

Testing individual inter-annual variations in flower production by means of retrospective analysis of meristem allocation in two tree species

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Abstract High inter-annual variations in flower and seed production have been frequently reported for wind-pollinated tree species. Such variations have been generally studied from a population perspective. The present study focuses on the meristematic cost of flower production in main branches of *Nothofagus obliqua* and *N. nervosa* through a retrospective analysis of flower production over 4–5 consecutive years. In both species, the percentage of flowering nodes in one year varied between 0 and 55 %. In *N. obliqua*, total flower production was bimodally distributed: years of high and low production were more common than years of intermediate production. Bimodality was not significant either for total flower production in *N. nervosa* or for each flower type separately in both species. Flower production affected branching negatively, which meant a meristematic cost. Periodicity in flower production was uneven among co-specific trees; more than two consecutive years of high flower production were rare in all trees. Inter-annual variations in the production of staminate and pistillate flowers were synchronized. In *N. nervosa*, the production of pistillate flowers tended to be more variable among

years than the production of staminate flowers. The applied technique helps in the assessment of individual variations of flower production and contributes to understanding factors promoting seed-production variations in tree species at population scale.

Keywords wind-pollinated trees · flower production · inter-annual variation · meristem allocation

Introduction

High inter-annual variations in the production of reproductive structures (flowers or cones) are common in wind-pollinated tree species. Such variations have often been associated with the intermittent production of large seed crops by a population of plants, a phenomenon described as masting or mast seeding (Kelly 1994; Herrera et al. 1998; Monks and Kelly 2006). The adaptive advantages so far proposed to explain inter-annual variations in flower/seed production, such as predator satiation or pollination efficiency, have most often been visualized from a population perspective. Nevertheless, in order to improve our understanding of such variations, studies at scales of observation smaller than population, e.g. inter-annual variations in flower production at individual level (Herrera 1998; LaMontagne and Boutin 2007; Żywiec et al. 2012), are essential.

Inter-annual variations in flower and seed production in individual plants may be explained by alluding at reproductive costs, i.e. the negative consequences of the development of reproductive structures for future plant

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growth. Mostly, reproductive costs are assessed through mass allocation to reproductive structures (Obeso 2002; Hirayama et al. 2007). In the case of trees, a more suitable approach to reproductive costs would involve the study of meristem allocation to reproductive structures and how this allocation impacts on further tree growth (Lehtilä and Larsson 2005). Both number and position of meristems with the potential to develop reproductive structures are related to the meristematic costs of reproduction, and depend on the tree species concerned: reproductive structures may be developed on shoots in a wide range of topological positions or only on shoots at specific topological positions (Hallé et al. 1978). In the former case, vegetative and reproductive functions may interfere strongly with one another, since meristems allocated to reproductive structures are unavailable for further growth and vice versa. In most tree species, the assessment of the meristematic cost of reproduction poses a challenge due to the large amount and variety of axes conforming the crown at adult (i.e. reproductive) ontogenetic stages (Barthélémy and Caraglio 2007). In these plants the costs associated with sexual reproduction can be estimated by the proportion of meristems of a branched system that generate reproductive structures (Fig. 1). The study of

meristem allocation to reproductive structures in individual trees may thus be useful for assessing variations in reproductive costs over time.

Nothofagus species (Nothofagaceae, Fagales) exhibit notable inter-annual variations in seed production (Allen and Platt 1990; Burrows and Allen 1991; Marchelli and Gallo 1999; Rosas et al. 2004). For populations of the South American species *Nothofagus obliqua* (Mirb.) Oerst. and *N. nervosa* (Phil.) Krasser [= *N. alpina* (Poepp. et Endl.) Oerst.], it has been suggested that events of relatively high flower production occur every three and two years, respectively (Donoso 1993; Marchelli and Gallo 1999; Donoso et al. 2006a,b). Nevertheless, inter-annual variations in the production of staminate and pistillate flowers have not been characterized at individual scale for either species. In *Nothofagus* species, reproductive shoots arise at main branches, secondary branches and short branches (Torres et al. 2012), and are able to produce both inflorescences and branches. Therefore, flowering implies a cost in terms of the number of meristems available for vegetative development (Fig. 1). Inter-annual variation of flower and seed production in these species has been studied, as in other anemophilous trees, through the counting of fruits/seeds in traps (Donoso 1993; Marchelli and Gallo 1999; Monks and Kelly 2006), which does not allow the estimation of the costs of reproduction in terms of individual meristem allocation. Variations over time in the production of flowers (especially staminate flowers) have been ignored so far. Estimating the proportional allocation of meristems to reproduction in successive years may improve our understanding of inter-annual variations in seed production.

