

Co-ordinated growth between aerial and root systems in young apple plants issued from in vitro culture

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E. Costes, E. Garcia-Villanueva, E. Jourdan, E. Regnard, Yann Guédon. Co-ordinated growth between aerial and root systems in young apple plants issued from in vitro culture. Annals of Botany, 2006, 97 (1) (1), pp.85-96. 10.1093/aob/mcj003 . hal-00018866

HAL Id: hal-00018866 https://hal.science/hal-00018866

Submitted on 27 Oct 2006

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15	3 tables
16	7 Figures
17	291 words in the abstract and 5487 in the remaining text
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1 Coordinated growth between aerial and root systems in young apple

2 plants issued from *in vitro* culture

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1 <u>Abstract</u>

Background and aims: In several species exhibiting a rhythmic aerial growth, the
existence of an alternation between root and shoot growth has been demonstrated.
The present study aims at investigating the respective involvement of the emergence
of new organs and their elongation in this phenomenon and its possible genotypic
variation in young apple plants.

Methods: Two apple varieties: X6407, recently named 'Ariane' and X3305 (Chantecler x Baujade) were compared. Five plants per variety, issued from *in vitro* culture, were observed in minirhizotrons over 4 months. For each plant, root emergence and growth were observed twice per week. Growth rates were calculated for all roots with more than two segments and the branching density was calculated on primary roots (R1). On the aerial part, the number of leaves, leaf area and total shoot length were observed weekly.

14 Key results: No significant difference was observed between varieties in any of the 15 final characteristics of aerial growth. Increase in leaf area and shoot length exhibited 16 a 3-weeks rhythm in X3305 while a weaker signal was observed in Ariane. The R1 17 growth rate was homogeneous between the plants and likewise between the varieties, while their branching density differed significantly. Secondary roots (R2) emerged 18 19 rhythmically, with a 3-week and a 2-week rhythm respectively in X3305 and Ariane. 20 Despite a high intra-variety variability, significant differences were observed 21 between varieties in the R2 life span and mean length. A synchronism between leaf 22 emergence and primary root growth was highlighted in both varieties while an 23 opposition phase was observed between leaf area increments and secondary root 24 emergence in X3305 only.

- *Conclusion:* A biological model of dynamics that summarises the interactions
 between processes and includes the assumption of a feedback effect of lateral root
 emergence on leaf emergence is proposed.
- 4
- 5 Keywords: plant architecture, root-shoot equilibrium, rhythmic growth, synchronism,
- 6 time series
- 7

INTRODUCTION

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1

3 In perennial plants, organogenesis can occur throughout ontogeny due to the 4 maintenance of cell divisions in meristems (Lyndon, 1998). The emergence of 5 successive leaves is usually characterized by the time between two successive 6 primordial, called plastochrone. On a longer time scale, many plants also exhibit 7 rhythmic growth with an alternation of organogenesis and rest periods (Hallé et al., 8 1978). At the whole plant scale, many studies devoted to growth rhythmicity have 9 demonstrated the existence of an alternation between root and shoot growth, for 10 instance in Pinus taeda (Drew and Ledig, 1980), Theobroma cacao (Greathouse et 11 al., 1971), Ligustrum sp. (Kuehny and Halbrooks, 1993), and Hevea brasiliensis 12 (Thaler and Pages, 1996). This has been interpreted as the result of competition for 13 assimilates between root and aerial growth, while root branching was promoted by 14 leaf development (Aguirrezabal et al., 1993).

15

16 In temperate fruit trees which exhibit rhythmic growth, a similar alternating pattern 17 has been demonstrated in adult trees, on an annual time scale, for instance in peach 18 trees (Williamson and Coston, 1989) and apple trees (Psarras and Merwin, 2000). 19 However, these studies did not distinguish between organogenesis and elongation 20 processes as previously done for other non fruiting species, probably because adult 21 trees are too complex for this purpose. Because of the practice of grafting in the fruit 22 industry, the interactions between root and aerial systems are of major interest. In the 23 apple tree, which is commonly grown on dwarfing rootstocks, these interactions have 24 been widely studied through the description of the effects of rootstock on the aerial part development (Schechter et al., 1991; Costes et al., 2001; Seleznyova et al., 25

2003), on fruiting (Avery, 1969; Larsen et al., 1992), or fruit characteristics (Drake, 1 2 1988; Daugaard and Callesen, 2002). The effects of rootstock on biomass allocation have also been reported (Zhu et al., 1999), and competition during the emergence 3 4 and expansion of new organs, including cambial growth, has been shown to interact 5 with fruit development (Costes et al., 2000; Berman and DeJong, 2003). However, 6 less information is available regarding the patterns in aerial and root growth within a 7 growth cycle. In addition, little information is available regarding the apple tree root 8 system structure, i.e. the combination of root growth, branching and life span 9 characteristics for several successive branching orders. This is probably due to the 10 difficulty in observing in situ root systems (Atkinson, 1974 and 1983). Because the root systems develop in an environment with less variation in temperature than the 11 12 air but which is physically heterogeneous, roots were often viewed as opportunist in 13 their development (Lynch, 1995). However, like the aerial part, roots have been 14 shown to develop according to architectural rules, by the architectural comparison of 15 both aerial and root systems of tropical trees (Atger and Edelin, 1994).

