Annals of Botany **100**: 347–357, 2007
doi:10.1093/aob/mcm114, available online at www.aob.oxfordjournals.org

OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Clarifying the Effects of Dwarfing Rootstock on Vegetative and Reproductive Growth during Tree Development: A Study on Apple Trees

E. COSTES[1,*] and E. GARCÍA-VILLANUEVA[2]

[1]*INRA, UMR Développement et Amélioration des plantes, Equipe Architecture et Fonctionnement des Espèces Fruitières, 2 Place Pierre Viala, Montpellier F-34060 Cedex 1, France and* [2]*Colegio de Postgraduados, Campus Montecillo, Especialidad de Fruticultura, km. 36·5 Carretera México-Texcoco, Chapingo, C.P. 56230, Estado de México, México*

Received: 18 February 2007 Returned for revision: 28 March 2007 Accepted: 30 April 2007

• *Background and Aims* Despite the widespread use of dwarfing rootstocks in the fruit-tree industry, their impact on tree architectural development and possible role in the within-tree balance between growth and flowering are still poorly understood, in particular during the early years of growth. The present study addressed this question in apple trees, through a detailed analysis of shoot populations, i.e. both vegetative and flowering shoots, during tree development.
• *Methods* Architectural databases were constructed for trees of two cultivars that were either own-rooted or grafted on dwarfing rootstock. Within-tree shoot demographics and annual shoot characteristics, i.e. their dimensions, number of laterals and flowering, were observed from the first to the fifth year of growth and compared among scion/root system combinations.
• *Key Results* Differences in axis demographics appeared among scion/root system combinations after the second year of growth. Differences were found (*a*) in the number of long axes and (*b*) the number of medium axes. Dwarfing rootstock reduced the total number of axes developed in a tree, and this reduction resulted from proportionally more medium axes and spurs than long axes. The life span of spurs was also shortened. These phenomena appeared after an increase in flowering that started in the second year of growth and involved both axillary and terminal positions. Flowering regularity was also increased in grafted trees.
• *Conclusions* These results confirm that the number of long shoots and flowering potential depend on the cultivar. They indicate that tree architectural plasticity in response to its root system mainly derives from the number of medium shoots developed and follows priorities within the whole tree axis population. There was also evidence for dwarfing rootstock involvement in adjusting the flowering abundance and that differences in flowering occurrence take precedence over those regarding vegetative growth during tree development.

Key words: Tree architecture, elongation, branching, flowering, return bloom, *Malus* × *domestica*, dwarfing rootstock.

INTRODUCTION

Controlling plant size has been an important goal for years in many plant species. Selection of dwarf genotypes has resulted in improvements of yield notably in grain crops (Khush, 2001). In fruit tree industries, tree vigour is mainly controlled by dwarfing rootstocks which are widely employed in intensive orchards to restrict tree volume and promote earlier flowering (Lockard and Schneider, 1981; Barritt *et al.*, 1995; Fallahi *et al.*, 2002). Several studies have been devoted to the analysis of dwarfing rootstock effects on the development of the aerial part of trees (for a recent review, see Costes *et al.*, 2006). In apple (Costes and Lauri, 1995) and peach cultivars (Weibel *et al.*, 2003) grafted on different rootstocks, the length of the growing period was shown to be reduced by dwarfing rootstocks. It also has been demonstrated that rootstocks reduce the internode length of fruiting branches (Seleznyova *et al.*, 2003; Weibel *et al.* 2003). But different results have been obtained regarding the effect of dwarfing rootstocks on the mean number of nodes per shoot. In peach, Weibel *et al.* (2003) indicated that differences in shoot length were related primarily to internode length rather than to the number of nodes, whereas Seleznyova *et al.* (2003) attributed the difference in apple branch size to a reduction in both the length of internodes and the number of nodes that are neoformed within long growth units. Average internode length per extension unit depended on unit node number, with internodes being shorter for units with fewer nodes.

The effect of rootstock on branching pattern was studied in apple relative to different axis types, with annual shoots sampled on 4- to 9-year-old trees (Hirst and Ferree, 1995), 3-year-old branches (Seleznyova *et al.*, 2003) and along 6-year-old trunks (Costes *et al.*, 2001). In all these situations, the percentage of budbreak of axillary buds on extension growth units was unaffected, regardless of the rootstock. Differences in the number of axillary annual shoots per branch were shown to result mainly from the number of nodes developed during the previous year (Costes *et al.*, 2001). This led to the interpretation that the effect of rootstock on aerial growth is cumulative and superimposed year after year. More recently, the changes induced in the floral development of the axillary shoots have been analysed by applying hidden semi-Markov chain models in 1-year-old 'Royal Gala' trees grafted on a range of rootstock/interstock combinations (Seleznyova

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

© The Author 2007. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

et al., 2004). In this study, dwarfing-associated effects were expressed as an increase in the number of floral axillaries as well as the positional distribution of floral axillary production along the trunk axis.

However, since all these previous studies have been performed either on young trees or on branches sampled on mature trees, they do not provide a comprehensive analysis of the dynamics of rootstock effect on tree architectural development, from young to adult stages. In particular, the interactions between the two main consequences of dwarfing rootstock on aerial development, i.e. early flowering and tree volume reduction, are not clear. This was the focus of the present study, paying special attention to different scion/rootstock combinations. Two scion cultivars were compared for aerial systems that were either own-rooted or grafted on a dwarfing rootstock. The scion cultivars were chosen for their different strategies of growth and branching in order to compare trees with different shoot demographics and tree architectures.

In the present study, the influence of rootstock was investigated on the within-tree shoot demographics and main annual shoot characteristics, i.e. their final dimensions, number of laterals, and flowering by describing whole apple trees from the first to the fifth year of growth. Architectural databases were *a posteriori* sampled to compare a set of variables among the different scion–root system combinations. We hypothesised that common trends or differences between cultivars could be examined along with the impact of root system on the development of the scion in order to clarify the root system effects on vegetative and reproductive growth during tree development.

MATERIALS AND METHODS

Plant material

Two cultivars, 'Ariane' and 'X3305', were chosen for their difference in growth and fruiting strategies. Ariane trees exhibit an upright habit with strong branches developing along the trunk leading to a hierarchic tree organization and a basitonic behaviour [as defined by Edelin (1991) and Lespinasse (1977), respectively]. This genotype produces three to four fruits per inflorescence. Therefore, early and severe thinning is necessary to obtain adequate fruit size and avoid alternate bearing. X3305 is an INRA hybrid, derived from a cross between 'Chantecler' and 'Baujade'. Trees exhibit an open canopy and tend towards a drooping habit which results from tip-bearing fruiting behaviour.

All trees, derived from *in vitro* culture, were planted in January 2000 after 1 year in a nursery. Trees were 5 m × 2 m apart and were grown without pruning. Half the trees were own-rooted while the other half was grafted on dwarfing M9 rootstock, clone Pajam 2® Cepiland. Three root systems (X3305, Ariane and M9) were thus used in order to compare their effects on scion development. Pajam 2® Cepiland clone presents medium to low vigour, 15–30 % lower than the initial M9 and is compatible with most apple cultivars (Masseron, 1989). Each half plot was located in two adjacent experimental plots with the same soil conditions. These plots were regularly irrigated using a microjet system monitored by tensiometers to avoid soil water deficits. All the experiments were carried out at the INRA Melgueil experimental station (Montpellier, South of France).

