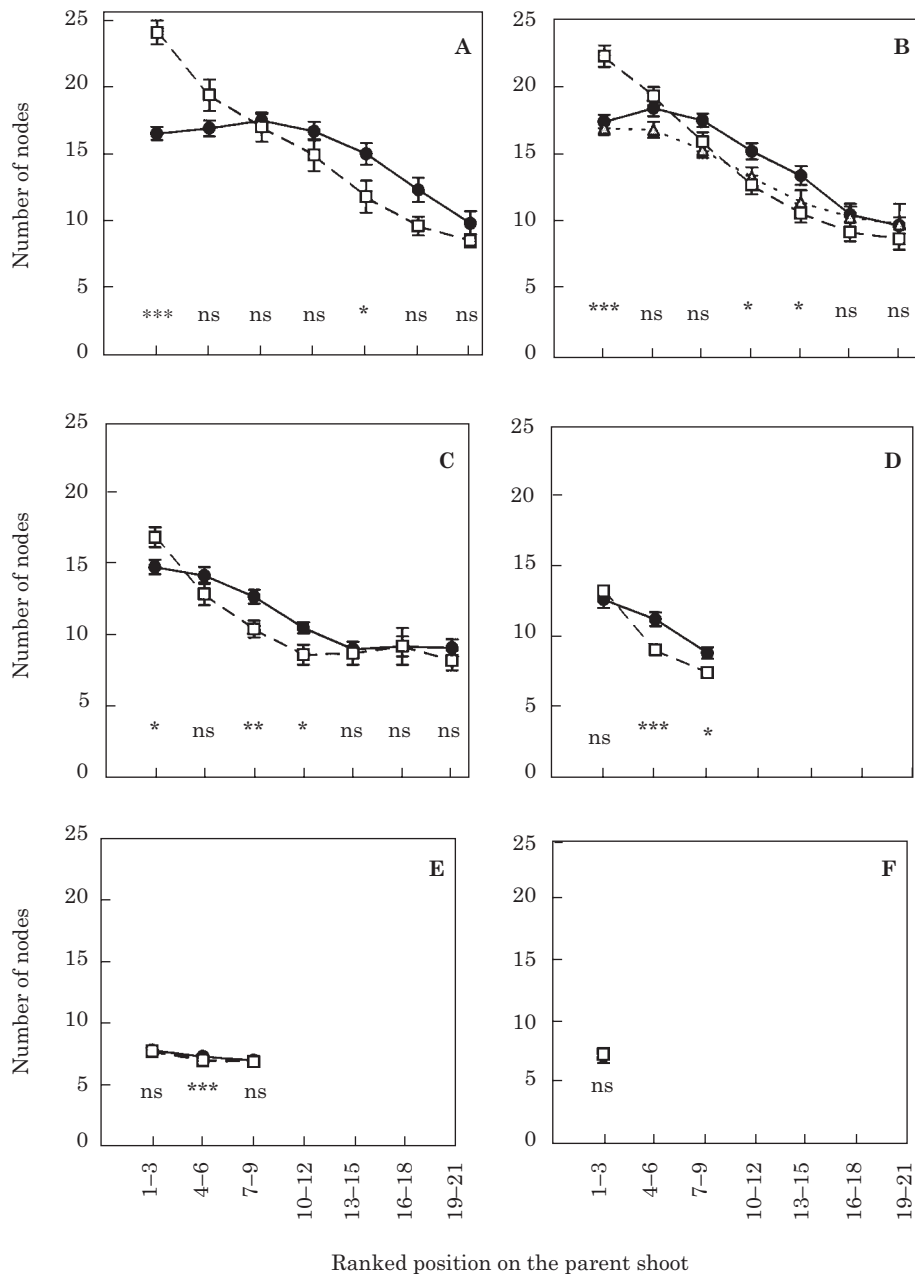
FIG. 4. Mean (±s.e.) number of nodes of the embryonic shoots in buds [(●): sample I, April 1997; (△): sample II, September 1997] and number of nodes in sibling shoots [(□): sample III, April 1998] according to bud or sibling shoot position on the parent shoot. Buds and sibling shoots were ranked from the parent shoot's distal end. A–F, groups 1 to 6, respectively. The results of the statistical between-sample comparisons are shown for each group and each set of ranked positions: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns $P > 0.05$.

Buds and sibling shoots of group 5 parent shoots had about seven nodes (Fig. 4E). The only bud and the only sibling shoot derived from group 6 parent shoots had seven nodes (Fig. 4F).

Sibling shoot length, diameter and apex survival

Shoots derived from terminal buds of sample III parent shoots had a mean length of 409 mm (±39 mm) and a mean basal diameter of 6·4 mm (±0·44 mm).

