ORIGINAL ARTICLE

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Crown architecture of grafted Stone pine (*Pinus pinea* L.): shoot growth and bud differentiation

Abstract The singular umbrella-like crown shape of Stone pine can be interpreted as a consequence of primary shoot-growth patterns and posterior axis differentiation due to differential secondary growth and down-bending of branches. This paper centres on the first aspect, analysing the growth, branching and flowering behaviour of about 5,000 individual shoots on 27 grafted Stone pines. The data measurement on standing trees allowed to study correlations of topologic and geometric variables in the shoot and their ancestors. The only significant correlations were found with parameters of the mother shoot formed the previous year and with the number of cones born 3 years before by the respective ancestor. The fitted relationships between geometric and topologic shoot and branch variables are the first step of a structural model construction that can be completed with functional components like a radiation and a carbon allocation submodel, stressing the importance of the heavy Stone pine cones as carbon sinks, with a total annual allocation similar to stem wood. In conclusion, the Stone pine crown shape emerges as consequence of the lack of initial vigour differentiation between stem and main-branch apical meristems that favour the generalized sylleptic reiteration in the open-grown trees.

Keywords Stone pine · *Pinus pinea* · Shoot growth · Bud differentiation · Crown architecture

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Introduction

The analysis and modelling of the crown architecture of trees has been the object of an increasing amount of research since the first descriptive works (cf. Rauh 1939; Halle and Oldeman 1970; Halle et al. 1978). They point both at empiric growth models (Reffye de et al. 1997) and at realistic simulations under the paradigm of "artificial life" with the aim of studying complex structures or traits as phenomena emerging from the reiterative application of simple algorithms (Prusinkiewicz and Lindenmayer 1990; Prusinkiewicz et al. 1995). Most models of plant growth and architectural development are based on an open, modular structure and its endogenous dynamics: birth, growth and senescence of the components, their topology and geometry (White 1979; Ford and Ford 1990; Kellomäki and Strandman 1995; Kurth and Anzola 1997). Functional–structural tree models approach crown development by processes at shoot level, using a framework of different interactive sub-modules which take into account as relevant factors for shoot morphology both branch position within the crown and light environment (Gavrikov and Sekretenko 1996; Kellomäki et al. 1999; Sievänen et al. 2000; Perttunen et al. 2001; Niinemets and Lukjanova 2003). Growth models based on simulations allow study of the plasticity of endogenous determinated shoot-growth patterns under micro-environmental dynamics and are available now for numerous conifers and broadleaf species and permit their analysis and visualization by computer graphics (cf. Barczy et al. 1997; Perttunen et al. 1998; Kurth 1999; Goulet et al. 2000).

Due to their preponderant importance for forest economy, conifers of temperate climate zones, especially Scots pine *Pinus sylvestris* L, have been the principal object of functional and/or structural models published until now. These models centred on growth as carbon allocation to tree compartments, with special attention to the stem wood as the principal forest product. Other components as branches, twigs, needles or root system, were taken into account only as far as their performance concerns the correct modelling of tree growth (light interception,

photosynthesis, carbon allocation, secondary growth) or wood quality (branchiness) (Reffye de et al. 1997; Kellomäki et al. 1999). Others like root architecture or reproductive structures were simplified or completely neglected. The attention in analysis and modelling has focused on the sensibility of axes' secondary growth on shading of the photosynthetic active leaves and the resulting plasticity of the growth and crown architecture in different light environments (O'Connell and Kelty 1994; Gilmore and Seymour 1996; Lo et al. 2001) and/or site fertilities (Niinemets et al. 2002; Niinemets and Lukjanova 2003).

On the other hand, in other climate zones light availability may not be the main limiting factor of plant growth and there cannot be expected to be a proportional response of growth to the light amount computed during the annual growth season for a given plant or shoot, paradigmatic of most published process-based growth models for boreal or temperate trees. In the Mediterranean climate, growth season is limited environmentally both by low temperatures in winter and by water stress in summer, thus even the avoidance of excessive irradiance has been shown to be an important evolutionary strategy that requires structural adaptations (Valladares and Pugnaire 1999). Under these conditions, other factors like hydraulic constrictions may have more relevance than light capture for predicting accurately shoot-growth differences within or between trees crowns (Goulet et al. 2000; Nikinmaa et al. 2003).

Among Mediterranean pines, Stone pine *Pinus pinea* is marked off by two outstanding adaptive traits that both motivated the present approach of architectural analysis and modelling. First, it presents less apical dominance than most other pine species with a conical crown shape. Stone pine ramification is polyarchic with "the lateral branches of a whorl growing in diameter and length as much as, if not more than the main axis" (Ruiz de la Torre 1979). The resulting crown shape is "wider than deep", spherical in youth and characteristically wide, umbrella-shaped in older trees (Lanner 1989), especially in open-grown trees in natural Stone pine habitats in sandy plains and dunes where competition is between root systems, not between crowns. On the other hand, Stone pine cones need 3 years to ripen and reach 250–300 g, and the large, nearly wingless seeds (0.6 g) are dispersed by animals, not by wind. In Stone pine forests the average amount of biomass allocation to cones is similar or even superior to stem growth, though with important crop variations between years (Cabanettes and Rapp 1981; Mutke et al. 2003b). Since the Palaeolithic, the large, edible seeds or pine nuts have been used for human consumption in the Mediterranean region, due to their high nutritional value (50% fats, 35% proteins). Currently, cones are the most important product of Stone pine forests, providing a higher income to the forest owners than timber. Moreover, in the last decades increasing efforts are dedicated to the domestication of this species as a nut producer in grafted orchards, and more information about its reproductive behaviour is required (Mutke et al. 2000).