Tree description

Four years after planting, at the end of 2003 growth, data were collected on eight trees, i.e. two trees per scion/root system combination. Tree topology was described by recording growth over 5 years (1999–2003; Fig. 1). A multi-scale representation of the trees and corresponding coding method were used (Godin *et al.*, 1997, Godin and Caraglio, 1998). The code used was similar to that defined for apple tree by Costes *et al.* (2003). In this study, three levels of organization were considered, each including different types of entities defined on the basis of morphological characteristics. Each tree (level 1) was decomposed into axes (level 2) which developed over four consecutive years. In apple tree, axes are sympodial due to the floral differentiation of apices (Fig. 1 and Crabbé, 1987). The trunk and three other axis types were considered, which were defined depending on the type of growth unit [GU, level 3 (a growth unit corresponds to a set of organs developed between two periods of growth cessation of an apical meristem; Hallé and Martin, 1968)] they contained. Four GU types were considered: a floral GU and three vegetative GU types which depended on their length (long GU with length >20 cm; medium GU with length 5–20 cm; and short GU with length <5 cm). Long axes contained as least one long GU, medium axes contained at least one medium GU but no long GU, and short axes or spurs contained only short GU. Coded data were input using 3A software (version 2·0; Adam *et al.*, 1999). Data acquisition was carried out in a predetermined order, i.e. from the base to the top of the tree, describing each long axis from its base to its apex, before coming back to its initial position to continue the description. Basal diameters were registered for long and medium GU only. The index of each GU corresponded to the year of its development. The architectural databases were then analysed by VPlant software (formerly AMAPmod) by means of aml programs (Godin *et al.*, 1999).

Definition of variables

Variables were extracted from the databases at two levels of detail – the entire tree and the annual shoots (AS). At the entire tree level, the total number of axes and the total number of floral AS were counted. Axes were classified according to their type and were counted per category. The floral AS and return bloom, i.e. the number of floral AS that were followed by another floral AS in the next year, were also counted according to the axis type to which they belonged. Along these axes, AS that were not explicitly coded in the databases, were considered as the sum of GU developed in a year along an axis (Fig. 1). AS contained either vegetative GU(s) only or a floral GU and vegetative GU(s). AS were classified based on the

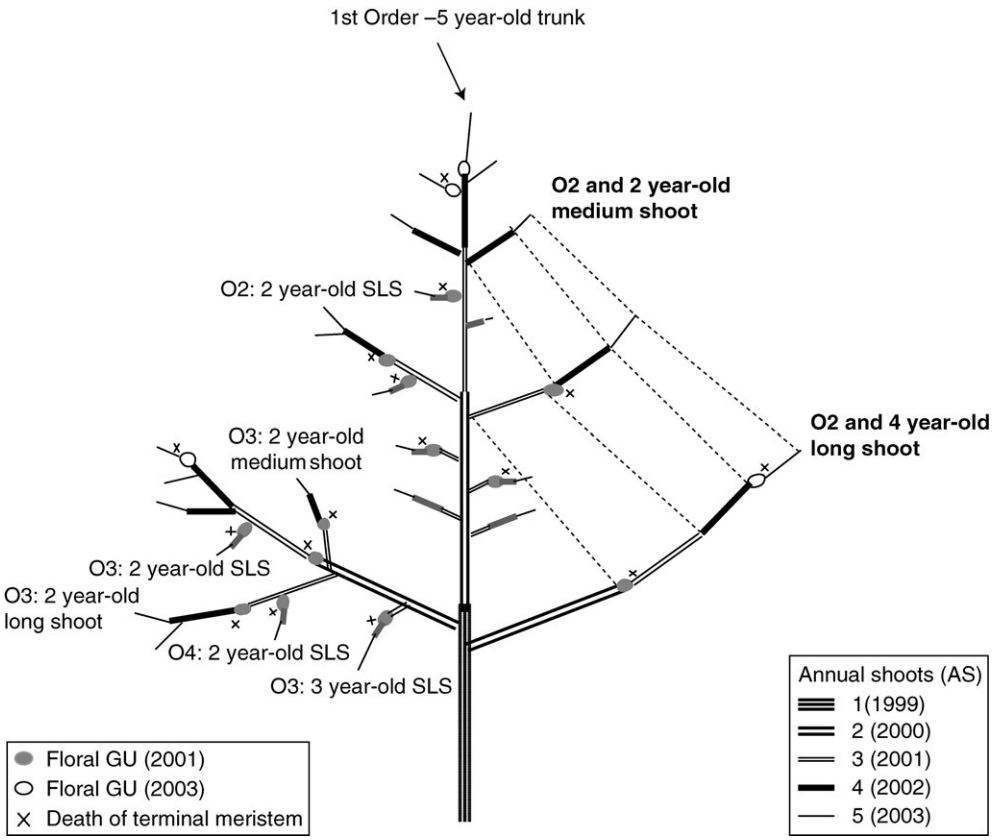


FIG. 1. Architecture of 5-year-old apple trees, as observed from the recorded databases. Growth units (GU) were classified by length (long, medium and short). Axes, composed of a succession of GU are sympodial after the occurrence of a floral GU. Annual shoots (AS), considered as the sum of GU developed during the same year, were gathered for analysis as indicated by the dotted lines; these AS had grown simultaneously but belonged to long, medium or short axes. SLS, short lateral shoot; O, branching order.

length of GU they contained, and, the main morphological characteristics of their vegetative parts were considered: length (in cm), basal diameter (in mm), number of lateral shoots (LS), number of lateral floral AS, and presence of a terminal flower. When several vegetative GUs developed in the same year of growth, the corresponding variables were summed. Since there were no differences in the variables when the AS were floral or not, the analyses did not distinguished these two categories.

Statistical analysis

Analyses were performed in successive steps: (*a*) the homogeneity between the two tree repetitions by scion-root system combination was evaluated, and (*b*) root system and cultivar effects were tested by an imbricate ANOVA, because own-rooted trees could not be considered as a single modality in comparison with grafted trees. The root systems were thus compared by pairs: X3305 own-rooted trees with X3305 trees grafted on M9; and Ariane own-rooted trees with Ariane trees grafted on M9. When counting variables and distributions in classes, the different distributions were compared by the chi-square test. Homogeneity tests were performed for comparing proportions. For continuous quantitative variables at the AS level, i.e. with large samples, different ANOVA tests were performed. In addition to root system and cultivar, other factors (axis type, year of growth and branching order) were included in the imbricate ANOVA when there was a significant effect on the variable considered. These additional effects were tested before focusing on the root system effects that were estimated by one way ANOVA performed on the mean values per cultivar. All these statistical analyses were performed with Stastistica® version 7·1 software.

