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RESEARCH PAPER

Effects of shoot bending on lateral fate and hydraulics: invariant and changing traits across five apple genotypes

Hyun-Hee. Han[1],*, Catherine Coutand[2], Hervé Cochard[2], Catherine Trottier[3] and Pierre-Éric Lauri[1,†]

[1] *UMR DAP, INRA-SupAgro-CIRAD-UMII, Equipe 'Architecture et Fonctionnement des Espèces Fruitières', 2 place P. Viala, F-34060, Montpellier, Cedex 1, France*

[2] *UMR 547 PIAF, INRA, UNIV BLAISE PASCAL, F-63100 Clermont-Ferrand, France*

[3] *Université Montpellier II, UMR I3M-5149, Equipe 'Probabilités et Statistique', Place Eugène Bataillon, F-34095 Montpellier Cedex 5, France*

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Abstract

The aim of this work was to study the variability of physiological responses to bending and the relationship with hydraulic conductance of the sap pathway to the laterals for five apple genotypes. The study focuses on the fate of the laterals. The genetic variability of bending can have two sources: a genetic variability of stem geometry which can lead to differences in mechanical state; and a genetic variability of sensitivity to bending. Since the aim was to check if some genetic variability of sensitivity to bending exists, the genetic variability of shoot geometry was taken into account. To do so, bending was controlled by imposing different bending intensities using guides of different curvature conferring a similar level of deformation to the five genotypes. Bending was done either in the proximal zone or in the distal zone of shoots, in June and in the following winter, respectively. A Principal Component Analysis comparing upright and bent shoots revealed that bending in the proximal zone stimulated vegetative growth of buds which would otherwise stay latent. A second Principal Component Analysis restricted to bent shoots revealed that bending increased the abortion of laterals in the lower face of the shoots. The abortion phenomenon was to the detriment of sylleptic laterals or of inflorescence, depending on the genotype. There was a strong effect of position around the shoot on within-shoot hydraulics. Hydraulic conductance was significantly decreased in the lower face of the shoot bent in winter. This result suggested a causal relationship between this phenomenon and lateral abortion.

Key words: Apple, bending, biomechanics, hydraulic conductance, lateral type, longitudinal strain, radial location, shoot tapering, topological location.

Introduction

The control of growth and branching of a fruit tree is monitored at two levels, at the whole-tree scale via the initial choice of rootstock and the yearly management of irrigation and fertilization, and at the branch or shoot scale via physical manipulations such as pruning and bending. Although plant growth regulators (PGRs) have been advocated partly to control branching density and flowering, their use is turning into an important societal and environmental problem in the context of sustainable horticulture. This topic re-updates the question of to what extent the use of classical environmentally neutral physical manipulations, i.e. based on a better knowledge of the genetic variability of shoot architecture, could be effective in order to monitor branching and flowering with precision. Bending is addressed here, which deserves more attention in some innovative fruit tree training systems but still remains based on empirical rules (Lauri and Laurens, 2005).

The main concepts in shoot architecture (e.g. apical dominance, acrotony) are well illustrated by the branching pattern of shoots in an upright (orthotropic) position (Champagnat, 1954*a*, 1965; Brown *et al.*, 1967; Crabbé,

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* Present address: Apple Experimental Station, NHRI (National Horticultural Research Institute), 286 Wiseong-ri, Sobo-myeon, Gunwi-gun, Gyeongbuk, South Korea 716-812.

† To whom correspondence should be addressed: E-mail: lauri@supagro.inra.fr

1985; Powell, 1995; Cook *et al.*, 1998; Lauri and Térouanne, 1998; Guédon *et al.*, 2001; Costes and Guédon, 2002). Moving the shoot from the vertical to any other direction and especially to a strictly horizontal position by leaning or bending changes the initial branching pattern (Champagnat, 1961; Salisbury, 1993). Wareing and Nasr (1961) proposed the term gravimorphism to refer to the biomechanical effects related to both gravity and mechanical manipulation. From an architectural viewpoint, a review of the studies on gravimorphism means that some general trends can be stated. First, branching topology is changed with a shift from acrotony toward mesotony or basitony (Wareing and Nasr, 1961; Champagnat and Crabbé, 1974; Lakhoua and Crabbé, 1975*a*; Lauri and Lespinasse, 2001). Second, branching frequency is increased on a bent shoot compared with an upright one (Naor *et al.*, 2003; Hampson *et al.*, 2004). Third, lateral type frequencies may also be modified with a controversial effect on flowering, i.e. either an increase in, or no consistent effect on, flowering (Longman *et al.*, 1965; Mullins, 1965; Tromp, 1970; Wareing, 1970). Eventually, an increased flowering precocity has been noticed on a bent shoot compared with an upright one (Meilan, 1997; Ito *et al.*, 1999). It is probable that the effects of bending on branch architecture are partly genotype dependent and also depend on the time of manipulation (Lauri and Lespinasse, 2001). In this latter case, the response of the branch to re-orientation may be fast, as shown by the increase of fruit set in apple branches trained to the horizontal during flowering (in this case fruit set enhancement is related to an increase in the proportion of healthy ovules; Robbie *et al.*, 1993). Changes in hormone levels in shoot and lateral buds as a reaction to bending have been shown (e.g. increase of zeatin-type cytokinins in the bent shoot; Ito *et al.*, 1999). Moreover, the stimulated bud growth in bent shoots is related to the increased sink capacity of the bud relative to the adjacent shoot tissues. This is suggested by the enhancement of the activities of several enzymes involved in sugar metabolism in the lateral bud, NAD-dependent sorbitol dehydrogenase (NAD-SDH), NADP-dependent SDH (NADP-SDH), and acid invertase (AI) (Ito *et al.*, 2004).

