Quantitative Analysis of the Phenotypic Variability of Shoot Architecture in Two Grapevine (Vitis vinifera) Cultivars

GAËTAN LOUARN¹, YANN GUEDON², JEREMIE LECOEUR¹ and ERIC LEBON¹,*

¹INRA, Montpellier SupAgro, UMR759 LEPSE, 2 place Viala, F-34060 Montpellier, France and ²CIRAD, UMR AMAP and INRIA, Virtual Plants, TA 40/PS2, F-34398 Montpellier, France

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INTRODUCTION

Crop canopy structure results from complex interactions between plant physiology (organogenesis, morphogenesis), architectural characteristics (leaf area distribution, branching pattern, growth habit), agronomic practices (plant density, training and pruning systems) and environmental conditions (Willame et al., 2004). Canopy structure affects light interception and plant microclimate, and therefore has consequences for many processes, including carbon acquisition (Monteith, 1977), fruit quality development (Smart et al., 1990; Haselgrove et al., 2000; Spayd et al., 2002) and plant disease control (Zahavi et al., 2001).

Major changes to plant architecture may be required to improve the overall performance of cultivated species (Burke et al., 2002). However, architectural traits have only recently been defined as possible selection criteria for varietal improvement [Bradshaw et al., 1995 (for poplar); Hemmat et al., 1997 (for apple trees); Costes et al., 2004]. Most of the world’s wine production is based on the use of a small number of grapevine (Vitis vinifera) cultivars. These cultivars originated from spontaneous crosses and have been subject to empirical selection over several centuries mainly for agronomic (earliness, drought tolerance, yield) and oenological (sugar concentration, polyphenols, aroma content) properties. This may account for the high level of phenotypic variability in architecture between the most widely used cultivars. However, light interception and penetration within the canopy, particularly in the fruiting zone, is one of the main determinants of grape berry composition (Gladstone and Dokoozlian, 2003). Sophisticated trellis systems and extensive summer pruning interventions resulting in high production costs have therefore been developed for certain cultivars that tend to be particularly vigorous or to have a ‘droopy’ habit. It may be possible to reduce these costs by taking genotypic architectural traits into account in the design of new training systems and/or in future genetic selection programmes. This requires a thorough analysis of canopy development, but the processes involved and their genotypic variability have not been extensively quantified for grapevine (Schultz, 1992; Fanizza and Castrigano, 1993). Such quantification requires the definition of a

* For correspondence. E-mail lebon@ensam.inra.fr

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comprehensive and relevant set of variables representing both the topology (the succession and branching relationships between organs) and the geometry (shape, size, position and orientation) of the organs comprising the structure (Godin et al., 1999).

The grapevine annual shoot consists of a branching system resulting from the development of axes consisting of three types of phytomer — P0, P1 and P2 (Bouard 1966; Jacquinet and Simon, 1971; Gerrath and Poslusnys, 1988) — generally following a regular pattern of succession, P0–P1–P2. P0 phytomers have short internodes and no tendrils. P1 phytomers immediately follow a P0 phytomer and have a tendril and a longer internode than P0. P2 phytomers have tendrils and generally have internodes longer than those of P0 or P1 phytomers. Based on the pattern of succession of phytomer types, the primary axis can be broken down into two parts: a basal preformed part (rank <8–10) in which the P0–P1–P2 pattern of succession may be perturbed, and a distal neo-formed part in which the regular P0–P1–P2 pattern is repeated with very few perturbations (Galet, 1993; Lebon et al., 2004). These three types of phytomer produce populations of branches with different development potentials (Ordovas et al., 1983; Lebon et al., 2004). Branches derived from P0 phytomers (R0) are consistently more highly developed than those derived from the P1 and P2 phytomers (R1 and R2) of the corresponding modular structure.

One of the aims of this study was to identify zones of homogeneity in terms of phytomer succession along the primary axes of the two cultivars and to quantify phytomer-type production by the apex within each zone. The first step in the analysis involved the use of hidden semi-Markov chains, a class of statistical models for the analysis of homogeneous zones within sequences. Hidden semi-Markov chains have been used in various biological contexts, including gene finding (Burge and Karlin, 1997; Lukashin and Borodovsky, 1998), protein secondary structure prediction (Schmidler et al., 2000) and the analysis of branching and flowering patterns in plants (Guédon et al., 2001). Hidden semi-Markov chains generalize hidden Markov chains (for a tutorial on hidden Markov models, see Ephraim and Merhav, 2002) with the distinctive property of explicitly modelling the length of each zone. In the second step of the analysis, the perturbations of the P0–P1–P2 pattern of succession were characterized within each zone, using variable-order Markov chains (Weinberger et al., 1995; Ron et al., 1996; Bühmann and Wyner, 1999). In variable-order Markov chains, the order, or memory length, is variable and depends on the context within the sequence instead of being fixed.