In the present study, we carried out a small-scale analysis of inter-annual variations in meristem allocation for flower production of individual trees by means of a retrospective evaluation in main branches over successive years. Previous observations on *Nothofagus* show that fallen staminate and pistillate inflorescences leave scars on the stem that may still be recognized several years after flower production (Puntieri et al. 2009; Torres 2013). This study was aimed at describing the variability in annual meristem allocation to flower production in *N. obliqua* and *N. nervosa* and the effect of flower production upon future branching. We also analysed the connection between inter-annual variations in meristem allocation to flowering on main branches and variations in seed production at population scale.

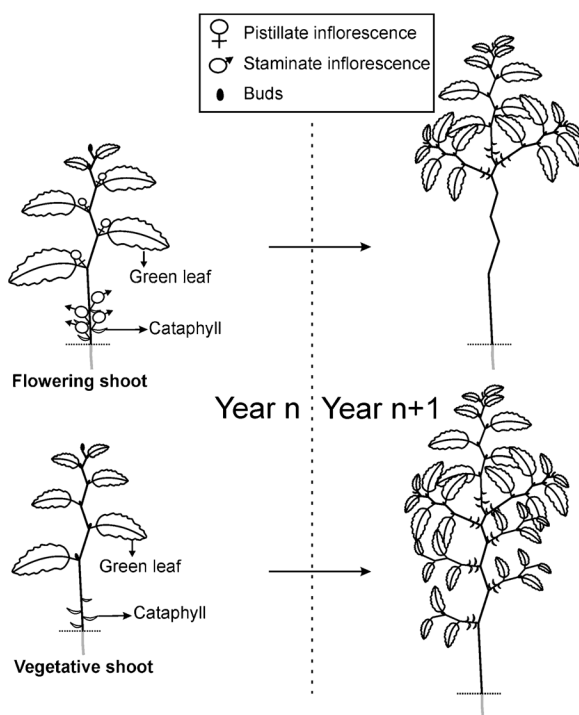


Fig. 1 Example of flowering and vegetative shoots of *N. obliqua* through two consecutive growing seasons. Vegetative buds at year n produce branches at year $n + 1$.

Material and methods

Flower distribution in *N. obliqua* and *N. nervosa*

These species are diclino-monoecious and have inconspicuous staminate inflorescences and pistillate inflorescences, each one composed of 1–3 flowers (trimerous pistillate inflorescences is the rule in both species). Flowering shoots usually develop both types of inflorescences at specific positions: staminate inflorescences arise from proximal nodes and pistillate inflorescences from intermediate to distal nodes. Non-flowering or vegetative nodes of flowering shoots in these species bear axillary buds with the potential to develop lateral shoots (branches; Fig. 1).

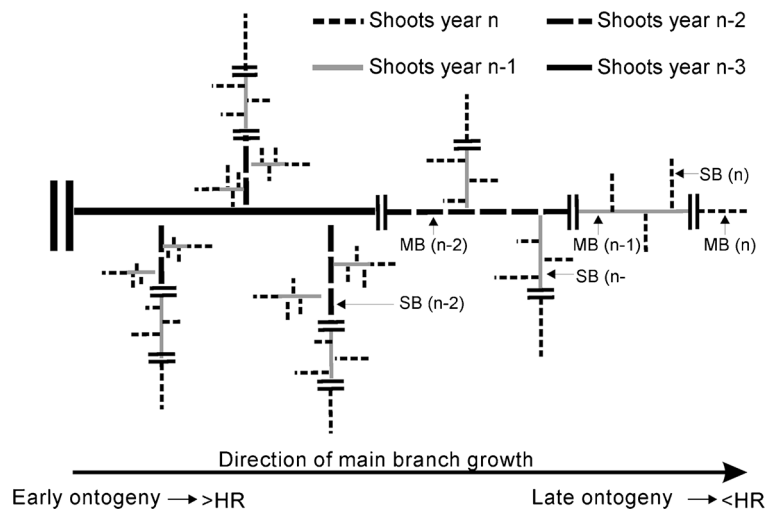
Sampling sites and trees

In order to assess the general pattern of periodicity in flower production of main branches, young-adult trees of *N. obliqua* and *N. nervosa* from different environments were selected at the end of the 2009–2010 growing season. We aimed at measuring as long a sequence of flowering shoots as possible for each tree, so that trees with flowering main branches at low positions (2–3 m high from the ground) were sought after. In addition, in order to avoid shaded branches, these trees had to be located in open areas and have undamaged flowering main branches on the sunniest side of the crown. Finding trees that fulfilled these requirements proved a difficult task, which explains why sample numbers were rather low. For *N. obliqua*, twelve individuals were

selected, six from a natural population located at Lanín National Park (hereafter Lanín NP; 40°09' S, 71°31' W; 687 m a.s.l.), and the other six from urban parks at San Carlos de Bariloche city (hereafter Bariloche; 41°08' S, 71°10' W; 878 m a.s.l.) and El Bolsón city (41°58' S, 71°32' W; 305 m a.s.l.). Regarding *N. nervosa*, eight individuals were selected, four of them from Lanín NP (40°00' S, 71°22' W; 930 m a.s.l.), and the other four from urban parks at Bariloche. Height and diameter at breast height of the sampled trees were, on average, 14.4 m ($SD = 1.7$) and 30.4 cm ($SD = 7.3$) for *N. obliqua*, and 21.9 m ($SD = 7.5$) and 67.1 cm ($SD = 27.0$) for *N. nervosa*. One flowering main branch was cut per tree.