RESULTS

Tree level

Within-tree axis demographics. After 5 years of growth, trees belonging to a similar scion/root system combination were only modestly different ($0{\cdot}01 < P < 0{\cdot}05$) (Table 1). Trees contained roughly 10 % long axes, 10 % medium axes and 80 % spurs. Both cultivar and root system had a significant effect on within-tree composition. In particular, Ariane trees contained proportionally more medium axes than X3305 trees, and this difference was more pronounced in grafted trees than in own-rooted trees. As expected the total number of axes per tree was reduced in both cultivars when grafted on M9 (Table 1). However, the magnitude of this reduction and the axis types that developed differed

TABLE 1. *Within-tree axis demographics of eight apple trees belonging to two apple cultivars either own-rooted (OR) or grafted on M9, and calculated in the fifth year of growth*

				Axis type			Statistical effect	
Roots	Cultivar	Tree	Total	Long	Medium	Short	Tree	Cultivar
OR	X3305	1	1669	8·03 %	8·15 %	83·82 %	0·13 ns	
		2	2229	9·74 %	8·75 %	81·52 %		
	Ariane	3	1339	7·99 %	10·9 %	81·11 %	0·42 ns	
		4	971	9·27 %	11·74 %	78·99 %		
M9	X3305	5	1132	13·34 %	7·51 %	79·15 %	0·015*	
		6	887	12·16 %	11·27 %	76·66 %		
	Ariane	7	861	8·71 %	15·33 %	75·96 %	0·053 ns	
		8	530	12·26 %	16·98 %	70·75 %		
OR	X3305	Mean	1949·0	8·88 %	8·45 %	82·82 %		0·04*
	Ariane	Mean	1155·0	8·53 %	11.26	80·05		
M9	X3305	Mean	1009·5	12·75 %	9·36 %	78·06 %		$6{\cdot}10^{-5}$**
	Ariane	Mean	695·5	10·06 %	15·96 %	73·98 %		
RS effect	X3305	0·004**						
	Ariane	0·005**						

The distributions of axis type percentages were compared between 5-year-old trees of the same cultivar by a chi-square test. Cultivar and root system (RS) effects were estimated by chi-square tests performed on the mean distributions calculated per cultivar with the same root system (root system effects are presented in the lower part of the table). The *P*-value for each test is indicated with its significance (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

depending on the cultivar. In X3305, the number of axes per tree was less reduced than in Ariane. In both cultivars the proportion of spurs decreased in the trees grafted with M9. In X3305, the number of long axes increased in proportion to the reduction in the number of spurs while the number of medium axes remained almost constant. By contrast, in Ariane the number of medium axes increased more than the number of long axes.

Dynamics of axis development. The time-course dynamics of axis development were compared among the four modalities (Fig. 2). Chi-square tests were performed pairwise, i.e. X3305 OR versus X3305 M9; Ariane OR versus Ariane M9; X3305 OR versus Ariane OR; and X3305 M9 versus Ariane M9. Surprisingly, the dynamics of the long-axis development was remarkably similar for percentage developed per year, regardless of the scion cultivar or root system, even though the number of long axes per tree differed (the number of long axes was always higher in X3305 than in Ariane). In both cultivars, differences in number of long axes per tree between own-rooted and grafted trees appeared in the third year of growth (2001). Differences among modalities were also noted in medium- and short-axis dynamics (Fig. 2). Own-rooted trees exhibited similar dynamics in medium axis development, with an increase up to the fourth year and a decline in the fifth. When grafted on M9, the dynamics of medium axes were different in both cultivars: a markedly different dynamics was found in X3305 trees, while only a slight difference was found between Ariane trees own-rooted or grafted on M9 ($0{\cdot}01 < P < 0{\cdot}05$). In grafted trees, the increase in the number of medium axes was less rapid than in own-rooted trees, though the maximum was still observed in the fourth year. In Ariane on M9 the decline in the fifth year was almost the same as for own-rooted trees while it was less rapid in X3305 trees. All spurs dynamics were statistically different but this was interpreted as resulting mainly from the very high number of observations. The spur dynamics observed in own-rooted trees were very similar, with a progressive increase in the number of spurs per tree. When grafted trees were compared with own-rooted trees, the number of spurs increased slowly up to the fourth year and then more rapidly in the fifth year, especially in X3305.

Dynamics of total number of floral AS per tree and bearing axis types. Floral AS were first observed in the second year of growth in X3305 trees grafted on M9. In 2001, i.e. the third year of growth, all the trees flowered and the mean number of floral AS per tree increased rapidly over the subsequent years (data not shown). In both cultivars the total number of floral AS developed from the third to the fifth years was greater in grafted than in own-rooted trees (Table 2). But, the difference was greater in Ariane trees than in X3305 trees: in Ariane trees, the total number of floral AS was double, whereas in X3305 trees the increase concerned only 10 % of the total number of floral AS.

Within-tree positions of floral AS were according to the type of axis to which they belonged. In a given cultivar, the proportion of floral AS belonging to long, medium and short axes differed between grafted trees (Table 2). Despite this heterogeneity, a larger proportion of floral AS were apparent on spurs in X3305 than in Ariane trees while, in Ariane trees, a larger proportion were part of medium axes. The proportion of floral AS belonging to long axes was almost the same in both cultivars (about 25 %). In X3305 trees the proportion of floral AS belonging to spurs was higher in the two grafted trees than in own-rooted trees, while the floral AS belonging to medium axes were proportionally lower and the proportion belonging to long