In this context, the aim of this paper is to study the growth, branching and cone-bearing patterns of individual shoots and axes and, as an emerging phenomenon, the crown shape of Stone pine. The analysis of the data, recollected in standing trees, stresses individual shoot parameters and their correlation along the axes or at branching points. It can be divided into three parts or submodels: the geometric relationships between terminal and lateral shoots of the same whorl, the autocorrelation of shoot vigour between years along the axes, and the topologic parameters of new whorls (Fig. 2). Available plant-architecture analysis tools like AMAPmod (Godin et al. 1997; Godin and Guédon 2001) allow the explicit calculation of implicit values like the absolute coordinates of each measured shoot, or the recovery of any topologic or biometric variable along the pathway below the studied point or in competing shoot axes. In consequence, all this additional information for individual shoots can be studied and included in the regression models, if a significant influence is found. The determination of the relevant relationships between different elements of plant topology [context sensitivity (Prusinkiewicz et al. 1995)] will allow the simplification of further field sampling, dropping non-significant covariables.

Thus, this study aims to be a first step in the construction of a functional–structural model for the studied species and focuses on apical growth and differentiation, neglecting for the moment carbon allocation and secondary growth, phenomena widely studied and discussed by other authors. Once laid out the architectural rules at shoot level, the model can be used to simulate the primary allocation patterns in the vegetative and regeneration tissues and the influence of different environmental variables on these phenomena. Later on, a model like LIGNUM that deals with the whole material balance of tree can become an efficient tool in studying the allocation patterns to secondary growth (Perttunen et al. 1998), taking into account the cones as important biomass sinks.

Materials and methods

Data measurement

The study was conducted on 27 young grafted Stone pines of two clone banks on the inland plateau of central Spain. The first plot is the clone bank B23PH1 of the National Forest Breeding Centre "Puerta de Hierro", Madrid, at 3°45′W, 40°28′N and 590 m a.s.l. (Mutke et al. 2003a). The other bank B23MN1 was planted in a clear-cut of mixed Stone pine and Holm oak woodland at 4°20′W, 41°35′N and 890 m a.s.l. in Quintanilla, province of Valladolid. Both orchards were planted in autumn 1992 with nursery-grown treelets that had been grafted homoplastically the spring before. Scions came from plus trees mass-selected for cone-production in Spanish Stone pine forests (Mutke et al. 2000).

The measured trees pertained to six different clones in the first plot (3–4 ramets per clone) and two clones in the

second one (2 and 5 ramets per clone). Measurements were done in the standing trees in autumn 2002, when average tree height was 2.60 m and 2.33 m in the respective plots. The growth and flowering phenology of the grafts in the second clone bank had been characterized in previous years (Mutke et al. 2003b).

The protocol for morphological measurements was adapted from Kurth and Anzola (1997) and Godin et al. (1997). The sampling unit was the tree segment or growth unit, corresponding in monocyclic species to an annual shoot (Barthélémy et al. 1997). In each shoot, the total length and the diameter near the base were measured (with 5 mm and 1 mm precision, respectively). The angle between the directions of the shoot and its bearing ("mother") shoot (approximately 5°) and its azimuth in the plane perpendicular to the latter (eight main directions at 45°) were estimated. The presence of needles and male strobili (or their scars) and the number of female strobili (or scars) and lateral branches (or buds or scars) in the terminal whorl were registered, as well as further observations such as the presence of mechanical or biotic damage from the European pine shoot moth (*Rhyacionia buoliana* Schiff.) and dead buds or shoots.

Measurements of each tree started from the grafting point on the rootstock upwards, thus the first measured shoot of each tree was the 1992 stem-segment corresponding to the originally grafted scion. The next measurements register all successive shoots of the axis up to the current 2002 apical shoot, labelling each shoot by a tree-and-axis code and the year of growth. Occasional summer shoots (lammas growths) were identified separately and labelled with an additional digit. This order of shoot notation withheld topological relationships between shoots. All principal axes of the crown, i.e. the stem and co-dominant,

upwards growing, "sylleptic reiterated" (sensu Halle et al. 1978) main branches were measured in all trees, whereas only samples of higher branching-order axes were measured (Table 1).

In particular, the lateral shoots of the last 2 years' whorls in the stems and main branches were measured. Thus, sampling was biassed towards the younger shoots in the upper part of the crowns, and all shoots bearing current first, 2nd or 3rd year female strobili were included. On the other hand, there were only a reduced number of measurements for the rare dichotomic branchlets borne by the normally non-branching fourth order axes (Gravelius order, assigning order 1 to the stem).

The shoots grown in the 1st year (before 1995) were excluded from the analysis due to their reduced number and in order to avoid the effects of the grafting and post-plantation stress, though the data were used as ancestor information of descendant shoots. Also summer shoots, dead buds and shoots or buds damaged by *Rhyacionia buoliana* were excluded from the analysis, as well as their direct descendants or lateral shoots of whorls with terminal shoots in any of these cases. The final number of registers in the database was 5,224 analysed shoots (Table 1).

Data codification and regression analysis

Measured tree data were coded both in dtd-format (Kurth 1994; Kurth and Anzola 1997) and in a MTG database for statistical exploration under the multiple-scale tree paradigm (Godin et al. 1997; Godin and Caraglio 1998). Statistical analysis of topology and morphology were performed using AMAPmod v1.8 (Godin and Guédon 2001), GROGRA v3.3 (Kurth 1994) and SAS v8 (SAS

**Table 1** Number of measured shoots by branching order or year of growth

Order	Total number	Number of analysed shoots[a]
1 (stem)	279	177
2 (main branches)	1,908	1,703
3 (lateral branches)	2,307	2,198
4 (branchlets)	1,251	1,114
5 (dichotomic branchlets)	44	32

Year	Total number	Number of analysed shoots[a]
1992	27	–
1993	55	–
1994	97	–
1995	144	126
1996	202	191
1997	262	253
1998	372	344
1999	530	513
2000	754	727
2001	1,281	1,213
2002	2,022	1,857
Summer shoots (different years)	43	–
	5,789	5,224

[a]Excluding shoots from 1992 to 1994, summer shoots, damaged or dead shoots and their direct descendants or laterals of the same whorl.