For parent shoots of groups 1 to 4, the length of sibling shoots derived from axillary buds decreased sharply from distal to proximal positions (Fig. 5A). Group 5 parent shoots developed longer shoots in the two most distal positions than in more proximal positions. The basal diameter of sibling shoots decreased from distal to proximal positions on the parent shoot (though less notably than their length) for all groups (Fig. 5B). Absolute differences in sibling shoot diameter between groups were less notable than for shoot length.
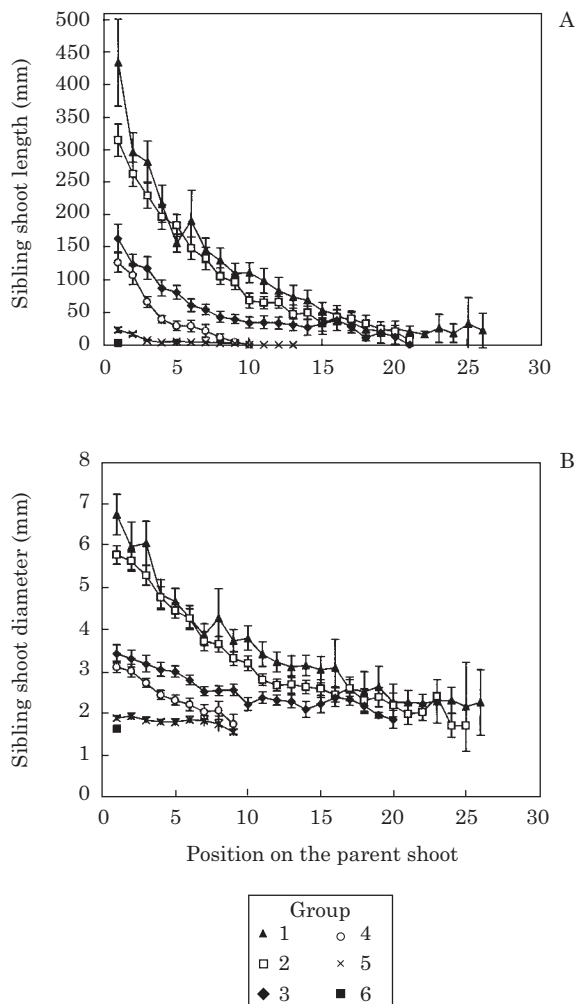
FIG. 5. Mean (±s.e.) length (A) and basal diameter (B) of sibling shoots ranked according to their position on the parent shoot (from the distal end of the parent shoot) for groups 1 to 6.

For groups 1, 2, 3 and 4 respectively, 16·0, 28·1, 4·1 and 5·5% of sibling shoots of rank numbers 1 to 3, and 12·5, 8·4, 3·2 and 0·0% of sibling shoots of rank numbers 4 to 6 had a surviving apex. For sibling shoots of rank numbers 7 to 12, the percentage of shoots with a surviving apex was lower than 5% for groups 1 and 2 and 0% for all other groups. More proximally-positioned sibling shoots of groups 1 to 4, and all shoots derived from groups 5 and 6 shoots had a dead apex.

DISCUSSION

Bud morphology in N. pumilio

In young *N. pumilio* trees, each bud contains an embryonic shoot with differentiated nodes, internodes, fully-developed cataphylls and green-leaf primordia. Within a bud, green-leaf primordia decrease in size from proximal to distal positions. A distal group of primordia with small laminae and a proximal group of primordia with large laminae may

sometimes be recognized in buds with many leaf primordia (Fig. 3B, unpubl. res.).

The present study indicates that most axillary buds have four basal cataphylls, irrespective of bud position on the tree. Terminal buds of *N. pumilio* have, on average, fewer cataphylls than axillary buds. These results are consistent with previous observations on this species (Raffaele *et al.*, 1998; Barthélémy *et al.*, 1999). The number of cataphylls in *N. pumilio* buds is low compared with that found for species of the closely-related Fagaceae family. For instance, numbers of cataphylls have been found to range between ten and 40 for *Quercus* spp. (Nitta and Ohsawa, 1998), between seven and 11 for *Fagus sylvatica* (Nicolini, 1997), between five and nine for *Castanopsis sieboldii*, and between ten and 15 for *Lithocarpus edulis* (Nitta and Ohsawa, 1998). In *N. pumilio*, the thick stipules of the outermost green leaves in each bud as well as the resinous secretions of the stipule colleters (Fig. 3) may play an important role in the protection of leaf blade primordia and the terminal meristem of the bud during winter.

The number of differentiated nodes in *N. pumilio* buds varies according to both the position of the parent shoot in the tree and the position of the bud on the parent shoot, as found for other tree species (Remphrey and Powell, 1984; Remphrey and Davidson, 1994). The most distal buds of the trunk and main branch shoots of a tree have the highest number of differentiated nodes per bud for this species (up to 24). The most proximal buds of trunk and main branch shoots, as well as all buds of secondary branch and short branch shoots, have at least seven nodes (four of them with cataphylls and three with green-leaf primordia); these buds develop seven-node shoots. The presence of buds with a relatively constant number of leaf primordia, eventually producing short shoots has also been found for *Betula papyrifera* (Macdonald and Mothersill, 1983) and *Fagus sylvatica* (Nicolini, 1997).