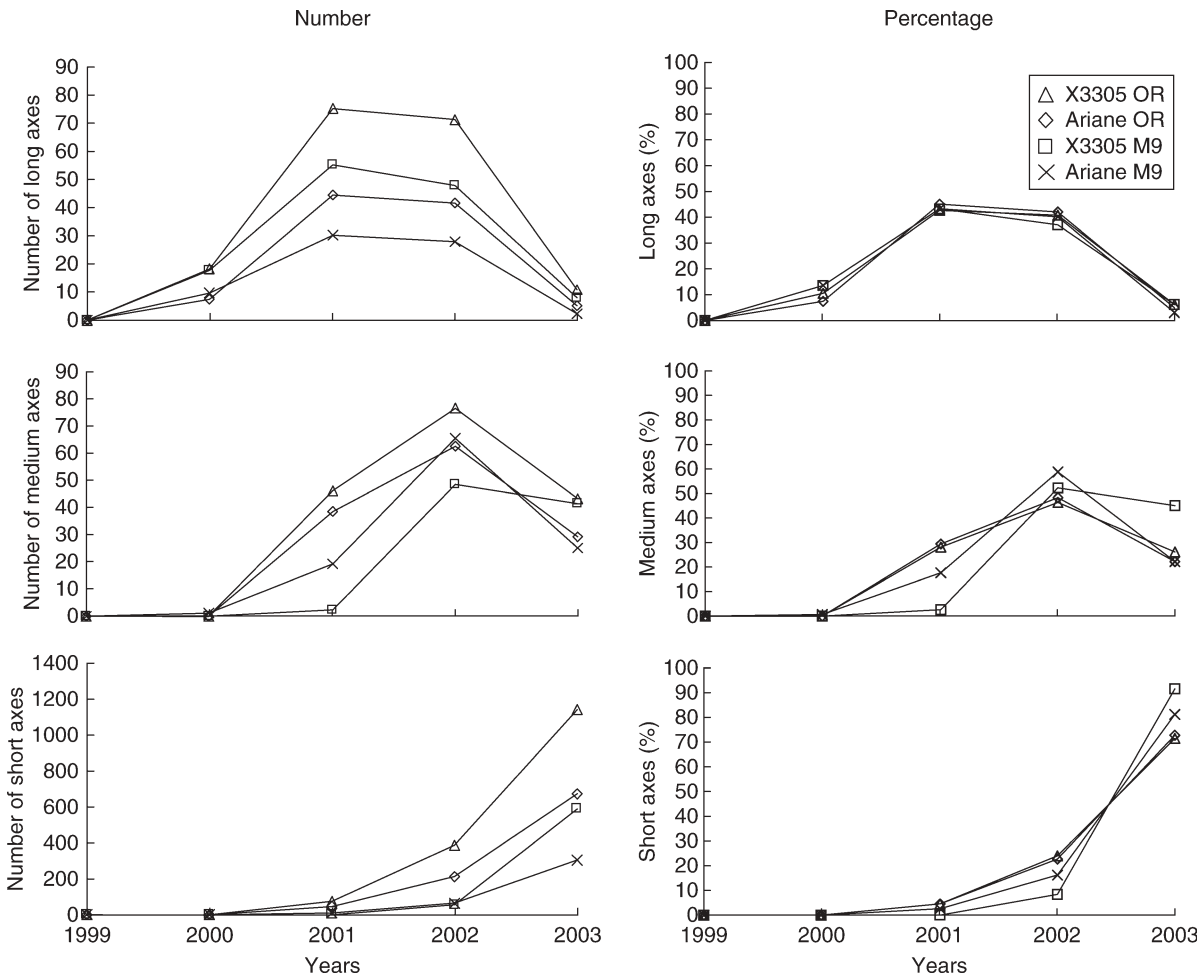


FIG. 2. Within-tree shoot demographics of two apple cultivars (X3305 and Ariane) either own-rooted (OR) or grafted on M9. Number (left) and percentage (right) of long, medium and short axes developed per year. The dynamics of axis development were compared pairwise by chi-square tests.

TABLE 2. *Distribution of floral annual shoots (AS) on the three axis types of eight apple trees belonging to two cultivars either own-rooted (OR) or grafted on M9*

				Axis type			Statistical effect	
Root system	Cultivar	Tree	Total no. of infloresences	Long	Medium	Short	Tree	Cultivar
OR	X3305	1	884	23·76 %	16·63 %	59·62 %	0·07 ns	
		2	1244	27·89 %	14·55 %	57·56 %		
	Ariane	3	189	32·80 %	26·98 %	40·21 %	0·06 ns	
		4	269	23·42 %	27·14 %	49·44 %		
M9	X3305	5	1245	23·12 %	8·16 %	68·32 %	0·005**	
		6	1024	22·45 %	12·24 %	64·82 %		
	Ariane	7	479	28·60 %	36·12 %	35·28 %	10^{-7}**	
		8	466	23·39 %	23·61 %	53·00 %		
OR	X3305	Mean	1064	25·83 %	15·59 %	58·59 %		10^{-4}**
	Ariane	Mean	229	28·11 %	27·06 %	44·83 %		
M9	X3305	Mean	1134·5	22·79 %	10·20 %	66·57 %		$2{\cdot}10^{-25}$**
	Ariane	Mean	472·5	26 %	29·87 %	44·14 %		
RS effect	X3305	$2{\cdot}10^{-5}$**						
	Ariane	0·73 ns						

Distributions were compared between 5-year-old trees of the same cultivar by a chi-square test. Cultivar and root system (RS) effects were estimated by chi-square tests performed on the mean distributions calculated per cultivar with the same root system (root system effects are presented in the lower part of the table). The *P*-value for each test is indicated with its significance (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

axes remained almost unchanged. The situation was less clear with Ariane trees.

Annual shoot level

Dynamics of AS dimensions. Mean AS dimensions did not differ significantly between trees belonging to a similar scion/root system combination (data not shown). Because medium axes were defined according to GU length, no effect of the root system was expected on AS belonging to this axis type. Thus, only long axes were analysed. In all combinations, the greatest mean length of long axis AS was observed during the early life of the tree, i.e when the trees developed their second growth (2000; Fig. 3A). Mean AS length decreased in the following years. Consequently, the year of growth had a significant effect on the mean AS length but no significant difference was found between the two cultivars. In X3305 trees, M9 rootstock reduced the mean AS length in 2001 only (i.e. during the third year of growth). This was observed regardless of the position of long axes in branching order (data not shown). In contrast, in Ariane trees, AS length was always longer in own-rooted trees than in grafted trees (Fig. 3A). The difference was significant three years among four, from 2000 to 2002. In these three years, M9 rootstock significantly reduced the mean AS length of AS belonging to axes born along the trunk but had no significant effect on axes located at higher branching orders (data not shown).

No significant difference in basal diameter of AS belonging to both medium and long axes was found between the cultivars. Similar patterns to those observed for AS length were found when basal diameters were compared for the successive AS of long axes (Fig. 3B). In both cultivars, few differences in AS diameter were found between own-rooted and grafted trees, and these differences did not corresponded to a reduction of AS diameter with M9 rootstock.

AS lateral branching. In order to compare lateral branching along AS independently of the AS length decrease over the years, the lateral shoots (LS) were counted per unit length (Table 3 and Fig. 4). In all modalities, AS belonging to long axes were more frequently branched than those belonging to medium axes (Table 3). In the following, the mean number of LS per unit length was studied for branched AS only. X3305 trees with a similar root system had similar numbers of LS per unit length while Ariane trees appeared heterogeneous in particular when they were own-rooted. Despite this heterogeneity, two effects were clear enough to be analysed. Indeed, both axis type and root system had a highly significant effect on the mean number of LS per unit length, while the cultivar had no significant effect. In fact, root system effect was significant on the mean number of LS per unit length in AS belonging to long axes but was not significant when the AS belonged to medium axes. In parallel, the mean number of LS per unit length was significantly different between AS belonging to long and medium axes but in own-rooted trees only. In grafted trees, the mean number of LS per unit length of AS belonging to long axes was significantly lower than in own-rooted trees and consequently there was no more difference between AS belonging to long and medium axes (Table 3).

Considering AS belonging to long axes only, a significant effect of the year of growth was observed on the number of LS per unit length and this variable increased progressively over the years in all modalities (Fig. 4A, test result not shown). This increase resulted from an increase in the number of short LS per unit length while the mean number of long and medium LS per unit length remained almost constant over the years (Fig. 4B). The change in the number of short LS in turn resulted from their death over years. All the spurs that had developed on 1-year-old wood (2002 AS) were still alive, while a