16

17 The main aims in the present study were to investigate (i) whether the growth of the 18 roots and shoots of the apple tree are coordinated and/or alternate, and when the 19 emergence of new organ and elongation are involved in this phenomenon (ii) the 20 characteristics of roots belonging to at least two successive branching orders. In these 21 two investigations, particular focus was given to the comparison between two 22 varieties in order to highlight which process could depend on genetic factors. This 23 study was carried out on young self-rooted trees issued from *in vitro* culture which 24 were small enough to observe the variations in new organ emergence and elongation 25 in both root and shoot growth.

MATERIALS AND METHODS

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1

Two newly released apple varieties which both showed scab resistance and offered good fruit quality were studied: X6407, named 'Ariane' after selection by INRA Angers; and X3305 (Chantecler x Baujade) selected by INRA Bordeaux (Roche *et al.*, 2003).

7

8 Both varieties were propagated vegetatively by *in vitro* culture on a culture medium 9 adapted from Murashige (1962). Roots were induced with an IBA application (5g/l) 10 and the plants were placed in darkness at 18°C for 5 days. The plants were then left 11 without any hormone in the culture medium for three weeks. After a total period of 12 three months of *in vitro* culture, five plants per variety, as homogeneous as possible 13 in their development, were selected to be transplanted in root observation boxes 14 (minirhizotron). The minirhizotrons were built as described in previous studies 15 (Neufeld et al., 1989; Thaler and Pagès, 1996). The box dimensions were 0,50 x 0,22 16 $x 0,03 \text{ m}^3$. The back and sides were made of opaque PVC while the front, for 17 observation purpose, was transparent. This side was covered by black plastic to keep 18 the roots in the dark. The boxes were filled first with gravel to 3cm and then with 19 sterilized compost. The roots were kept separated from the substratum and 20 sandwiched against the transparent side by nylon mesh (Neufeld et al., 1989).

21

Immediately after transplantation, the ten observed plants were placed in a climatic chamber at 25°C, with 100% relative humidity and light of 55 μ moles m²/s in PAR for fourteen hours per day. During the first week after transplantation, they were provided with the nutritive solution that was in the *in vitro* medium, but without

growth regulators. After one week and during the experimentation follow-up, the
 plants were watered twice per week. Fungicide treatments against powdery mildew
 were applied only if necessary.

4

After transplantation, all the roots initiated during in vitro culture died and turned 5 6 brown. Within fourteen days, new white roots developed from the dead roots and 7 grew straight downwards. In the following, these new roots are referred as R1, their 8 lateral roots as R2 and the laterals borne on R2, if any, are called R3. To avoid root 9 growth constraints, the growth dynamics of both the aerial and root systems were 10 observed until the longest R1 roots reached the box bottom. This led us to observe 11 the plants for 111 days after transplantation. The root growth dynamics were 12 recorded twice per week on a transparent plastic sheet by drawing the roots with 13 different coloured permanent markers (Neufeld et al., 1989).

14

15 The drawings were then digitised in 2D using Rhizodigit 1.2 software developed at 16 CIRAD, France, by Jourdan and Mailhe. During digitising, each new root was 17 labelled with a specific number, and its type and initial 2D coordinates were recorded at its insertion point. On each segment corresponding to the root length developed 18 19 between two observation dates, the top spatial coordinates and the observation date 20 were recorded. After digitising, different variables were computed with macro 21 functions developed in Excel software. For each plant, the number of newly 22 developed roots was counted per unit of time and per type (R1, R2 and R3). The 23 growth rate was calculated for all roots with more than two segments as the ratio 24 between the length and the difference in the number of days between the first and the

last observation dates. The branching density was calculated as the ratio between the
 number of lateral roots per unit length of the parent root.

3

On the aerial part, at each observation day, the number of nodes and leaves, and the total shoot length were noted. The length and width of each leaf were measured once per week. Then, leaf area was precisely measured on a sub-sample of leaves. A linear relationship was then calculated to correlate the length and width to the area (data not shown). A single relationship (y = 0.69 x) was proved to be suitable to represent both cultivars with an acceptable r^2 coefficient (0.88).

10

Because of the low number of plants per variety, intra- and inter-variety variations in 11 12 the final characteristics were analysed by a Kruskal-Wallis non-parametric ANOVA 13 using Statistica® version 6.0. At the end of the observation period, the aerial growth was characterised by four variables: the total shoot length, number of leaves, leaf 14 15 area and the basal diameter. R1 were characterised by their total length, mean growth rate and mean branching density. R2 roots were characterised by their length and life 16 17 span, and by the percentage of R2 roots with more than one segment (poly-18 segmented R2 roots).

19

Growth dynamics were studied with time series analysis methods on three variables on the aerial system, i.e. weekly increase in the number of leaves, leaf area and shoot length using the statistical module of AMAPmod software (Godin *et al.*, 1997). Five variables were considered on the root systems in order to compare their development and dynamics to the aerial part: mean total length and growth rates of both R1 and R2 roots, and weekly increase in number of R2 roots. For analysing the relationships

between growth and branching in primary roots, the only R1 selected were those which emerged during the first month after transplantation, grew all along the observation time and reached at least 30 cm. One to seven roots per plant corresponded to these three criteria. On these roots, the number of R2 roots per week and the growth rate of the parent R1 during the corresponding week were analysed as time series.

