

Relative Extents of Preformation and Neof ormation in Tree Shoots: Analysis by a Deconvolution Method

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- **Background and Aims** Neof ormation is the process by which organs not preformed in a bud are developed on a growing shoot, generally after preformation extension. The study of neof ormation in trees has been hindered due to methodological reasons. The present report is aimed at assessing the relative importance of preformation and neof ormation in the development of shoots of woody species.
- **Methods** A deconvolution method was applied to estimate the distribution of the number of neof ormed organs for eight data sets corresponding to four *Nothofagus* species and a *Juglans* hybrid.
- **Key Results** The number of preformed organs was higher and less variable than the number of neof ormed organs. Neof ormation contributed more than preformation to explain full-size differences between shoots developed in different positions within the architecture of each tree species.
- **Conclusions** Differences between the distributions of the numbers of preformed and neof ormed organs may be explained by alluding to the duration of differentiation and extension for each of these groups of organs. The deconvolution of distributions is a useful tool for the analysis of neof ormation and shoot structure in trees.

Key words: Preformation, neof ormation, shoot, leaf primordia, deconvolution, *Nothofagus*, *Juglans*.

INTRODUCTION

In many woody species from both temperate and tropical regions, foliated axes grow rhythmically (Hallé and Martin, 1968; Hallé *et al.*, 1978; Bell, 1991). This implies that the extension of an axis portion or shoot is followed by the formation of a resting terminal bud consisting, very frequently, of a set of partially developed organs and one to several groups of undifferentiated cells (i.e. meristems). These primordial organs are called preformed organs and, usually, complete their development in the growth period following that of their inception. A shoot may also grow in length by developing neof ormed organs, i.e. a shoot portion or set of organs, each of which differentiates and extends without ever integrating a resting bud. For these species, three types of shoots may, in theory, be distinguished based on the times of initiation and extension of their constituting organs: a shoot may be entirely preformed or entirely neof ormed, or may be a mixed shoot, consisting of a proximal set of preformed organs and a distal set of neof ormed organs (Caraglio and Barthélémy, 1997). Entirely preformed shoots have been reported for *Ginkgo biloba* (Critchfield, 1960), *Fraxinus excelsior* (Gill, 1971), *Juglans regia* (Sabatier *et al.*, 1995), *Fagus sylvatica* (Roloff, 1987; Nicolini, 1998) and *Nothofagus* spp. (Puntieri *et al.*, 2000, 2002a, b; Souza *et al.*, 2000). Entirely neof ormed shoots seem to be relatively uncommon, but have been described for some tropical trees (Borchert, 1969). Mixed shoots seem to be frequent in the vigorous axes of several tree species (Critchfield, 1960;

Kozłowski, 1971; Nitta and Ohsawa, 1997; Puntieri *et al.*, 2000; Souza *et al.*, 2000; Gordon *et al.*, 2006), although their existence in shorter axes has been proved at least once (Puntieri *et al.*, 2002b).

Despite their importance for the understanding of tree architecture and physiology, relatively little information is available about the relevance of preformation and neof ormation and their relative extent in the architecture of a tree species. There is evidence that preformed and neof ormed leaves of the same shoot may differ in their developmental and functional attributes (Critchfield, 1960; Carles *et al.*, 1964; Steeves and Sussex, 1989; Koike, 1990; Seleznyova and Greer, 2001). Such dissimilarities may be justified by the fact that these two kinds of leaves differentiate and/or extend at different times and, therefore, under different environmental conditions. The contribution of either type of leaves to the future development of the plant and the productivity of the population may indeed be different. A detailed and comparative description of preformation and neof ormation and the analysis of their relative extent in trees would thus improve knowledge of plant growth and functioning.

Methodological restrictions have so far hindered scientific progress on the quantitative assessment of preformation and neof ormation in woody plants. Whereas the study of preformation may be achieved through bud dissection during the resting period, that of neof ormation poses methodological restrictions. The mean number of neof ormed organs of a set of shoots may be estimated by subtracting the mean number of preformed organs in a sample of dissected buds from the mean number of organs

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of a sample of fully extended shoots derived from buds similar to those that were dissected (Puntieri *et al.*, 2000; Souza *et al.*, 2000). The result may be too coarse an estimation of neof ormation, as the number of preformed organs in buds for a given species and architectural position is usually less disperse and asymmetric than that of the total number of organs of fully extended shoots derived from equivalent buds (de Reffye *et al.*, 1991; Puntieri *et al.*, 2000; Souza *et al.*, 2000). No methods have so far allowed more detailed descriptions of neof ormation. The aim of the present study was to apply a deconvolution method to estimate the neof ormation distributions (rather than simply the mean number of neof ormed organs) of shoots of five woody species known to develop both preformed and neof ormed organs. For each of eight data sets, neof ormation distributions were estimated from two independent distributions: that of the number of preformed organs and that of the total number of extended organs.