Morphological markers known for these and other *Nothofagus* species were recognized so as to identify inter-annual growth limits (Barthélémy et al. 1999; Passo et al. 2002). By this means, axis segments developed in successive growth periods, i.e. annual shoots, were identified starting from the most recently extended annual shoot, developed in the 2009–2010 growing season (Fig. 2). For each annual shoot, we assessed the number of axillary meristems by counting the number of nodes (one axillary meristem arises at each node). The axillary production at each node of each annual shoot was assigned to one of the following categories: (1) vegetative structure, (2) staminate inflorescence, (3) pistillate inflorescence, and (4) axillary production not evident. The first category includes axillary buds and branches either standing or detached at the time of sampling. Most of the axillary structures that had been developed two or more years before sampling were identified by the scar left on the stem by each structure

Fig. 2 Schematic representation of a four-year section of a main-branch indicating annual shoots developed in different years. Note the decreasing of the size of main-branch shoots relative to the most vigorous secondary-branch shoots as ontogeny progresses. MB – main branch; SB – secondary branch. HR (hierarchy ratio) = No. nodes MB ($n - x$) / No. nodes SB ($n - x$).



(Fig. 3). In *Nothofagus*, the development of fruits and cupules occurs independently of ovule fertilization (Riveros et al. 1995), so the scar left by a cupule after its abscission is an accurate indicator of the presence of a pistillate inflorescence. The scar left by a cupule is deep and wide, and has a circular protruding vestige of vascular tissues at its centre (Fig. 3A). The scar left by a staminate inflorescence after falling is shallow, narrow and elliptical (Fig. 3B). The scar left by a fallen branch is similar in outline to that left by a cupule, but is shallower and devoid of the central protruding vestige of vascular tissues (Fig. 3C). An accurate retrospective analysis was possible for shoots extended four or five years back, depending on the tree. In older shoots, the scars of different axillary structures on the stem become indistinguishable from one another as secondary growth forces bark cracking and shedding. The total numbers of shoots and nodes observed per species and year are indicated in Table 1.

Data analysis

Equation 1 was employed to compute, for each branch and year (x), the meristematic allocation to flower development (MAF), expressed as the proportion of nodes assigned to flower production in one year. This calculation was made separately for staminate inflorescences, pistillate inflorescences and total (staminate + pistillate) inflorescences, considering

$MAF_{year\ x}$

$$= \frac{\sum_{i=1}^n N^{\circ} \text{ inflorescences of shoot}_i \text{ in year } x}{\sum_{i=1}^n N^{\circ} \text{ nodes of shoot}_i \text{ in year } x} \quad (1)$$

Where n represents the total number of shoots produced in the year concerned. A similar equation,

replacing number of inflorescences by number of vegetative structures, was applied to assess the meristematic allocation to vegetative structures (MAV). The frequency distribution of the MAF was computed including information of all sampled trees of each species for all years of shoot development, as this provided an indication of the degree of variation in MAF. In this regard, a unimodal distribution of MAF would indicate that variation in flower production fluctuates around a mean value. A significant deviation from a unimodal distribution, on the contrary, would support the idea that years of high and years of low MAF would take place. Hartigan's diptests were applied so as to assess if the observed MAF distributions differed significantly from unimodality (Hartigan and Hartigan 1985).

It was considered that variability in flower production among main branches may depend upon ontogenetic factors. Main branches of *Nothofagus* start their development from the trunk as hierarchical axes, in which the straight main axis is clearly differentiated, both in size and orientation, from its branches (Fig. 2); as a branch progresses along ontogeny, differences between its main axis and its branches get gradually less notable, so that the main branch becomes a less hierarchical structure (Barthélémy et al. 1999; Stecconi et al. 2010). Therefore, the degree of hierarchical structure of a main branch was considered an accurate indicator of the ontogenetic stage of a main branch. We computed the ratio between the number of nodes of the annual shoot that follows the direction of growth of the main branch in one year and the number of nodes of the most vigorous lateral shoot developed from the main branch in the same year. Hereafter, this ratio will be referred to as hierarchy ratio (HR). For the main branches of the sampled trees, HR varied between 1.0 and 2.2 in *N. obliqua* and between 0.8 and 2.0 in *N. nervosa*. Based on the frequency distribution of HR, the trees

Fig. 3 Illustrations showing *Nothofagus* shoot nodes with scars left by a fallen cupule (A), a staminate inflorescence (B) and a branch (C). SI – staminate inflorescence; VT – vascular tissues; LS – leaf scar.