7

8 For analysing the rhythmic variations of variables, trends i.e. long-term changes in 9 the mean level, were removed in order to obtain stationary time series (Chatfield, 10 2003). Different filtering procedures were tested based on either first-order 11 differencing or symmetric smoothing filters such as the binomial distributions B(n, n)12 p) with p = 0.5. Residual values, i.e. (original value – smoothed value) for each rank, 13 were then extracted with a symmetric smoothing filter corresponding to the 14 probability mass function of the binomial distribution B(4, 0.5). As proposed by 15 Brockwell and Davis (2002), the first and the last terms of the series were repeated 16 when applying this symmetric smoothing filter in order to overcome end-effect 17 problem. Sample autocorrelation and cross-correlation functions were then computed on residual values. Given τ observations in a sequence, $x_1, \dots, x_{\tau}, \tau - k$ pairs of 18 observations $(x_1, x_{k+1}), (x_2, x_{k+2}), \dots, (x_{\tau-k}, x_{\tau})$ can be formed, considering the first 19 observation in each pair as one variable, denoted by $X_{(1)}$, and the second observation 20 as a second variable, denoted by $X_{(k+1)}$. The autocorrelation coefficient at lag k for a 21 22 single sequence of length τ is thus given by (Chatfield, 2003; Brockwell and Davis, 2002). 23

24
$$r(k) = \frac{\sum_{t=1}^{\tau-k} (x_t - \mu)(x_{t+k} - \mu)/(\tau - k)}{\sum_{t=1}^{\tau} (x_t - \mu)^2 / \tau},$$

1 where $\mu = \sum_{t=1}^{\tau} x_t / \tau$. When generalizing to a sample of sequences, the 2 autocorrelation at lag k is computed from the $\sum_i (\tau_i - k)$ pairs of observations 3 extracted from all the sequences. The usual rule of thumb to indicate the uncertainty 4 in an autocorrelation coefficient is that under the null condition of no correlation 5 (purely random sequence), the autocorrelation coefficient at lag k has standard error 6 which is roughly $\pm 1.96\sqrt{\sum_i (\tau_i - k)}$ in the case of a sample of sequences (Diggle *et* 7 *al.*, 2002).

8

9 Correlation properties of multivariate sequences can be investigated by sample cross-10 correlation functions which are a direct generalization of sample autocorrelation 11 functions in which the pair of variables is either $X_{(1)}$ and $Y_{(k+1)}$ or $Y_{(1)}$ and $X_{(k+1)}$ 12 (Chatfield, 2003; Diggle, 1990). Hence, while the sample autocorrelation function is 13 an even function of the lag in that r(k) = r(-k), the sample cross-correlation 14 function is not an even function of the lag.

15

16 Since the application of linear filters for trend removal induces an autocorrelation 17 structure (Diggle, 1990), we chose to show the autocorrelation function induced by 18 the selected filter on the reference model (deterministic trend + white noise). A white 19 noise series is a series of independent Gaussian random variables with zero mean and 20 common variance parameter. The following convention was chosen to compute 21 cross-correlation functions between variables 1 and 2: variable 1 was considered as 22 reference and variable 2 shifted for increasing and decreasing lags. Consequently, the 23 number of pairs of observations decreased with increasing absolute lag values and the maximum absolute lag value considered was fixed at 10. 24

1 RESULTS

2

3 Shoot growth

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No significant difference was observed between the two varieties regarding the final
mean values of the variables selected to characterise their aerial growth (Table 1).
However, at the end of the observation period, Ariane exhibited more variability in
all variables than X3305.

9

10 Sample autocorrelation functions showed significant coefficients at different lags 11 depending on both the variable and variety (Fig. 1). In X3305, a rhythmic and 12 particularly stable pattern appeared for both leaf area and shoot length (Fig. 1a). 13 Significant and positive coefficients were observed at lags 3 and 6, while negative at 14 lags 4 and 7. Thus, the variations in leaf area and shoot length were synchronised 15 with a rhythm of three weeks, and were in opposition phase the following week. No 16 significant autocorrelation coefficients were observed for the number of new leaves. 17 In Ariane, the number of new leaves tended to alternate, i.e. exhibited a two-week rhythm, but only the first autocorrelation coefficients were significant and the pattern 18 19 died out rapidly (Fig. 1b). The variations in leaf area and shoot length were only 20 negatively correlated at lag 2 and those in leaf area tended to be synchronised each 21 three weeks. Finally, in X3305, the elongation variables had higher autocorrelation 22 coefficients than the number of new leaves and exhibited a similar rhythm of three 23 weeks. By contrast, in Ariane, leaf emergence had higher autocorrelation coefficients 24 than leaf and shoot elongation, and tended to alternate.

