Apple shoot architecture: evidence for strong variability of bud size and composition and hydraulics within a branching zone

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Summary

- In the apple tree (*Malus domestica*), shoot architecture – the distribution of lateral bud types and growth along the parent shoot – has been extensively investigated. The distal zone of a shoot is characterized by a high proportion of vegetative or floral axillary branches mixed with latent buds and aborted laterals. The hypothesis tested here was that bud development was related to hydraulic conductance of the sap pathway to the bud, independently of an acrotonic (proximal vs distal) effect.
- The distal zone of 1-yr-old shoots was studied on five cultivars for bud size and composition (number of appendages) and hydraulic conductance before bud burst.
- Bud size, composition and hydraulic conductance were highly variable for all cultivars. A positive correlation was demonstrated between both the number of cataphylls and green-leaf primordia, and hydraulic conductance. Cultivar and bud size affected the intercept of these relationships more than the slope, suggesting similar scaling between these variables, but different hydraulic efficiencies. A great proportion of small buds were also characterized by null values of hydraulic conductance.
- This study suggests that hydraulically mediated competition exists between adjacent buds within the same branching zone, prefiguring the variability of lateral types in the following growing season. It is hypothesized that this developmental patterning is driven by hydraulic characteristics of the whole metamer, including the subtending leaf, during bud development.

Key words: branching, bud size, cataphylls, green-leaf primordia, *Malus domestica* (apple), metamer, shoot architecture, xylem hydraulic conductance.

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Introduction

Tree architecture is the expression at a given time of the balance between endogenous factors and exogenous constraints (Hallé *et al.*, 1978). Endogenous factors determine characters that are not influenced by the environment. In the apple tree, all cultivars belong to the architectural model of Scarrone: all axes are orthotropic with rhythmic growth and sexuality in the terminal position (Hallé *et al.*, 1978), with a similar location of branching zones, each being characterized by a homogeneous composition of lateral types (vegetative, floral, latent) along the 1-yr-old shoot (Costes & Guédon, 2002). However, several features differ depending on the cultivar, including branching density (Lespinasse & Delort, 1986; Forshey *et al.*, 1992) and frequency of lateral flowering on 1-yr-old wood (Lauri & Lespinasse, 2001). For a given genotype, branching and lateral flowering frequency may be modulated by environmental factors such as temperature, which affects dormancy completion of buds (Cook & Jacobs, 1999; Labuschagné *et al.*, 2003; Naor *et al.*, 2003), or orientation of growth of the parent shoot, such as vertical vs horizontal positioning (Lauri & Lespinasse, 2001; Naor *et al.*, 2003).

The growth potential of a branch is generally considered to be positively related to the number of primordia in the overwintering bud to which it belongs (Kozlowski, 1973). However, at shoot level the physiological mechanisms underlying branching frequency (ratio of latent vs growing laterals, whether vegetative or floral) and the type (latent, vegetative, floral) and final length of the lateral are still not understood, even though the interplay of genetic programmes with the environment has been investigated intensively in model plants, both dicots and monocots (McSteen & Leyser, 2005). The involvement of the water-transport system (hydraulic architecture; Tyree & Ewers, 1991) in shoot growth and branching has been investigated in relation to growth in height (Koch *et al.*, 2004; Woodruff *et al.*, 2004), transport efficiency (McCulloh & Sperry, 2005) and mechanical properties (Rosner *et al.*, 2007) but, to our knowledge, scarcely in relation to bud morphogenesis. A recent attempt has been made by Cochard *et al.* (2005), on *Fagus sylvatica*, showing a positive correlation between the number of leaf primordia in the bud before bud burst and the hydraulic conductance (K_x) of the xylem vascular system connected to this bud, whether in the lateral or terminal position. The study was conducted on the whole shoot, thus including a variability in potential bud development related to the topological position along the shoot, specifically a lower growth potential of laterals situated in proximal position compared with buds situated more distally along the parent shoot (Fisher, 1984; Nicolini, 1997; Le Bris *et al.*, 1998). Therefore this hydraulic study on *F. sylvatica* does not permit us to separate the effects on hydraulic conductance of the topological position along the shoot from the intrinsic size and composition of the buds. Indeed, small buds are frequent in the proximal part of the shoot, and this positional effect might be related more to the lower K_x of the xylem vascular system connected to these buds, than to bud size and composition *per se*.