Although the relatively poor vegetative and fruiting development of laterals located on the underside of the bent shoot has been noticed in apple (Crabbé, 1969; Champagnat and Crabbé, 1974; Lakhoua and Crabbé, 1975*a*, *b*; Rom, 1992) and rose (Zieslin and Halevy, 1978), there is a lack of quantitative analysis of the effect of position around the bent shoot, hereafter referred to as radial location, on lateral development. Indeed, the relative part played by bud latency and lateral abortion has not yet been investigated.

Some authors noticed a reduced water transport in the bent shoot compared with the upright shoot of annuals (*Helianthus annuus*, Smith and Ennos, 2003), as well as for woody plants (apple, Cristoferi and Giachi, 1964; *Vitis vinifera*, Schubert *et al.*, 1995). A more severe reduction was also noticed at the bending point compared with the other portions of the downward or horizontal portions of the shoots (Schubert *et al.*, 1995). In rose, it has been suggested that the reduced water conduction of the bent shoot may be, in part, responsible for the lower net photosynthesis, transpiration, and stomatal conductance of water vapour of the leaves projecting downward (Kim *et al.*, 2004). This phenomenon can be related to reaction wood (i.e. xylem fibres with a thick extra G-layer at the inner side of the secondary wall) differentiation which reduces water conduction (Woodrum *et al.*, 2003; Pilate *et al.*, 2004). On the other hand, on a 30-year-old trunk of *Pinus taeda*, it was found that bending did not affect hydraulic conductivity (Fredericksen *et al.*, 1994). As far as is known, the effect of bending on water transport just beneath the bud and depending on radial location is not documented.

This study was carried out on apple for which shoot architecture is well documented (Lauri and Térouanne, 1998; Guédon *et al.*, 2001; Costes and Guédon, 2002; Renton *et al.*, 2006). Generally speaking, the various lateral types follow an orderly sequence from the bottom to the top of the annual growth unit with a predominance of latent buds and vegetative laterals in the proximal zone, and a predominance of vegetative and flowering laterals in the distal zone (Renton *et al.*, 2006). Sylleptic laterals (i.e. which develop in the same year as the parent shoot) are usually found in a medial position (Champagnat, 1954*a*; Costes and Guédon, 1997). The objectives of this study were therefore (i) to document across a range of genotypes the change in frequency of lateral types on bent shoots compared with upright ones taking into account the effect both of the topological zone on which bending was applied and of the radial location, and (ii) to analyse the relationships with pre-bud burst hydraulic conductance (k_{LAT}) of the vascular system immediately beneath the bud.

Materials and methods

Plant material and determination of zone and time of bending

Five genotypes with a range of 1-year-old shoot dimensions (length, diameter) and shape (slenderness) were chosen: Ariane, Braeburn, Fuji, Gala, and Granny Smith (Table 1). One-year-old trees, grafted on Pajam rootstock, were planted in two adjacent rows in February 2004 in the INRA experimental field in Montpellier, France. Trees were pruned at planting to leave 3–5 buds at the bottom of the scion. The most vigorous 2004 shoot was then selected after bud burst for the experiment.

Each tree was dedicated to bear either an upright (control) or a bent shoot. In the latter case, following previous observations (data not shown), bending was carried out on two shoot zones with, presumably, the most contrasting branching patterns: proximal zone

Table 1. *Length and basal diameter (mean ±SE) of shoots at time of bending, i.e. spring for proximal zone and winter for distal zone, according to the genotype*

ANOVA is performed to separate the effects of genotype. Within the same column, different letters indicate significant differences at P=0.05, Duncan multiple means comparison test. n is the number of shoots.

Genotype	Bending in proximal zone		n	Bending in distal zone		N
	Shoot length (cm)	Shoot basal diameter (mm)		Shoot length (cm)	Shoot basal diameter (mm)	
Ariane	37.9±1.1 b	7.3±0.1 a	11	143.8±9.7 a	17.7±0.9 b	13
Braeburn	39.9±1.8 b	7.0±0.2 a	16	124.7±4.1 b	20.3±0.8 ab	21
Fuji	54.4±1.6 a	7.4±0.2 a	22	149.6±3.6 a	22.0±0.6 a	22
Gala	38.6±1.7 b	6.1±0.2 b	12	131.6±4.3 b	18.9±0.7 b	12
Granny Smith	37.3±1.6 b	7.2±0.3 a	11	131.9±4.7 b	19.7±0.8 ab	12

(P) on growing shoots of approximately one-third of the final length (38–54 cm depending on the genotype), and distal zone (D) on fully grown shoots (125–150 cm depending on the genotype) (Table 1; Fig. 1). These shoots will hereafter be referred to as P- and D-shoots, respectively. For each treatment, there were about 20 trees for Fuji and Braeburn, and about 10 trees for Ariane, Gala, and Granny Smith (Table 1) in a completely randomized design. The two bending treatments were done at two different times, during active growth (June 2004) for P-shoots, and during dormancy (January 2005) for D-shoots. In the former case bending was done during active organogenesis and would potentially lead to a change in lateral bud development. In the latter case bending was done during dormancy on already pre-formed buds and would potentially lead only to post-organogenesis processes. This system made it difficult to separate the effects of the topological zone along the shoot and time of year, because the proximal part of a shoot always developed before the distal part. First, bending in June could only be done on the proximal zone at a time when the distal zone was not yet fully elongated. Second, bending of the proximal zone during dormancy, i.e. at the time when bending of the distal zone was done, could not be done because of a high risk of breakage due to the large diameter of the bottom part of the shoots. All shoots were kept during the whole of the 2005 growing season for morphological observations.