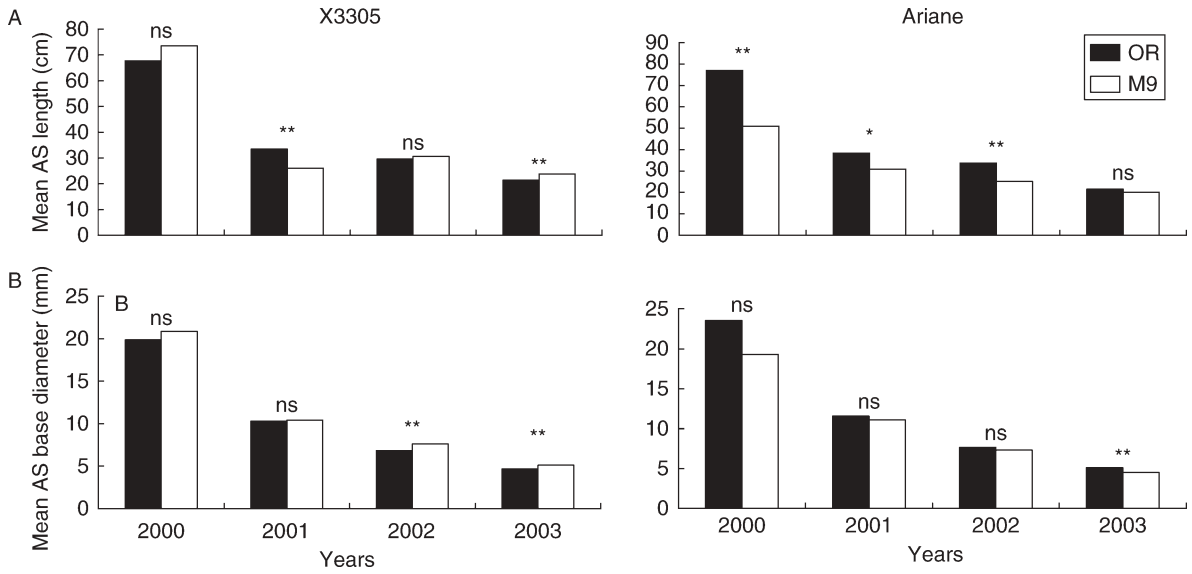


FIG. 3. Annual shoot dimensions along long axes of two apple cultivars (X3305 and Ariane) either own-rooted (OR) or grafted on M9. Mean length (upper graphs) and mean basal diameter (lower graphs) of annual shoots (AS). Root system effects were estimated by a one-way ANOVA performed on the mean values calculated per cultivar and year (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

TABLE 3. *Percentage of branched annual shoots (AS) and mean number of lateral shoots per unit length (LS/L) belonging to long and medium axes of two apple cultivars either own-rooted (OR) or grafted on M9 rootstock*

			Long axis			Medium axis				
RS	Cultivar	Tree	% branched AS	Mean no. LS/L	Tree effect	% branched AS	Mean no. LS/L	Tree effect	Axis type effect	Cultivar effect
OR	X3305	1	0·83	0·26	ns	0·58	0·21	ns		
		2	0·79	0·23		0·51	0·20			
		Mean	0·81	0·24		0·54	0·20		**	ns
	Ariane	3	0·75	0·27	**	0·56	0·17	ns		
		4	0·64	0·22		0·48	0·17			
		Mean	0·70	0·24		0·53	0·17		**	
M9	X3305	5	0·72	0·16	ns	0·53	0·19	ns		
		6	0·69	0·17		0·45	0·10			
		Mean	0·71	0·17		0·49	0·15		ns	ns
	Ariane	7	0·72	0·16	*	0·73	0·17	ns		
		8	0·68	0·20		0·69	0·20			
		Mean	0·70	0·18		0·71	0·18		ns	
RS effect	OR	Mean	0·77	0·24		0·54	0·19	ns		
	M9	Mean	0·71	0·17	**	0·65	0·17			

Tree effect on LS/L was tested by a one-way ANOVA. Axis type, cultivar and root system (RS) effects were tested by an imbricate ANOVA (RS imbricate in cultivar; ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

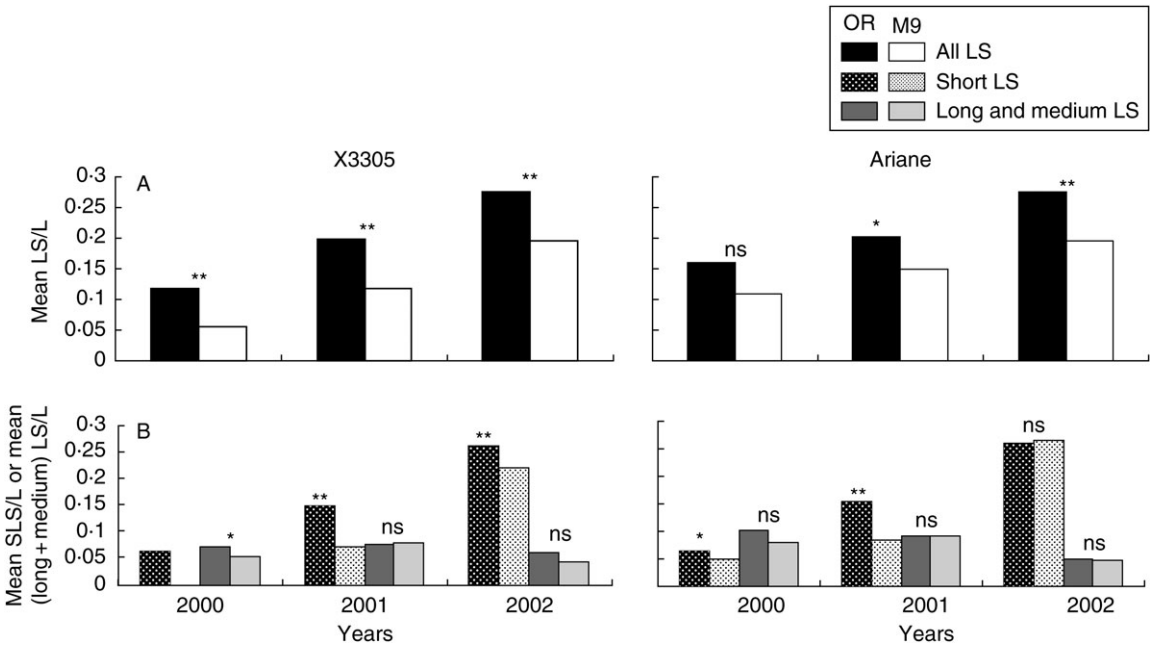


FIG. 4. Branching characteristics along long axes of two apple cultivars (X3305 and Ariane) either own-rooted (OR) or grafted on M9. (A) Mean number of lateral shoots per unit length (LS/L); (B) mean number of short lateral shoots per unit length (SLS/L) and long and medium lateral shoots per unit length of annual shoots (AS) belonging to long axes. Root system effects were estimated by a one-way ANOVA performed on the mean values calculated per cultivar and year (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

proportion on older wood (2001 and 2000) died. In all modalities, the decrease in the mean number of short LS from 2002 to 2000 can be fitted by an exponential function $y = a\ e^{b}$, where b represents a constant probability of short shoot death over the years. In own-rooted trees, similar parameters were found in both cultivars (in X3305 $a = 0{\cdot}034$, $b = 0{\cdot}78$ with $r^2 = 0{\cdot}99$; in Ariane $a = 0{\cdot}033$, $b = 0{\cdot}71$ with $r^2 = 0{\cdot}98$). In both cultivars, lower a values and greater b values were obtained in grafted trees (in X3305 grafted $a = 0{\cdot}008$, $b = 1{\cdot}13$ with $r^2 = 1{\cdot}0$; in Ariane grafted $a = 0{\cdot}02$, $b = 0{\cdot}83$ with $r^2 = 0{\cdot}96$), indicating that spur death was more rapid when the trees were grafted on M9.