Our study was developed in apple trees, for which shoot architecture (the distribution of lateral bud types and growth along the parent shoot) has been investigated extensively (Costes *et al.*, 2006). Long shoots of apple trees are usually defined by a high frequency of latent buds in the proximal zone, and a distal zone with a high frequency of vegetative and floral buds (Greene & Autio, 1994; Guédon *et al.*, 2001; Brunel *et al.*, 2002; Costes & Guédon, 2002; Renton *et al.*, 2006; Lauri, 2007). Buds, usually latent, in the proximal zone are small, whereas buds in the distal zone, which are usually vegetative or floral, are large (Brunel *et al.*, 2002). Latent buds can also be present in the distal zone, although less frequently (Lauri & Térouanne, 1998; Costes & Guédon, 2002; Lauri, 2007). In addition to these bud types, a proportion of buds that would otherwise develop as vegetative or floral laterals physiologically abort within their first year of development (Lauri & Térouanne, 1998). This physiological abortion of laterals is known as lateral extinction in a horticultural context (Lauri *et al.*, 1995, 1997). The heterogeneity of bud potential within the distal zone offers a unique opportunity to validate the relationships between the hydraulic conductance of the xylem vascular system connected to the lateral bud, hereafter referred to as K_{LAT}, and bud size and composition independent of the topological position.

Our analysis was developed on lateral buds in the distal zone of shoots before bud burst for a range of apple cultivars. Our objectives were: (1) to search for differences in bud size and composition that would predict actual development of the lateral in spring, and especially the existence of latent and aborted buds; (2) to assess relationships between bud size and K_{LAT} and to analyse the possible differential effects of cataphylls and green-leaf primordia on these relationships; and (3) to examine whether these relationships are affected by the cultivar.

Materials and Methods

Plant material

Five apple (*Malus domestica* Borkh.) cultivars (Ariane, Braeburn, Fuji, Gala and Granny Smith) were grafted on M.9 rootstock and planted in a completely randomized design in a single row in the INRA experimental field in Montpellier, France. These cultivars are characterized by differences in branching frequency, frequency of floral buds in the 1-yr-old shoot, and shoot length and shape (Han *et al.*, 2007). In spring 2006, trees were pruned at planting to leave three to five buds at the bottom of each scion and the most vigorous shoot was later selected for the experiment, leading to a single shoot per tree directly stemming from the grafting point (Fig. 1). In the following winter, before the estimated time of bud burst (from 19 March to 4 April 2007), six to 10 healthy 1-yr-old 2006 shoots per cultivar, depending on the cultivar, were selected (Table 1). The idea was to take morphological and hydraulic measurements (see below) before new cambial growth occurred. Every 2 d, three to five shoots were randomly selected and cut off in the field. The cut end was immediately immersed in water and transported to the laboratory for bud observations and hydraulic measurements. Each cultivar was sampled four to eight times. Previous year observations showed that bud burst occurred with a time-lag between cultivars with the following ranking, from early to late: Braeburn, Granny Smith, Fuji, Ariane, Gala. In 2007, Braeburn actually burst from 4 April 2007 onwards. The sampling method took into account this time-lag, and early cultivars were preferentially sampled earlier than late cultivars.

The distal third of each shoot was identified (excluding the most distal five nodes, which were characterized by very short internodes and having tiny or no buds). This portion had between 15 and 26 nodes depending on the total number of nodes of the shoot (Table 1). Only true axillary buds were

Table 1 Number of shoots per apple (*Malus domestica*) cultivar, total number of nodes in the distal third based on number of nodes of each shoot, and actual number of lateral buds sampled per shoot for morphological and hydraulic studies (excluding sylleptic shoots and damaged buds)

		Per shoot	
Cultivar	Number of shoots	Number of nodes of the distal one third	Number of buds studied
Ariane	6	26 ± 3.63	19.1 ± 4.50
Braeburn	9	15.8 ± 0.60	9.3 ± 1.87
Fuji	10	17.5 ± 0.70	13.6 ± 3.53
Gala	9	15.2 ± 1.09	11.3 ± 2.87
Granny Smith	8	20.3 ± 6.02	13.1 ± 6.98

Data are means ± SD.

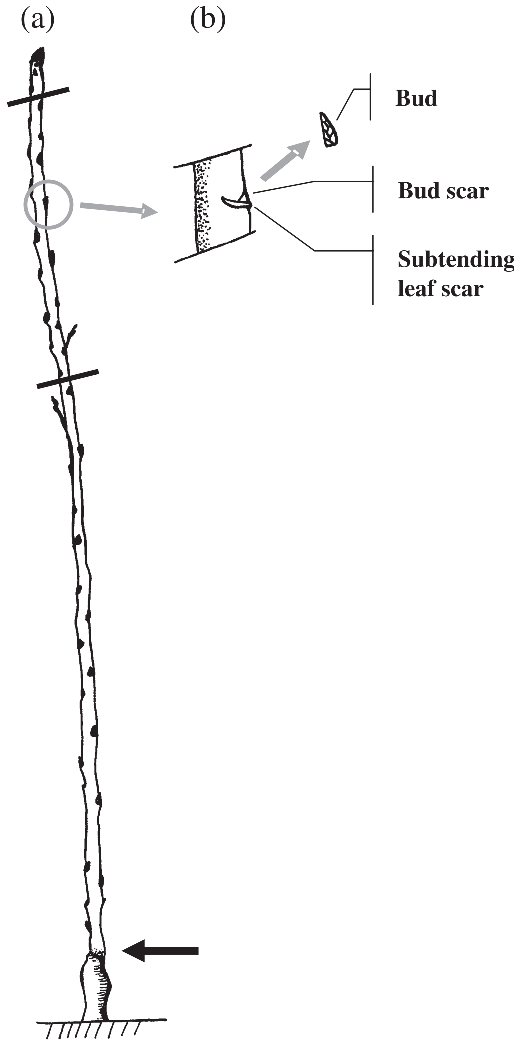

Fig. 1 (a) An apple shoot after leaf fall stemming from the grafting point, with the studied distal third excluding the uppermost buds; (b) detail of a node bearing an axillary bud, and after bud removal, bud scar on which K_{LAT} is measured. Black arrow, grafting point.