Plant topology results from the pattern of organ production by each apex of the shoot. In grapevine, both the main shoot and the various branches (R0–R1–R2) display indeterminate, hierarchical growth (Lebon et al., 2004). The growth of each axis can be analysed using a kinematic approach initially designed for use with annual plants (Varlet-Grancher et al., 1996; Moulia et al., 1999) and recently adapted to grapevine shoots (Lebon et al., 2004). In this approach, changes in the number of phytomers on the main shoot over time are expressed as a linear function of thermal time. The application of such an analysis to the various axes forming the shoot is the second key element in the comparison of plant topology between different cultivars.

The aim of this study was to analyse the characteristics determining the phenotypic variability of grapevine shoot architecture, a highly complex structure. Shoot architecture results from interactions between many processes. Therefore two very different cultivars, ‘Grenache N’ and ‘Syrah’, were compared to identify the relevant traits related to the observed phenotypic variability of architecture and to quantify their relative importance. Experiments were carried out on de-fruited shoots, to prevent complex interaction with fruits. Topology was analysed for each genotype, using a hidden semi-Markov chain and variable-order Markov chains for the patterns of succession of phytomer types on the main shoot and a combination of four variables (leaf appearance rate on the main shoot, branching probability, leaf appearance rate on the branches, duration of branch development) for the kinematic analysis of whole-shoot growth. Plant geometry was assessed by evaluating the final size of the leaves (surface area) and internodes (length, diameter, volume).

**MATERIALS AND METHODS**

**Experimental design and culture conditions**

Four experiments were carried out in 2002 (expt 1), 2003 (expt 2), 2004 (expt 3) and 2005 (expt 4) at the Agro-M – INRA Campus in Montpellier (France) (43°38′N, 3°53′E) on two grapevine (Vitis vinifera L.) cultivars (‘Grenache N’ and ‘Syrah’; Fig. 1) with different architectures grafted on Fercal rootstocks. Plants were grown outdoors, in large pots (0.3 m diameter, 0.7 m high pots with a volume of 0.050 m³) filled with a 16 % clay, 30 % loam, 54 % sand mixture (proportions based on volume). Each pot contained one plant, except in expt 1, in which one plant of each cultivar was planted in each pot. Plants were pruned before bud burst such that each plant retained two or three latent buds. Reproductive organs were eliminated as soon as they appeared. At the ‘five separated leaves’ stage (stage 12 on the modified Eichorn and Lorenz scale; Coombe, 1995), the plants were thinned to one branch and trained vertically. The pots were treated once per month with 25 g complex fertilizer (Osmocote 18:11-10 NPK; Scotts, France SAS) and completed with ammonium nitrate (25 g at stage 19 when flowering begins and 50 g at stage 32 when bunches close in expts 2–4). Soil water content was measured daily, with a time domain reflectometry device (Trase System I; Soil Moisture
Equipment Corp., Santa Barbara, CA, USA) (expts 1 and 2) and a dielectric probe (Echo2; Decagon Devices, Inc., Pullman, WA, USA) connected to a datalogger (CR10X, Campbell Scientific Ltd, Shepshed, Leics, UK) (expts 3 and 4). Both devices were calibrated by comparison with simultaneous direct measurements of soil water content from an additional set of pots. Transpirable soil water content was maintained above 70–75 % of pot capacity. These levels are much higher than the thresholds of 25–35 % (Sinclair and Ludlow, 1986) or 35–55 % (on grapevine, Bindi et al., 2005, Lebon et al., 2006) below which no physiological or developmental processes are affected.

Air temperature and relative humidity were measured on the experimental site with a capacitive hygrometer (HMP35A Vaisala, Oy, Helsinki, Finland) placed in a standard, naturally aspirated radiation shield, at a height of 2.5 m from the soil. Photosynthetic photon flux density (PPFD) was also measured with a PPFD sensor (LI-190SB; LI-COR, Lincoln, NB, USA). All data were stored in a datalogger (CR10X, Campbell Scientific Ltd), with measurements taken every 30 s and an average calculated over 1800 s. Mean diurnal values ranged from 23.2 (expt 1) to 25.2 °C (expt 2) for air temperature with average minimal temperature from 13.3 (expt 2) to 20.0 °C (expt 1) and average maximal temperature from 27.4 (expt 1) to 30.6 °C (expt 2). Average maximum PPFD and vapour pressure deficit per day ranged, respectively, from 53.6 (expt 1) to 63.3 mol m⁻² d⁻¹ (expt 3) and from 2.47 (expt 1) to 2.81 kPa (expt 2). Measurements were taken during the period month–month (expt 1), month–month (expt 2), month–month (expt 3) and month–month (expt 4).