MATERIALS AND METHODS

In this study, eight published or unpublished data sets were used for which the development of neof ormed organs was identified. Five of these data sets refer to South American species of *Nothofagus* (Nothofagaceae) and the other two to walnut interspecific hybrid *Juglans major* (Torr.) A. Heller 209 × *Juglans regia* L. (Juglandaceae). Each data set consisted of two samples taken in two successive years from randomly chosen trees within each population: sample I included buds and sample II consisted of fully extended shoots derived from buds equivalent, in terms of position in the trees, to those of sample I. Buds of sample I of each data set were dissected manually under a stereo-microscope, and the number of organs of each bud was counted. Shoots of sample II were observed in detail with magnifying glasses so as to identify the number of organs present. Buds and shoots with evident signs of damage were excluded. Depending on the data set, the sampled shoots were positioned at the distal end of the main vertical axis of the tree (trunk) and/or the largest horizontal branches (main branches) derived from the trunk. In the case of *Nothofagus* trees, shoot growth may be definite or, less frequently, indefinite. Nevertheless, it has been shown that trunk and main branch shoots derived from terminal buds and those derived from distal axillary buds are morphologically similar (Puntieri *et al.*, 1998). Therefore, buds and shoots originating at terminal and distal axillary positions were taken as equivalent for the analysis of *Nothofagus* spp. data. On the contrary, the growth of the trunk of young *Juglans* spp. trees is indefinite and, since terminal and axillary buds in these trees produce shoots with different morphology (Sabatier and Barthélémy, 2000), only buds and shoots in terminal positions were sampled. The numbers of sampling units of each sample are indicated in Table 1. Data sets may be described as follows (whenever available, published reports for further information are indicated in parentheses).

Data set Nal1

Samples corresponded to the most distal trunk buds of 1-year-old shoots of 2-year-old seedlings of *Nothofagus alpina* (Poepp. et Endl.) Oerst. cultivated at the experimental nursery of the Instituto Nacional de Tecnología Agropecuaria (INTA) at Bariloche, Argentina (41°04'S, 71°10'W, 780 m altitude). These seedlings were growing under natural sunlight and were watered daily.

Data set Nal2

Data were collected concerning the composition of terminal buds and shoots of the trunk and main branches of 7- to 15-year-old saplings of *N. alpina* of a natural regeneration in a managed forest at Pucará, Parque Nacional Lanín, Argentina (41°08'S, 71°43'W, 1100 m altitude). These saplings derived from seeds and were growing under the thinned canopy of a mixed *N. alpina*–*Nothofagus obliqua* (Mirb.) Oerst. forest.

Data set Nd1

Data were collected concerning bud and shoot composition for the trunk (buds and shoots in positions 1–10 starting from the trunk's distal end) and the main branches (positions 1–5) of 10- to 15-year-old trees of *Nothofagus dombeyi* (Mirb.) Oerst. growing at Villa Mascardi, Argentina (41°10'S, 71°10'W and 850 m altitude; Puntieri *et al.*, 2000). The selected trees correspond to the roadside regeneration cohort from seeds after the forest was cut down for road construction. In order to increase the numbers of buds or shoots for the estimation of distributions, buds or shoots of the trunk in different positions were pooled as follows: positions 1–4, 5–7, 8–9 and 10.

Data set Nd2

Data were collected from the four most distal buds and shoots of the main branches of 7- to 20-year-old *N. dombeyi* trees growing at Villa La Angostura, Argentina (41°47'S, 71°40'W and 770 m altitude; Puntieri *et al.*, 2002a). These trees are growing at the margin of a road and derived from seeds after road construction.

Data set Nan

Data corresponded to the five most distal buds and shoots of the main branches of about 8- to 22-year-old *Nothofagus antarctica* (G. Forster) Oerst. trees growing at the Centro de Salmonicultura, Bariloche, Argentina (41°10'S, 71°25'W and 880 m altitude; Puntieri *et al.*, 2002b). This population derived from stumps and represents the major woody cover of an area which was affected by an extensive fire.

Data set Np

Data were collected from the two most distal buds and shoots of main branches of 10- to 21-year-old trees of *Nothofagus pumilio* (Poepp. et Endl.) Krasser growing at

TABLE 1. Mean, standard deviation (s.d.) and coefficient of variation (CV) of the estimated probability mass functions of preformed and neoformed organs and index of dispersion (ID) for the estimated probability mass functions of neoformed organs for samples of tree shoots (see text for further details)

Data set	Position	N_1	N_2	Preformation (X)			Neoformation (Y)				$P(Y=0)$	Data shift
				Mean	s.d.	CV	Mean	s.d.	CV	ID		
<i>Nal1</i>	Trunk	176	37	6.24	1.26	0.20	3.70	1.79	0.48	0.87	0.068	–
<i>Nal2</i>	Trunk	20	21	13.00	0.95	0.07	10.55	5.54	0.52	2.91	0.045	4
	Branch	20	20	12.70	2.41	0.19	1.60	2.51	1.57	3.94	0.330	2
<i>Nd1</i>	Trunk 1–4	53	24	13.48	4.22	0.31	8.50	4.02	0.47	1.90	0.000	–
	Trunk 5–7	42	22	13.60	3.24	0.24	5.37	4.34	0.81	3.51	0.034	–
	Trunk 8–9	47	26	12.67	3.07	0.24	5.24	4.89	0.93	4.55	0.067	–
	Trunk 10	46	26	11.85	3.56	0.30	3.33	3.65	1.10	4.01	0.100	–
	Branch 1	46	65	9.76	3.29	0.34	4.65	4.41	0.95	4.18	0.124	1
	Branch 2	42	55	9.05	3.31	0.37	3.78	4.17	1.10	4.61	0.157	1
	Branch 3	43	57	8.28	2.34	0.28	3.05	2.91	0.95	2.78	0.171	1
	Branch 4	38	52	8.05	2.47	0.31	3.04	2.47	0.81	2.01	0.129	1
	Branch 5	35	46	7.63	2.11	0.28	2.70	2.77	1.03	2.85	0.162	1
<i>Nd2</i>	Branch 1	101	217	13.66	3.33	0.24	5.29	3.59	0.68	2.44	0.048	–
	Branch 2	105	216	13.87	2.90	0.21	3.32	2.59	0.78	2.02	0.104	–
	Branch 3	103	217	13.84	2.73	0.20	2.50	2.07	0.83	1.72	0.113	–
	Branch 4	95	218	13.81	2.73	0.20	1.75	1.14	0.65	0.73	0.148	–
<i>Nan</i>	Branch 1	49	89	8.94	0.96	0.11	6.09	3.31	0.54	1.80	0.037	1
	Branch 2	50	86	8.84	0.90	0.10	4.42	2.89	0.66	1.90	0.062	1
	Branch 3	50	89	8.64	0.82	0.09	2.20	2.00	0.91	1.83	0.208	1
	Branch 4	50	87	8.56	0.83	0.10	1.91	2.02	1.06	2.13	0.205	1
	Branch 5	50	85	8.34	0.81	0.10	1.48	1.77	1.19	2.12	0.304	1
<i>Np</i>	Trunk distal	47	71	19.96	1.84	0.09	7.35	4.95	0.67	3.33	0.059	4
	Trunk proximal	43	47	14.91	3.01	0.20	6.19	3.06	0.49	1.51	0.057	4
<i>JH1</i>	Trunk	27	27	14.22	1.57	0.11	8.72	3.36	0.38	1.29	0.000	–
<i>JH2</i>	Trunk	55	29	16.98	1.53	0.09	12.30	4.64	0.38	1.75	0.000	–