1996) software packages, in order to obtain biometric parameters and rules of Stone pine shoot growth, branching pattern and strobili development. The realistic graphic display of the reconstructed trees with GROGRA v3.3 allowed error checking by comparison with each real tree in the field (Fig. 1). The exploratory statistical analysis searched functional relationships based on correlation and regression analysis, using logarithm transformations to achieve normality when necessary, or general linear models when class variables were included.

Variables at shoot level taken into account were: (1) the actual measured shoot length, diameter, angle and azimuth referred to the bearing shoot, (2) the implied topologic information like the shoot position (terminal or lateral) on the mother shoot or its branching order, the axis' age in the year of the shoot growth or the number of lateral branches and strobili borne by the shoot, and (3) geometric parameters like the cross-section area, surface and volume, or others derived from the shoot coordinates like its inclination from vertical, its height from ground, the length of the pathway from the stem base to the shoot base, the depth of the shoot in the crown (computed as tree height in the year of the shoot growth minus shoot height from ground) and the horizontal distance of shoot base from the stem. Also the values of these parameters in the bearing ("mother") shoot and, in the case of lateral shoots, of the central shoot borne by the same mother shoot were considered as predictive variables for shoot growth. Fixed effects at higher scales were assigned hierarchically to the sample plot, the clone and the ramet that the shoot pertained to, and finally to the year of shoot growth.

The effect of shoot position within the crown on growth and lateral bud differentiation had been assessed in other species by fixed or even linear effects of the branching order or by other morphogenetic gradients (Kellomäki and Strandman 1995; Perttunen et al. 1996; Sabatier and
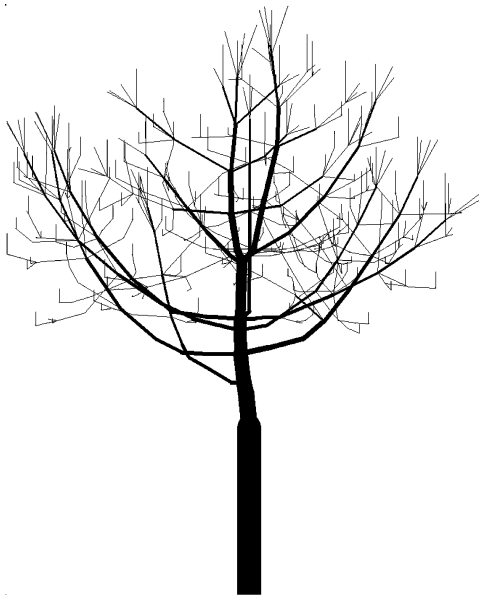
Barthélémy 1999) or in a vigour-oriented approach, e.g. expressed as dimensionless vigour index (VI) for each axis or shoot as defined by Goulet et al. (2000) and Nikinmaa et al. (2003). The value in 2002 of this vigour index VI was calculated for each shoot of the present sample, following the woody axes from the base of the tree ($VI=1$) up to this shoot i, multiplying it at each of the n branching points along the pathway by the ratio of its cross-section (S_i) above and the thickest one of the dividing axes at the branching point (S_{Mi}): $VI = \prod_{i=0}^{n} S_i/S_{Mi}$ thus $V \in [0; 1]$ (Goulet et al. 2000). The axis vigour can also be taken into account intrinsically by the correlation with the same or other variables (e.g. length, diameter) in the mother shoot or in the terminal shoot borne by the same mother (Barthélémy et al. 1997; Kurth 1999).

Following geometric and topologic shoot-growth model subcomponents were studied:

- The shoot length–diameter ratio of first-year shoots
- The shoot length decrease with branching
- The lateral branch and flower/cone number borne by the shoot
- The shoot length autocorrelation within the same axis
- The angle and azimuth reference to the mother shoot

For each of these components, the possible effects of the above indicated shoot variables in a multiple regression or general linear model were evaluated by their biological significance, the influence on changes in R^2 and least square means (LSM), taking into account the presence of possible multicollinearity. A regression model for shoot diameter was established for current 2002 shoots, in order to estimate the initial (1st year) diameter of the older shoots. The shoot length decrease by branching was estimated by regression of lateral shoot length against the length of the terminal shoot of the same whorl, avoiding biases between years (Kurth 1999).

Results

The results are structured following the scheme of three submodels: the geometric relationships between terminal and lateral shoots in the same whorl, the autocorrelation of shoot vigour along the axes, and the topologic parameters of new whorls (Fig. 2). A fourth, stochastic submodel analysing the meristematic fate (meristematic damage or death due to pine shoot moth or other reasons) was not included in the present paper.

Length/diameter ratio of new shoots

The relationship between shoot length and diameter was studied in the 1,857 shoots elongated on 2002. Simple linear regression of shoots diameter D on shoot length L achieved a coefficient of determination $R^2=0.76$, but was under-specified because of significant changes in the