Calculation of thermal time

Thermal time ($T_d$) was calculated by the daily integration of mean air temperature ($T_m$) minus a base temperature ($T_b$) of 10 °C common to both cultivars (Winkler et al., 1974; Lebon et al., 2004). It was expressed in degree-days (°Cd) for the period 0–$n$ days. The duration of the experiments, $n$, ranged respectively from 60 d (expt 1) to 114 d (expt 4):

$$T_d = \int_0^n \max(0; (T_m - T_b)) \, dt$$  \hspace{1cm} (1)

Plant measurements

Analysis of the structure of the main stem. For expts 2–4, the phytomer sequences of the primary axis of each shoot were recorded according to the P0–P1–P2 classification proposed by Bouard (1966). In total, 60 shoots per genotype were observed. Nodes bearing tendrils were classified as P1 or P2, depending on their position relative to the previous tendril-less node. The approach used to analyse this sequence is illustrated in Fig. 2 for the ‘Grenache N’ cultivar. Initially, a subclass of hidden semi-Markov chains in which some non-absorbing states were Markovian was used (Gue´don, 2005). These Markovian states were grouped into threes, mimicking the P1–P2–P0 pattern, and used to identify perturbed patterns in the preformed part of the shoot. They were preceded by a semi-Markovian state corresponding to an initial P0 zone and followed by an ‘end-effect’ state (see below) and an absorbing state for the distal less-perturbed P1–P2–P0 zone (Fig. 2C). This hidden hybrid Markov/semi-Markov

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Fig. 1. Photographic overview of (A) ‘Grenache N’ and (B) ‘Syrah’ commercial vineyards, with grapevines trained on a single-wire bilateral cordon.
chain was designed as an instrumental model for segmenting the various zones of interest along the sequences: (a) initial P0 zone; (b) perturbed P1–P2–P0 zone; (c) distal (non-perturbed) P1–P2–P0 zone.

Once the hidden hybrid Markov/semi-Markov chain had been estimated (Guédon, 2003, 2005), the most probable state sequence was computed with the Viterbi algorithm (Guédon, 2005) for each sequence observed (Fig. 2D). The very specific structure of the hidden hybrid Markov/semi-Markov chains estimated (all observation distributions are deterministic with the exception of that attached to the final absorbing state) results in there being

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**Fig. 2.** Schematic representation of the successive steps in analysis of the sequences of phytomer types on the primary axis: (A) initial sample of shoots; (B) types of phytomer sequence determined; (C) hidden hybrid Markov/semi-Markov chain estimated from the sequences for segmentation into homogeneous zones; (D) phytomer-type sequences and their corresponding restored state sequences defining each zone; (E) length distribution (zone A) and variable-order Markov chains (zones B and C) estimated for each zone.
only one state sequence that can account for a given observed sequence. With these restored state sequences, it was possible to segment the corresponding observed sequences into three (for ‘Grenache N’) or four zones (for ‘Syrah’). An ‘end-effect’ state had to be included before the final absorbing state for estimation of the transition probabilities based on the segmented sequences. So, as the second zone for ‘Grenache N’ corresponded to the second, third and fourth states (Fig. 2D), an additional state (the fifth state) was included for estimation of the transition probabilities for this zone.

Within each zone, perturbation of the P1–P2–P0 pattern can be analysed using a second-order Markov chain. The three most-represented memories, 01, 12 and 20, correspond to the normal succession of phytomers, whereas memories 00 and 10 are necessary for the modelling of possible perturbations. As state 1 is always preceded by state 0 and state 2 by state 1, the resulting model can be interpreted as a variable-order Markov chain (Weinberger et al., 1995; Ron et al., 1996; Bühlmann and Wyner, 1999) with memories {00, 10, 20, 1, 2} (see Fig. 2E). Thus, memories 11, 21, 02 and 22 are never observed.

Multiple sequence alignment. A multiple sequence alignment method was used to synchronize the sequences before extracting the branch characteristics for each rank (number of phytomers). Multiple sequence alignment is a well-known generalization of pair-wise sequence alignment. Iterative alignment is the most widely used technique (Gusfield, 1997; for the application of multiple alignment to branching sequences, see also Guédon et al., 2003). In this case, a succession of pair-wise alignments (in which the pairs of objects aligned may be simple sequences or previously computed alignments) is generated. Iterative alignment methods are heuristic, but the quality of the results obtained depends on the ‘guide tree’ determining the order of the alignments. A guide tree is a binary tree in which the leaves correspond to sequences and the interior vertices correspond to alignments. In the present case, the guide tree was generated by the application of a hierarchical clustering method to the distance matrix between sequences resulting from their pair-wise alignment.

Leaf appearance rate and duration of development. The number of unfolded leaves of five to six shoots per genotype was determined once a week for the main stem (expts 2 and 3) or of a subsample (expts 1 and 4). These measurements were also carried out on a sample of 30 branches per treatment. Individual leaf areas (S\textsubscript{lf}) were estimated from the quadratic relationship between the length of the leaf lamina (L\textsubscript{lf}) and the corresponding leaf area (Schultz, 1992). The parameters of the relationship were estimated for each experiment, from an independent data set corresponding to leaves collected from additional plants (Table 1):

\[ S_{lf} = a_3 L_{lf}^2 + b_3 L_{lf} \]  \hspace{1cm} (3)

Internode volume (V\textsubscript{in}) was calculated, assuming that internodes were approximately cylindrical in shape, as follows:

\[ V_{in} = a_4 \pi L_{in} (D_{in}/2)^2 \]  \hspace{1cm} (4)

where D\textsubscript{in} is the internode diameter and L\textsubscript{in} is the internode length. A corrective parameter (a\textsubscript{4}) was introduced to take into account the asymmetry of the internode length.
cross-section and variations in diameter at the node level. This parameter was estimated in expt 3 for the main stem and the branches on both cultivars, by directly measuring the volume of water displaced on immersion (Table 1).