The probability of a shoot consisting exclusively of preformed organs (i.e. neoformation, $Y=0$) is indicated for each sample of each data set. Depending on the data set, Position refers to the position of the shoot on the tree (trunk or branch) and/or to the position of the shoot on its parent shoot counted from the parent shoot's distal end. N_1 and N_2 indicate, respectively, the number of dissected buds of sample I or fully extended shoots of sample II of each data set. The data shift column indicates the number of scale units (organs) of shift applied to the total number of organs so as to account for the death of distal primordia.

Cerro Otto, Bariloche, Argentina (41°09'S, 71°10'W and 1350 m altitude; Souza *et al.*, 2000). The sampled trees constitute the regeneration cohort among dispersed, older trees of *N. pumilio*.

Data set JH1

Data were collected concerning the composition of terminal buds and shoots of the trunk of 2-year-old hybrid walnut saplings freely growing in an open field plantation at Poët-Laval (44°32'N, 5°0'W, Drôme, France; Sabatier and Barthélémy, 2000).

Data set JH2

Data were collected concerning the composition of terminal buds and shoots of the trunk of 3-year-old hybrid walnut saplings freely growing in an open field plantation at Antagnac (44°21'N, 0°0'E, Lot et Garonne, France; Sabatier and Barthélémy, 2000). $n = 29$ for both samples.

Data analysis

The working model is of the form $Z = X + Y$ where X represents the number of preformed organs and Y represents the number of neoformed organs. The aim was

to estimate the distribution of the number of neoformed organs, P_Y , from two independent samples, one corresponding to the distribution of the total number of organs (in fully extended shoots of sample II), P_Z , and the other to the distribution of the number of preformed organs, P_X (in buds of sample I). If it is assumed that the random variables X and Y are independent, the distribution of the total number of organs is the convolution of two distributions (Feller, 1968): that of the number of preformed organs and that of the number of neoformed organs, expressed as:

$$P(Z = z) = \sum_k P(X = z-k)P(Y = k)$$

with $\mu_Z = \mu_X + \mu_Y$ and $\sigma_Z^2 = \sigma_X^2 + \sigma_Y^2$ (μ denotes the mean and σ the standard deviation of the corresponding random variable; the latter relationship is a consequence of the independence assumption).

This independence assumption is reasonable in cases where the dispersion of the number of preformed organs is small with respect to that of the number of extended organs, while it may be more questionable in other cases (for instance, if shoots with a higher number of preformed organs are assumed to produce a higher number of neoformed organs). The estimation problem is a deconvolution problem for discrete distributions.

The deconvolution problem was extensively studied both in statistics and in signal processing; see Eggermont and LaRiccia (2001) and references therein. The deconvolution algorithm applied to the count data is summarized in the Appendix.

One output of the deconvolution method is the estimate of the probability $P(Y = 0)$ of occurrence of shoots only composed of a preformed part. Because of the degree of incompleteness of the data, the individual probability masses $p_y = P(Y = y)$ cannot be precisely estimated, while the estimates of the mean μ_Y and the standard deviation σ_Y (s.d.) and, consequently, of the coefficient of variation σ_Y/μ_Y (CV) are far more reliable (because of the strength of the convolution assumption). Hence, the estimates of $P(Y = 0)$ should be interpreted with more caution than the estimates of μ_Y and σ_Y in Table 1. The deconvolution procedure is implemented in the AMAPmod software (Godin *et al.*, 1997).

For each sample of each data set, the observed distribution for preformed organs and the estimated distribution for neoformed organs were described by means of the following descriptors: mean, s.d., skewness coefficient and CV. The index of dispersion (ID) defined as σ_Y^2/μ_Y (Johnson *et al.*, 1993) was also computed for each neoformation distribution. This index, which equals 1 for a Poisson distribution, is widely used for counting distributions as a measure of data overdispersion ($\sigma_Y^2 > \mu_Y$) or underdispersion ($\sigma_Y^2 < \mu_Y$).