considered, excluding those in the terminal position on sylleptic shoots, whether short or long (Table 1).

Hydraulic and morphological measurements

For the distal third of each shoot, each lateral bud was numbered following a proximal-to-distal sequence and cut off with a razor blade perpendicular to the main axis of the parent shoot, just below the bud cataphylls, leaving a bud scar on the stem. Each bud was then put in alcohol 70% in a 2-ml Safe-Lock Eppendorf tube for later bud investigations. To avoid any possible effect of bud removal on the rate of water exudation (F, see below) from the other bud scars, all studied buds of a shoot were removed at the beginning of each shoot study (Cochard *et al.*, 2005).

Hydraulic conductance (K_{LAT}) of the xylem sap pathway to each previously removed bud was measured using a high-pressure flow meter (HPFM, Dynamax, Houston, TX, USA) apparatus, which is based on the perfusion of deionized and filtered water at a given pressure at the bottom of the cut parent shoot (P, MPa) and measurement of the rate of water exudation (F, µmol s^{-1}) at each individual bud scar (Cochard *et al.*, 2005; Han *et al.*, 2007). Water exudation was measured using a weighted piece of dry cotton applied for 1 min to the bud scar surface. The difference in weight, to the nearest tenth of a milligram, before and after measurement gave the amount of water exuded. In a preliminary work, the strongly positive relationship ($r^2 \approx 0.99$) between P and F of a sample of excised buds was assessed for a range of water pressures, 0.1–0.5 MPa.

For each lateral bud (Fig. 1), the following were investigated: bud size was measured and buds were ranked into three length classes, small (S, < 2 mm), medium (M, 2–4 mm) and large (L, > 4 mm); the number of cataphylls and green-leaf primordia were counted using a stereomicroscope (×40).

Data analysis

Three types of analyses were performed During the 1-min K_{LAT} study, a proportion of bud scars did not exude a measurable amount of water. In this case, K_{LAT} was considered as equal to 0. The relationship between bud size (small, medium, large) and exudation (with, without) was analysed through a Bartholomew test. This test compares the proportions of exuding bud scars in each bud size group (p_S, p_M, p_L) by testing the equality of proportions (H_0: $p_S = p_M = p_L$) against an order-restricted hypothesis (H_1: $p_S \le p_M \le p_L$ with at least one strict inequality).

For exuding bud scars, the effects of cultivar and bud size on both total number cataphylls and green-leaf primordia, and K_{LAT} were first analysed through a parametric two-way ANOVA and followed by Duncan's multiple range test when F was significant ($P < 0.05$). For percentage of cataphylls, a Kruskal–Wallis (H) nonparametric ANOVA was carried out.

The relationship between K_{LAT} and the number of cataphylls and green-leaf primordia was then explored using regression models. Because of an important underdispersion in Poisson modelling, the linear model framework was used to relate the number of appendages (ranging from 0 to 13) with the $\log_e(K_{LAT})$. A covariance analysis was performed, taking into account the effects of the two factors, cultivar and bud size. A backward model construction strategy was adopted, starting from the richest model with all order 2 interactions on the intercept and slope on $\log_e(K_{LAT})$ and removing step-by-step nonsignificant terms (for each level of each factor).

We then completed these two covariance analyses for number of cataphylls and number of green-leaf primordia with a comparison of the regression slopes. To achieve that, we calculated the difference between these two dependent variables and similarly constructed a linear regression model on $\log_e(K_{LAT})$.

Results

Cultivar and bud size effects on number of appendages

The number of appendages varied between cultivars, with similar trends for cataphylls and green-leaf primordia. Gala had the highest number of total appendages with *ca.* 14 appendages and the highest proportion of large buds as opposed to Granny Smith with *ca.* 9 appendages and with the lowest proportion of large buds (Table 2). Moreover, the number of total appendages of large buds differed between the two cultivars, with 17.1 and 11.5 appendages for Gala and Granny Smith, respectively (Table 2). Ariane, Braeburn and Fuji had intermediate values. The percentage of cataphylls among all appendages was always above 50%, with differences between cultivars. Highest values were observed for Granny Smith (69%) and lowest values for Gala (57%).