**Estimation of axis leaf area**

The leaf area carried by the axis ($S_{ax}$) was estimated by summing the individual leaf areas.

$$S_{ax} = \sum_{i=1}^{n} (S_{lf,i})$$  \hspace{1cm} (5)

where $n$ is the number of phytomers under consideration.

Individual leaf areas ($S_{lf,i}$) were estimated according to rank of insertion with respect to the apex ($r$), as follows:

$$S_{lf,i}(r) = a_6 + \frac{b_6}{1 + \exp[-((r-c_6)/d_6)]}$$  \hspace{1cm} (6)

The independent parameters were set according to estimates obtained in expts 2 and 3 for the main and secondary axes (Table 1).

**Statistical analysis**

The ANOVA/MANOVA procedure of Statistica 6.0 (Statsoft, Tulsa, OK) was used to test for significant differences between means. Linear and logistic regression lines were fitted to the data, using the ‘optimize’ procedure of SciPy (http://scipy.org).

Hidden semi-Markov chains and variable-order Markov chains were estimated with AMAPmod software (http://amap.cirad.fr). The parameters of the variable-order Markov chains were compared in pairs, based on their 95% confidence intervals. The length of the initial P0 zone was extracted directly from the observed sequence of phytomer types. The two discrete empirical zone length distributions were compared, using the Wilcoxon–Mann–Whitney test.

**RESULTS**

**Main characteristics of canopy morphology and shoot development**

The main characteristics of shoot morphology (reported in Table 2) differed significantly between cultivars. At the end of expts 3 and 4, the primary axis was significantly longer in ‘Syrah’ than in ‘Grenache N’ ($P < 0.001$), whereas the two cultivars had similar total numbers of primary leaves and primary leaf areas ($P > 0.21$). Even

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Cultivar</th>
<th>Axis</th>
<th>$y$</th>
<th>$x$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$d$</th>
<th>$n$</th>
<th>$r^2$</th>
<th>CVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>Syr</td>
<td>$S_{lf}$ (cm$^2$)</td>
<td>$L_{lf}$ (mm)</td>
<td>0.0134</td>
<td>-0.0762</td>
<td>248</td>
<td>0.971</td>
<td>0.119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Gre</td>
<td></td>
<td></td>
<td></td>
<td>0.0100</td>
<td>0.1620</td>
<td>233</td>
<td>0.956</td>
<td>0.106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3, 4]</td>
<td>Syr</td>
<td></td>
<td></td>
<td></td>
<td>0.0111</td>
<td>0.1494</td>
<td>182</td>
<td>0.953</td>
<td>0.137</td>
<td></td>
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<td></td>
<td>0.0091</td>
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<td>0.972</td>
<td>0.216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Syr</td>
<td>$V_{in}$ (cm$^3$)</td>
<td>$D_{in}$ (cm)</td>
<td>1.315</td>
<td>0.95</td>
<td>160</td>
<td>0.95</td>
<td>0.122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Gre</td>
<td></td>
<td></td>
<td></td>
<td>1.510</td>
<td>0.98</td>
<td>75</td>
<td></td>
<td>0.084</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1. Empirical relationships obtained for ‘Syrah’ (Syr) and ‘Grenache N’ (Gre) cultivars for the estimation of leaf area ($S_{lf}$ and $S_{lf,i}$) and internode volume ($V_{in}$)**

<table>
<thead>
<tr>
<th>Equations no.</th>
<th>Expt</th>
<th>Cultivar</th>
<th>Axis</th>
<th>$y$</th>
<th>$x$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$d$</th>
<th>$n$</th>
<th>$r^2$</th>
<th>CVE</th>
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<td>$L_{lf}$ (mm)</td>
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<td></td>
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<td>0.95</td>
<td>160</td>
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<tr>
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<td>75</td>
<td></td>
<td>0.084</td>
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</table>

$\text{CVE} = \text{error coefficient of variation.}$

**TABLE 2. Main shoot characteristics at the end of the various experiments (1200 °Cd)**

<table>
<thead>
<tr>
<th>Axis type</th>
<th>Main characteristic of axis</th>
<th>‘Grenache N’</th>
<th>‘Syrah’</th>
<th>‘Grenache N’</th>
<th>‘Syrah’</th>
<th>Cultivar effect</th>
<th>Expt effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary axis</td>
<td>Phytomer number</td>
<td>50-8</td>
<td>52-9</td>
<td>56-0</td>
<td>55-2</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td>Secondary axes</td>
<td>Leaf area (m$^2$)</td>
<td>0-59</td>
<td>0-65</td>
<td>0-79</td>
<td>0-77</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Axis length (m)</td>
<td>3-76</td>
<td>4-34</td>
<td>4-52</td>
<td>5-04</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Secondary axes</td>
<td>Phytomer number</td>
<td>347</td>
<td>423</td>
<td>473</td>
<td>500</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Leaf area (m$^2$)</td>
<td>2-00</td>
<td>2-58</td>
<td>3-54</td>
<td>3-81</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Axis length (m)</td>
<td>9-83</td>
<td>12-93</td>
<td>—</td>
<td>—</td>
<td>***</td>
<td>—</td>
</tr>
</tbody>
</table>

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$, n.s., not significant.
larger differences between the cultivars were observed for the branches. The number of phytomers and the leaf area of the secondary axis were significantly greater for ‘Syrah’ than for ‘Grenache N’ ($P < 0.05$). The differences were larger in expt 3 ($+22$ and $+29\%$, for number of phytomers and leaf area of the secondary axis, respectively) than in expt 4 ($+6$ and $+8\%$, respectively). The cumulative metric length of the secondary axis was also $32\%$ greater ($P < 0.001$) for ‘Syrah’ than for ‘Grenache N’.