Fig. 1 Visualization of the completely measured grafted Stone pine A-96-1-29 in plot 2 [created with GROGRA software (Kurth 1994)]
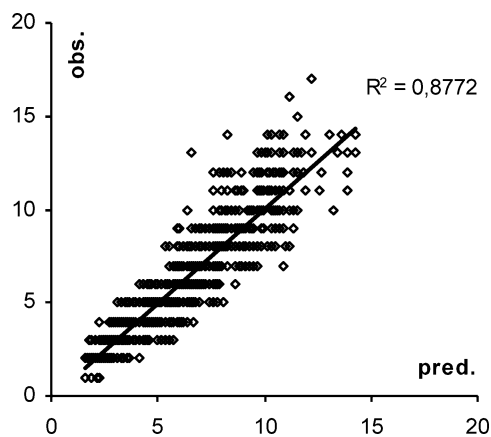
Fig. 2 Pathway for the geometric and topologic submodels of the vigour-based shoot-growth model in grafted Stone pine
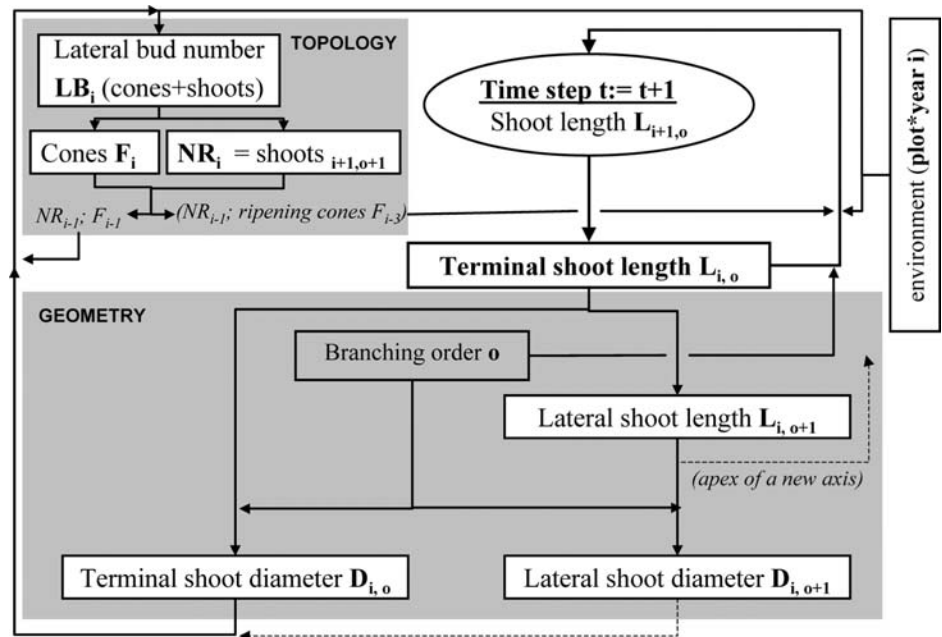
regression slope depending on shoot position on the mother shoot V ($V\doteq0$ if lateral, 1 if terminal) and significant additive effects of the branching order o. The regression model for shoot diameter in mm (R^2=0.88) included therefore length L (mm), shoot position V and branching order o of the shoot i (Fig. 3)

$$D_i = (0.0149 + 0.0057V_i)L_i + a_{oi} + \varepsilon_i \qquad (1)$$

where branching-order effect was a_1=4.6 mm, a_2=3.1 mm, a_3=2.0 mm, a_4=a_5=1.5 mm (fourth and fifth order effect were not significantly different, thus pooled data for these shoots were used). Residual values ε of the model were randomly normal distributed with deviation (MSE) 0.98 mm and not correlated significantly with any other shoot variables.
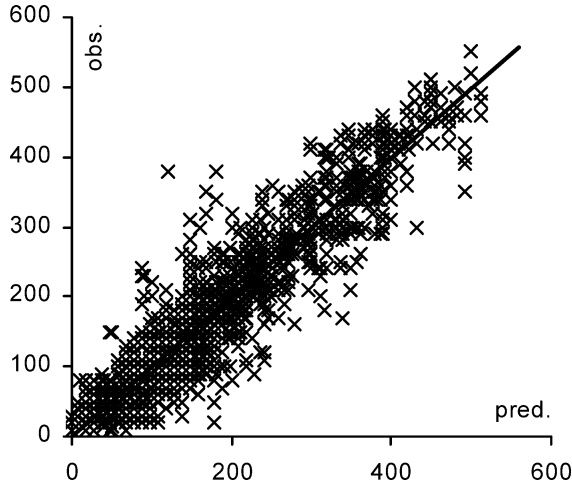
Length decrease by branching

The length of the basal shoot of each branch was compared with the terminal shoot of the bearing axis in the same year. The regression model of lateral shoot length in relation to the terminal shoot of the same whorl was established on the base of 1,827 lateral shoots elongated between 1995 and 2002. The model obtained by regression analysis achieved a coefficient R^2=0.87 [proc GLM (SAS 1996)]

$$L_{\text{lateral } i} = a_o + a_1 L_{\text{terminal } i} + \varepsilon_i \qquad (2)$$

where $L_{\text{lateral } i}$ was the length of the lateral shoot i in mm, a_o was the additive effect of the branching order $o \in \{2, 3, 4, 5\}$, a_1 the linear effect of the predictive variable $L_{\text{terminal } i}$ (length of the corresponding terminal "brother" shoot) and ε_i the error term. Branching effect was positive in main branches and decreased from second to fifth order (+41, −11, −53 and −73 mm, respectively), whereas regression coefficient a_1 did not differ significantly from 1.0 (Fig. 4). Residual values of this model were randomly normal-distributed (deviation 41.2 mm).