The total number of appendages was positively related to bud size, and both variables were inversely related to the percentage of cataphylls, with large buds having the highest number of appendages (*ca.* 16) and the lowest percentage of cataphylls (*ca.* 53%), whereas small buds showed an inverse trend: 8 and 73% for both variables, respectively (Table 2).

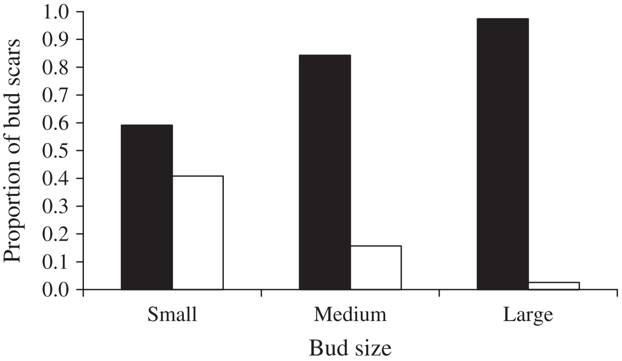

Fig. 2 Proportions of bud scars with (closed bars) and without (open bars) exudation after bud removal according to bud size (small, medium, large) for the merged five apple (*Malus domestica*) cultivars. Probability associated with the Bartholomew test, $P < 10^{-10}$.

Relations with hydraulics

Around 20% of all bud scars for all cultivars did not exude. No clear pattern of differences between cultivars and between individual shoots of each cultivar were found concerning the proportions of exuding bud scars (data not shown). The proportions (p_S, p_M, p_L) of exuding bud scars increased from small to large buds (Fig. 2).

Between bud scars that exuded, K_{LAT} was significantly higher for Granny Smith (0.062 µmol MPa^{-1} s^{-1}) than for the other cultivars (between 0.018 µmol MPa^{-1} s^{-1} and 0.039 µmol MPa^{-1} s^{-1}) (Table 3). However, it is likely that the high K_{LAT} values obtained for cv. Granny Smith were mainly influenced by the notably high value obtained for the large buds (Table 3). K_{LAT} also differed strongly between bud scars of buds of different size, with an 8-fold increase in large buds compared to small buds (Table 3). Supporting this relationship, there were positive correlations between the number of cataphylls and green leaf primordia in buds and $\log_e(K_{LAT})$ (Fig. 3). However these relationships varied with both cultivar and bud size. The significantly lower K_{LAT} of both small and medium-sized buds as opposed to large buds (Table 3) prompted us to group small and medium-sized buds.

For cataphylls, the significant terms retained in the model gave the following results (Table 4). Intercepts differed significantly between cultivars, with lower values for cvs Braeburn, Fuji and Granny Smith and a higher value for Gala compared with Ariane ($P < 0.05$ in all cases), and were lower for small and medium-sized buds compared with large buds (−1.76, $P < 0.001$). However, there was a significant interaction between cultivar and bud size for cvs Braeburn and Granny Smith, resulting in a differential increase of the intercept for small and medium-sized buds compared with large buds (+1.08, $P < 0.001$ and +4.48, $P < 0.001$, respectively). Slopes were nonsignificantly different between cultivars (+0.29) except for cv. Granny Smith (with a lower slope, −2.12, $P < 0.01$), but only for large buds, and this effect was cancelled out for small

Table 2 Effects of apple (*Malus domestica*) cultivar and bud size on bud composition