The distributions of secondary leaf area, pooled by the $P_0–P_1–P_2$ module along the main stem (expt 2), differed between cultivars (Fig. 3). The secondary leaf area of the proximal part of the shoot was higher in ‘Syrah’ than in ‘Grenache N’ (approx. $0.35\ m^2$ in experiment 2, $+26\%$). Conversely, leaf area in the median part of the shoot was higher in ‘Grenache N’ than in ‘Syrah’ (approx. $0.10\ m^2$ in expt 2, $+23\%$).

**Main shoot structure**

Hidden semi-Markov chains were used for segmentation of the primary axis phytomer-type sequences for both cultivars (Fig. 4). The preformed perturbed part of the shoot corresponded to two (A and B) and three (A, B1 and B2) homogeneous zones for ‘Grenache N’ and ‘Syrah’, respectively. The final zone (C) corresponds to the neoformed part of the shoot. The pattern of succession of phytomer types is much more regular in this zone. The segmentation led to the identification of particular sites at which the normal $P_0–P_1–P_2$ pattern was perturbed (B and B1 for the $0–0$ transition and B2 for the $1–0$ transition). These sites differed between cultivars. In addition, whereas zone B was shorter in ‘Grenache N’ than in ‘Syrah’ (three or four phytomers rather than nine), the initial zone of $P_0$ (zone A) appeared to be significantly longer (‘Grenache N’, $4.0\pm0.8$, $n = 58$; ‘Syrah’, $2.7\pm0.6$, $n = 56$; Wilcoxon–Mann–Whitney test, $P < 0.01$).

Variable-order Markov chains with memories $\{00, 10, 20, 1, 2\}$ (zones B and C) were estimated from the segmented sequences for both cultivars (Fig. 5). Whatever the zone considered, the probability of a perturbed pattern was slightly higher in ‘Grenache N’ than in ‘Syrah’. As a result, the mean number of $P_0$ initiated in ‘Grenache N’ over the ten first phytomers was also higher ($6.2\pm0.18$, $n = 58$) vs. $5.1\pm0.14$, $n = 58$; Wilcoxon–Mann–Whitney test, $P < 0.001$). This difference corresponds roughly to the difference in the length of zone A between the two cultivars. The perturbed $P_1–P_2–P_0–P_0–P_1$ pattern
predominated in the ‘Grenache N’ zone B but was less frequent in zone B1 of ‘Syrah’ (see Table 3). The perturbed P1–P0–P1 pattern occurred frequently in zone B2 of ‘Syrah’ and rarely in zone B1 of ‘Syrah’ and zone C of ‘Grenache N’.

**Distribution of branches along the main stem**

Mean branch leaf number and branch length profiles were computed after multiple phytomer-type sequence alignment. Consequently, at each rank, branch characteristics were determined for one phytomer type only. Figure 6 shows the influence of main shoot structure on branch development. At the shoot scale, in both cultivars, branch size increased from the bottom of the shoot up to phytomer 5 and then gradually decreased, reaching a minimum close to the shoot tip. More locally, the P0–P1–P2 pattern gave a high level of variability in branch size, with P0 phytomers systematically bearing the longest branches (R0) and P2 phytomers bearing the shortest branches ($P < 0.001$). The R0 branches located within zone A were systematically shorter than the other R0 branches of the preformed part. At the local R0–R1–R2 scale, the two cultivars differed in terms of the development of R1–R2 branches relative to R0 branches. R1–R2 branches attained about one-third the size of R0 branches in ‘Grenache N’ and about one-half that in ‘Syrah’. The overall branching patterns of the two cultivars were compared by analysing final branch leaf number profiles distinguishing the three types of branch (R0–R1–R2). These patterns were fitted beyond rank 5, for each individual shoot by a four-parameter logistic function (eqn 2). Significant differences were detected between branch types ($P < 0.01$) and between cultivars ($P < 0.05$) for the positional parameter $a_2$, but not for the damping parameter $b_2$. This difference between cultivars resulted from the larger number of short branches at the bottom of the shoot in ‘Grenache N’, leading to an earlier decrease in mean branch leaf number along the main shoot (the mean value of parameter $a_2$ was 4.9 for R1 branches and 3.1 for R2 branches in ‘Grenache N’ whereas the equivalent values were 9.0 and 6.8, respectively, in ‘Syrah’).