Frequency of axillary and terminal floral AS. Both the percentage of AS with terminal flowering and bearing axillary floral AS were homogeneous between trees belonging to the same scion/root system combination, except between

Ariane own-rooted trees in which flowering occurred rarely (data not shown). In all modalities, the percentage of AS bearing axillary floral AS was higher on long axes than on medium axes where floral axillary AS occurred rarely (Table 4). In Ariane trees, the percentage was higher in grafted trees than in own-rooted trees in all years and axis types while in X3305 a higher percentage was observed in 2002 only, i.e. in 1-year-old AS.

Terminal flowering occurred frequently and regularly in all X3305 trees, whether own-rooted or grafted and in both long and medium axes: >75 % of AS set terminal flowers in all years (Table 4). By contrast, in Ariane trees, terminal flowering occurred twice as often when the trees were grafted on M9 than when they were own-rooted, in both medium and long axes.

The mean number of axillary floral AS per unit length was considered only on AS belonging to long axes and bearing axillary floral AS. Both own-rooted and grafted X3305 trees were homogeneous for this trait while Ariane trees exhibited a slight heterogeneity ($0{\cdot}01 < P < 0{\cdot}05$, data not shown). In all root system–cultivar combinations, an increase in the mean number of axillary floral AS per unit length was observed over the years (Fig. 5). This increase was more pronounced in grafted trees than in own-rooted trees, and in X3305 than in Ariane trees. As a consequence, no significant difference was observed between cultivars and years in own-rooted trees while both these factors had a significant effect in grafted trees (tests result not shown). Regarding root system effect, the mean number of axillary floral AS per unit length was significantly higher in grafted trees than in own-rooted trees, in 2001 and 2002 years. This difference was observed in both cultivars (Fig. 5).

TABLE 4. *Proportion of annual shoots (AS) that bore axillary floral AS (% AFAF) and occurrences of terminal flowering (TF) along long and medium axes in two apple cultivars either own-rooted (OR) or grafted on M9 rootstock*

		Long axes			Medium axes	
Root system	Cultivar	2000	2001	2002	2001	2002
% AFAS						
OR	X3305	0·54	0·41	0·42	0·04	0·03
M9	X3305	0·46	0·51	0·79	–	0·18
		ns	ns	**	–	**
OR	Ariane	0·07	0·11	0·16	0·00	0·01
M9	Ariane	0·53	0·48	0,50	0·05	0·12
		**	**	**	ns	**
% TF						
OR	X3305	0·81	0·90	0·90	0·79	0·74
M9	X3305	0·91	0·89	0·91	–	0·86
		ns	ns	ns	–	**
OR	Ariane	–	0·39	0·40	0·36	0·37
M9	Ariane	0·32	0·84	0·81	0·87	0·73
		–	**	**	**	**

Proportions were compared by a homogeneity test (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

Return bloom. Return bloom was calculated from 2001 to 2002 and then from 2002 to 2003. Since the difference among the years was not significant (data not shown), return bloom was studied for inflorescences developed either in 2001 or 2002 (Table 5). All the trees exhibited homogenous behaviour with regard to return bloom except Ariane own-rooted trees. However, these trees had the lowest number of floral AS which caused large fluctuations in return bloom ratios. Both cultivar and root system had a significant effect on return bloom. X3305 trees had higher return bloom ratios than Ariane trees and this difference was significant in own-rooted trees. In both cultivars, the trees grafted on M9 exhibited a higher return bloom than own-rooted trees. In X3305 trees, this difference was not significant while it was significant in Ariane trees. As a result of this increased return bloom ratio in grafted trees, the difference between the cultivars was not significant for grafted trees. Finally, grafting on M9 led to greater homogeneity between the cultivars with regard to return bloom ratios. In all the combinations the return bloom ratio was higher on spurs than on medium axes, and higher on medium than on long axes (data not shown).

DISCUSSION

Differences in within-tree shoot demographics

The two cultivars studied differed in terms of both whole tree composition and AS characteristics. The absence of

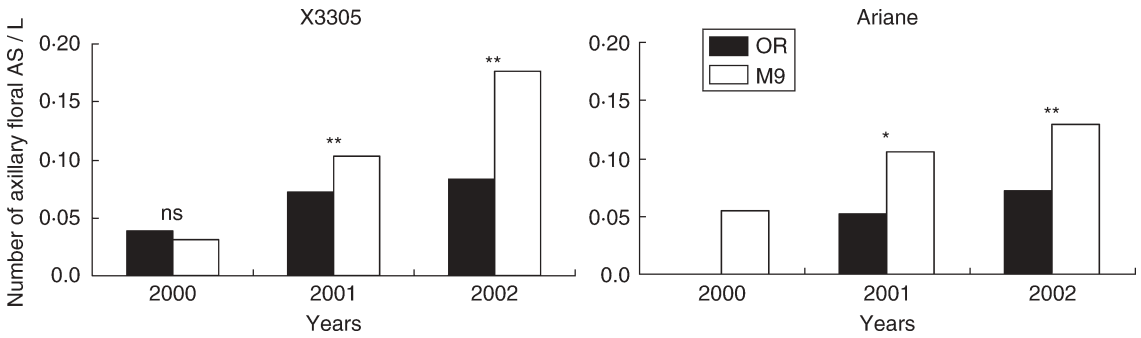


FIG. 5. Mean number of axillary floral annual shoots (AS) on successive AS belonging to long axes of two apple cultivars (X3305 and Ariane) either own-rooted or grafted on M9. Root system effects were estimated by a one-way ANOVA performed on the mean values calculated per cultivar and year (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

TABLE 5. *Total number of floral annual shoots (AS) per tree that developed in a given year (Nb I) and number of floral AS that returned to bloom the next year (Nb II) in eight apple trees belonging to two cultivars either own-rooted (OR) or grafted on M9*

RS	Cultivar	Tree no.	Nb I	Nb II	%	Statistical effect: Tree	Statistical effect: Cultivar
OR	X3305	1	270	181	0·67	0·47	
		2	301	222	0·74	ns	
		Mean	285·5	201·5	0·71		0·003
	Ariane	3	78	15	0·19	0·005	**
		4	42	23	0·55	**	
		Mean	60	19	0·32		
M9	X3305	5	183	175	0·96	0·4	
		6	207	175	0·85	ns	
		Mean	195	175	0·90		0·11
	Ariane	7	205	129	0·63	0·15	ns
		8	117	95	0·81	ns	
		Mean	161·5	112	0·69		
RS effect	X3305	0·08 ns					
	Ariane	0·006**					

Return bloom was compared between trees of the same cultivar by a chi-square test. Cultivar and root system (RS) effects were estimated by chi-square tests performed on the mean proportions calculated per cultivar with the same root system (root system effects are presented in the lower part of the table). The *P*-value for each test is indicated with its significance (ns, non-significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

pruning allowed this comparison to be focused on the genetic potential of the cultivars, without accounting for their interactions with tree reactions to pruning. X3305 trees developed more axes, especially more long axes, and proportionally less medium axes than Ariane trees. Lateral branching per unit length was also less intense in X3305 than in Ariane. A higher probability of terminal flowering was observed in X3305 than in Ariane and the occurrences of axillary floral GU seemed more regular in X3305 than in Ariane. Moreover, X3305 had a higher return bloom than Ariane. This combination of traits is consistent with a classification of Ariane and X3305 as type III and type IV in Lespinasse's classification (Lespinasse, 1977).