			Number of appendages			
Cultivar	Bud size	No. of buds	Total	Green-leaf primordia	Cataphylls	Percentage of cataphylls†
Ariane	Large	17	17.1 ± 2.5	8.3 ± 1.6	8.8 ± 1.5	51.6 ± 5.3
	Medium	71	13.0 ± 2.7	6.1 ± 1.7	6.9 ± 1.6	53.8 ± 7.5
	Small	27	8.1 ± 3.1	2.5 ± 2.2	5.5 ± 1.3	74.9 ± 19.9
Braeburn	Large	23	13.9 ± 2.3	6.4 ± 1.5	7.5 ± 1.7	53.7 ± 8.5
	Medium	55	11.9 ± 2.9	5.2 ± 2.0	6.7 ± 1.3	58.0 ± 11.7
	Small	6	6.3 ± 2.1	1.3 ± 0.8	5.0 ± 1.4	81.0 ± 10.7
Fuji	Large	2	14.0 ± 0.0	6.5 ± 0.7	7.5 ± 0.7	53.6 ± 5.0
	Medium	96	10.7 ± 2.5	4.4 ± 2.0	6.4 ± 1.4	61.2 ± 13.4
	Small	38	8.4 ± 2.7	2.9 ± 2.0	5.4 ± 1.3	69.0 ± 17.8
Gala	Large	29	17.1 ± 2.6	7.9 ± 1.5	9.1 ± 1.8	53.3 ± 6.3
	Medium	62	12.8 ± 2.9	5.4 ± 1.7	7.4 ± 1.9	58.1 ± 8.4
	Small	11	10.7 ± 3.5	4.7 ± 2.6	6.0 ± 1.5	58.8 ± 14.1
Granny Smith	Large	6	11.5 ± 4.3	5.2 ± 1.9	6.3 ± 2.5	54.8 ± 5.9
	Medium	40	10.8 ± 2.7	4.4 ± 1.9	6.4 ± 1.3	61.0 ± 11.6
	Small	59	6.8 ± 3.4	2.1 ± 1.9	4.6 ± 1.8	75.5 ± 18.4
Cultivar mean						
Ariane		115	12.5 ± 3.9b	5.6 ± 2.6b	6.9 ± 1.8b	58.4 ± 14.6c
Braeburn		84	12.1 ± 3.2b	5.3 ± 2.2b	6.8 ± 1.5b	58.2 ± 12.5b
Fuji		136	10.1 ± 2.8c	4.0 ± 2.1c	6.1 ± 1.4c	63.3 ± 15.0ab
Gala		102	13.8 ± 3.6a	6.0 ± 2.1a	7.7 ± 2.1a	56.7 ± 8.9c
Granny Smith		105	8.6 ± 3.7d	3.2 ± 2.3d	5.4 ± 1.8 d	68.8 ± 17.3a
Cultivar effect						
F – H			17.2***	12.7***	11.4***	42.3***
Bud size mean						
Large		77	15.6 ± 3.2a	7.3 ± 1.8a	8.3 ± 1.9a	53.1 ± 6.7c
Medium		324	11.8 ± 2.9b	5.1 ± 1.9b	6.8 ± 1.5b	58.4 ± 11.2b
Small		142	7.7 ± 3.3c	2.6 ± 2.2c	5.1 ± 1.6 c	72.6 ± 18.5a
Bud size effect						
F – H			125.8***	102.6***	65.3***	36.0***

Data are means ± SD.

†The percentage of cataphylls was computed as the ratio of the number of cataphylls among the total number of appendages.

A two-way ANOVA was performed to separate the effects of cultivar and bud size. Within a column, different letters indicate significant differences at $P = 0.05$ (F-test followed by Duncan's multiple mean comparison test for numbers; Kruskal–Wallis H-test followed by multiple mean comparison test for percentages). ***, Significant differences between means at the 0.001 level.

and medium-sized buds because of the interaction with bud size (+2.19, $P < 0.01$).

For green-leaf primordia, the significant terms retained in the model gave the following results (Table 5). Intercepts differed between cultivars and bud sizes, with similar grouping of cultivars as for cataphylls: lower values were observed for cvs Braeburn, Fuji and Granny Smith ($P < 0.001$ in all cases) than for Gala and Ariane, which had similar values, and small and medium-sized buds had lower values compared with large buds (–2.03, $P < 0.001$). There were significant interactions between cultivar and bud size for Braeburn, Fuji and Granny Smith, resulting in an increase in intercept values for small and medium-sized buds ($P < 0.001$ in all cases). Slopes were the same for Ariane, Braeburn and Gala and differed for Fuji and Granny Smith, where the latter two cultivars had an interaction with bud size. Indeed, whereas slope values decreased (–1.14, $P < 0.01$ and –2.12, $P < 0.01$, for Fuji and Granny Smith, respectively), this effect was only for large buds because of the positive interaction with bud size for the small and medium-sized buds (+1.58, $P < 0.001$ and +2.14, $P < 0.01$ for Fuji and Granny Smith, respectively).

The differential effects of K_{LAT} on the number of cataphylls and green leaf primordia were analysed by computing the relationships between K_{LAT} and the difference between the numbers of cataphylls and green-leaf primordia in each bud. The significant terms retained in the model gave the following results (Table 6). The common intercept did not differ from 0 ($P > 0.05$), but was higher for Fuji (+3.23, $P < 0.05$), meaning a higher difference between number of cataphylls and number of green-leaf primordia for this cultivar compared with the others. However, there was a significant interaction between cultivar and bud size for Fuji, with a negative interaction (–5.52, $P < 0.01$), that is, lower difference, for Fuji small and medium-sized buds. The common slope was significantly negative (–0.37, $P < 0.001$), meaning that for a given increase in K_{LAT} the increase in the number of cataphylls was lower

Table 3 Effects of apple (*Malus domestica*) cultivar and bud size on hydraulic conductance of the xylem vascular system connected to the bud, K_{LAT}, for exuding bud scars