For data sets *Nal2*, *Nd1* (branches), *Nan* and *Np*, some fully extended shoots had fewer organs than the buds with lowest preformation, which is explained by the spontaneous death of a number of preformed primordia, together with the apex of the shoot, before the extension of neoformed organs. Based on previous information concerning the number of preformed organs usually aborting with the apex of each species (Souza *et al.*, 2000; Puntieri *et al.*, 2002b; J. Puntieri, unpubl. res.), the distributions of the total number of organs for these data sets were shifted 1–4 units to the right (depending on the species and position concerned; Table 1).

RESULTS

For all data sets analysed, the mean number of preformed organs was higher than the mean number of neoformed organs. Within each data set, the s.d. tended to be either higher for neoformed organs than for preformed organs (*Nal1*, *Nal2*, *Nan*, trunk of *Np*, *JH1* and *JH2*) or similar for both distributions (*Nd1*, *Nd2*, branch of *Np*; Table 1).

For data set *Nal1*, shoots had a low probability of consisting only of preformed organs. The mean number of preformed organs was similar for trunk and main branch shoots of *Nal2*, but the s.d. was higher for the latter (Table 1). For this data set, neoformation had a higher mean and s.d. for the trunk than for the branches. Trunk shoots had a much lower probability of not including neoformed organs than branch shoots. The mean number of

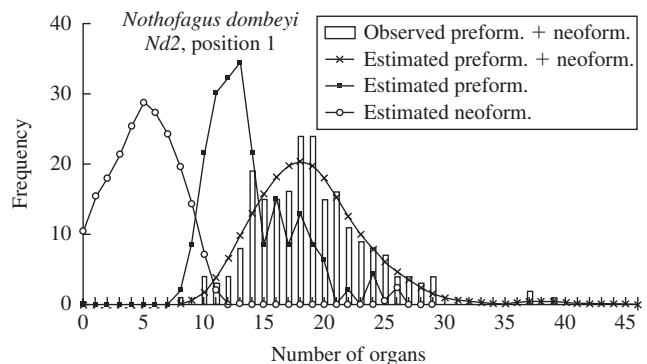


FIG. 1. Observed and estimated distributions of the numbers of preformed and preformed + neoformed organs and deconvolution-estimated distribution of the number of neoformed organs for distal branch shoots of *Nothofagus dombeyi* (*Nd2* data set).

preformed organs was about twice as high for saplings (*Nal2*) as for seedlings (*Nal1*) of *N. alpina* (Table 1).

For position 1 of data set *Nd2*, the observed and estimated distributions for the numbers of preformed and neoformed organs are shown (Fig. 1). In this case, the mean number of preformed organs was higher than that of neoformed organs, whereas the s.d. was slightly higher for neoformed than preformed organs (Table 1). For these shoots, there was a low probability of shoots consisting exclusively of preformed organs.

For data sets *Nd1*, *Nd2* and *Nan*, the mean and s.d. of the number of preformed organs was similar for all positions on the same shoot type (i.e. trunk or branch; Table 1). The mean number of neoformed organs diminished from distal to proximal positions for all three data sets (Figs 2–5). The s.d. decreased in a similar way for *Nd2*, *Nan* and the branches of *Nd1*, but showed little variation for the trunk of *Nd1* (Table 1). The CV of the number of neoformed organs was relatively constant (approx. 1) for all positions of trunk shoots and branch shoots of *Nd1*, except for the most distal trunk shoots (positions 1–4), for which it was lower. This coefficient also showed little variation among positions of *Nd2*. The CV of the number of neoformed organs increased from distal to proximal positions in *Nan*. The ID decreased from distal to proximal positions in branch shoots of *Nd2*. The ID was close to 2 for all positions of *Nan*. The probability of a shoot consisting exclusively of preformed organs increased as the position of the shoot approached the proximal end of its parent shoot (Table 1).

The probability mass functions of preformed organs for data sets *Nd1*, *Nd2* and *Nan* were more bell-shaped and symmetrical than the corresponding probability mass functions for neoformed organs. In consonance, cumulative distribution functions had more sigmoid outlines for preformed than for neoformed organs. These differences were more notable as the shoot position approached the proximal end of a parent shoot (Figs 2–5).

For data set *Np*, the number of preformed organs had a higher mean and lower s.d. for the distal position compared with the proximal position (Table 1). Regarding