Differences in the number of long axes appeared between cultivars from the second year of growth (2000). This suggests that the number of axes permitted to develop into long axes along the trunks was genetically determined, as previously suggested in set apple cultivars (Costes and Guédon, 2002). In subsequent years, despite differences in the number of axes in own-rooted trees, the proportions of long, medium and short axes within the trees were similar in both cultivars and whatever the root system. By contrast in grafted trees, the reduction in the total number of axes developed per tree was not equally distributed among the three categories. As expected, M9 rootstock reduced the total number of axes per tree and thus also reduced the whole tree volume as previously described by other authors (Lockard and Schneider, 1981; Hirst and Ferree, 1995). But, the number of long laterals remained unchanged in 2000, i.e. in the second year of growth, irrespective of root system. In the present study, the earliest differences in the number of long axes between grafted and own-rooted trees appeared in 2001, i.e. when the trees were 3 years old. In addition, in 2001, while the proportion of long axes remained similar, the number and the proportion of medium axes were reduced. This indicated that the total number of axes and the proportion permitted to develop into long and medium axes are determined at the whole tree level, with a hierarchy within the axis population; the long axes development had priority, and then only a limited proportion developed into medium axes, the remaining class corresponded to spurs. Consequently, the number of medium axes appears to be the main factor that gives a tree plasticity in its architectural development in response to its root system. This is consistent with previous studies which demonstrated the plasticity of neoformation in trees (Costes, 1993; Costes *et al.*, 2003; Gordon *et al.*, 2006), but also indicated that the capability of a given axis to develop neoformation was dependent upon priorities within the whole tree axis population.

Differences in AS dimensions and branching

The decrease in AS dimensions over successive years, observed in both cultivars, is consistent with previous architectural studies in apple (Costes *et al.*, 2001, 2003) and in other species (Costes, 1993; Nicolini, 1998). AS length was reduced especially in trees grafted on M9 rootstock but the reduction was less pronounced than in previous studies (Costes *et al.*, 2001; Weibel *et al.*, 2003). This probably resulted from different sampling strategies. In the study carried out by Costes *et al.* (2001), AS were measured along trunks, while in the present study all axes were considered. Moreover, the reduction in AS length was more pronounced in Ariane than in X3305 trees. By contrast, base diameters were not significantly modified by M9 rootstock.

In both cultivars, AS branching was reduced in grafted trees. This reduction mainly resulted from a reduced number of lateral shoots per unit length while the percentage of branched AS remained almost unchanged. These

results provide new information regarding the proportion of branched AS at the whole tree level and complement results previously acquired on the dwarfing rootstock effect on branching (Hirst and Ferree, 1995; Costes *et al.*, 2001; Seleznyova *et al.*, 2003). Indeed, the analysis of two types of axes highlighted that M9 rootstock reduced branching per unit length in AS belonging to long axes only, while branching along medium axes was unchanged. Moreover, the changes observed in the mean number of LS mainly involved a reduction in short LS while the number of long and medium LS was similar between own-rooted and grafted trees. This was observed over three successive years and in both cultivars. This result connects observations at AS and tree levels. However, it contrasts with those obtained in Rome Beauty and Starkrimson cultivars (Costes *et al.*, 1991), where the dwarfing rootstock reduced the proportion of lateral buds that developed into long shoots along the trunks. The different resulted obtained in the present study were probably because the mean number of lateral shoots was analysed per unit length. This indicates that, although both AS length and branching were both reduced by dwarfing rootstock, branching was proportionally more reduced than length. The concomitant reduction of growth and branching agrees with the interpretation that the dwarfing rootstock effect corresponds to a faster physiological ageing of trees (Lauri *et al.* 2006).

Clarifying rootstock effect on vegetative and reproductive growth

Besides the M9-induced reduction in the number of axes per tree, the main effects exerted by the root system on the aerial part of the tree concerned its flowering behaviour. Dwarfing rootstock impacted on all flowering-related processes: (*a*) axillary flowering occurrences; (*b*) the probability of terminal flowering; and (*c*) return bloom probability. The positive effect of dwarfing rootstock on axillary flowering resulted from an increase in both the percentage of AS bearing axillary floral AS and the mean number of these axillary floral AS per unit length, and was observed over three consecutive years. The effect was more pronounced in Ariane than in X3305 trees that had a high flowering potential even when they were own-rooted. These results extend those obtained on 1-year-old trunks (Seleznyova *et al.*, 2004). They are also consistent with studies performed of branches of trees grown on a broad range of rootstock (Hirst and Ferree, 1995) or from different cultivars (Lauri *et al.*, 2006). But, in previous studies, differences were significant only for the first year of branch development whereas the present results suggest that dwarfing rootstock had a persistent effect on flowering occurrence over several years. This difference may result from different sampling strategies since only a certain number of branches, probably with homogeneous behaviour, were sampled in the studies cited above. It can be assumed that the samples in the previous studies were homogeneous and that strong branches near the base of the trunk were avoided. Finally, even though two trees per cultivar may appear to be a small sample at an orchard level, the description of entire trees, taking all the branches into account, provided a global understanding of tree development which may have remained partially hidden if within-tree sampling had been performed and the most extreme branches of the trees had not been considered.

Dwarfing rootstock also increased the terminal flowering occurrence in all axis types, with within-tree heterogeneity for this trait being reduced in both cultivars. As previously demonstrated in other apple cultivars (Costes *et al.*, 2001; Seleznyova *et al.*, 2004), the flowering enhancement occurred early during the life of the tree. In the present study, it occurred in 2001 spring, in both axillary and terminal positions, before any change developed in the number of long and medium axes. Thus, the high flowering occurrences observed in grafted trees preceded the reduction in the number of axes. Due to competition between vegetative and reproductive growth, these flowering occurrences are likely to have impacted on further vegetative growth (Abbott, 1984; Costes *et al.*, 2006). Two arguments converge to support this hypothesis: the reduction in the number of long and medium axes in the third and fourth years of growth, and the higher spur mortality observed in grafted trees than in own-rooted trees. Thus, the high flowering potential observed in grafted trees from 2001 bloom is likely to have had an impact on the number of AS able to develop, as well as their growth potential (here represented by AS length); and the number of short axes able to remain alive.

Higher return bloom, which was the third character enhanced in grafted trees compared with own-rooted trees, likely resulted from a combination of more flowering and higher spur mortality. Indeed, a positive relationship has been observed between spur death and return bloom in a range of apple cultivars (Lauri *et al.*, 1997) that could result from different intensities in within-tree competition between reproductive and vegetative processes. In this relationship, the root system appears to be involved in the adjustment of flowering potential at the whole tree level. A similar interpretation of dwarfing rootstock effect *via* the enhancement of flowering and greater competition between reproductive and vegetative growth has already been proposed in citrus by Lliso *et al.* (2004). This enhancement of flowering potential by dwarfing rootstock could rely on graft union effects (including the degree of compatibility between the scion and the rootstock) but also on different hormonal balances between roots and shoots as proposed by different authors (Lockard and Schneider, 1981).