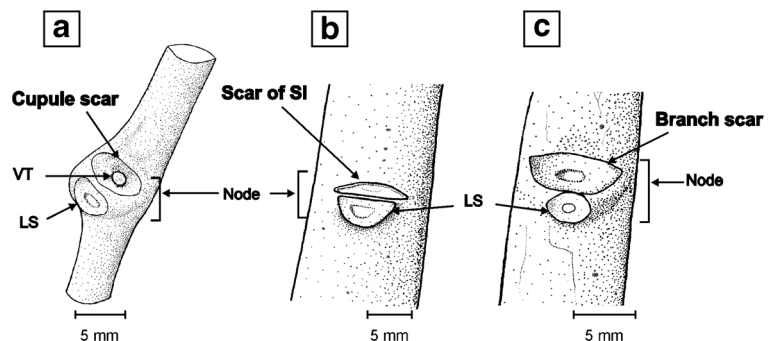


Table 1 Total numbers of annual shoots, the sum of their nodes and number of nodes per shoot corresponding to each growing season for each species.

Growing season	<i>N. obliqua</i>			<i>N. nervosa</i>		
	N° shoots	N° nodes	N°nodes/shoot	N° shoots	N° nodes	N°nodes/shoot
2005–2006	6	92	15.3	4	63	15.7
2006–2007	35	683	19.1	22	312	14.2
2007–2008	124	1,932	15.6	93	1,220	13.1
2008–2009	460	6,411	13.9	309	4,023	13.0
2009–2010	1,428	17,856	12.5	671	6,767	10.1

selected for this study were differentiated in two groups: those with HR higher than 1.3 (hereafter HR1), i.e. with the main branch at an earlier ontogenetic stage, and those with HR lower than 1.3 (hereafter HR2), i.e. with the main branch at a later ontogenetic stage.

In order to quantify the extent to which flower production restricts branching, the relationship between the number of inflorescences and the number of vegetative structures per shoot was assessed by means of hierarchical generalized linear mixed models (GLMMs). In this analysis, the number of inflorescences was considered as a predictor variable and the number of vegetative structures as a response variable, assuming a Poisson error distribution and a log-link function; tree was included as a random factor. For each species, both groups of trees discriminated by HR were considered independently. Such analysis was performed in R using the lme4 package (Bates et al. 2009). For each species, we registered the number of trees in which flower production in two or more consecutive years was higher than the individual average.

Inter-annual variations in the production of staminate and/or pistillate inflorescences were assessed for each tree by means of the coefficient of variation ($CV = \text{standard deviation} / \text{mean}$) of the MAF. In order to compare the magnitude of inter-annual variation in each flower type, the CVs of staminate and pistillate inflorescences were compared for each species by means of non-parametric Wilcoxon tests (Sokal and Rohlf 1981). Inter-annual variations of MAF and MAV were compared with those in precipitations and mean temperature in each year. According to previous studies on *Nothofagus* species, the differentiation of preformed organs (including all flowers) takes place in the warmest summer months, i.e. January and February (Puntieri et al. 2009). Therefore, mean annual values of daily

minimum and maximum temperatures and total precipitations during the January–February period of each year were computed for data recorded at regional meteorological stations at Lanín NP and Bariloche (no data available for El Bolsón).

The degree of synchrony of inter-annual variations in the productions of staminate inflorescences, pistillate inflorescences and vegetative structures was evaluated by applying an analysis employed by Lepš (2004) in the assessment of synchrony in biomass production among species of a community. In our case, we compared the variance of total axillary production with the sum of the variances of the production of each structure. If different axillary productions vary independently from one another, then Eq. 2 should be confirmed:

$$\text{Var} \left(\sum_{i=1}^n xi \right) \approx \sum_{i=1}^n \text{Var}(xi) \quad (2)$$

, where xi is the axillary production (number of staminate inflorescences, pistillate inflorescences and vegetative structures) and n is the number of all types of axillary productions considered in the analysis. In order to avoid variations associated with the different numbers of shoots that were observed for each growing season, in this analysis the axillary productions of the growing seasons 2005–2006 to 2008–2009 were recalculated proportionally to the number of nodes observed in the last growing season (2009–2010). A higher variance of total axillary production (left side of the equation) means synchronous variation (positive correlation), whereas a higher value of the sum of variances of the production of each structure (right side) means asynchronous variation. By this means we estimated the degree of synchrony between the productions of staminate and pistillate flowers, and between the productions

of total flowers and vegetative structures (in both analyses $n = 2$ in Eq. 2). Variances were calculated for each tree and then a Wilcoxon matched-pairs test were applied for intra-specific comparisons.