**Phytomer characteristics**

Differences in the morphological characteristics of the various types of axis between cultivars were investigated (Table 4). Primary axis internodes were systematically longer for ‘Syrah’ than for ‘Grenache N’ ($P < 0.001$), whereas the diameters of these internodes were greater for ‘Grenache N’ ($P < 0.001$). No significant difference in internode volume was observed. Similar trends were observed for the secondary axes, except that the difference in internode diameter between the two cultivars was not significantly different. Individual leaf areas were similar for the two cultivars. The impact of the P0–P1–P2 pattern

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**Table 3.** Frequencies of patterns observed within each zone for both cultivars

<table>
<thead>
<tr>
<th></th>
<th>‘Grenache N’</th>
<th>‘Syrah’</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>P1–P0–P1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>P1–P2–P0–P1</td>
<td>15</td>
<td>612</td>
</tr>
<tr>
<td>P1–P0–P0–P1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>P1–P2–P0–P0–P1</td>
<td>43</td>
<td>29</td>
</tr>
</tbody>
</table>

---

**Fig. 5.** Length distribution (zone A) and variable-order Markov chains (zones B and C) estimated for each homogeneous zone of ‘Grenache’ and ‘Syrah’ cultivars. Figures in brackets are confidence intervals for the transition probabilities at $P = 0.05$. 
on morphological characteristics was also investigated. This pattern directly influenced internode length but had no significant effect on internode diameter or individual leaf area. The internodes of P2 phytomers were systematically longer than those of P0 and P1 phytomers ($P < 0.001$). Internode volume varied in a similar manner, but the differences were not significant. Secondary axes displayed the same characteristics. The P0–P1–P2 pattern influenced both cultivars in the same way.

**Kinetics of axis development**

**Primary axis.** The rates of leaf production on main stems were compared for ‘Syrah’ and ‘Grenache N’, in expts 1–4. Similar values of approx. 0.044 leaf $\text{cm}^{-2} \text{day}^{-1}$ were obtained for the two cultivars.

**Secondary axis.** The thermal time at which the sylleptic axillary buds burst is shown in Fig. 7A. Budburst systematically occurred beyond the fourth position on the primary axes. Chronologically, budburst never occurred before the shoot had reached a critical size of ten phytomers (Fig. 7B). A short lag period was systematically observed between the appearance of a phytomer and axillary budburst, keeping phytomer appearance and the burst of the corresponding axillary bud separated by about four to six phyllochrons. No differences could be found between the cultivars on these points.

Once branches had appeared, their development depended on their insertion rank on the main stem and their position in the local P0–P1–P2 pattern. Branch development in the two cultivars was compared on the basis of two variables: the mean rate of leaf appearance and the mean duration of branch development (Table 5). The main differences between the cultivars concerned zones B and C. Within zone B, leaves unfolded at a similar rate on R0 (approx. 0.034 leaves $\text{cm}^{-2} \text{day}^{-1}$ and R2 branches (0.026–0.030 leaves $\text{cm}^{-2} \text{day}^{-1}$) in both cultivars. However, the rate of leaf appearance on R1 did not differ significantly from that on R0 in ‘Syrah’, whereas it did not differ significantly from that on R2 in ‘Grenache N’. In this zone, the duration of development followed the same trends as leaf appearance rate for both cultivars.

In zone C, the rate of leaf appearance and the duration of development decreased strongly for all types of branches, from the proximal to the median part of the zone, with further decreases observed from the median to the distal part ($P < 0.001$). The branches in the proximal part of zone C behaved strictly similarly to zone B, whereas significant differences were observed for the median part. The rate of leaf appearance on R0 branches was systematically higher than that on R1 and R2 branches. Furthermore, the rate of leaf appearance on R0 was significantly lower in ‘Syrah’ than in ‘Grenache N’. The duration of development followed the same basic trend. The differences in leaf appearance rate and duration of development between R0 and R1–R2 branches were smaller in ‘Syrah’ than in ‘Grenache N’.

Finally, no significant cultivar or branch type effects were detected at both ends of the shoot (zone A and distal part of zone C). Branches in these parts were weak, and grew more slowly and for shorter periods than those in other zones.

| Table 4. Morphological characteristics of primary and secondary phytomers of both cultivars |
|----------------------------------------|---------|---------|---------|---------|
| Axis type                              | Morphological parameters | 'Grenache N' | 'Syrah' |
|                                       | P0      | P1      | P2      | P0      | P1      | P2      |
| Primary axis                          | Individual leaf area (cm$^2$) | 166.6$^a$ | 167.6$^a$ | 163.0$^a$ | 168.9$^a$ | 169.6$^a$ | 170.8$^a$ |
|                                       | Internode length (mm)      | 67.3$^a$ | 68.7$^c$ | 80.4$^b$ | 86.3$^b$ | 82.1$^b$ | 100.4$^a$ |
|                                       | Internode diameter (mm)    | 8.6$^a$  | 8.1$^a$  | 8.3$^a$  | 7.8$^b$  | 7.8$^b$  | 7.7$^b$  |
|                                       | Internode volume (cm$^3$)  | 3.0$^a$  | 2.8$^a$  | 3.1$^a$  | 3.1$^a$  | 2.9$^a$  | 3.4$^a$  |
| Secondary axes                        | Individual leaf area (cm$^2$) | 117.1$^a$ | 121.5$^a$ | 122.0$^a$ | 121.6$^a$ | 121.3$^a$ | 124.3$^a$ |
|                                       | Internode length (mm)      | 48.4$^a$ | 52.0$^b$ | 60.5$^b$ | 64.3$^b$ | 61.8$^b$ | 77.0$^b$ |
|                                       | Internode diameter (mm)    | 5.6$^a$  | 6.0$^a$  | 5.6$^a$  | 5.7$^a$  | 5.9$^a$  | 5.5$^a$  |
|                                       | Internode volume (cm$^3$)  | 0.8$^a$  | 1.0$^a$  | 1.0$^a$  | 0.9$^a$  | 0.9$^a$  | 1.0$^a$  |