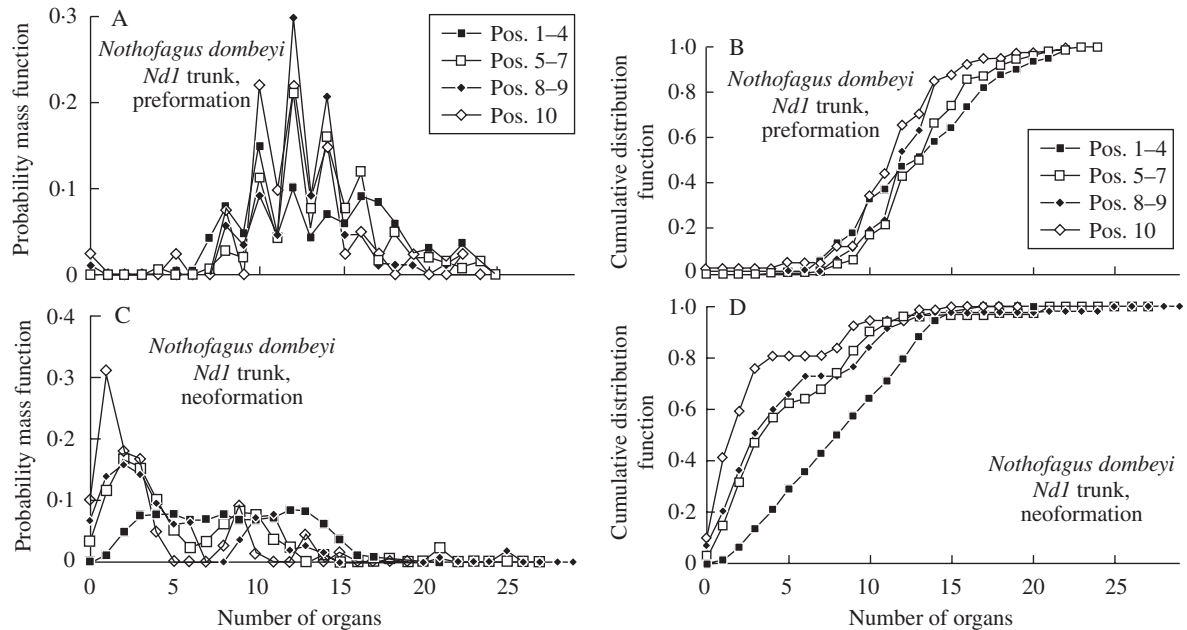


FIG. 2. Preformation (A, B) and neoformation (C, D) of trunk shoots of *Nothofagus dombeyi* (data set *Nd1*) according to shoot position numbered from the distal node of the parent shoot. Estimated probability mass (left column) and cumulative distribution functions (right column) are shown.

neoformation, a higher mean and s.d. were found for the distal than for the proximal positions. The probability of a shoot consisting exclusively of preformed organs was low and similar for both positions (Table 1).

The mean numbers of preformed and neoformed organs were higher for data set *JH2* than for *JH1*. In the case of preformed organs, little difference in s.d. was detected between both data sets. With regards to neoformation, the s.d. was higher for *JH2* than for *JH1*, which rendered the CV similar between data sets (Table 1). The probability of shoots consisting exclusively of preformed organs was zero (Table 1).

DISCUSSION

Preformation and neoformation as components of shoot size

One of the outcomes of the present study is that, for the species under survey, preformation is more relevant than neoformation in the mean number of organs developed by shoots at a specific position within a tree's architecture. The number of preformed organs depends both on the individual tree and on the position of the bud on the tree (Figs 3 and 4). The present and several previous contributions indicate that the distribution of the number of preformed organs per bud, although basically unimodal, varies depending on the position of the bud on the tree as well as on tree age and growth conditions (Gill, 1971; Remphrey and Powell, 1984; Remphrey and Davidson, 1994; Puntieri *et al.*, 2000, 2002a, b; Souza *et al.*, 2000; Costes, 2003; Gordon *et al.*, 2006). According to these results, preformation is the first determinant of axis differentiation and tree architecture. In contrast, other

studies remarked on the relative constancy of the mean number of primordia per vegetative bud in each tree species (Ohlert, 1837 and Assaf, 1965, quoted in Rivals, 1965; Thorp *et al.*, 1994). Two explanations may be proposed for this discrepancy. One of them is the criteria of tree and bud selection: buds in similar architectural positions and collected from trees at a similar growth stage and under similar conditions may have similar numbers of primordia. This could be the case for the studies on orchard trees cited above. The second explanation could be the number of axis types characterizing the architecture of a particular tree species: tree species with a low number of axis types could have less variation in terms of preformation. This justification may only be partially valid, since some of the species presented by Assaf (in Rivals, 1965) as exhibiting a constant mean number of primordia in buds, e.g. *Carya olivaeformis*, *Castanea sativa* and *Juglans regia* var. *parisienne*, resemble architecturally those included in the present study.

For the species studied here, neoformation contributed less than preformation to the total number of organs of shoots, with the exception of trunk shoots of young *N. alpina* (data set *Nal2*) and *Juglans* (*JH1* and *JH2*) trees for which almost as many neoformed organs as preformed organs developed. For long shoots of *Prunus armeniaca* (Costes, 1993), *Actinidia* spp. (Snowball, 1997) and *Prunus persica* (Gordon *et al.*, 2006), neoformation has been shown to contribute to the total number of nodes per shoot to a larger extent than preformation. The results indicate that, although neoformation development was a likely event in all data sets analysed here, it may not occur in shoots of most data sets concerning *Nothofagus* species. In