All statistical analyses were performed using R v.2.12.0 (R Development Core Team 2012). A 5 % significance level was adopted in all comparisons.

Results

For both species, the MAF for a main branch in one year varied between 0.00 and 0.55 (Fig. 4). For *N. obliqua*, the MAF for staminate inflorescences varied between 0.00 and 0.35, with peaks at 0.00–0.10 and 0.16–0.25. The MAF for pistillate inflorescences in one year reached up to 0.35; MAFs lower than 0.06 and between 0.11 and 0.25 were the most common. Nevertheless, the frequency distributions of MAF for staminate and pistillate inflorescences did not differ significantly from unimodality (Hartigan's diptest, $P = 0.17$ and 0.60 , respectively; Fig. 4A,B). On the other hand, low (0.01–0.05) and high (0.31–0.45) values of the MAF for both flower types together (staminate + pistillate) were more frequent than intermediate values, which was supported by the significant deviation of the frequency distribution of MAF from a unimodal distribution ($P < 0.01$; Fig. 4C). In *N. nervosa*, MAF varied between 0.00 and 0.35 for staminate inflorescences, between 0.00 and

0.25 for pistillate inflorescences, and between 0.00 and 0.55 for all inflorescences (Fig. 4F); deviations of MAF from unimodality were not significant ($P = 0.69$, 0.83 and 0.91 , respectively).

The number of inflorescences per shoot had a significant negative effect upon the number of vegetative structures (branches or buds) for both species, more notably so for *N. obliqua* (Fig. 5). The ontogenetic stage of the main branches did not affect the significance level of this result in any of the two species. GLMMs results for *N. obliqua*: $\chi^2 = 1122.1$ in HR1 trees and $\chi^2 = 568.55$ in HR2 trees; for *N. nervosa*: $\chi^2 = 28.9$ in HR1 trees and $\chi^2 = 70.8$ in HR2 trees; $P < 0.001$ in all four tests (Fig. 5).

Annual MAF did not exhibit a uniform trend over time for all trees of either species (Fig. 6A,B). Half of the observed *N. obliqua* trees presented at least two consecutive years with MAF above the individual mean (Fig. 6A). In the remaining *N. obliqua* trees, a year of relatively high MAF was preceded and followed by one year of low or null flowering (Fig. 6A). More than two consecutive years of nearly constant MAF were uncommon. Only two trees of *N. nervosa* presented MAF higher than their individual average in two consecutive years (Fig. 6B). Overall, trees of HR2 were more likely to present consecutive years with MAF above their respective means. In both species and both sample sites, several individuals coincided in having high flower production in the 2008–2009 growing season

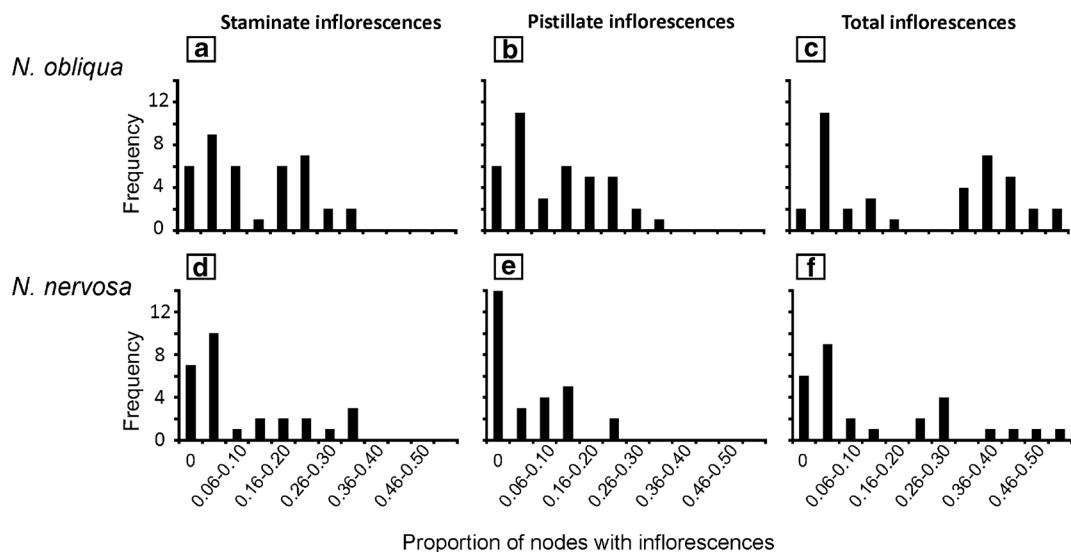


Fig. 4 Frequency distributions of the proportion of nodes producing staminate inflorescences, pistillate inflorescences and total inflorescences in one year (see Eq. 1) for *N. obliqua* (A–C) and *N. nervosa* (D–F).