Number of lateral vegetative (branch) and reproductive (cone) organs

Less than 60% of the analysed shoots branched at all, resulting a bimodal distribution of the lateral shoot number per shoot. Excluding the non-branching shoots, the distribution was symmetric with a very regular number of lateral vegetative buds per shoot (86% bore 3±1 shoots), while only 19% formed also flowers. The probability of carrying a strobilus was very low in shoots with less then

Fig. 3 Observed versus predicted shoot diameter (prediction based on shoot length, branching order and position) (mm)

Fig. 4 Observed versus predicted length of lateral shoot (prediction based on terminal shoot length and branching order (mm))

three lateral branches, but increased with the total lateral bud number (Fig. 6).
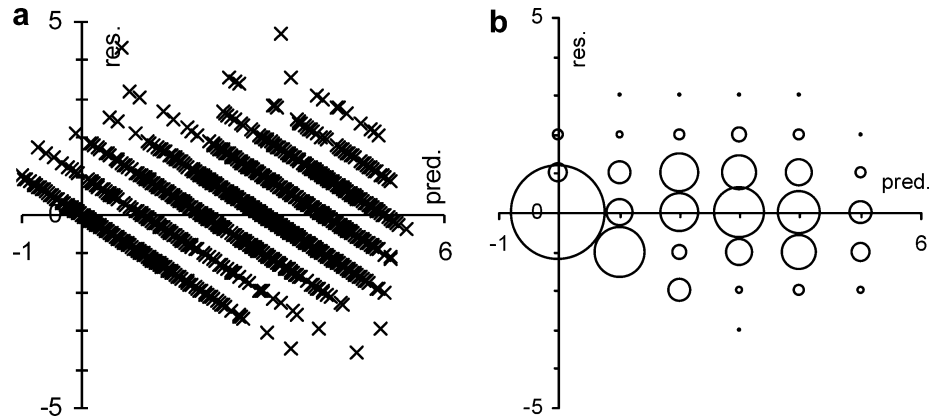
All stem shoots and nearly all second order-axes shoots branched, whereas the third order axes presented both branching and non-branching shoots. Fourth order twigs branched very rarely at all: only 30 of 1,251 shoots bore a fifth order axis, 10 of them due to traumatic reiteration after damage to the bearing axis. No higher order axes were produced in the sampled young graftlings, though in adult Stone pines with developed crowns, branchlets up to order 8 can be observed in sylleptic reiteration complexes.

In order to predict both branch (NR) and strobili number (F) borne by a shoot i, a general linear regression was first performed to predict the total number of lateral buds LB= (NR+F) by the model (R^2=0.79)

$$LB_i
= \left(a_{pi} + a_{(br \times p)i} + a_{(fl \times p)i}\right)
+ \left(b_{pi} + b_{(br \times p)i} + b_{(fl \times p)i}\right) \ln(D_i) + a_{y \times p(i)}
+ a_{vi} + \varepsilon_i \tag{3}$$

where a_p was the effect of the plot, the effect of the branching habit in the mother shoot was estimated by a fixed effect (a_{br} if it bore any lateral shoot, zero if not) and by an analogous a_{fl} if it had produced a female strobilus the year before, both effects varying significantly between plots. Also the slope for the natural logarithm of initial diameter of the shoot $\ln(D)$ as covariable of the bud number depended on the plot and the mothers branching/flowering habit (b_p, b_{br}, b_{fl}). Finally, the model took into account the environmental influence as fixed effect of the year by plot $a_{y \times p} \in [-0.1143; 0.4713]$, and the effect of terminal or lateral shoot position on the mother shoot (a_v=0.7469 if terminal). The residual value ε_i of the prediction (including the rounding to integer value, Fig. 5) fulfilled the basic hypothesis of independence, homoscedasticity and normal distribution.
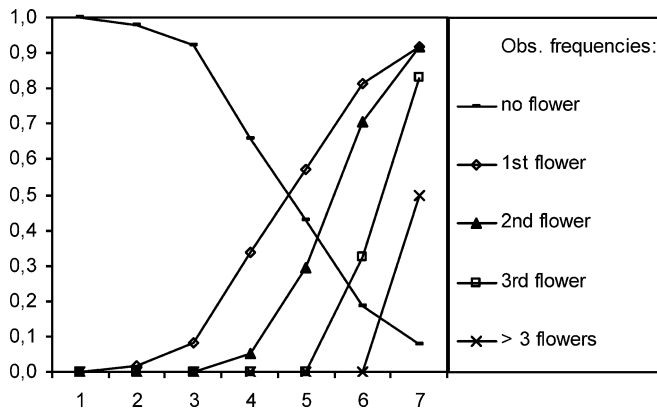
In a second step, the proportion of vegetative shoots NR/LB on shoot i was predicted in dependence on its total lateral bud number LB_i and other variables by the log-normal regression model (R^2=0.45)

$$\ln(NR/LB)_i
= a_o LB_i + b_{o \times p} \ln(D_i) + c_o LB_i \ln(D_i) + a_{y \times p(i)}
+ a_{clon(i)} + \varepsilon_i \tag{4}$$

where $a_o \in [-0.099; 0.112]$ was the branching-order depending coefficient for lateral bud number LB_i (Fig. 6), $b_{o \times p} \in [-0.034; 0.279]$ was the coefficient for natural logarithm of initial diameter of the shoot $\ln(D)$ varying significantly between axis orders and between plots and $c_o \in [-0.063; -0.021]$ was the axis order depending coefficient for the interaction between both. Additionally, there were significant fixed effects of the environment $a_{y \times p(i)} \in [-0.174; 0.218]$ and of the clone $a_{clon(i)} \in [-0.23; -0.116]$.

Autocorrelation of shoot size within the same axis

The best predictive variable for terminal shoot length was found to be the mother shoot's vigour, expressed either by its length, by its initial diameter (estimated by Eq. 1) or by its vigour index *VI*. As the three variables presented strong

Fig. 5 **a** Residual values versus predicted lateral bud number per shoot. **b** Residual values versus rounded predictions (circle size proportional to case number)

Fig. 6 Observed frequencies of female strobili borne by a shoot (*y*-axis) in dependence on its total lateral bud number, summing branches and strobili (*x*-axis)