Values followed by different letters are significantly different ($P < 0.05$).
DISCUSSION

Primary axis organogenesis was very similar for both genotypes

The present results confirm that the timing of primary axis development is constant. The rate of leaf appearance in three successive years (0.044 ± 0.002 leaves °Cd−1) was similar to that previously reported for ‘Grenache N’ (Lebon et al., 2004) and Riesling (Schultz, 1992). This remarkably stable rate of leaf appearance, expressed as a function of air temperature, is probably the expression of a physiologically imposed minimal plastochron reached in non-limiting environments. Genetic variability has been shown for this trait in various species [Turc and Lecoeur, 1997 (for Pisum sativum); Granier and Tardieu, 1999 (for Helianthus annuus); Chena et al., 2005 (for Arabidopsis thaliana)]. However, the data available suggest that the level of variability between cultivars of Vitis vinifera is low (Duchêne, 1998).

As a consequence of this stability, the number of phytomers on the main stem was the same for both cultivars at the end of each experiment and throughout the growing period. Thus, the same number of axillary sylleptic buds was initiated in both cultivars. The present results also show that the probability and timing of budburst were identical in both genotypes. Thus, shoot architecture characteristics were mostly determined by thermal time, branch development and geometry.

Slight differences in main shoot structure can significantly affect secondary leaf area

Main shoot structure was analysed in detail, due to its impact on subsequent secondary-shoot development, as the secondary shoots are the main contributors to total leaf area (Lebon et al., 2004). Statistical models were used to segment the sequences of phytomer types (hidden semi-Markov chain) and to quantify the extent to which phytomer-type patterns were perturbed in the various zones of interest (variable-order Markov chains with associated pattern frequencies). In the grapevine, main stem structure was mostly predetermined and therefore directly apparent in the data. In most cases, statistical models are used to identify less-apparent structures from background noise (Gue´don et al., 2001; Heuret et al., 2003). In this study, such quantification provided no additional information about the structure itself, but did make it possible to quantify the level of pattern perturbation precisely. Thus, the smaller secondary leaf area of ‘Grenache N’ could be linked with the supplementary P0 of this genotype in zone A. Indeed, although P0 phytomers usually bear the stronger branches of the module.

Table 5. Branch development characteristics as a function of branch type (R0–R2) and position on the primary axis for the ‘Grenache N’ and ‘Syrah’ cultivars

<table>
<thead>
<tr>
<th>Development character</th>
<th>Zone</th>
<th>‘Grenache N’</th>
<th>‘Syrah’</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R0</td>
<td>R1</td>
</tr>
<tr>
<td>Leaf appearance rate (leaves °Cd−1)</td>
<td>Zone A</td>
<td>0.0117a</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Zone B</td>
<td>0.0334a</td>
<td>0.0235b</td>
</tr>
<tr>
<td></td>
<td>Zone C, proximal</td>
<td>0.0341a</td>
<td>0.0288b</td>
</tr>
<tr>
<td></td>
<td>Zone C, median</td>
<td>0.0268a</td>
<td>0.0138c</td>
</tr>
<tr>
<td></td>
<td>Zone C, distal</td>
<td>0.0145a</td>
<td>0.0097a</td>
</tr>
<tr>
<td>Branch development duration (°Cd)</td>
<td>Zone A</td>
<td>404a</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Zone B</td>
<td>967a</td>
<td>631b</td>
</tr>
<tr>
<td></td>
<td>Zone C, proximal</td>
<td>950a</td>
<td>717b</td>
</tr>
<tr>
<td></td>
<td>Zone C, median</td>
<td>562a</td>
<td>301b</td>
</tr>
<tr>
<td></td>
<td>Zone C, distal</td>
<td>200a</td>
<td>48a</td>
</tr>
</tbody>
</table>

Values followed by different letters are significantly different (P < 0.05).
(Lebon et al., 2004), it has been shown that those on the very first phytomers produced on the main shoot (zone A) are significantly smaller than subsequent branches. As the R0 branch of the first P0–P1–P2 module bears leaves with a total area of about 0.25 m², this single phytomer gap may account for >50 % of the difference in secondary leaf area between the two cultivars studied. This quantitative approach also revealed significant differences in pattern perturbation between the two genotypes. Although these differences may lead to differences in the spatial distribution of the most important branches, it has been shown here that they have only a small impact on the balance between the different phytomer types, particularly if the shoots are thinned, as in vineyards.