Cultivar	Bud size	Number of buds	K_{LAT} (μmol MPa^{-1} s^{-1})
Ariane	Large	17	0.086 ± 0.062
	Medium	70	0.031 ± 0.024
	Small	16	0.014 ± 0.012
Braeburn	Large	22	0.061 ± 0.079
	Medium	40	0.027 ± 0.035
	Small	1	–
Fuji	Large	2	0.009 ± 0.004
	Medium	69	0.020 ± 0.018
	Small	20	0.013 ± 0.010
Gala	Large	28	0.045 ± 0.069
	Medium	61	0.024 ± 0.023
	Small	11	0.018 ± 0.018
Granny Smith	Large	6	0.519 ± 0.270
	Medium	33	0.038 ± 0.044
	Small	36	0.007 ± 0.013
Cultivar mean			
Ariane		103	0.038 ± 0.040b
Braeburn		63	0.039 ± 0.056b
Fuji		91	0.018 ± 0.017b
Gala		100	0.029 ± 0.042b
Granny Smith		75	0.062 ± 0.156a
Cultivar effect			
F			8.4***
Bud size mean			
Large		75	0.096 ± 0.159a
Medium		273	0.027 ± 0.028b
Small		84	0.012 ± 0.014b
Bud size effect			
F			45.2***

Data are means ± SD.
A two-way ANOVA was performed to separate the effects of cultivar and bud size. Within a column, different letters indicate significant differences at $P = 0.05$ (*F*-test followed by Duncan's multiple mean comparison test). ***, Significant differences between means at the 0.001 level.

Table 4 Effects of apple (*Malus domestica*) cultivar and bud size on the relationships between $\log_e(K_{LAT})$ and the number of cataphylls in the following model: number of cataphylls ~ (common intercept + B + F + G + GS + SM + B:SM + GS:SM) + (common slope + GS + GS:SM) × $\log_e(K_{LAT})$

Factors/levels of factors		Effects	*t*-test	*P*
Common intercept		+9.75	29.48	***
	B	−1.01	−2.52	*
	F	−0.63	−2.82	**
	G	+0.43	1.98	*
	GS	−4.96	−6.70	***
	SM	−1.76	−6.93	***
	B: SM	+1.08	2.33	*
	GS: SM	+4.48	4.91	***
Common slope (×$\log_e(K_{LAT})$)		+0.29	3.66	***
	GS	−2.12	−3.14	**
	GS: SM	+2.19	3.22	**

Cultivars: B, Braeburn; F, Fuji; G, Gala; GS, Granny Smith.
Bud size: SM, small and medium.
*, **, ***, Significant effects at the 0.05, 0.01 and 0.001 levels, respectively.

Table 5 Effects of apple (*Malus domestica*) cultivar and bud size on the relationships between $\log_e(K_{LAT})$ and the number of green-leaf primordia in the following model: number of green-leaf primordia ~ (common intercept + B + F + GS + SM + B:SM + F:SM + GS:SM) + (common slope + F + GS + F:SM + GS:SM) × $\log_e(K_{LAT})$

Factors/levels of factors		Effects	*t*-test	*P*
Common intercept		+10.67	29.74	***
	B	−1.68	−4.26	***
	F	−5.90	−4.60	***
	GS	−6.34	−8.29	***
	SM	−2.03	−7.53	***
	B: SM	+1.91	3.99	***
	F: SM	+7.21	4.72	***
	GS: SM	+5.24	5.63	***
Common slope (×$\log_e(K_{LAT})$)		+0.77	8.49	***
	F	−1.14	−3.04	**
	GS	−2.12	−3.08	**
	F: SM	+1.58	3.84	***
	GS: SM	+2.14	3.09	**

Cultivars: B, Braeburn; F, Fuji; G, Gala; GS, Granny Smith.
Bud size: SM, small and medium.
, *, Significant effects at the 0.01 and 0.001 levels, respectively.

than the increase in the number of green-leaf primordia (Fig. 3). However, there was a significant interaction between cultivar and bud size for Fuji with a global increase in slope (+0.84, $P < 0.05$) but an additional decrease for small buds (−1.32, $P < 0.01$).

Discussion

It has been hypothesized that the organogenesis of the lateral bud, which accurately forecasts the type and growth of the branch deriving from it, is influenced by the structural proportions of the 1-yr-old parent shoot: length, proximal and distal diameters, and slenderness (ratio of length to diameter) (Lauri & Trottier, 2004). In apple trees, as in most temperate species, bud organogenesis occurs in the summer and autumn preceding actual spring growth (Bijhouwer, 1924; Pratt, 1988). Cochard *et al.* (2005) suggested a positive relationship between cambial activity in the year of parent shoot growth and the primary growth of buds, whether in terminal or lateral position, in the following year. Our study showed a high variability of apple bud development within the distal branching zone, independent of position along the shoot, and documented relationships with hydraulics taking into account the number and types of appendage included in the bud.