Fig. 7 Branching angle in dependence on branch age (down-bending took place mainly during first 3 years)

correlations, only one of them could be used for daughter-shoot length prediction: we chose the mother-shoot length, the only one that stays unaltered since shoot formation. The regression required the logarithm transformation of both lengths to achieve homoscedasticity of the residuals. Furthermore, this shoot-length autocorrelation showed dependence on branching order o, introducing in the model a different slope for each order. Significant environmental influences were taken into account by the class variable Plot × Year and there was also significant negative effect of the number of maturing cones F_{i-3} below the shoot during the bud differentiation (these strobili are born and pollinated by the segment elongated 2 years ago, i.e. 3 years before the elongation of the studied shoot). Finally, the weakest axes were penalized, identified by a non-branching mother shoot. The complete model with four independent factors ($R^2=0.81$) of the regression was

$$\ln\left(L_i\right) = a_o \ln\left(L_{i-1}\right) + a_{p\times y} + a_f F_{i-3} + a_{\mathrm{br}} + \varepsilon_i \qquad (5)$$

where ln(*L*) was the natural logarithm of length of shoot expressed in millimetres, $a_o \in [0.361; 0.617]$ was the linear coefficient for logarithm of the mother-shoot length in dependence on the axis order o, $a_{p\times y} \in [-0.710; 0.406]$ stood for the fixed environment effect (plot × year), $a_f = -0.095$ was the effect of the cone number F borne by the ancestor shoot $i-3$, $a_{\mathrm{br}}=0.547$ was an additive factor if the mother shoot had branched, and ε was the residual.

Initial branching angle, azimuth and posterior down-bending

A log-linear model predicting branching angle (measured with approximation of 5°) by branch age as class variable (non-lineal effect) absorbed about 53.8% of total variation of the transformed variable, though the fixed effect of the branch age did not differ significantly between branches older than 3 years. The average initial branching angle (in
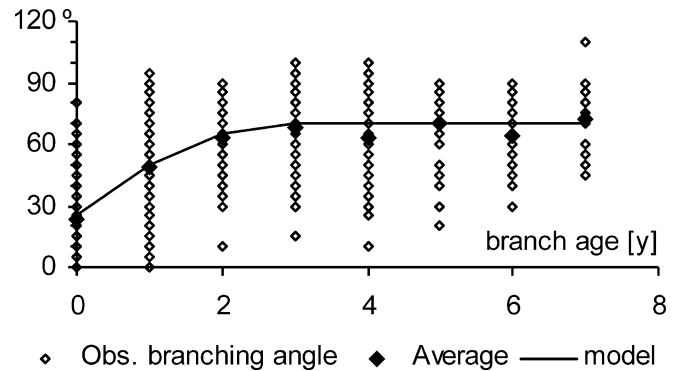
lateral shoots elongated in 2002) referred to the direction of mother shoot (rounded to nearest 5°) was 25°, and the average down-bending during the first 3 years (estimated on 2001-, 2000- and 1999-shoots) about +25°+15°+5° summed up to a final average branching angle of 70° (Fig. 7). After the 4th year (shoots formed before 1999), there was no increment of the average angle.

The initial branching angle did not differ significantly between axis orders. Bending was not limited to the branching point, but seems to have taken place along the branch axis. Within the same axis, the azimuth in the normal plane of the mother shoot was normally upwards (in 79% of the 3,398 measured cases, less than 22.5° from verticality, in 93% less than 67.5°). New, tender shoots, in particular, that elongated vertically in spring of year *I* incline somewhat during the 1st year under their own needle weight and even more in the next spring (*I*+1) due to the distal flexion moment produced by the weight of the new daughter shoots. As a result, along the main branches the new shoots were about 22° more vertical than their mother segments, whereas older segments remained only slightly more vertical (average 10°) than their mother segments.

Discussion

Geometric variables

The relationships among the geometric variables (length, diameter) of the terminal and lateral shoots within the same whorl depended only on the branching order, and the regression models could not be improved by other explicative variables. Other models published for different pine species had handled this vigour or length decrease by

– A fixed shoot length depending only on branching order (*q*×*o*), modified by a intercept model that simulates the light environment of the mother shoot and by a global carbon balance adjustment at tree level (Perttunen et al. 1996, 1998)

Fig. 8 a Coefficients of environment factor (plot × year) on shoot length variation (*x*-axis: year); *x* - plot 1; *o* - plot 2. **b** Same coefficients in dependence on water stress (*x*-axis: mm rainfall during the June of bud formation).
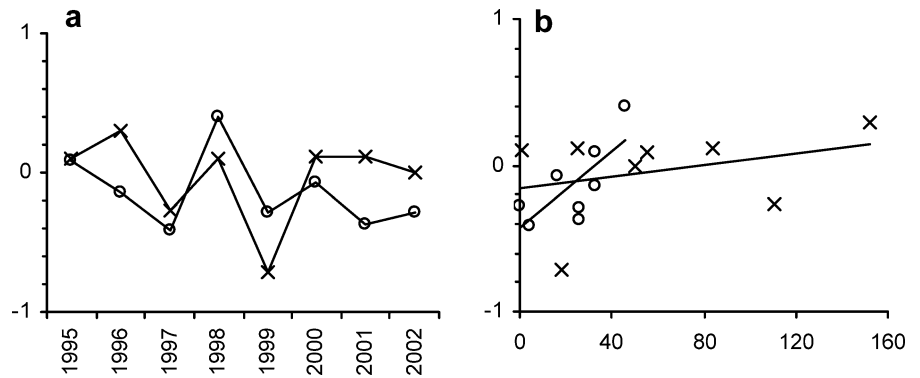
– A branching-order and branching-position depending autoregressive factor (Kellomäki and Strandman 1995; Kellomäki et al. 1999)
– A vigour index calculated for each shoot, combined with a light environment model (Goulet et al. 2000; Lo et al. 2001; Nikinmaa et al. 2003)

On the other hand, the autocorrelation of shoot vigour (quantified by shoot length) within the same axis observed in the present study varied significantly depending not only on the branching order but also on ancestors' branching (the year before) and flowering habit (3 years before, putatively due to the strong growth competition of the ripening cones, besides possible phytohormonal interactions). The (macro-)environmental effect on shoot-length variation, estimated here as fixed factor for plot and year, seemed to be related in plot 2 with the rainfall during bud formation (linear R^2=0.54) (Fig. 8). In plot 1, there may be other additional predominant factors; namely the first heavy cone yield of this plantation was in fall 1996 and the best crop in 1998, both followed by poor flowering in the next year. Hence, the negative effects of the ripening cones as strong carbohydrate and nutrient sinks at overall tree level (not only in the same axis as the scale included in the model) may have rested vigour at the moment of terminal bud formation and flower induction (Lee 1979).