1 In X3305, the cross-correlation function between the variations in leaf area and shoot 2 length appeared to be an even function of the lag since $r(k) \cong r(-k)$ (Fig. 2a). Indeed, 3 the cross-correlation coefficient was highly significant at lag 0 while, at higher 4 positive or negative lags, the coefficients were successively positive and negative 5 with a symmetric rhythm of three weeks (negative at lags $\pm 1, \pm 4, \pm 7$, positive at lags 6 ± 3 , ± 6). This remarkable structure indicates that these two variables followed a 7 similar rhythm. Their respective cross-correlation functions with the number of new 8 leaves (Fig. 2b) were also quite similar with significant coefficients observed for 9 positive lags while few or no significant coefficients were observed for negative lags. 10 This dissymmetry of the cross-correlation function indicates that the elongation 11 processes is correlated to the number of leaves that emerged in the weeks before 12 while the inverse is not true. The elongation process, in both leaves and shoots, was 13 positively correlated with the number of leaves emerged one week before (positive 14 coefficients at lags 1) while in opposition phase with the number of leaves emerged 15 two weeks before (negative coefficients at lags 2). This pattern tended to repeat with 16 a three-week rhythm. In Ariane, the variations in leaf area and shoot length were 17 synchronised in the current week and four weeks before, with an opposition phase 18 the week after the synchrony (Fig. 2c). The number of new leaves and the variations 19 in both leaf area and shoot length were not significantly correlated regardless the lag 20 value and sign (Fig. 2d).

21

22 Root growth

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24 Seven to forty roots per plant developed directly from the *in vitro* dead roots and 25 were labelled R1. The total number of secondary roots per plant, labelled R2, also

exhibited a large variation between plants (Table 2). A six-fold coefficient was observed between the plants that had the lowest and largest number of secondary roots. Basically, the fewer the leaves, the fewer the roots each plant had. The converse was also true. Few tertiary roots developed for any plant (Table 3) and most had only one segment, which meant that they lived less than one week. Thus, these roots were not analysed in the following.

7

8 Root system architecture. Among R1, a large variation was observed in the final 9 length. However, the selected roots were homogeneous in their growth rate between 10 plants and no difference was observed between varieties (Table 2). By contrast, their mean branching density was significantly different between X3305 and Ariane, with 11 a higher density observed in X3305. As a consequence, the total number of R2 per 12 13 plant as well as the mean number of R2 per selected R1 root was significantly lower 14 in Ariane than in X3305 (Table 2). About 50% of R2 stopped growing during their 15 first three days of growth, these roots developing a single segment. The number of 16 R2 which were still growing from one observation date to the following decreased 17 exponentially. Because of this exponential decrease, R2 life span was analysed through median and centiles values. In both varieties, significant differences were 18 19 observed between plants (Table 3). Despite this intra-variety heterogeneity, 20 significant differences were also observed between varieties: the R2 life span was 21 significantly shorter in Ariane than in X3305. R2 mean length was also significantly 22 different between both plants and varieties, with R2 slightly longer in Ariane than in 23 X3305. The mean R2 growth rate, life span and total length were calculated on the 24 sub-sample of R2 which developed more than 2 segments (Table 3). In both 25 varieties, these R2 grew nearly twice as slowly as R1 and their growth rate was

significantly higher in Ariane than in X3305. Their total length was also significantly 1 2 different between varieties, while their life span was not different (data not shown). Finally, the differences in R2 between varieties can be summarized as follows: in 3 4 X3305, the R2 were more numerous than in Ariane and more than 50% developed at 5 least during two successive observations (i.e. more than 3 days), these roots growing relatively slowly and remaining quite short. By contrast, in Ariane, few R2 6 7 developed, less than 50% of them remained alive for more than 3 days but these 8 roots grew faster and became longer.

9

10 Root growth dynamics. The growth dynamics of primary and secondary roots were 11 studied per plant through five variables which were calculated weekly: (i) the mean 12 total length and the mean growth rate of both R1 and R2, and (ii) the number of new 13 R2. In both varieties, the sample autocorrelation functions of the variables related to 14 R1 did not show a clear pattern and few or no coefficients were significant regardless 15 of the lag (cross-correlation functions not shown). By contrast, sample 16 autocorrelation functions of the variables related to R2 showed rhythmic patterns 17 with significant coefficients (Fig. 3). In both varieties, R2 emerged rhythmically. Only one significant coefficient was observed at lag 3 in X3305 while an alternation, 18 19 i.e. a two-week rhythm with significant coefficients until lag 5, was observed in 20 Ariane. R2 growth rates exhibited a quite similar pattern in both varieties with an 21 opposition phase observed each two weeks (negative coefficients at lag 2). In X3305, 22 a synchrony was also observed each 3 weeks for this variable. In both varieties, the 23 mean length of R2 did not show significant autocorrelation coefficients.

1 In both varieties, the cross-correlation functions between the mean length and mean 2 growth rate of R1 did not show significant coefficients and these two variables were also poorly correlated to the equivalent variables in R2 (cross-correlation functions 3 4 not shown). By contrast, the cross-correlation functions between the three variables 5 considered on R2 showed significant coefficients (Fig. 4). In X3305, these three 6 variables were synchronous in the current week (Fig. 4a). The cross-correlation 7 function between R2 growth and both the number of R2 and their mean total length 8 were dissymmetric, with higher coefficients for negative than positive lags. Thus, the 9 variations in R2 growth rate were correlated to the two other variables observed in 10 the weeks before, with a rhythm of three weeks (negative coefficients at lags -1, -4 and -7 and positive coefficients at lags -3, -5 and -8). The cross-correlation function 11 12 between the number of R2 and their mean total length exhibited an even structure, 13 with a rhythm of three weeks until lags ± 3 or ± 4 , and which rapidly died out at higher lags. This particular structure was also observed on the cross-correlation 14 15 function between the number of R2 and their mean total length in Ariane (Fig. 4c), even though the coefficients were less significant and the rhythm was different (two-16 week). 17

18

In X3305, the emergence of new R2 was correlated to both the R1 growth rate and mean length for negative lags only (Fig. 4b). A positive correlation was observed in the current week and three weeks before (lags 0 and -3), while an opposition phase was observed one week after (negative coefficients at lags -2 and 1). In Ariane, the number of R2 was significant correlated only to the R1 mean length and for negative lags (Fig. 4d). A positive coefficient was observed in the current week and with a four-week rhythm towards negative lags. In both varieties, high correlation coefficients were observed for high negative lags suggesting relatively long term
 effects of the R1 growth rate in X3305 and the R1 total length in Ariane, on the
 number of new R2.

