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The Analysis of the Impact of Carbon Source-sink Relationships on Flowering Patterns Reveals That Apple Tree Growth and Functioning are Determined by Mechanisms Occurring at the Tree and Shoot Scales

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Abstract

In apple, external (temperature and water stress) and internal (hormonal signaling, C source-sink relationships) factors are assumed to affect floral induction and, consequently to determine the regular or biennial fruiting behavior. Nevertheless, no clear consensus exists on the role of each factor and on the level of plant organization at which the production patterns are determined. This study thus aimed at analyzing the C source-sink relationships from shoot to tree scales, in a set of genotypes displaying a large variability in flowering and production patterns.

Within an apple tree population issued from a bi-parental cross, three groups of genotypes were identified: biennial genotypes, regular genotypes resulting from a high production of floral growth units over years (“bourse over bourse” genotypes), regular genotypes displaying, each year, an almost equal proportion of vegetative and reproductive growth units (“desynchronized” genotypes). Photosynthesis and growth measurements were carried out in 2014 and 2015, during spring and summer.

No difference in photosynthesis was observed between “bourse over bourse” compared to biennial genotypes in “ON” years. For biennial genotypes, a higher photosynthesis rate was observed in shoots of “ON” trees compared to “OFF” ones, suggesting a positive feed-back of fruit presence (sink activity) on sources. All shoots (vegetative or reproductive) of “desynchronized” genotypes reached the same photosynthesis activity, suggesting a regulation of photosynthesis at the tree scale. Conversely, in those genotypes, vegetative shoots were longer than reproductive shoots and reached the same length than the vegetative shoots of biennial genotypes in “OFF” years.

These results give new insights into the within-tree variability of source-sink relationships in apple depending on either local or global fruiting conditions. They provide evidence that shoot growth seems to be determined by the local context whereas photosynthesis is regulated by the carbon demand, at the plant scale.

Keywords: photosynthesis, genotypic variability, shoot growth, architecture, biennial bearing.

INTRODUCTION

In apple, external (temperature and water stress) and internal (carbohydrate allocation and hormonal signals) factors affect flower formation (Hanke et al., 2007). Seeds are known to contain large amounts of hormones, and previous studies have shown that auxins (IAA) and gibberellins (GA) may act together or independently to inhibit floral

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induction (FI), whereas cytokinins are likely to be the hormones enhancing FI. GAs are produced by seeds and could be transported to apical buds where FI occurs or could enhance polar IAA transport to inhibit FI (Bangerth, 2006). However, the negative effect of seed number on FI can be overcome by a high vegetative growth rate of the bourse shoot, which develops as a lateral sympodial shoot on the fruiting unit, also suggesting a role of carbon competition between organs on FI (Neilsen and Dennis, 2000). The involvement of source-sink relationships on FI is also supported by molecular studies showing an over-expression of transcripts involved in response to carbohydrate starvation in meristems of ON trees compared to OFF trees (Guitton et al., 2016).

Large variability in flowering patterns has been observed in different apple trees commercial cultivars (Lauri and Lespinasse, 1993) or in segregating populations (Durand et al. 2013). The flowering patterns ranged from biennial bearing to regular bearing patterns. It was also observed that regular bearing patterns can be achieved with a high flowering rate each year (bourse over bourse) or with desynchronized alternating shoots or branches. This latter case can lead to a production of almost 50% of vegetative and reproductive buds each year and thus to a regular production over years. Former studies have demonstrated the impact of crop load on source-sink relationships in apple trees (Palmer et al., 1997; Wünsche et al., 2000). However, how this impact could be modulated in the different types of shoots (vegetative and reproductive) within the branching structures and for genotypes displaying different bearing patterns has not been investigated so far.

In this study three questions were addressed: (1) can the different bearing patterns be related to the variability in source activities (photosynthesis) among genotypes? (2) What is the impact of the tree crop load and fruit presence at shoot level on source activity and shoot growth? (3) At which scale of plant organization (shoot vs tree scales) photosynthesis and growth are adjusted with respect to crop load and how these processes can be related to contrasted bearing patterns?

MATERIALS AND METHODS

Plant material and growing conditions

The experiment was carried out in 2014 and 2015 on genotypes belonging to an apple tree F1 progeny derived from a cross between the INRA hybrid X3263 and the cultivar 'Belrène'. The parents of the progeny were chosen because they displayed contrasted architecture and bearing habits. 'Belrène' exhibits erected tree architecture and is prone to biennial bearing whereas X3263 is considered to have an intermediate growth habit and to be insensitive to biennial bearing (Celton et al., 2013).