Bending treatment and biomechanics

The variability of reaction to bending can come from two sources: a genetic variability of shoot diameter that will lead to a variability of the mechanical state of the bent branch if bending is the same (Brüchert and Gardiner, 2006); and a genetic variability of reaction to the mechanical state imposed by bending. Until now, the mechanical state of the bent shoot has been poorly controlled: angle of the tip (Lauri and Lespinasse, 2001), natural shoot and fruit load (Alméras *et al.*, 2002, 2004), and weight of artificial mass added (Barritt, 1992). Because of intraspecific variability of shoot tapering, the same tip angle or the same mass added to the shoot can lead to a very different mechanical state of the bent shoot. In order to decorrelate the two genetic variabilities concerning bending, all bent shoots were set in a similar mechanical state (see below). That way, if differences between genotypes were observed they would indicate a genetic variability of shoot sensitivity to mechanical state imposed by bending. In order to do that, a quantification of bending was required. Studies have demonstrated that the mechanical variable which is sensed by the plant submitted to mechanical constraint is the level of strain and not the applied force (Coutand and Moulia, 2000). In this study, the rationale was to take into account the variability of shoot geometry and tapering, and to adapt the intensity of bending to each genotype in order to impose the same average mechanical strain on the different genotypes.

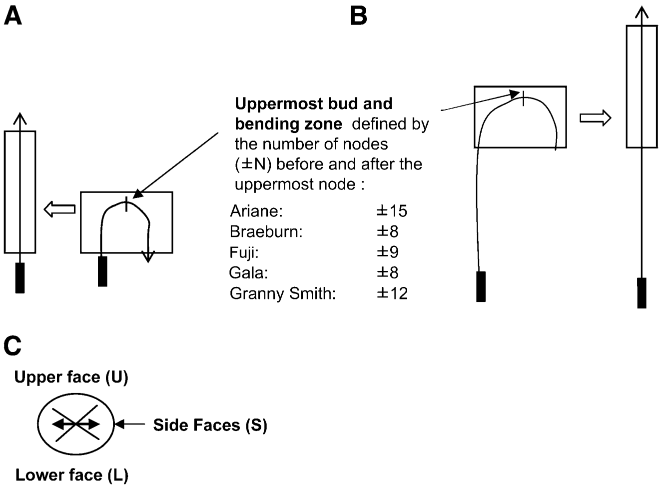

Fig. 1. Quantitative control of bending and determination of the bent portion in shoots bent in the proximal zone in spring (A) and in shoots bent in the distal zone in the following winter (B). Bending is done in order to place the apical bud in a vertical position towards the ground. The bent zone corresponds to the portion of the shoot rolled on the guide. The uppermost bud is located at the middle of the bent zone. The three faces around the bent shoot are illustrated in (C).

From a mechanical point of view, as shoots are slender structures, they can be considered as beams. The level of maximal longitudinal strain at a point located at the stem periphery and at a distance i from the stem base (ε_{LL}, i) is given by the product of the imposed curvature (C_i) and the radius of the stem (r_i) at point i:

$$\varepsilon_{LL,i} = C_i \times r_i$$

So, in order to get the same level of strain, the stoutest shoots have to be curved less than the most slender ones. Therefore, to set the genotypes at the same average level of strain, different bending must be done. A study of shoot tapering between genotypes was done and showed, first, a linear evolution of diameter from the apex for P- as well as for D-shoots, and, second, a significant variability between genotypes: statistical tests on differences between slopes clustered the genotypes into three groups for P-shoots: (i) Ariane, Granny Smith; (ii) Braeburn and Gala; (iii) Fuji (Fig. 2A). For D-shoots, the same procedure also led to three groups: (i) Ariane, (ii) Granny Smith, (iii) Braeburn, Fuji, and Gala (data not shown).

In practical terms metallic guides were designed to control the level of applied curvature and longitudinal strain. The shoot was rolled on the guide (beginning from the apex toward the stem base) and then attached to wires behind the shoot in order to maintain the shoot at the imposed bending and to set the guide free for another shoot.