4 Comparison between aerial and root system dynamics

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6 This comparison was performed at the whole plant scale. The three variables studied 7 in the aerial part were plotted against the five variables studied on root systems, and 8 cross-correlation functions were considered for each pair of variables. Since the 9 cross-correlation functions were too numerous, we chose to present only the main 10 results.

11

12 In both varieties, the variables related to R1 were not correlated to the variations in 13 leaf area and shoot length (cross-correlation functions not shown). By contrast, 14 significant cross-correlation coefficients were observed with the number of new 15 leaves (Fig. 5). In X3305, the number of leaves was positively correlated to the R1 16 mean length and growth rate that occurred one week after leaf emergence (lag -1) 17 and this repeated with a three-week rhythm. However, the pattern died quite rapidly 18 with increasing lags (Fig. 5a, b). In Ariane (Fig. 5c), the number of new leaves was 19 alternatively positively and negatively correlated to the R1 mean length for negative 20 lags, with a rhythm of two weeks beginning from a synchrony in the current week. 21 Cross-correlation coefficients with the R1 growth rate were not significant (Fig. 5d). 22 Finally, leaf emergence was synchronised with the R1 mean length in both varieties, 23 and with the R1 growth rate only in X3305.

Regarding the variables related to R2, in both varieties, the cross-correlation 1 2 functions between the number of new leaves and the number of new R2 exhibited a rhythmic pattern, with significant coefficients only for positive lags (Fig. 6a, c). 3 4 Thus, leaf emergence was correlated to the number of new R2 that developed before 5 them, with a rhythm of three weeks in X3305 and two weeks in Ariane. In X3305, no 6 significant correlation was observed in the current week and the pattern died out after 7 lag 5. In Ariane, leaf and R2 emergence were synchronous in the current week and 8 each two weeks afterwards, with significant coefficients until high positive lags. The 9 cross-correlation functions between the number of new leaves and R2 growth rate 10 showed a rhythmic pattern only in X3305, with a three-week rhythm (Fig. 6b). Similarly, higher correlation coefficients were observed between the R2 variables 11 12 and the variations in both leaf area and shoot length in X3305 than in Ariane (cross-13 correlation functions shown for leaf area only, Fig. 6). In X3305, the cross-14 correlation function between the leaf area and the number of R2 exhibited an 15 rhythmic pattern for both positive and negative lags. These variables were negatively 16 correlated in the current week and were positively correlated the week after, this 17 pattern repeating each three weeks afterwards (negative coefficients at lags $0, \pm 3$ and 18 -6, positive at lags ± 2 and ± 5). Significant coefficients were observed between the 19 shoot length and both the number of R2 and R2 growth rate, but only for negative 20 lags and still with a three-week rhythm (cross-correlation functions not shown).

DISCUSSION

1 2

3 The present study highlighted the existence of rhythmic patterns on the studied 4 plants, which involved different processes and periods depending on the variety. A 5 three-week rhythm was observed in X3305 and concerned mainly the elongation 6 processes in the aerial part (leaf expansion and increases in shoot length, Fig. 1) 7 while a two-week rhythm was observed in Ariane, mainly in R2 emergence and in a 8 lesser extent in leaf emergence (Fig. 1 and 3). However, due to the low number of 9 studied plants and to the development stage of the young plants that were just issuing 10 from *in vitro* culture and still in acclimation phase, the fluctuations in growth observed in the present study are not equivalent to previous observations in the field 11 12 (for instance Abbott, 1984 or Costes and Lauri, 1995). In particular, the number of 13 leaves developed as well as their rhythm of emergence were very low. In Ariane, the 14 fact that the successive leaves tended to emerge each two weeks while the total 15 number of leaves per plant was not different between the varieties indicates that the 16 number of new leaves which developed at once, and consequently the "signal" 17 recorded, was particularly low. On the other hand, leaves emerged more synchronously in Ariane than in X3305. The differences in the leaf emergence 18 19 rhythm between varieties are consistent with a stable leaf emergence rate for a given 20 variety as demonstrated previously in peach (Kervella et al., 1995). These 21 differences could also be due to different adaptations of the varieties to the 22 experimental conditions. Indeed, environmental conditions such as temperature, light, water and nitrogen supply have been shown to influence growth components in 23 24 many crops (Médiène et al., 2002; Zhu et al., 1999). The interaction between

- environmental conditions and variety behaviour could thus be further investigated
 since, in the present study, different environmental conditions were not compared.
- 3