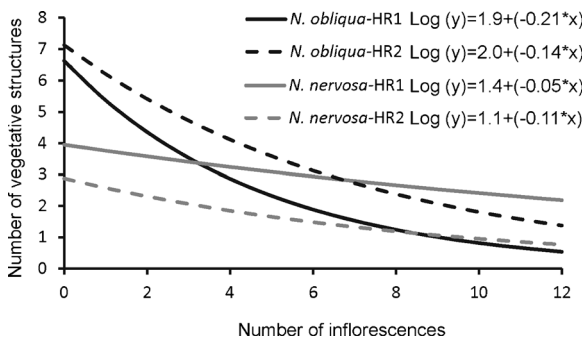


Fig. 5 Model relating the number of inflorescences and the number of vegetative structures per shoot for *N. obliqua* and *N. nervosa*. For each species, trees of HR1 and HR2 are distinguished (for details, see M&M section). The functions provided for each species result from hierarchical GLMMs, considering tree as random factor.

(Fig. 6A–C). In the preceding summer (January and February 2008) mean maximum temperature was high and precipitations low at Lanín NP (Fig. 6D). In Bariloche, total precipitation registered in January and

February in 2008 was higher than at Lanín NP, but such precipitation was concentrated just in two days (29 January and 26 February), while there were no rainfall in the remaining days (data not shown). In Lanín NP, the mean maximum temperature in 2008 reached the highest level for the study period. Less variation in this regard was registered at Bariloche. A peak of summer rain was registered in 2006, preceding a growing season (2006–2007) in which most trees had low flower production and high vegetative production (Fig. 6C,D).

For *N. obliqua*, inter-annual variations in the MAF for staminate and pistillate inflorescences, estimated by the CV per tree, were, on average, 1.02 (*SE* = 0.12) and 1.11 (*SE* = 0.12) respectively, and did not differ between them statistically (Wilcoxon test, *P* = 0.31). In the case of *N. nervosa*, the MAF for pistillate inflorescences tended to be more variable among years (*CV* = 1.47; *SE* = 0.21) than the MAF for staminate inflorescences (*CV* = 1.14; *SE* = 0.19; Wilcoxon test, *P* < 0.05). The