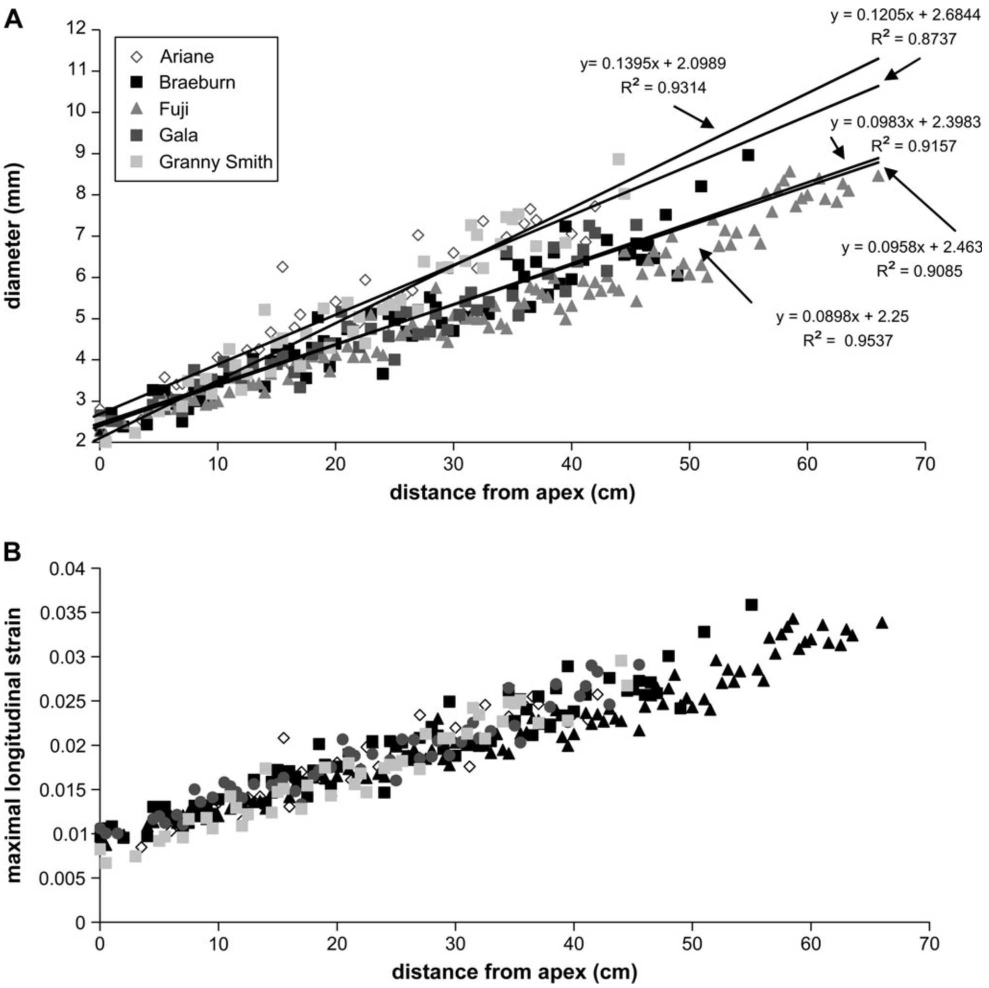

Fig. 2. Geometry and state of strain of shoots. The example of shoots bent in spring. In (A) change in diameter of shoots from the apex. Each symbol corresponds to a genotype. The tapering of the shoots was well fitted by a linear equation. The equation and determination coefficient are given for each genotype. In (B) imposed deformation along the shoots for the five genotypes. Taking the geometry of shoots into account in bending resulted in a similar imposed strain state.

The control of the curvature applied is given by the curvature of the guide. This rationale was used for bending on both proximal and distal zones. (i) For P-shoots, as Fuji tapering was very close to those of Braeburn and Gala, the same metallic guide (137 mm in radius) was used for the three genotypes. The guide for Ariane and Granny Smith was 162 mm in radius. The use of a circular guide led to a longitudinal gradient of strain, but all the genotypes were set at the same average level of strain (Fig. 2B). (ii) For D-shoots, the 1-year-old shoot was stouter than in the previous case and a stronger tapering led to the building of other guides. As the taper exhibited differences compared with shoots bent in the proximal zone, setting the same strain state meant designing new guides. The use of circular guides in spring led to a strain gradient along the shoots. Setting the shoots bent in winter at the same state of strain as the shoots bent in spring (i.e. with respect to the imposed gradient of strain) required the guides to be non-circular. Three guides were used according to the analysis of slopes (as for P-shoots, see above): (i) Ariane; (ii) Granny Smith; and (iii) Braeburn, Fuji, and Gala.

Description of shoot architecture

In the spring of 2005, the bent zone of the bent shoots was first determined on P-shoots including all nodes from the grafting point upwards to the uppermost node, and the same number of nods downwards (Fig. 1A). The same number of nodes was then determined on the bent zone of D-shoots (Fig. 1B). To compare the branching patterns of the bent zone of P- and D-shoots with their topological counterparts on upright shoots, the mean number of nodes from the bottom bounding the P and D zones on the bent shoots was then compared with upright shoots (Fig. 1A, B). Each lateral was characterized by its type and radial location. Five types of laterals were considered: sylleptic (S), latent bud (L), vegetative bud (V), inflorescence (I), and aborted lateral (AL). AL types were seen in both situations: 2004 sylleptic lateral, usually short, whose terminal bud failed to grow in 2005; and a bud which began to grow in spring 2005 and soon died. The radial location was considered by dividing the cross-section circumference of the shoot into four quarters. The two lateral quarters were merged, determining three faces hereafter referred to as upper (U), lower (L), and side (S) faces (Fig. 1C).

Hydraulic studies

Studies were carried out on two genotypes chosen from the five genotypes previously studied for architecture, Fuji and Braeburn. Since hydraulic measurements were destructive and had to be done before bud break, they were done on a separate shoot sample. Eleven and nine 1-year-old shoots exhibiting similar lengths to the shoots used for architectural studies were selected for Braeburn and Fuji, respectively. In these samples, eight and six shoots for Braeburn and Fuji, respectively, were bent in December 2005 using the same methodology as for the D-shoots in the architectural study, and three shoots per genotype were left as controls. In March 2006, 10–15 d before the estimated bud burst, all shoots belonging to the two genotypes were cut off in the field, with their cut end immediately immersed in water, and transported to the laboratory. For bent shoots, the cord linking the upward and the downward portion of the shoots was maintained in order to avoid possible passive uprighting.

Hydraulic conductance (k_{LAT}) of the sap pathway to the different buds was measured using the High Pressure Flow Meter apparatus (HPFM, Dynamax, USA; Tyree *et al.*, 1995; Salleo *et al.*, 2002) which is based on the perfusion of deionized and filtered water at a given pressure at the bottom of the cut shoot (P, MPa) and the measurement of the rate of water exudation (F; mmol s^{-1}) at the base of each lateral bud just below bud scars (Cochard *et al.*, 2005). Buds could be in a strictly axillary position or ending a sylleptic lateral. The buds were excised with a razor, permitting water to exude, and F was measured by using a weighed piece of dry cotton applied for 1 min on the cut surface of the shoot where the bud had been removed. The difference in weight before and just after measurement gave the amount of water exuded. In a preliminary work, the strong positive relationship ($r^2 \approx 0.99$) between P and F of a sample of excised buds was assessed for a range of water pressure of 0.1–0.5 MPa. To avoid any possible effect of bud removal on F of the other buds, all studied buds of a shoot were removed at the beginning of each shoot study (Cochard *et al.*, 2005). On all shoots, k_{LAT} was measured for every two buds within zone D of both bent and upright shoots. On the latter shoots, radial location (U, L, S) was noted as for the architectural analysis.

Data analysis

Three types of analysis were carried out. A first analysis aimed at modelling the effects of genotype (GEN), zone of branching along the shoot (ZON), and bending status (BST) on the proportion of lateral types (LAT). For this, multinomial models were constructed using the canonical logarithmic link (a linear predictor combining factors is used to explain the ratio of probabilities of each lateral type to one reference type category, here L). The effects of the three factors and of interactions between factors up to order 3 were considered. A backward model construction strategy was adopted, beginning from the richest model containing all effects and order 2 and 3 interactions, and removing step by step non-significant elements by testing embedded models. Finally, since this analysis revealed a highly significant order 3 interaction (Table 2; Model 0), a Principal Component Analysis (PCA), based on a covariance matrix, was carried out on fitted values in order to help the interpretation of the model obtained.