4 The variations in leaf area and shoot length were rhythmic only in X3305, with a 5 three-week rhythm (Fig.1). In Ariane, these processes were in opposition phase every 6 two weeks. In both varieties, leaf and shoot elongation occurred synchronously (Fig. 7 3). This synchrony was symmetric and very clear in X3305 while it was only a 8 tendency in Ariane. Increase in leaf area and shoot length occurred with a delay of 9 one week after leaf emergence in X3305 while no clear synchronism between leaf 10 emergence and elongation processes appeared in Ariane. This lack of synchronism in 11 Ariane may be due to the weakness of the leaf emergence signal. The existence of 12 dependency between leaf emergence and shoot elongation was established many 13 years ago through defoliation experiments which suppressed further shoot elongation 14 (Abbott, 1984). Further investigations have shown the role of hormones in this 15 dependency, in particular auxin and gibberellins (Steffens and Hedden, 1992; Zhao et 16 al., 2002). The present study provides information on the relative timing of the 17 processes and suggests that both this timing and the intensity of the signal could be variety dependent. 18

19

The growth rate observed in primary roots varied from 0.2 to 1 cm per day without any significant difference between varieties. These values are similar to those found in young citrus trees (Bevington, 1985), peach trees (Pagès *et al.*, 1993) and oil palms (Jourdan and Rey, 1997) but lower than those observed in rubber tree taproots (Thaler and Pagès, 1996). In many other studies, root growth rates have been measured using different methods such as the selective placement of ³²P (Atkinson,

1 1974) or the dried and fresh weight (Kuehny and Halbrooks, 1993; Zhu *et al.*, 2003) 2 which would not have allowed us to do a direct comparison of the results. The 3 growth rate of secondary roots was about half as low as that of primary roots. This 4 decrease in growth rates with the successive branching orders is consistent with 5 previous observations carried out in different species, for instance on oak tree 6 (Riedacker *et al.*, 1982), peach tree (Pagès *et al.*, 1993) or oil palm (Jourdan and Rey, 7 1997).

8

9 In addition, our results highlighted a significant difference in the growth rate of 10 secondary roots between the two studied varieties even though a large heterogeneity 11 between plants of the same variety was also observed. On average, secondary roots 12 grew faster in Ariane than in X3305. Similarly, the life span of the secondary roots 13 was different between the two varieties. In Ariane the secondary roots had a slightly 14 lower longevity than those of X3305. In both varieties, the life span of secondary 15 roots decreased exponentially over time and, consequently, most of the secondary 16 roots died within the first seven days of emerging. Despite different experimental 17 conditions, the range of life spans of the secondary roots is consistent with previous studies and with about 20% of secondary roots surviving more than 14 days, apple 18 19 secondary roots appear to have a low to intermediate longevity, as previously stated 20 for fine roots by Wells and Eissenstat (2001). For comparison, 40% and 6% of the 21 roots survived more than fourteen days in Prunus avium and Picea sitchensis 22 respectively (Black et al., 1998).

23

24 The branching density, calculated along selected primary roots which grew 25 throughout the observation period, was also significantly different between the two

1 studied varieties. The values obtained in the present study varied from 0.8 to 0.4 2 secondary roots per cm on average, according to the variety, and were in the same range of values as those for the peach tree, as mentioned by Pagès et al. (1993). The 3 4 difference in root branching density between varieties showed that the root systems 5 of Ariane plants were half as branched than those of X3305 plants. This result 6 combines with the difference in growth rate to show that each variety has specific 7 rules that lead to a different hierarchy between the primary and secondary roots and 8 thus to different root architecture. These structural differences may lead to different 9 capabilities in terms of soil exploration and possibly in nutrient uptake since fine root 10 physiology has been shown to depend upon root age and branching order (Wells and Eissenstat, 2003; Comas and Eissenstat, 2004). Moreover, recent studies have 11 12 demonstrated that different internal hormone concentrations and transports, as well 13 as external concentrations in ions and plant susceptibility could explain differences in 14 root system architecture (Bhalerao et al., 2002, Lopez-Bucio et al., 2003). Our 15 experimentation was designed to provide similar nutrient availability to all plants, 16 ensuring a negligible effect of external concentrations of ions on root elongation and 17 lateral root initiation. Thus, two main assumptions remain: that there are differences 18 in variety susceptibility to external concentrations in ions and differences in hormone 19 concentration or transport. However, we are not able at this stage of our investigation 20 to focus on one particular hormone among those possibly involved, i.e. auxin, 21 cytokinines, ABA and gibberellins (e.g. Casson and Lindsey, 2003).

22

In terms of the dynamics of root growth, fluctuations were observed in the number of
R2 and in their growth rates, in both varieties, while R1 growth did not exhibit any
evident rhythm (Fig. 3). Even though, as far as we know, no study on apple tree roots

1 has focused on the root dynamics depending on branching order, similar fluctuations 2 in root growth rates were observed in other perennial species (Drew and Ledig, 1980; Kuehny and Halbrooks, 1993; Thaler and Pagès, 1996). The relationships between 3 4 the emergence of secondary roots and the fluctuations in the growth of primary roots 5 was indicated mainly through positive cross-correlation coefficients between the 6 number of new R2 and the R1 mean length during the current week (Fig. 4). Such 7 relationships between growth and branching of primary roots are consistent with 8 previous results obtained in the peach tree (Pagès et al., 1993).