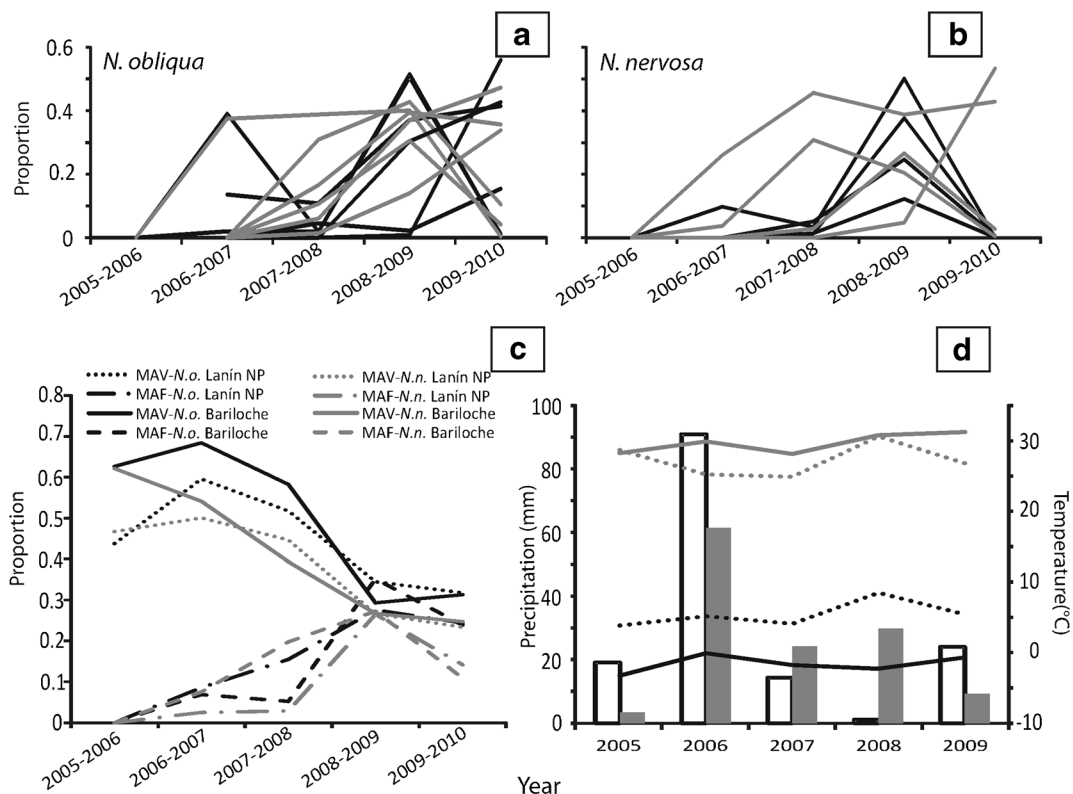


Fig. 6 Inter-annual variation of the meristematic allocation to flower development (MAF) of 12 *N. obliqua* trees (A) and eight *N. nervosa* trees (B). (C) Mean MAF and MAV of the trees of both species sampled at Lanín NP and at Bariloche. (D) Mean maximum (grey lines) and minimum (black lines) temperature and

precipitation (bars) of January and February at Lanín NP (dotted lines, empty bars) and Bariloche city (solid lines; grey bars). For each species, black lines correspond to trees of HR1 and grey lines correspond to trees of HR2.

variance of total inflorescences production was significantly higher than the sum of the variances of staminate and pistillate inflorescences separately (Wilcoxon test: *N. obliqua* – $P < 0.01$, *N. nervosa* – $P < 0.05$), suggesting synchronous variations for both flower types. Concerning the productions of total inflorescences and vegetative structures, asynchronous variations were found for both species: i.e. sum of variances of total inflorescences and vegetative structures higher than the variance of total axillary productions (Wilcoxon test: *N. obliqua* – $P < 0.01$, *N. nervosa* – $P < 0.05$).

Discussion

We studied inter-annual variations in the flower production of trees through the novel application of the retrospective analysis of branch growth. For both *Nothofagus* species under survey here, the percentage of main-branch axillary meristems allocated to flower production in one year varied between 0 and 55 %. In the case of *N. obliqua*, years of high and years of low flower production (~30–45 % and < 5 % of flowering nodes, respectively) were more common than years of intermediate production. This bimodal pattern has been associated to species in which reproduction occurs at the expense of vegetative growth (Kelly 1994). Deviation from unimodality was not demonstrated statistically either for the yearly flower production of *N. nervosa* or for staminate and pistillate inflorescences separately examined in either species.

Inflorescences production per shoot and the number of meristems able to generate vegetative organs were negatively related. This was also evidenced by the fact that inter-annual variations in inflorescence production were asynchronous with those in the production of vegetative structures. The more negative effect of flower production upon future branching in *N. obliqua* than in *N. nervosa* could be related to the longer time-gap between mast years documented in the former than in the latter species (Donoso 1993; Marchelli and Gallo 1999; Donoso et al. 2006a,b). It has been demonstrated for *Nothofagus* and other woody species, that heavy flowering and/or seed production impact negatively on radial wood increment (Kelly and Sork 2002; Monks and Kelly 2006) and leaf production (Alley et al. 1998). The observed decline in the number of branches that *Nothofagus* trees are able to develop during a year of heavy flowering could explain the patterns just

mentioned. Since flowering in *Nothofagus* is not constrained to particular axes, the impact of a year of high flower production upon branching – and therefore upon leaf production – should affect further growth which, in turn, would mean fewer sites for future flower production. It may be argued that years of low or nil flower production should allow plants to accumulate photosynthates necessary for future flowering.

The measure of reproductive cost employed in this study considered all axillary meristems as equals, which assumes that all inflorescences have a similar influence on future growth. This is unlikely to be the case for several reasons. First of all, only pistillate inflorescences are tied to fruit and seed development, which makes them stronger nutrient sinks (Obeso 2002; Case and Ashman 2005). In addition, because of the acrotonic gradient of branch sizes that characterizes *Nothofagus* species (Barthélémy et al. 1999), each pistillate inflorescence would mean a sharper restriction to future branch growth than a staminate inflorescence, as the former are more distally positioned than the latter on the flowering shoots (see Fig. 1; Puntieri et al. 2009). Based on these ideas, we expected to find higher CVs in the production of pistillate flowers than in that of staminate flowers. This prediction was supported for *N. nervosa* but not for *N. obliqua*. This inconsistency between both species could be related with the more distal position, and thus higher cost, of pistillate inflorescences in *N. nervosa* than in *N. obliqua* (Puntieri et al. 2009). A previous study on *Carya* species (Juglandaceae) reported that inter-annual variations in the production of staminate flowers were higher than those in pistillate flowers (McCarthy and Quinn 1989). Nevertheless, the meristematic costs associated with the production of each flower type were not evaluated for these species, as far as we know.