A second analysis using the same modelling tools and following the same strategy was carried out on bent shoots only. Here, the effects on the proportion of lateral types (LAT) of the three factors: genotype (GEN), zone of branching along the shoot (ZON), and radial location (RAD) were considered. As for the first analysis, the model was obtained by removing the order 3 interaction and the order 2 interaction between genotype and radial location (GEN: RAD) (Table 3; Model 4′), and was then interpreted by a PCA on fitted values.

Eventually, analyses on hydraulic conductance were performed with Duncan's Multiple Range Test, at the 5% level of confidence.

Results

The effect of bending on shoot architecture

The effects of GEN, ZON, and BST on the probabilities of lateral types were clearly interacting, resulting in a highly significant order 3 interaction (Model 1, $P=2.34\times10^{-12}$; Table 2). This prevented any simple and general interpretations. The only general and consistent trend across the genotypes was observed for inflorescences (I) which were in a higher proportion in the distal zone compared with the proximal zone (36–68% versus 0–2%; Fig. 3). The same picture was not observed for the other lateral types whose proportions varied according to genotype, zone, and bending status, for example, sylleptic laterals which were higher in the proximal (Granny Smith)

Table 2. *Effects of genotype (GEN), zone along the shoot (ZON), and bending status (BST) on proportion of lateral types (LAT) of* Malus×domestica

The multinomial model is constructed by selection of factors and interactions. For each model, '~' separates the dependent variable on the left from the list ('+') of dependent variables on the right; an 'asterisk' indicates the proper effect of each factor and interactions between them; ':' indicates interaction between two variables.

Models, factors, and interactions	Model structure	Deviance test	P
Model 0 – LAT~GEN*ZON*BST			
Model 1 – LAT~GEN+ZON+BST+ GEN:ZON+GEN:BST+ZON:BST	M1 ⊂ M0	90.16	2.34×10^{-12}

Table 3. *Effects of genotype (GEN), zone along the shoot (ZON), and radial location (RAD) on proportion of lateral types (LAT) of bent shoots of* Malus×domestica

The multinomial model is constructed by a selection of factors and interactions. For each model, '~' separates the dependent variable on the left from the list ('+') of dependent variables on the right; an 'asterisk' indicates the proper effect of each factor and interactions between them; ':' indicates interaction between two variables.

Models, factors, and interactions	Model structure	Deviance test	P
Model 0' – LAT~GEN*ZON*RAD			
Model 1' – LAT~GEN+ZON+RAD+GEN:ZON+GEN:RAD+ZON:RAD	M1′ ⊂ M0′	48.65	0.03
Model 2' – LAT~GEN+ZON+RAD+GEN:RAD+ZON:RAD	M2′ ⊂ M1′	361.61	$<10^{-12}$
Model 3' – LAT~GEN+ZON+RAD+GEN:ZON+GEN:RAD	M3′ ⊂ M1′	23.24	3×10^{-3}
Model 4' – LAT~GEN+ZON+RAD+GEN:ZON+ZON:RAD	M4′ ⊂ M1′	30.35	0.55

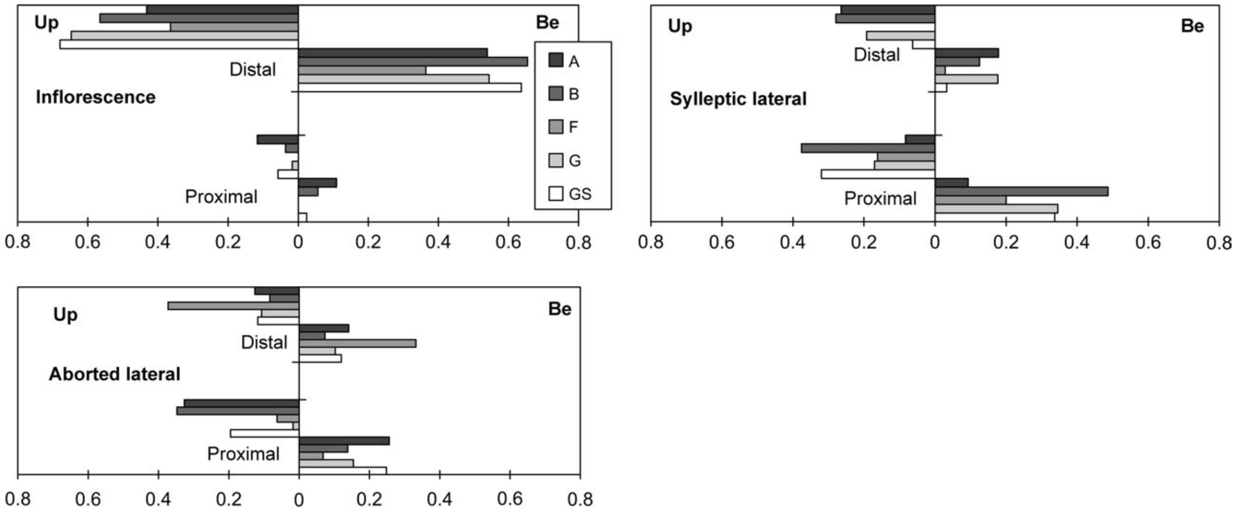

Fig. 3. Observed proportions of the lateral types on distal and proximal zones of upright (Up) and bent (Be) shoots for the five genotypes. For legibility only three types are shown (inflorescence, sylleptic lateral, and aborted lateral).

or in the distal (Ariane) zones, or aborted laterals which were increased (Gala) or decreased (Braeburn) by bending the same zone (Fig. 3).