9

10 The fluctuations in R1 length were synchronous with the emergence of leaves but 11 with different rhythms and synchronisms between varieties (Fig. 5). The existence of 12 synchronisms between these two processes in the current week in Ariane, or in the 13 week before R1 elongation in X3305, suggests that leaf emergence may be involved 14 in the timing of primary root elongation. Furthermore, R2 emergence also exhibited a 15 rhythmic synchronism with leaf emergence but their cross-correlation coefficients 16 were higher for positive lags than for negative lags (Fig. 6). Thus, in contrast to 17 primary roots, secondary roots were synchronous with the leaf emergence that 18 occurred after them or inversely new leaves were correlated to the number of new R2 19 developed in the previous weeks. This suggests that new secondary roots enhanced 20 the emergence of new leaves, possibly via a feedback effect which could result from 21 an increase in water and nutrient uptake and supply towards the aerial part.

22

R2 emergence also showed significant cross-correlation coefficients with the
variations in leaf area (Fig. 6). This relationship was more pronounced in X3305 than
in Ariane, the lack of clear synchronism in Ariane being interpreted previously as a

1 consequence of the weakness of the aerial signals. In X3305, an opposition phase 2 between the variations in leaf area and both the R2 emergence and growth rate was observed in the current week. This result is consistent with a previous study carried 3 4 out in the rubber tree by Thaler and Pagès (1996) and with the assumption of a 5 possible dependence between root branching and leaf development. Indeed, the 6 initiation of secondary roots may be enhanced by growth factors synthesised in 7 growing leaves such as auxin (Pagès et al., 1993) which has also been reported to be 8 involved in shoot-root relationships in apple trees, especially via xylem 9 differentiation (Soumelidou et al., 1994; Kamboj, 1997).

10

In conclusion, the synthesis of the results obtained in the present study led us to 11 12 propose a conceptual model which represents the root-shoot relationship as a cyclic 13 process including three steps (Fig. 7). Indeed, leaf emergence was followed by 14 variations in R1 length (or synchronous with them in Ariane) which was followed by 15 new R2 emergence, while this latter event impacted on further leaf emergence. A 16 parallel loop concerns the fluctuations in leaf area which follow leaf emergence and 17 may, as R1 growth, enhance R2 emergence. This integrated view of the plant is consistent with the existence of a functional equilibrium between shoots and roots at 18 19 the whole plant scale which is usually interpreted as a consequence of water status 20 (Diaz-Perez et al., 1995), limitation in carbon acquisition (Drew, 1982) or carbon 21 and nitrogen partitioning (Marcelis, 1996). This equilibrium has also been assumed 22 to develop models of partitioning (e.g. Minchin and Thorpe, 1996; Thornley, 1998). 23 Even though the proposed scheme needs further confirmation at the adult stage, on 24 other crops and in other environmental conditions, it supports the assumption that the

1	root-shoot equilibrium could involve the relative dynamics of both aerial and root
2	parts and could have consequences on the structural organisation of the root system
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4	ACKNOWLEDGEMENTS : E. García-Villanueva Ph.D. was financially supported
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3	by CONACY I-Mexico. We gratefully thank G. Garcia and S. Feral for technical
6	assistance in field observations and H. Boukcim for his help in minirhizotrons
7	building. We acknowledge K. Renton for improving the English.
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TABLE 1: Total number of leaves, leaf area (LA), shoot length, basal diameter and number of primary roots per plant, of young 'X3305' and 'Ariane' apple plants sixteen weeks after transfer from in vitro culture. Standard deviation are indicated in italics between brackets. Significant differences between varieties were tested by a Kruskal-Wallis non-parametric ANOVA (ns: non-significant; * : significant with p < 0.05; ** : significant with p < 0.01).

Variety	Nb of leaves	Leaf area (mm ²)	Shoot length (mm)	Basal diameter (mm)	Nb of R1
X3305 Ariane	16 <i>(4.85)</i> 19 <i>(5.61)</i>	122.82 <i>(86.42)</i> 122.36 <i>(102.99)</i>	56.70 <i>(41.90)</i> 49.16 <i>(47.93)</i>	3.10 <i>(0.41)</i> 2.84 <i>(0.47)</i>	19.2 <i>(12.95)</i> 18.6 <i>(9.02)</i>
Varietal effect	ns	ns	ns	ns	ns

TABLE 2: Mean length, growth rate, branching density, and number of secondary roots of a selection of primary roots (R1), which grew all over the observation period (sixteen weeks after transfer from in vitro culture), on young 'X3305' and 'Ariane' apple plants. Standard deviations are indicated in italics. Significant differences between plants and varieties were tested by a Kruskal-Wallis non-parametric ANOVA (ns: non-significant; *: significant with p < 0.05; **: significant with p <0.01).

Cv	Nb of	Total Length		Growth rate		Branching		Nb of R2		
	Selected R1	(cm	l)	(cm/	day)	den	sity			
						(nb R2	2 / cm)			
		М	Std	М	Std	Μ	Std	Total nb	Mean nb per	Std
								per plant	R1	
X3305	16	43.64	8.55	0.51	0.11	0.8	0.4	747	34.00	17.61
Plant effect		ns		ns		ns		ns		
Ariane	19	40.42	8.14	0.46	0.10	0.4	0.3	519	16.95	12.95
Plant effect ns ns		ns		ns						
Varietal effect		ns		n	s	*	*		**	

TABLE 3: Total number of secondary (R2) and tertiary roots (R3) per plant. Mean and standard deviation of the total length (mm), median and centiles (25% and 75%) of the life span (days) are indicated for all R2 roots. The mean growth rate (mm/day) was calculated for R2 roots with more than one segment (Standard deviations in italics). Significant difference between plants and varieties were tested by a Kruskal-Wallis non-parametric ANOVA for the life span and by a hierarchic ANOVA with plant effect within the variety effect, for both length and growth rate (ns: non-significant, * : significant with p < 0.05; ** : significant with p < 0.01).