The behaviour of the R0 branches in zone A is consistent with reports based on in vitro cultures and describing the production of a series of P0 phytomers as a juvenile trait of the meristem (Mullins et al., 1979; Fournié and Bessis, 1993). Genetically, it is interesting to note the consistency between the longer zone A observed in ‘Grenache N’ and the higher probability of the perturbed P1–P2–P0–P0–P1 pattern in the neoformed part of the shoot for this genotype (zone C; see Table 3). Genetic variability in the ability of the meristem to produce the different types of phytomers has already been demonstrated for various species of the Vitaceae (Gerrath et al., 1998). However, this study provides the first demonstration of variability between varieties of the same species.

The parameter values of the statistical models are certainly not environment-independent but it is reasonable to expect that the structural properties highlighted in this study have a genetic basis. To check this point, it would be interesting to evaluate if the same perturbed patterns could be identified in similar positions along the primary axis for other cultivars or other samples of ‘Grenache N’ and ‘Syrah’ growing in different conditions (even if the perturbed pattern frequencies may depend on the environment).

The ontogenic properties of branches in P0–P1–P2 patterns result in differences in secondary leaf area distribution along the main axis between cultivars

For branches, the rate of leaf emergence and the duration of development depend on genotype. The two cultivars had similar individual leaf areas. Thus, differences in branch development are the principal factor accounting for differences in leaf area at the axis and whole-shoot scale. Kinematic analysis also showed that both leaf appearance rate and the duration of development were affected by position on the main stem and, as highlighted in previous studies (Ordovas et al., 1983; Lebon et al., 2004), by local position within the P0–P1–P2 pattern. Both leaf appearance rate and the duration of development decreased strongly from the base to the tip of the main shoot. However, the difference between leaf appearance rate in R0 and R1–R2 was always smaller in ‘Syrah’ than in ‘Grenache N’. All these factors resulted in differences in vertical secondary leaf area distribution between genotypes. The preferential development of R1 and R2 branches at the bottom of the shoot in ‘Syrah’, but not in ‘Grenache N’, may account for much of the difference in secondary leaf area between the two cultivars (around 0.25 m², corresponding to about 50 % of the difference in expt 2). This may also account for the earlier decline of vigour in the median branches of the shoots in ‘Syrah’ than in ‘Grenache N’. Indeed, competition between branches increases during shoot development. Recent studies on pea (Pisum sativum) have shown that the hierarchical organization of branches depends on both the growth rate and the size of individual axes (Novoplansky, 2003). The presence of longer, more vigorous branches at the bottom of the shoots in ‘Syrah’ may increase competition in the distal part of the shoot, resulting in a concomitant decrease in the rate of leaf emergence and the duration of development. These results suggested that the distribution of secondary axis development at shoot scale is a consequence of differences in the rates of leaf emergence of R0 and R1–R2 within the P0–P1–P2 pattern.

Internode geometry may play a key role in the genotypic variability of shoot shape

The ‘Grenache N’ and ‘Syrah’ cultivars have very different architectures (OIV, 1983). ‘Grenache N’ shoots tend to be quite erect, whereas those of ‘Syrah’ tend to be droopy. These differences may be due to the greater length/smaller diameter of the internode of the main stem in ‘Syrah’ than in ‘Grenache N’. Indeed the systematically higher length to diameter ratio of ‘Syrah’ internodes than of ‘Grenache N’ internodes probably results in a much higher bending momentum of the entire shoot in ‘Syrah’. These variables were recently shown to play a key role in the natural curvature of apricot branches (Alméras et al., 2004). However, other factors affecting the biomechanical properties of the stem, including the rheological properties of the wood and radial growth dynamics, may also be involved.

CONCLUSIONS

Topological analyses, based on Markovian models and a kinematic approach, were combined with geometric analysis in studies of the annual shoots of two cultivars of grapevine with very different growth habits. This is, as far as is known, the first time that such a quantitative analysis has been applied to aspects of the shoot architecture of different grapevine cultivars. In favourable environments, these cultivars differ in terms of total shoot leaf area, leaf area distribution along the main axis and axis length. Differences in leaf area result principally from slight differences in the patterns of phytomer succession in the preformed part of the shoot and in the rate and duration of development of R1–R2 branches with respect to R0 branches. However, more detailed investigations of the spatial developmental gradient of branches along the main shoot with respect to changes in competition between sinks for resources, and in the developmental physiology of branches, are required to go beyond this analysis phase and develop rules accounting for differences in secondary
leaf area distribution along the stem between genotypes. In terms of geometry, internode morphology was found to play a key role in determining axis length, potentially accounting for the well-known differences in growth habit between the cultivars. This study, based on a limited range of cultivars, provides a relevant framework in which to analyse the genotypic variability of grapevine shoot architectural traits on a broad scale. Further development of this work will include an evaluation of the relative importance of canopy-level architectural traits in terms of light interception and canopy microclimate. Such studies could be carried out using three-dimensional representations of virtual plant associated with radiative transfer models.

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LITERATURE CITED