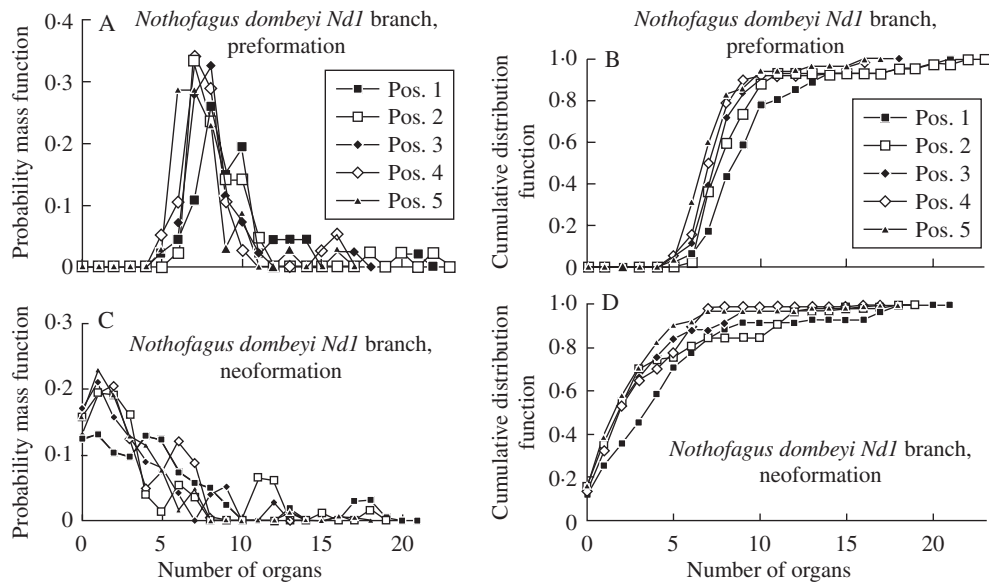


FIG. 3. Preformation (A, B) and neoformation (C, D) of branch shoots of *Nothofagus dombeyi* (data set *Nd1*) according to shoot position numbered from the distal node of the parent shoot. Estimated probability mass (left column) and cumulative distribution functions (right column) are shown.

general terms, the probability of a shoot including only preformed organs is lower for distal than for proximal shoots of the same trees (data sets *Nd1*, *Nd2* and *Na*; Table 1) and lower for the trunk than for the branches of the same tree (data set *Nd1*). At least under some conditions, the most distal shoots derived from the trunk of *N. dombeyi* trees (data set *Nd1*) have a very high probability of including preformed and neoformed organs (Table 1). This seems to be the case also for *Juglans* hybrids (data sets *JH1* and *JH2*).

The differences in the number of neoformed organs among shoots in different positions on the same parent shoot are larger than those in the number of preformed organs. The more distal a shoot, the higher the number of neoformed organs it would be able to develop. This indicates that proximal-to-distal trends of increasing size among shoots derived from a common parent shoot would be linked to variations in both neoformation and preformation.

Distributions of preformed and neoformed organs

The present as well as previous information on several tree species support the view that the distribution of the number of preformed organs in a bud follows approximately symmetric frequency distributions with low dispersion around a mean value (e.g. Rivals, 1965; Champagnat, 1983; Puntieri *et al.*, 2000, 2002a; Sabatier and Barthélémy, 2000; Souza *et al.*, 2000; Seleznyova *et al.*, 2002). Agreement among studies in this respect is more general whenever bud selection observes architectural criteria (e.g. position on the tree, tree age and developmental stage). On the contrary, distributions of the number of neoformed organs deviated from symmetric distributions for all data sets included in the present study.

Preformation and neoformation distributions for each data set and shoot position differed not only in their mean values, as discussed above, but also regarding their dispersion as measured by the s.d. The s.d. of the number of neoformed organs estimated through deconvolution was higher than that of the number of preformed organs, which may be partly explained by alluding to the time of differentiation of preformed and neoformed organs. All preformed organs of a bud would be initiated in a single event of cell differentiation from a shoot's apical meristem at the end of the period of shoot extension (Steeves and Sussex, 1989; Puntieri *et al.*, 2002a). After bud-break, which tends to occur more or less simultaneously within a population (Kozłowski, 1971; Puntieri *et al.*, 1998, 2000; Sabatier *et al.*, 1998; Sabatier and Barthélémy, 1999; Steconi *et al.*, 2000), all preformed organs complete their development as shoots extend. The simultaneous extension of these organs corresponds to the spring peak in shoot growth (Kozłowski, 1971; Puntieri *et al.*, 1998, 2002b). This implies that both initiation and extension of preformed organs take place, for each population, within narrow periods of time. After preformation extension, the shoot's apex may either cease growth (by aborting or entering a dormancy period, *sensu* Borchert, 1991) or produce neoformed organs. Both initiation and extension of neoformed organs would take place in a stepwise fashion during longer periods of time than those of preformed organs (Critchfield, 1960; Kozłowski, 1971). For *N. dombeyi*, neoformed organs start differentiating and extending in late spring and stop extending in mid- to late summer (depending on shoot position); from then until early autumn, primordia accumulate in the terminal bud (Puntieri *et al.*, 2002a). The hypothesis could be proposed that the development of each neoformed organ would depend on the local conditions (both internal and external)