Cv	Total Nb of R2	Total Le (cm	ength)	Life span (days)		Nb of R2 with more than 1	Growth rate (cm/day)		Total nb of R3
	K2	М	Std	Ме	ed Cent.	segment	М	Std	
X3305	747	2.01	1.63	3	0-10	410	0.24	0.13	24
Ariane	519	2.34	2.76	0	0-8	242	0.30	0.16	20
Hierarchic	Variety		*				*	*	
ANOVA	Plant(Variety) **						*	*	
Kruskal-	Plant effect (X3305)				**				
Wallis	Plant effect (Ariane)				**				
ANOVA Varietal effect				*					

1 FIGURE CAPTIONS

2

FIG. 1.Sample autocorrelation functions of the variables characterising the aerial growth: number of new leaves (nb N), variations in leaf area d(LA), and shoot length d(L) calculated per plant, for two apple varieties, X3305 (a) and Ariane (b). Each unit on the x-axis corresponds to one lag of seven days. Autocorrelation function induced by the selected filter on the reference model, i.e. deterministic trend + white noise (- -) and randomness 95% confidence limits (---) are indicated with dashed lines.

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FIG. 2. Cross-correlation functions between variables of aerial growth calculated per plant and for two apple varieties: between leaf area d(LA) and shoot length d(L) for X3305 (a) and Ariane (c); between leaf area d(LA) or shoot length d(L) and the number of new leaves (nb N), for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines.

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FIG.3. Sample autocorrelation functions of the variables characterising secondary roots, for two apple varieties X3305 (a) and Ariane (b): Number of new secondary roots (nbR2), their growth rate (GR R2) and mean total length (long R2). Each unit on the x-axis corresponds to one lag of seven days. Autocorrelation function induced by the selected filter on the reference model, i.e. deterministic trend + white noise (--) and randomness 95% confidence limits (---) are indicated with dashed lines.

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FIG. 4. Cross-correlation functions between root variables: between the number of new secondary roots (nb R2), their growth rate (GR R2) and mean total length (long R2), calculated on selected primary roots for two apple varieties X3305 (a) and Ariane (c); between the number of new secondary roots (nb R2) and the growth rate of primary roots (GR R1) and their mean total length (long R1), for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines.

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FIG. 5. Cross-correlation functions between the number of new leaves (nbN) and the mean total length of primary roots (long R1) for two apple varieties X3305 (a) and Ariane (c); between the number of new leaves (nbN) and the growth rate of primary roots (GR R1) for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines.

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FIG. 6. Cross-correlation functions between variables characterising the aerial systems and secondary roots: between the number of new leaves (nbN), leaf area d(LA), and number of new secondary roots (nb R2), for two apple varieties X3305 (a) and Ariane (c); between the same variables of aerial systems and the growth rate of secondary roots (GR R2), for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines.

- FIG. 7. Schematic representation of cyclic succession of events between aerial and root systems with the corresponding time delays which were highlighted in time-series analysis (in italics). 2 3 4 5



FIG. 1. Sample autocorrelation functions of the variables characterising the aerial growth: number of new leaves (nb N), variations in leaf area d(LA), and shoot length d(L) calculated per plant, for two apple varieties, X3305 (a) and Ariane (b). Each unit on the x-axis corresponds to one lag of seven days. Autocorrelation function induced by the selected filter on the reference model, i.e. deterministic trend + white noise (-) and randomness 95% confidence limits (---) are indicated with dashed lines.

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FIG. 2. Cross-correlation functions between variables of aerial growth calculated per plant and for two apple varieties: between leaf area d(LA) and shoot length d(L) for X3305 (a) and Ariane (c); between leaf area d(LA) or shoot length d(L) and the number of new leaves (nb N), for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines.



FIG.3. Sample autocorrelation functions of the variables characterising secondary roots, for two apple varieties X3305 (a) and Ariane (b): Number of new secondary roots (nbR2), their growth rate (GR R2) and mean total length (long R2). Each unit on the x-axis corresponds to one lag of seven days. Autocorrelation function induced by the selected filter on the reference model, i.e. deterministic trend + white noise (--) and randomness 95% confidence limits (---) are indicated with dashed lines.







FIG. 5. Cross-correlation functions between the number of new leaves (nbN) and the mean total length of primary roots (long R1) for two apple varieties X3305 (a) and Ariane (c); between the number of new leaves (nbN) and the growth rate of primary roots (GR R1) for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines.



FIG. 6. Cross-correlation functions between variables characterising the aerial systems and secondary roots: between the number of new leaves (nbN), leaf area d(LA), and number of new secondary roots (nb R2), for two apple varieties X3305 (a) and Ariane (c); between the same variables of aerial systems and the growth rate of secondary roots (GR R2), for X3305 (b) and Ariane (d). Each unit on the x-axis corresponds to one lag of seven days. Randomness 95% confidence limits (---) are indicated with dashed lines