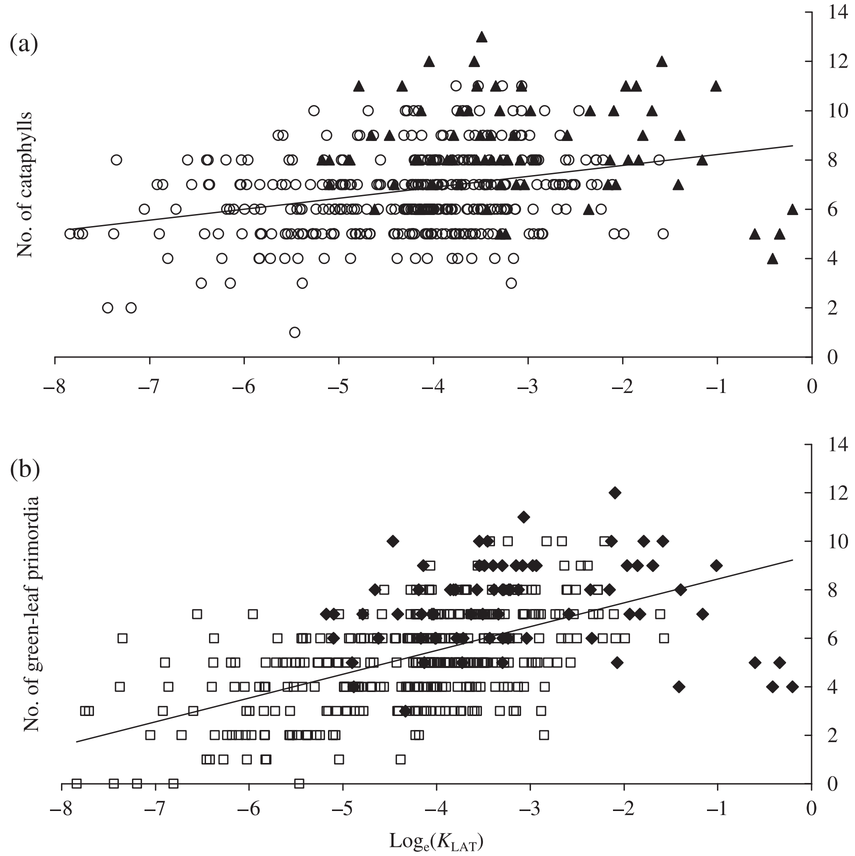

Fig. 3 Relationships between $\log_e(K_{LAT})$ of the xylem vascular system connected to the bud and the number of (a) cataphylls; (b) green-leaf primordia of this bud, for all buds and for all apple (*Malus domestica*) cultivars and bud size categories. For each appendage category, buds are split into 'small and medium' and 'large'. (a) Triangles, large; circles, small and medium; (b) diamonds, large; squares, small and medium. Regression lines are computed for cataphylls in (a) (number of cataphylls = 0.443 × $\log_e(K_{LAT})$ + 8.662, $R^2 = 0.09$, $n = 432$) and for green-leaf primordia in (b) (number of green-leaf primordia = 0.981 × $\log_e(K_{LAT})$ + 9.425, $R^2 = 0.31$, $n = 432$).

Table 6 Effects of apple (*Malus domestica*) cultivar and bud size on the relationships between $\log_e(K_{LAT})$ and the difference between the number of cataphylls and the number of green-leaf primordia in the following model: number of cataphylls – number of green-leaf primordia ~ (common intercept + F + F:SM) + (common slope + F + F:SM) × $\log_e(K_{LAT})$

Factors/levels of factors		Effects	*t*-test	*P*
Common intercept		−0.44	−1.62	ns
	F	+3.23	2.25	*
	F: SM	−5.52	−3.15	**
Common slope (×$\log_e(K_{LAT})$)		−0.37	−16.17	***
	F	+0.84	1.98	*
	F: SM	−1.32	−2.75	**

Cultivars: B, Braeburn; F, Fuji; G, Gala; GS, Granny Smith.
Bud size: SM, small and medium.
*, **, ***, ns, Significant effects at the 0.05, 0.01 and 0.001 levels, and nonsignificant, respectively.

Bud size and composition variability within the distal zone

Over a large range of shoot sizes, there is a positive relationship between the size (e.g. diameter) of a shoot and the size of its laterals (Marcelis-van Acker, 1994). However, at a smaller scale, our results clearly showed a great variability of bud size and composition within the distal zone of a same shoot, although shoot diameter did not vary significantly along the shoot (data not shown). These differences between buds were therefore independent of a positional effect. Differences could be observed between adjacent buds without a clear pattern, for example, no phyllotactic pattern could be demonstrated from our data compatible with the leaf hydraulic sectoriality hypothesis (Orians *et al.*, 2005; data not shown). The variability in bud development within the distal zone mirrored the architectural pattern of the whole 1-yr-old shoot: small – potentially latent – buds have a lower total number of appendages and a higher percentage of cataphylls than large – potentially vegetative and floral – buds (Brunel *et al.*, 2002; Puntieri *et al.*, 2007).