Irrespective of the position of the shoot on the tree, the most proximal leaves of each shoot in *N. pumilio* do not bear visible buds. As shown for other tree species, cells capable of division might be present at these positions, even though they would not develop into a shoot in the growing season following that in which their parent shoot extended (Fink, 1984; Nicolini, 1997).

Relationships between bud content and shoot size

Since most shoots of *N. pumilio* consisted of nodes, internodes and leaves which had been differentiated in buds by the end of the extension period of their parent shoots, they may be considered entirely preformed. These shoots may include fewer leaves than the number of leaf primordia present in the corresponding buds because of the death of the most distal leaves which usually accompanies apex abortion in *Nothofagus* species (Puntieri *et al.*, 1998; Barthélémy *et al.*, 1999).

Nothofagus pumilio shoots derived from the terminal, or the most distal axillary, buds of trunk shoots, and vigorous main branch shoots may develop more leaves than those present in the buds from which they derived. The present data indicate that this additional initiation of leaves takes

place during shoot extension. The most vigorous shoots of this species would therefore include a preformed portion and a neoformed portion. Because determination of the number of preformed organs involved destruction of the bud, no exact account of the number of neoformed organs developed in each shoot was possible. Nevertheless, the difference between buds and sibling shoots in terms of number of nodes suggests a maximum neoformation of 13 nodes per shoot.

Neoformation has also been reported for vigorous shoots of species of *Salix*, *Populus*, *Acer*, *Betula*, *Fraxinus*, *Abies* and *Picea* (Critchfield, 1960; Kozlowski, 1971; Remphrey and Powell, 1984; Davidson and Remphrey, 1994). In contrast, all shoots seem to be entirely preformed in species of the related Fagaceae genera *Quercus* and *Fagus* (see Collin, Badot and Millet, 1996; Nicolini, 1997). In species of the latter two genera, vigorous shoots may be polycyclic, i.e. they go through several cycles of bud formation and budbreak during one growing season (Thiébaut, 1982; Nicolini, 1998). Polycyclism, so far not reported for *Nothofagus* spp. (Puntieri *et al.*, 1998; Barthélémy *et al.*, 1999), was not observed in the present study.

In addition to the possibility of neoformation, shoots derived from the distal portion of trunk and main branch shoots of young *N. pumilio* trees have a higher apex survival after shoot extension than both proximal shoots of trunk and main branch shoots, and all sibling shoots derived from secondary branches and short branches. Nevertheless, apex death is a frequent event for all shoots in this species, as in other *Nothofagus* species (Puntieri *et al.*, 1998; Barthélémy *et al.*, 1999). Preformation, neoformation, and potential for length and diameter growth are similar for shoots derived from terminal buds and shoots derived from the most distal axillary buds of trunk and main branch shoots. Therefore, apex death in these shoots does not represent a severe limitation to the future growth of the axis concerned.

Axis and shoot differentiation in *N. pumilio*

Young *N. pumilio* trees exhibit a clear differentiation of axes and a clear gradient of sibling shoot size for each parent shoot. The largest parent shoots produced in this species (those with >20 nodes), which are formed both by the trunk and by the main branches, bear sibling shoots corresponding to any of the possible shoot sizes developed by this species, i.e. shoots characteristic of trunk, main branches, secondary branches and short branches. Shoots of intermediate size (those with eight to 15 nodes) may be part of main branches and secondary branches, and bear intermediate or short shoots. Short shoots (those with <eight nodes) produce only short shoots. Therefore, the size of a shoot developing from a particular bud will depend as much on the position of the bud on its parent shoot as on the position of the parent shoot on the tree.

In *N. pumilio*, the size of sibling shoots developed at different positions from the same parent shoot is determined by a series of components: (1) the extent of preformation; (2) the probability and extent of neoformation; (3) shoot length growth; and (4) shoot diameter by the end of shoot extension. These components give rise to a gradient of increasing size towards the distal end of the parent shoot. Such a gradient is associated with a decreasing probability of sibling shoot apex death from the proximal to the distal end of each parent shoot. The size gradient among sibling shoots becomes sharper over time because of the more marked diameter growth of larger than smaller shoots (compare the diameters of sample I and sample III parent shoots; Table 2). All four components affect the development of the most vigorous shoots. As the vigour of the parent shoot decreases, fewer of these components are expressed and shoots derived from the same parent shoot tend to resemble each other. The present study suggests that the gradient in vigour among sibling shoots derived from each parent shoot in young *N. pumilio* trees results from a set of endogenous developmental rules. External conditions, such as nutrient availability, might affect the expression of each of these endogenous rules as shown for other tree species (Brown and Sommer, 1992; Costes, 1993; Davidson and Remphrey, 1994).

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