The chosen autocorrelation approach had a practical advantage over the use of the vigour index *VI*, because the latter changes between years due to secondary growth of each axis. Thus, its determination requires each year the recalculation of the new diameter relationships at all branching points of the pathway from root up to each meristem, and the calculation effort increments each time step more than proportional to meristem number. In computer-based shoot-growth models, local autocorrelations are straightforward (e.g. by the use of pointers in object-oriented program languages as C++) and thus easier to handle than global sensitivity functions that require overall calculations in each iteration (Kurth 1999). In the present study on Stone pine grafts, only correlations with shoot length and branching habit of the mother shoot and flowering 3 years ago were found to be significant. Thus, in model programming, these few shoot variables can even be "inherited" as additional shoot attributes to the successor shoots and in this case explicit context-sensitivity would not be required at all.

Topologic variables

As well as the shoot length, the topologic shoot variables (number of borne lateral shoots and cones) were influenced by the branching and flowering behaviour of the mother shoot and by a covariable related to shoot size. Additionally, these coefficients varied significantly between years, and finally there was a weak clonal effect on the proportion of vegetative or reproductive lateral buds—the only significant genotype effect observed in the analysed data. Former models for other pine species assumed a fixed *bifurcation ratio* resulting in a geometric increase of the tree's shoot number between years (Niinemets and Lukjanova 2003), or a lateral bud number as function of the shoot size. The latter had been estimated by the shoot length [with different coefficients for branching orders (Kellomäki and Strandman 1995; Kellomäki et al. 1999)], by the shoot diameter (Gavrikov and Sekretenko 1996), by the needle mass estimated from shoot length (Perttunen et al. 1996) or by the sapwood area (Lo et al. 2001).

In this context, it is interesting that the number of female strobili of Stone pine could be estimated in this work more accurately by an indirect approach, predicting first total lateral bud number dependent on shoot size and position and then the proportion of vegetative buds, than by a direct regression analysis of strobili number based on the other shoot parameters. Furthermore, the proportion of flower or vegetative buds did not depend significantly on the ripening cones of the ancestor shoot ($I-3$) the year before—this negative effect was putatively absorbed already in the shoot vigour (length) prediction that determined the total lateral bud number. The biological sense may be that the predictive variables like shoot vigour or competition from ripening cones determine the total length of the time interval for differentiation of lateral long shoot primordia (for both shoots and strobili) during bud formation. Thus, assuming a constant plastochron in time (or thermal time) for apical meristematic activity, the interval length (in continuous scaled time) would be directly correlated with the (discrete) number of formed

Fig. 9 Rauh's model of plant architecture (Halle et al. 1978) (*left*) and two examples of measured Stonepine grafts (*right*)
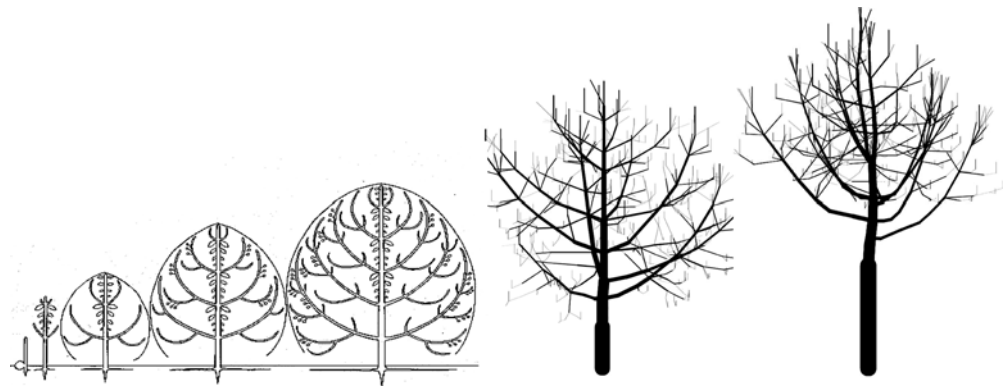
primordia of lateral buds. On the other hand, the timing of the switch from the formation of reproductive to vegetative primordia would need a detailed sampling of these sequences at growth-unit level and an analysis by methods such as cellular automata or Markov's chain (Guédon 1997). At the chosen sampling and analysis scale (shoot level), the approximation of the binomial-distributed bud number by a normal distribution was satisfactory due to the great number of analysed shoots. Also the log-normal regression for the proportion of vegetative buds may reflect the underlying Poisson processes.

Crown shape

The primary growth and branching rules might explain, together with the down-bending processes of the initially vertical branch tips, the main mechanisms that determine the crown architecture and shape of Stone pine, even without taking into account possible traumatic reiterations like active upward-bending produced by differential growth stresses, as observed in top-pruned or girdled pine trees (Wilson 1998). The autocorrelation of shoot length allowed the prediction of new shoot parameters independent of structural restrictions, without taking into account explicitly the light environment of the shoot (in this paper poorly estimated by the crown depth). However, also some "autocorrelation" might probably exist in the light conditions between the shoot and its mother shoot (in absence of pruning that would liberate shaded meristems), thus the functional relationships have been "absorbed" by the auto-regression analysis.