Relations with hydraulic conductance: common trends and differences between cultivars

Although shoot sampling lasted 10–15 d for each cultivar, there was no significant change in K_{LAT} over this period (data not shown). This would *a posteriori* support our presumption that measurements were carried out before new cambial growth occurred. Our study showed, for a range of apple cultivars, a positive relationship between bud size and number of appendages, and a positive relationship between these two variables and the hydraulic conductance of the vascular system connected to the buds. It confirmed and expanded on previous results at the whole-shoot scale on *F. sylvatica* by Cochard *et al.* (2005). Based on our results, we suggest a two-step process for the relationships between K_{LAT} and bud development. First, the absence of any exudation ($K_{\mathrm{LAT}} = 0$ in our experimental conditions) was observed mainly for small and medium-sized buds. This would apply well to buds that remain latent, or that burst in spring and soon abort, leaving a scar (Lauri & Térouanne, 1998). Second, for bud scars that exuded, the positive correlation between K_{LAT} and the number of bud appendages would explain the range of sizes reached by vegetative and floral laterals.

According to Marcelis-van Acker (1994), increasing the assimilate supply during lateral bud development increases the mass of the bud and the number of leaf primordia in the bud. Our results showed that K_{LAT} correlated positively with bud size, and not only with green-leaf primordia but also with cataphylls. However, it was shown here that slopes of the relationships with K_{LAT} were higher for green-leaf primordia than for cataphylls, indicating that for a given increase in K_{LAT}, the number of green-leaf primordia increased more than the number of cataphylls. The slopes of the relationships between K_{LAT} and number of appendages were not affected by bud size or cultivar. However, these relationships were differentiated by their allometric constant, meaning that the efficiency of the vascular system for bud organogenesis differed depending on bud size and cultivar: large buds and Gala had higher efficiencies compared with small and medium-sized buds and the other cultivars, respectively.

It has been hypothesized that the formation of cataphylls is a result of the slowing down of the plastochron (in apple trees, cataphylls begin to form when the plastochron becomes longer than 5 d; Crabbé & Escobedo-Alvarez, 1991). These cataphylls are likely to play a key role in bud formation in buffering the apex against a resumption of growth, possibly via the abscisic acid they contain (Abbott, 1970; Crabbé, 1994; Brunel *et al.*, 2002). According to Brunel *et al.* (2002), proximal (potentially latent) buds have a significantly higher number of cataphylls than distal buds, and both the number of cataphylls and *KNAP2* (*KN1*-like gene family) expression are negatively related to the growth potential of the bud. Our results showed that, in the distal zone, small buds had fewer cataphylls and green-leaf primordia compared with large buds. It was also shown that small buds had a higher percentage of cataphylls than large buds (Table 2), meaning that the differences in the number of appendages between small and large buds were caused by differences in the number of green-leaf primordia (2.8-fold) rather than by differences in the number of cataphylls (1.6-fold). Based on these results, it may be proposed that differences between small and large buds in the distal zone did not result from a higher number of cataphylls of the former, which would hamper further organogenesis within the bud. Rather, the process would begin with the development of the first cataphylls and increase with bud development, and at any time was positively related to K_{LAT}.

A potential role of the whole metamer in the relationships between K_{LAT} and lateral bud development

The acrotony concept is used to interpret the differential branching pattern of the proximal as opposed to the distal zone of the parent shoot (Crabbé, 1985; Bell, 1991; Cook *et al.*, 1998; Wilson, 2000). At this scale, architectural gradients may result from competitive interactions between distal and proximal zones, and also with secondary growth (Lauri, 2007). In our study, secondary growth could hardly be advocated as a main competition factor between buds, because buds of different sizes were mixed in a distal zone with a similar diameter. However, it may be hypothesized that hydraulically mediated competition for assimilates between adjacent territories along the shoot are set as soon as metamers unfold. Relationships between leaf and lateral bud developments have rarely been investigated, and conclude to either a positive (Larson & Pizzolato, 1977) or a negative (Schmitz & Theres, 1999) relationship. However, it is likely that relationships between the development of both the leaf and its axillary bud are more complex and should include the whole metamer (leaf, node and subtending internode), and should be considered in a time scale. First, a minimum leaf size has to be reached to develop a visible axillary meristem (Lauri & Térouanne, 1998). Second, the growth dynamics of the leaf (e.g. in peach; Kervella *et al.*, 1995) or of the whole metamer (e.g. in apple; Lauri & Térouanne, 1995, 1998) plays a crucial role in the axillary bud type, floral vs vegetative. These relationships at the morphological level suggest that, within the distal zone, all factors favouring early and sustained growth of the leaf, compared with the internode, also favour growth of the axillary bud. The involvement of hydraulic conductance in these dynamic relationships is poorly documented. Correlations between hydraulic traits of adjacent territories have generally been established between stem and leaf (Preston & Ackerly, 2003; Edwards, 2006), and scarcely between stem and buds (Cochard *et al.*, 2005). According to Lo Gullo *et al.* (2004), apical dominance, the absence of branching during growth of the terminal bud, and hydraulic dominance, higher leaf hydraulic conductance for distal leaves, are positively related. Considering adjacent metamers within the same branching zone, however, the involvement of hydraulic conductance in the cross-relationships between metamer components (internode, leaf and axillary meristem) is an interesting avenue for further studies.

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