Finally, comparing two apple cultivars confirmed that the number of long axes developing in the first year of growth and flowering potential are genotypic traits and that there are two main effects of dwarfing rootstock on the aerial development of trees (even though those effects were expressed with different intensity in the two cultivars). These effects are a reduction in the number of axes developed per tree and an enhancement of flowering. The present study provides additional and consistent information on the dwarfing rootstock effect at both the AS and the whole tree level, with a lesser impact on long axes than in medium and

short axes. It establishes a comprehensive pattern of dwarfing rootstock effects over years since the flowering occurrences preceded the reduction in the number of long and medium axes. This pattern confirms the cumulative nature of dwarfing rootstock effects over years since the first differences in flowering appeared early in tree development and persisted in subsequent years.

ACKNOWLEDGEMENTS

This research was funded by INRA and E. Garcia-Villanueva's PhD was financially supported by Conacyt-México. We are grateful to G. Garcia and J. C. Salles for their contribution to field measurements, and to S. Feral for technical assistance in the orchard. We also wish to thank Mr Mark Jones for improving the English.

LITERATURE CITED

Abbott DL. 1984. *The apple tree: physiology and management*. London: Grower Books.

Adam B, Sinoquet H, Godin C. 1999.. *Un logiciel pour l'Acquisition de l'Architecture des Arbres, intégrant la saisie simultanée de la topologie au format AMAPmod et de la géométrie par digitalisation 3D. Guide de l'utilisateur*, 3A version 1·0. Clermont-Ferrand: INRA-PIAF.

Barritt BH, Konishi BS, Dilley MA. 1995. Performance of 3 apple cultivars with 23 dwarfing rootstocks during 8 seasons in Washington. *Fruit Cultivars Journal* **49**: 158–70.

Costes E. 1993. Architecture aérienne de l'abricotier en développement libre. *Acta Botanica Gallica* **140**: 249–261.

Costes E, Guédon Y. 2002. Modelling branching patterns on 1-year-old trunks of six apple cultivars. *Annals of Botany* **89**: 513–524.

Costes E, Lauri PE. 1995. Processus de croissance en relation avec la ramification sylleptique et la floraison chez le pommier. In: Bouchon J, ed. *Architecture des arbres fruitiers et forestiers*. Montpellier: INRA Editions, 41–50.

Costes E, Salles JC, Garcia G. 2001. Growth and branching pattern along the main axis of two apple cultivars grafted on two different rootstocks. *Acta Horticulturae* **557**: 131–138.

Costes E, Sinoquet H, Kelner JJ, Godin C. 2003. Exploring within-tree architectural development of two apple tree cultivars over 6 years. *Annals of Botany* **91**: 91–104.

Costes E, Lauri PE, Regnard JL. 2006. Analyzing fruit tree architecture: implications for tree management and fruit production. In: Janick J, ed. *Horticultural reviews*. Hoboken: John Wiley and Sons, 1–61.

Crabbé J. 1987. *Aspects particuliers de la morphogenèse caulinaire des végétaux ligneux et introduction à leur étude quantitative*. Bruxelles: Editions IRSIA (Institut pour l'encouragement de la recherche scientifique pour l'industrie et l'agriculture).

Edelin C. 1991. Nouvelles données sur l'architecture des arbres sympodiaux: le concept de plan d'organisation. In: Edelin C, ed. *L'arbre, biologie et développement, Actes du 2ème Colloque International sur l'Arbre*, Montpellier, 10–15 September 1990, 127–154.

Fallahi E, Colt WM, Fallahi B, Chun I. 2002. The importance of apple rootstocks on tree growth, yeld, fruit quality, leaf nutrition and photosynthesis with an emphasis on 'Fuji'. *HortTechnology* **12**: 38–44.

Godin C, Caraglio Y. 1998. A multiscale model of plant topological structures. *Journal of Theoretical Biology* **191**: 1–46.

Godin C, Guédon Y, Costes E, Caraglio Y. 1997. Measuring and analyzing plants with the AMAPmod software. In: Michalewicz M, ed. *Advances in computational life science*. Vol. I. *Plants to ecosystems*. Melbourne: CSIRO, Chapter 4,

Godin C, Guédon Y, Costes E. 1999. Exploration of a plant architecture database with the AMAPmod software illustrated on an apple tree hybrid family. *Agronomie* **19**: 163–184.

Gordon D, Damiano C, DeJong TM. 2006. Preformation in vegetative buds of *Prunus persica*: factors influencing number of leaf primordia in overwintering buds. *Tree Physiology* **26**: 537–544.

Hallé F, Martin R. 1968. Etude de la croissance rythmique chez l'hévéa *Hevea brasiliensis* Mull. Arg. Euphorbiacées–Crotonoidées. *Adansonia* **8**: 475–503.

Hirst PM, Ferree DC. 1995. Rootstock effects on the flowering of 'Delicious' apple. I. Bud development. *Journal of the American Society for Horticultural Science* **120**: 622–634.

Khush GS. 2001. Green revolution: the way forward. *Nature Reviews Genetics* **2**: 815–822.

Lauri PE, Térouanne E, Lespinasse JM. 1997. Relationship between the early development of apple fruiting branches and the regularity of bearing: an approach to the strategies of various cultivars. *Journal of Horticultural Science* **72**: 519–530.

Lauri PE, Maguylo K, Trottier C. 2006. Architecture and size relations: an essay on apple (*Malus domestica* Borkh.) tree. *American Journal Botany* **93**: 357–368.

Lespinasse JM. 1977. *La conduite du Pommier.* I. *Types de fructification. Incidence sur la conduite de l'arbre*. Paris: I.N.V.U.F.L.E.C.

Lliso I, Forner JB, Talon M. 2004. The dwarfing mechanism of citrus rootstocks F&A 418 and #23 is related to competition between vegetative and reproductive growth. *Tree Physiology* **24**: 225–232.

Lockard RG, Schneider GW. 1981. Stock and scion growth relationships and the dwarfing mechanism in apple. In: Janick J, ed. *Horticultural reviews*. Hoboken: John Wiley and Sons, 315–375.

Masseron A. 1989. *Les porte-greffe, pommier, poirier et nashi*. Paris: CTIFL.

Nicolini E. 1998. Architecture et gradients morphogénétiques chez de jeunes hêtres (*Fagus sylvatica* L. Fagaceae) en milieu forestier. *Canadian Journal of Botany* **76**: 1232–1244.

Seleznyova A, Thorp G, White M, Tustin S, Costes E. 2003. Structural development of branches of 'Royal Gala' apple grafted on different rootstock/interstock combinations. *Annals of Botany* **91**: 1–8.

Seleznyova A, White M, Tustin S, Costes E. 2004. Application of Markovian models to study root/interstock effects on flowering of young apple trees. In: Godin *et al*., eds. Proceedings of the 4th International Workshop on Functional-Structural Plant Models, 7–11 June 2004, Montpellier, France. 311–314.

Weibel A, Johnson RS, Dejong TM. 2003. Comparative vegetative growth responses of two peach cultivars grown on size-controlling versus standard rootstocks. *Journal of American Society for Horticultural Science* **128**: 463–471.