The PCA on fitted values revealed that 78.8% of all variability was taken into account by the first two factors (64.1% and 14.7% for factors 1 and 2, respectively; Fig. 4A). Factor 1 was essentially explained by I, whereas factor 2 opposed L and V. S and AL had little influence on these two factors (grey symbols; Fig. 4A). Factor 1 clearly opposed the proximal zone on the right part of the graph, i.e. with low I, to the distal zone on the left part of the graph, i.e. with high I (Fig. 4B). Factor 2 discriminated between upright and bent shoots, with more L in the former and high V in the latter whatever the genotype (grey arrows; Fig. 4B). This effect was higher in the P zone compared with the D zone.

The asymmetric effect of bending on lateral type distribution around the shoot

The effect of GEN, ZON, and RAD on the proportion of lateral types was studied on bent shoots only. There was only a weak order 3 interaction (+48.65, P=0.03; Table 3) compared with order 2 interactions between ZON and RAD, and ZON and GEN which were highly significant (+23.24, $P=3\times10^{-3}$ and +361.61, $P<10^{-12}$, respectively; Table 3). There was no significant order 2 interaction between GEN and RAD (+30.35, P=0.55; Table 3).

The PCA on fitted values revealed that 83.5% of all variability was taken into account by the first two factors (61.3% and 22.2% for factors 1 and 2, respectively; Fig. 5A). Factor 1 was strongly explained by I, whereas factor 2 opposed S and AL with a lower impact of L and V on these two factors (grey symbols; Fig. 5A). Moving from U to L consistently increased AL (grey arrows; Fig. 5B). However, this was to the main detriment of S for Granny Smith and Gala, whereas it was to the main detriment of I for Braeburn and Ariane (vertical and oblique grey arrows, respectively; Fig. 5B). Fuji was in an intermediate position.

Hydraulic measurements

There was a strong effect of the genotype on k_{LAT}, with Fuji values 2- to 6-fold those observed in Braeburn (Table 4). k_{LAT} was not significantly influenced by bending for Fuji and Braeburn. There was no significant interaction between genotype and bending (Table 4). For both genotypes, k_{LAT} varied significantly according to radial location: laterals situated in U of the bent shoot had k_{LAT} values about 4-fold higher than laterals situated in L, with intermediate values for laterals in S (Fig. 6). For both genotypes, this asymmetric distribution of k_{LAT} on bent shoots was significantly affected by the re-uprighting of shoots, resulting in equivalent k_{LAT} in faces which were previously in the U, L, and S positions (Fig. 6).

Discussion

This study compared branching on shoots of five apple genotypes either in an upright position or in a bent position. In the latter case the same level of mechanical strain was applied to all shoots, taking into account the mean geometrical properties of each cultivar. As a method for unravelling the respective effects of position along the parent shoot and bending on the fate of the lateral shoots, the data analyses developed here, i.e. multinomial modelling followed by a PCA, appeared to be efficient. In the first step of this study, the architectural analysis of upright

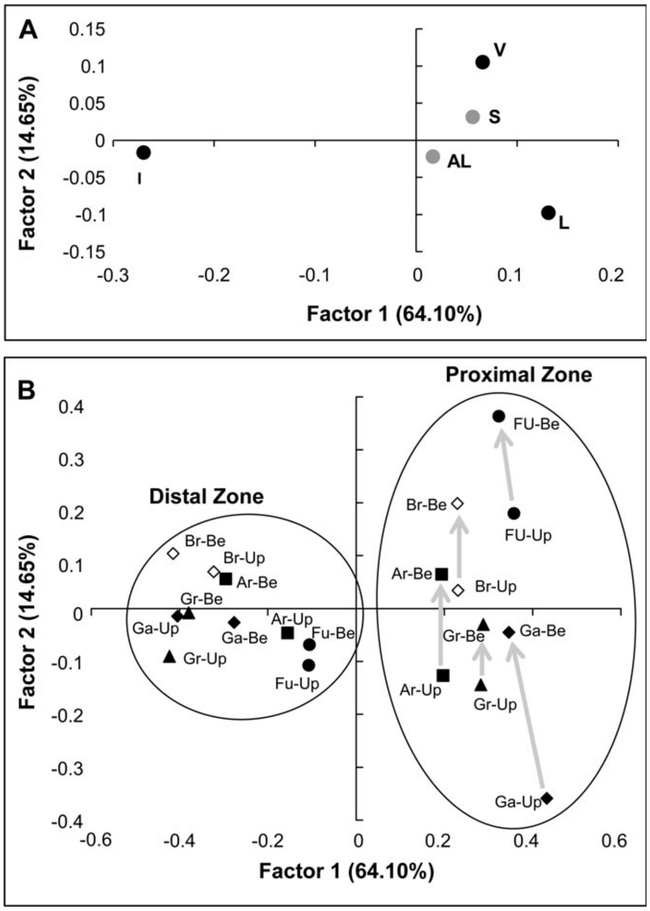

Fig. 4. Principal Component Analysis of fitted values of lateral probabilities in Model 0. Projection of the variables (A) and of individuals (B). In projection of the variables, the labels refer to the five lateral types (AL, aborted lateral; I, inflorescence; L, latent; S, sylleptics; V, vegetative). Black and grey symbols indicate variables with a high versus a low link with factor 2, respectively. In projection of the individuals, each label is composed of the following items: genotype [GEN, Ariane (Ar); Braeburn (Br); Fuji (Fu); Gala (Ga); and Granny Smith (Gr)], and bending status [BST, upright (Up), bent (Be)]. Within the same zone (proximal, distal), the same symbols belong to the same genotype. Within the proximal zone, grey arrows link the upright to the bent status of the same genotype.