Based on previous data on seed production of *Nothofagus* spp., the occurrence of one year with high flower production every several years with low or nil flower production was expected (Marchelli and Gallo 1999; Kelly and Sork 2002; Rosas et al. 2004; Monks and Kelly 2006). Nevertheless, this pattern was not consistent for all *N. obliqua* and *N. nervosa* trees observed: for some trees, MAF was similar in consecutive years. This result suggests that some degree of intra-specific variation regarding the periodicity of flower production should be expected. Previously, Marchelli and Gallo (1999) reported variations of the periodicity of seed production among populations of *N. nervosa*. In

each individual, the productions of staminate and pistillate inflorescences were, according to our results, synchronized, which means that years of high seed production in populations of these species should be also years of high pollen production.

Inter-annual individual variations in flower production obtained here by a retrospective analysis are comparable to those in seed production previously reported for populations of *Nothofagus* species (*CV* between 1.44 and 2.73; Schaubert et al. 2002; Monks and Kelly 2006). By re-analysing data reported by Marchelli and Gallo (1999), we estimated that the *CV* of seed production for different populations of *N. nervosa* varied approximately between 0.77 and 1.84 (mean \approx 1.33), matching the mean inter-annual *CV* of 1.47 computed in this study for the production of pistillate inflorescences on main branches of *N. nervosa*. Therefore, at least for *N. nervosa* part of the inter-annual variation in seed production found for natural populations may reflect variations in the flower production of individual trees. No previous information in the *CV* of seed production for *N. obliqua* was found to compare with the *CV* = 1.11 found here for pistillate flower production.

The following question may be posed: which factors rule variability in flower production among co-specific trees? The ontogenetic stage of the tree may be among these factors (e.g. Heuret et al. 2006). The present study provides preliminary support to this idea: main branches at earlier and later ontogenetic stages were slightly different regarding between-year variations in meristematic allocation to flower development. This issue would be worth of further, more exhaustive studies in which higher numbers of individuals at different ontogenetic stages would be sampled. Considering the high proportion of trees of both species with high MAF in the 2008–2009 growing season, it may be proposed that environmental conditions may contribute to increase flower production. Long-term studies in populations of mast tree species showed that warm temperatures and/or low precipitation during summer could be among the flower-inducing environmental cues (Allen and Platt 1990; Schaubert et al. 2002; Kon et al. 2005; Övergaard et al. 2007). Although the period of time covered in the present study was rather short, the high temperatures and very low precipitations registered at Lanín NP in the summer preceding the period with high flower production registered here, are consistent with the cited studies. The high level of precipitations recorded regionally for the 2006 summer period (Fig. 6D)

could have been involved in the relatively high production of vegetative organs in the 2006–2007 growing season.

The retrospective analysis applied here means a novel contribution to the assessment of inter-annual variations of flower production in mast tree species. Retrospective analyses of primary growth have been previously performed to evaluate ontogenetic trends in the development of trees (Heuret et al. 2006) and, for herbaceous species, proved to be an alternative to dendrochronology for assessing the effects of inter-annual climatic variations on plants growth (Havström et al. 1995; Johnstone and Henry 1997; Štátná et al. 2012; Klimešová et al. 2013). The technique applied here allows a very accurate evaluation of meristem allocation to flower production, discriminating between pistillate and staminate flowers. Moreover, year-to-year variations in flower production could be assessed at a single point of time, avoiding long-term observations. The retrospective technique may be considered to have a weakness due to the fall of shoots derived from older main-branch shoots. Although we cannot be certain about the factors that caused shoot falls, it may be proposed that fallen shoots would not have been different, in terms of meristem allocation to flowers, from those that were standing at the time of sampling. Therefore, the main results may not have been significantly different had all developed shoots been standing. On the other hand, it may be argued whether the conclusions of this study may be extrapolated to entire trees, considering that only one main branch per tree was examined. This query may be answered positively in the case of trees that are fully exposed to open conditions. Supporting this assumption, a recent study on *N. obliqua* and *N. nervosa* trees showed that lower and upper branches tended to develop similar numbers of flowers (relative to the total number of nodes; Torres 2013). In addition, the periodicity in seed production mentioned in previous studies on *Nothofagus* sp. div. (e.g. Rosas et al. 2004) is coherent with the idea that flower production in a particular year reaches a similar level throughout the crown of a tree.

Conclusions

The present study shows that flower production in main branches of *N. nervosa* and *N. obliqua* trees is highly variable between years. Inter-annual variations in flower

production would not follow a similar pattern in all trees developed in the same region. Some individuals seem to be able to reach high flower production in two or more successive years, whereas others have low flower production both before and after a year of high flower production. Ontogenetic differences among trees may be involved in this difference among trees. This study supports the hypothesis that variations in flower production could be related to the costs of flowering in terms of meristem allocation. Inter-annual variations in the productions of staminate and pistillate flowers follow similar patterns in *N. obliqua* and *N. nervosa*. In the latter species, the production of pistillate flowers tends to exhibit sharper between-years variations than that of staminate flowers.

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