The sampled Stone pine crowns, though hierarchically structured by different effects of the branching order, showed a lack of apical dominance, i.e. of initial vigour decrease between stem and main branches (which basal segments are in average even somewhat longer than the correspondent stem segment). This result is in accordance with the cited polyarchic character of the species that results in a spherical crown in youth that becomes umbrella-shaped in adult trees, when basal branches are lost or pruned (Ruiz de la Torre 1979; Lanner 1989).
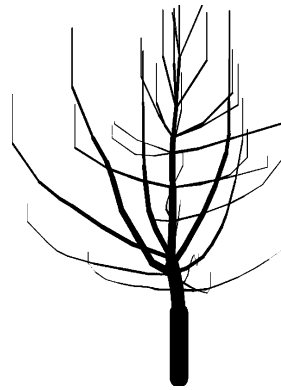
Thus, the architectural base model of Stone pine follows strictly Rauh's model defined by Halle and Oldeman (1970) as a tree architecture model determined by a monopodial trunk which grows rhythmically developing tiers of branches, the main branches themselves morphogenetically identical with the trunk, and the flowers lateral, without any effect on the topology of the shoot system. The development sequence of the Rauh's model given in Halle et al. (1978) is strictly emulated by juvenile Stone pine (Fig. 9). This includes a lateral vigour symmetry (dorso-ventrality) of lateral branching systems due to the "escape asymmetry" of higher-order branches, which develop stronger in the lower (i.e. oriented to the exterior) than in the upper face of the main branches. This tendency of generalized sylleptic reiteration leads to crowns with co-dominant main branches that lose the dorso-ventrality of their branching habit once stabilized by the verticality of their distal part (stiffened by secondary growth that stops their down-bending), constituting "first order" axes of new architectural units and bearing new "second order" axes. Hence, the latter ones are the observed flowering shoots of third branching order.

Stone pine is an extreme case of continuous sylleptic reiteration of Rauh's model, modifying the model's life strategy of "easy apical meristem replacement in case of stem apex destruction" (Halle et al. 1978) into a polyarchic branching system and crown structure without any necessity of replacement if lost. In fact, the apical control and regulation of lateral meristem vigour is reduced even in the normal crown development of Stone pine: the loss of the leader shoot due to European pine shoot moth or to top pruning does not produce a simple substitution by the most distal and vigorous lateral shoot as a new leader (traumatic reiteration), typical in other pine species, but it hardly modifies the growth pattern of the rest of the polyarchic crown.

Nevertheless, most Stone pines do present a single main stem and a tree shape, and not a bushy form without differentiation between stem and main branches. As it seems not to be regulated by an initial vigour decrease from stem to main branches, it should be produced later by positive feed-back of secondary growth and axis vigour. Beside the leader shoot of the stem, only the tips of those co-dominant branches remain in the canopy surface in the best light conditions that have stiffened more by greater secondary growth and consequently suffer less down-bending than shaded, less vigorous ones (Fig. 10). Thus, these positional advantages lead to a higher photosynthesis
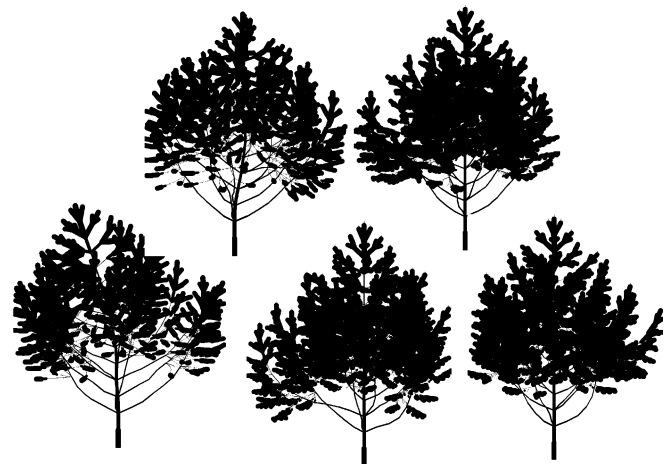
Fig. 10 Positive feed-back between axis vigour, secondary growth and stiffening in the measured Stone pine graft B-22-4 (only the stem and 2nd-order axes are shown): less vigorous branches remain finer and bend down to horizontality, whereas co-dominant ones are stiffened by secondary growth and stay more vertical with their tips in full-light upper crown layers

Fig. 11 Examples of different runs of a non-sensitive, stochastic Lindenmayer-system based on the results of this paper

rate and greater secondary growth. Moreover, the greater the shoot's vigour is, the higher the branching ratio, and the greater the needle biomass, photosynthetic production and secondary growth. Saplings of Stone pine grown in high plantation density show less generalized sylleptic reiteration than open-grown graftlings, bending most of their main branches down to the horizontal, due to lateral shading that reduces branches' secondary growth.

The relationships fitted in this paper between geometric and topologic shoot and branch variables are the first step to obtain the subcomponents of a structural model that can be completed with functional components like a radiation submodel. Data measurement on standing trees allowed to establish correlations between shoot variables and ancestors' topologic and geometric parameters. Significant relationships included only mother shoot's parameters and the cones born 3 years before. As well, this non-destructive sampling method allows re-measurements for model validation in following years. The future implementation of a light environment submodel and the quantification of global sensitivity on environmental factors (rainfall) or on covariables at tree level will allow the construction of a sensitive growth model, using versatile modelling techniques like Lindenmayer-systems (Fig. 11) (Prusinkiewicz and Lindenmayer 1990; Kurth 1999).

We conclude that the spherical Stone pine crown shape emerges as consequence of the lack of vigour differentiation between apical meristems of stem and main branches that favour the generalized sylleptic reiteration in open-grown trees. This may be interpreted as a life strategy, in the absence of lateral competition for light, to enhance maximal seed production by maximizing co-dominant branch number, taking into account that only the tips of the strongest axes are able to sustain the heavy cones.

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