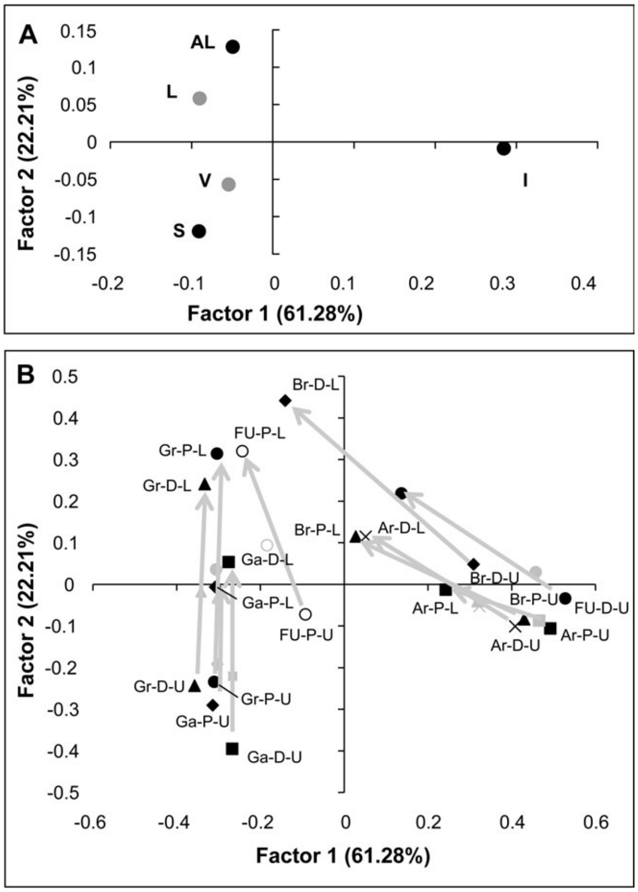

Fig. 5. Principal Component Analysis of fitted values of lateral probabilities in Model 4′. Projection of the variables (A) and of individuals (B). In projection of the variables, labels refer to the five lateral types (AL, aborted lateral; I, inflorescence; L, latent; S, sylleptics; V, vegetative). Black and grey symbols indicate variables with a high versus a low link with factor 2, respectively. In projection of the individuals, each label is composed of the following items: genotype [GEN, Ariane (Ar); Braeburn (Br); Fuji (Fu); Gala (Ga); and Granny Smith (Gr)], zone along the shoot [ZON, proximal (P); distal (D)], and radial location [RAD, upper (U); lower (L)]. The same symbols belong to the same combination of genotype–zone. For legibility, laterals in the side faces are not labelled (grey symbols). Grey arrows link the upper and lower faces of the same genotype–zone combination.

and bent shoots confirmed on the five genotypes that the proximal and distal zones were characterized by contrasting branching patterns. Two main conclusions could be drawn. First, an ontogenetic effect was indicated by the first factor of the PCA, discriminating between the proximal and the distal zones by the proportion of inflorescences. These results confirmed existing literature on shoot architecture (see Introduction). Second, an effect of bending was shown by the second factor of the PCA opposing latent buds and vegetative laterals. It is shown here that bending could change the original branching patterns by stimulating the growth of latent buds giving rise to vegetative laterals. This result agrees with the literature, showing a global increase of lateral shoot development in response to bending (Naor *et al.*, 2003; Hampson *et al.*, 2004) probably related to an increase of cytokinin in buds (Ito *et al.*, 1999). According to classical hypotheses, axillary bud outgrowth is determined by a balance among several hormones, in particular the basipetal flow of auxin and the locally and/or root-derived cytokinin (Salisbury, 1993; Shimizu-Sato and Mori, 2001; Bennett and Leyser, 2006). In our experiment, the increase in vegetative lateral outgrowth was observed mainly in the proximal part and to a far lesser extent in the distal part. This could be explained by the fact that, in our experimental setting, bending in the proximal zone in spring was able to affect bud organogenesis during the rest of the growing season whereas bending in the distal zone during winter dormancy was unable to alter the course of bud

Table 4. *Hydraulic conductance of the vascular system connected to the lateral (k_{LAT}; mmol $s^{-1}MPa^{-1}$; mean ±SE) of upright and bent shoots for the two genotypes, Braeburn and Fuji*

ANOVA is performed to separate the effects of genotype and bending treatment. Within the same column, different letters indicate significant differences at P=0.05, Duncan multiple mean comparison test. *n* is the number of laterals.

Genotype	Treatment	*n*	Hydraulic conductance
Braeburn	Bending	52	0.092±0.021 ab
	Upright	20	0.024±0.010 b
Fuji	Bending	35	0.166±0.030 a
	Upright	24	0.130±0.037 a
Genotype effect		*F*	9.644
		P	*0.0023*
Treatment effect		*F*	3.239
		P	*0.074*
Genotype×Treatment		*F*	0.321
		P	*0.572*

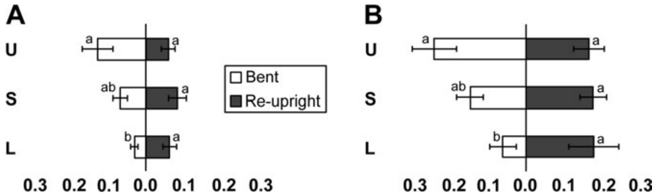

Fig. 6. Effects of radial location (upper, side, and lower faces) on bent and re-uprighted shoots, on hydraulic conductance of the vascular system connected to the bud (k_{LAT}; mmol s^{-1} MPa^{-1}; mean ±SE) for the two genotypes, Braeburn (A) and Fuji (B). Within the same side part of each graph different letters indicate significant differences ($P < 0.05$, Duncan multiple means comparison test).

organogenesis which was already set. It should also be noticed that within the proximal zone, bending affected all the genotypes in the same way, i.e. an increased number of vegetative buds to the detriment of latent buds. It may be that this one-way trend was found because of taking care to apply a similar mechanical strain to the five genotypes. It would be of interest to carryout the same type of study, but to compare the effect of controlled bending versus uncontrolled bending on bud fate.