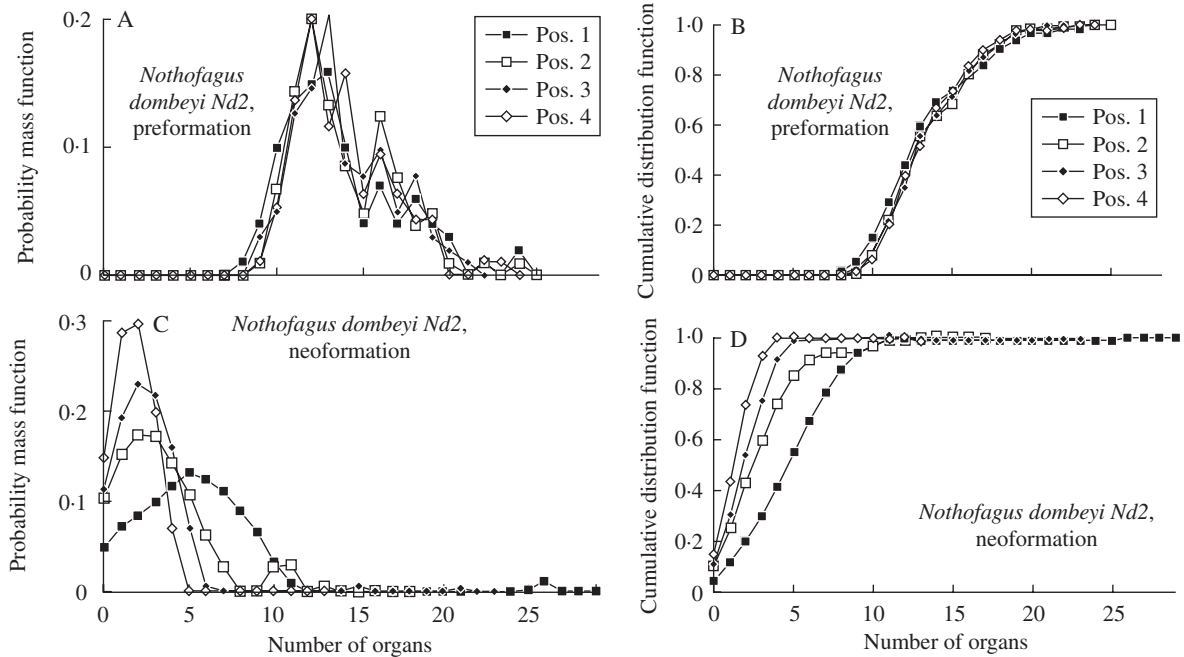


FIG. 4. Preformation (A, B) and neoformation (C, D) of trunk shoots of *Nothofagus dombeyi* (data set Nd2) according to shoot position numbered from the distal node of the parent shoot. Estimated probability mass (left column) and cumulative distribution functions (right column) are shown.

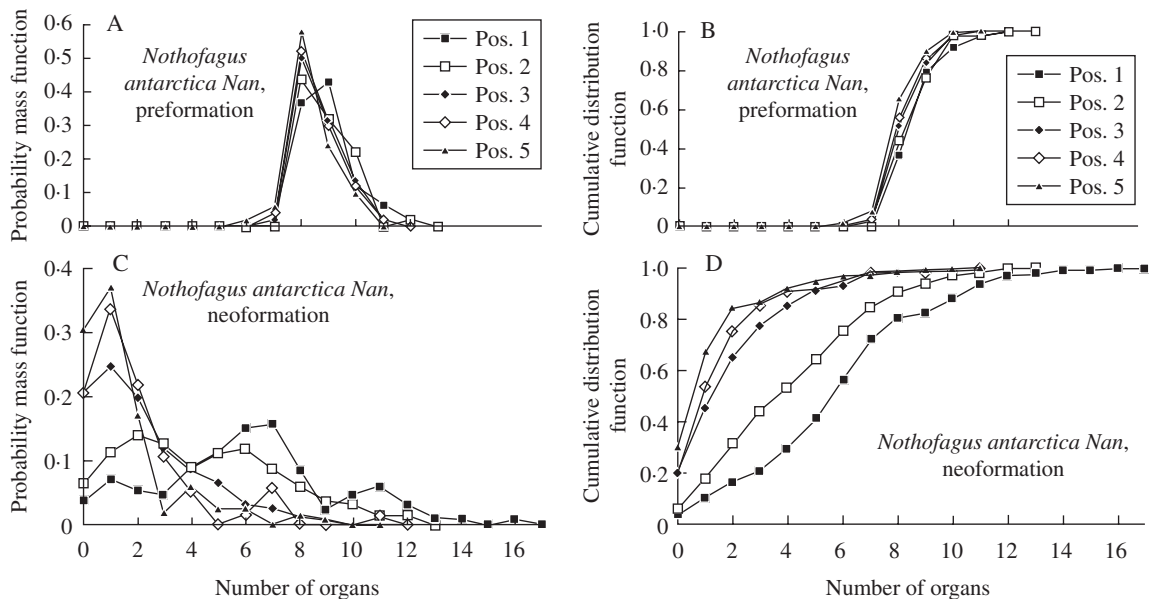


FIG. 5. Preformation (A, B) and neoformation (C, D) of trunk shoots of *Nothofagus antarctica* (data set Nan) according to shoot position numbered from the distal node of the parent shoot. Estimated probability mass (left column) and cumulative distribution functions (right column) are shown.

affecting the shoot concerned, such as architectural position (including topology and geometry), radiation incidence, and water and nutrient supply to the shoot, rather than those affecting the whole tree, such as daylength and water availability in the soil. Whereas shoots in some architectural positions and facing favourable local conditions

(e.g. with high incident radiation and nutrient supply) would continue adding neoformed organs to their structure after preformation extension, those in other positions and/or under less favourable conditions would stop growing. This would partly explain the large differences in growth cessation time among shoots in the same architectural

positions on similar plants (Puntieri *et al.*, 1998; Stecconi *et al.*, 2000). By the time all shoots have entered dormancy, the number of neoformed organs per shoot would differ notably among shoots even within the same architectural position of similar trees. Since terminal buds of the studied species always include at least some leaf primordia, the differentiation of distal primordia would proceed further on than their extension. This uncoupling of differentiation and extension indicates the end of the neof ormation and the beginning of next year's preformation.

Gradients of decreasing numbers of neoformed organs from distal to proximal branches on the same parent shoot (such as those shown here for data sets *Nd1* and *Nd2*) have been explained in terms of endogenously determined gradients of meristem functioning (Barthélémy *et al.*, 1997; Barthélémy, 2003). The variations in the number of neoformed organs within each position observed in the present study could be interpreted as resulting from different shoots being subject to different local conditions (as defined above; see Davidson and Remphrey, 1994; Souza *et al.*, 2000) and would account for shoot plasticity as observed in other species (Gordon *et al.*, 2006). The probability of development of exclusively preformed shoots indicates that the potentiality of a shoot to develop neoformed organs may not necessarily be expressed. This may be the case on most occasions for *Juglans* spp. and hybrids, where preformation seems to be the rule whereas neof ormation occurs in particularly favourable conditions (e.g. cultivated trees such as those studied here and grafted scions in irrigated conditions; Sabatier *et al.*, 1999).