As mentioned earlier, the effect of bending on flowering is still controversial. In the present study flowering was not increased by bending. This could be related to shoot type, i.e. a vertical trunk directly stemming from the graft point in this study versus oblique branches on fruiting trees in previous studies. Furthermore, it may be suggested from our results that the zone along the shoot interacting with time of bending may play a role in the effect of bending on bud fate and flowering in particular. Future studies should then investigate if other possible changes in lateral type (e.g. latent to inflorescence, vegetative to inflorescence) may be related to various combinations of these two traits.

The second step of the analysis was done on bent shoots only. It took the radial location of the lateral into account. It clearly showed that in all cases bending increased the probability of aborted laterals in the lower face of the parent shoot. However, a genotypic effect was evident with a concomitant decrease in number of inflorescences for Ariane and Braeburn, and a concomitant decrease in sylleptic laterals for Gala and Granny Smith. This phenomenon was observed whatever the zone along the shoot (i.e. the same trend was observed for proximal and distal zones) except for Fuji which resembled the first two genotypes for the distal zone, and resembled the latter two genotypes for the proximal zone. Although, in the former case, abortion occurred mostly on buds which would otherwise give rise to an inflorescence, in the latter case abortion could appear on already existing sylleptic laterals (usually short; data not shown) corresponding to the death of the terminal buds. Our results, therefore, showed that lateral abortion and not bud latency played a consistent role in the asymmetric branching patterns of the bent shoot. To the best of our knowledge this point is not documented in the literature.

The fact that bud abortion was enhanced in the lower face of shoots bent in spring, as well as in shoots bent in the following winter, suggests that the abortion mechanism may intervene at various moments in the growth cycle. In shoots bent in the proximal zone in spring, abortion may occur during bud organogenesis or later, namely during dormancy. In shoots bent in the distal zone in winter, it can only occur post-organogenesis on already completely pre-formed lateral buds. Tromp (1970) suggested that the enhancement of branching frequency on the bent shoot may be, in part, related to a reduced bud abortion. It is shown here that this phenomenon is true only at a local level. Indeed, vegetative branching was generally enhanced by bending in the proximal zone to the detriment of latent buds without any effect of AL on the first two factors of the PCA (first analysis). However, there was a clear increase of AL in the lower face of the bent shoot compared with the upper face, as seen by the strong impact of AL on factor 2 in PCA on bent shoots (second analysis).

Our study on k_{LAT} did not agree with the findings of Cristoferi and Giachi (1964) and Schubert *et al.* (1995), that showed a reduction of shoot hydraulic conductance in the bent shoot compared with the upright one. However, this study revealed a strong asymmetry between the opposite two faces of the shoot. Indeed, mirroring the effects of bending on AL probabilities, k_{LAT} was significantly decreased in the lower face compared with the upper face of the bent shoot. Although the reduced leaf traits noticed by Kim *et al.* (2004) and the higher lateral abortion (present results) could be attributed to the anisotropy of the physical environment (e.g. possible lower light irradiance on the lower face of the bent shoot compared with the upper face), the present study strongly suggested a causal relationship between the reduced k_{LAT} and the increased AL probabilities. The fact that the asymmetrical k_{LAT} distribution could be reversed by returning the shoot to an upright position could show that the relationships between hydraulics and lateral fate, especially abortion, could be overcome by shoot re-orientation. These results should, however, be restricted to the effects of bending without secondary growth, which was the case in the present hydraulic study. In this case the mechanical constraints experienced by the shoot (e.g. extended versus compressed wood on the upper and lower face, respectively) may satisfactorily explain the results. To what extent secondary growth occurring on a bent shoot is able to change the radial distribution of hydraulic conductance remains to be documented. Indeed, both the local reduction of hydraulic conductance in tension wood, i.e. in the upper face (Woodrum *et al.*, 2003; Pilate *et al.*, 2004), and the higher number of vessels in the opposite wood, i.e. in the lower face, compared with tension wood (Pruyn *et al.*, 2000; Ruelle *et al.*, 2006) suggest that, on shoots kept in a bent position during a growing season and developing tension wood, hydraulic conductance is enhanced in the lower face of the bent shoot compared with the upper face. In our experiment, therefore, the increase of aborted laterals in the lower face of shoots bent in the spring could not be caused by a reduction of hydraulic conductance and could be better related to environmental factors. A comparative study of branching patterns and hydraulics in response to bending at various times of the year and of different durations would permit the respective effects of mechanical strain (with bending, one side is compressed and one side is set under tension), biomechanical reaction of the bent shoot (when secondary growth occurs tension wood is produced on the upper side), and environment anisotropy (light for example) to be disentangled. Furthermore, the temporal variability of herbaceous plant sensitivity to mechanical stress has been described by Lefèvre *et al.* (1994) and Beyl and Mitchell (1977). It may be suggested that the sensitivity of the shoot to bending varies during the year. It would be interesting to carryout a factorial experiment in order to assess the effect both of the intensity of bending and of the time at which bending is applied.

The effect of bending has often been addressed in a horticultural context, i.e. focusing on the intensity of vegetative growth, and flowering and fruiting (Lakhoua and Crabbé, 1975*a*; Robbie *et al.*, 1993; Ito *et al.*, 1999; Lauri and Lespinasse, 2001). It was shown here that bending could also affect branching frequency through lateral abortion. Indeed, shoot and bud mortality, i.e. cladoptosis, is usually described as an adaptation to environmental stresses (shade, drought; Bell, 1991; Davis *et al.*, 2002). Compared with other cases of cladoptosis that have been described, the lateral abortion observed in our experiment presented two possible conclusions. First, it might be induced in a relatively short time: bending applied a few weeks before bud burst triggered significant lateral mortality in the following spring. In this case lateral mortality would probably be caused by a significant reduction in hydraulic conductance. Second, it could happen on already developed sylleptic short laterals as well as on pre-formed inflorescence buds, depending on the genotype.

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