Deconvolution as a method of neof ormation analysis and characterization

The deconvolution method applied here allowed the estimation of the distribution of the number of neoformed organs per shoot from two observed distributions: that of the number of preformed organs in buds and that of the number of organs of fully extended shoots derived from equivalent buds. In the present study, this was achieved by defining data sets with two architectural criteria: the type of axis, often associated with functional roles; and the position of buds and shoots along their parent shoot, which allows size gradients within each axis to be dealt with (Barthélémy *et al.*, 1997; Puntieri *et al.*, 2003). Data set *Nd1* is a good example of a case in which both preformation and neof ormation differ in mean value between two axis types (trunk and main branches). Data sets *Nd1*, *Nd2* and *Nan* exemplify within-axis gradients, especially in terms of the number of neoformed organs. After taking these sources of variation into account, the deconvolution technique proved a useful tool for the description of preformation and neof ormation, even for data sets which, *a priori*, might be considered insufficient (e.g. data sets *JH1* and *JH2*).

CONCLUSIONS

For each axis type of a tree species, there seems to be little variation in the number of leaf primordia of a bud and,

thus, in the number of preformed leaves of the shoot originating from this bud. As a result, an accurate estimation of the distribution of these organs results from bud dissection following architectural criteria. According to the deconvolution method applied, distributions of neoformed organs would be more variable and less symmetric both within and between positions on trees of the same species. This indicates that neof ormation responses within a specific position of a tree would be involved in the plastic response of trees to factors acting locally at the time of shoot extension. This hypothesis could be tested through the assessment of neof ormation in shoots subject to known and controlled conditions.

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APPENDIX

Deconvolution algorithm

Recall that the preformation distribution P_X is supposed to be known. One may compute an estimate of the neoformation distribution $P_Y = \{p_y\}$ by the following algorithm essentially due to Shepp and Vardi (1982). Let $\{p_y^{(0)}\}$ be an initial guess for the neoformation distribution and let $\theta^{(k)}$ denote the current value of the parameters of the neoformation distribution at iteration k . The iteration of the non-parametric deconvolution algorithm is

$$p_y^{(k+1)} = \frac{1}{r} p_y^{(k)} \left\{ \sum_{j=1}^r \frac{P(X_j = z_j - y)}{P(Z_j = z_j; \theta^{(k)})} \right\} = \frac{\alpha_y^{(k)}}{r}, \quad (\text{A1})$$

where r is the sample size and $\alpha_y^{(k)}$ is the expected multiplicity of y neoformed organs given the total number of organs and the current parameters at iteration k . This is an example of the EM algorithm (Dempster *et al.*, 1977; McLachlan and Krishnan, 1997) applied to solve a non-parametric maximum likelihood estimation problem.

Unfortunately, direct application of the above-defined algorithm generally shows that fewer and fewer smooth distributions are obtained as the iteration progresses. The standard solution then consists of stating the deconvolution problem as a maximum penalized likelihood estimation problem; see Eggermont and LaRiccia (2001) and references therein. It should be noted that the count data context is far more simple than the general deconvolution problem (also referred to as the contamination problem) since, in this case, the distribution of interest is a discrete distribution defined on a finite support and not a continuous distribution.

For the regularization of the estimated neoformation distribution, a penalty term is thus incorporated in the likelihood. In the framework of the EM algorithm, the E-step is unchanged but for the M-step, the maximization of

$$Q(\theta|\theta^{(k)}) = \sum_y \alpha_y^{(k)} \log p_y$$

is replaced by the maximization of

$$Q(\theta|\theta^{(k)}) - \lambda J(\theta), \quad (\text{A2})$$

where λ is a tuning constant that determines the relative importance of $Q(\theta|\theta^{(k)})$ and $J(\theta)$, and $J(\theta)$ is a roughness penalty. In the present case, $J(\theta)$ will be the sum of squared second differences:

$$J(\theta) = \sum_y \{(p_{y+1} - p_y) - (p_y - p_{y-1})\}^2.$$

Green (1990) demonstrated the computational economy and accelerated convergence yielded by employing the one-step-late (OSL) algorithm. The OSL algorithm solves

$$DQ(\theta|\theta^{(k)}) - \lambda DJ(\theta^{(k)}) = 0, \quad (\text{A3})$$

where D denotes the derivative operator.

The only difference between equation (A3) and equating the derivatives of expression (A2) to 0 is that in equation (A3), the derivatives of the penalty are evaluated at the current value $\theta^{(k)}$. Both expression (A2) and equation (A3) have the same fixed point so the OSL algorithm converges to a maximum penalized likelihood estimate.

Let

$$\eta_y^{(k)} = \lambda \frac{\partial J(\theta^{(k)})}{\partial p_y^{(k)}}.$$

The M-step (A1) is replaced by

$$p_y^{(k+1)} = \frac{\alpha_y^{(k)}}{\xi^{(k+1)} + \eta_y^{(k)}},$$

where $\xi^{(k+1)}$ is the unique solution for ξ in the equation

$$\sum_y \frac{\alpha_y^{(k)}}{\xi + \eta_y^{(k)}} = 1.$$