This progeny was planted in 2008 at the INRA experimental unit in Mauguio (France, 43°36'N 3°58'E) and consisted of 261 genotypes with one or two individuals per genotype. From planting, the trees were irrigated and fertilized according to commercial practices to avoid any soil water or mineral depletion. Trees were neither pruned nor thinned in order to observe an unmodified architectural developmental and bearing habit.

Determination of sub-sets of genotypes

Previous published indexes were used to characterize the bearing patterns within the population (Durand et al., 2013). Three indexes were used, (i) the *BBI-res-norm* defined as the biennial bearing index computed on the residuals between the observed harvested fruit number and the general trend over time to account for the increase in production during the first years after planting, (ii) the autocorrelation (*autocor*) between these residuals, (iii) the *entropy* which represents the synchronization in flowering among shoots within the tree during each year. To estimate these indicators, the number of harvested fruits was recorded from planting until 2013 on each tree. The number of harvested fruits

was then used to determine the BBI_res_norm and $autocor$. $entropy$ was estimated from data recorded in spring 2014 on successions of floral and vegetative GU along 16 axes arising from the trunk and from first-order branches.

These indicators were used to define three different bearing patterns (Figure 1), (1) 'biennial' genotypes (high value of BBI_res_norm , high negative value of $autocor$ and low value of $entropy$), (2) 'regular' genotypes with a high production of floral bud each year (low BBI_res_norm , and low value of $entropy$) that correspond to a 'bourse over bourse' behavior (Lauri et al. 1995), (3) 'regular' genotypes with an almost equal proportion of vegetative and reproductive buds each year (low BBI_res_norm , high value of $entropy$). Genotypes of groups 2 and 3 are called afterwards 'bourse-over-bourse' and 'desynchronized' genotypes, respectively. Irregular genotypes (i.e. genotypes with an irregular production but without a biennial bearing pattern) were not considered in this study.

For the 'bourse over bourse' and 'desynchronized' genotypes, four genotypes with one tree per genotype were selected, with mean values of BBI_res_norm , $autocor$ and $entropy$ equal to [0.51, 0.31,-0.13] and [0.37, 0.61, 0.60], respectively. For biennial genotypes, two subclasses corresponding to trees that were in ON or OFF years in 2014 were defined with mean values of BBI_res_norm , $autocor$ and $entropy$ equal to [1.40, 0.33, -0.85] and [1.99, 0.36, -0.86], respectively. For each subclass, four genotypes with one tree per genotype were chosen.

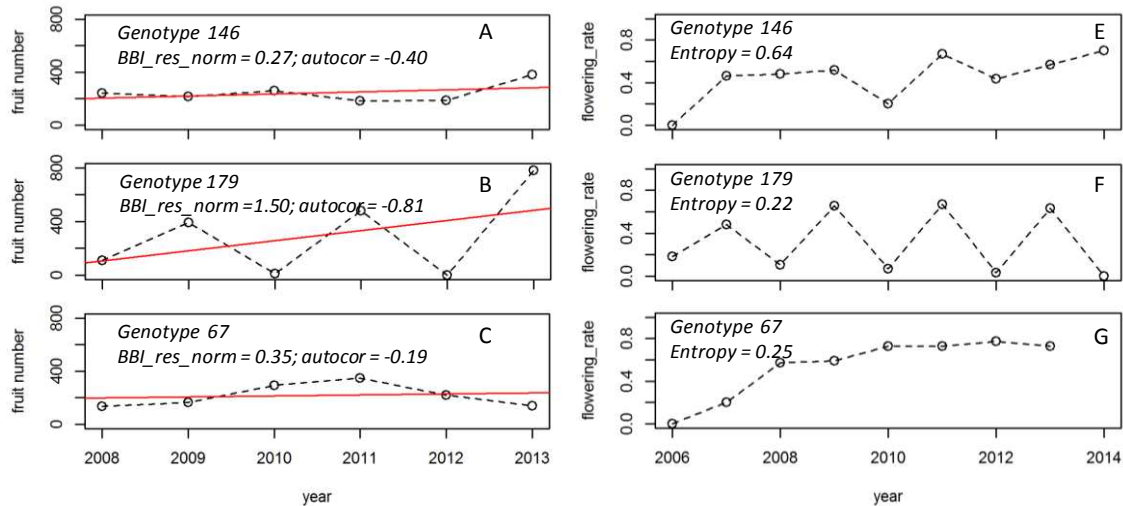


Figure 1. Graphical representation of genotypes with contrasted fruiting patterns: 'desynchronized' genotype (A, E), 'biennial' genotype (B, F) and 'bourse over bourse' genotype (C, G). A, B, C: harvested fruit number over years used to compute BBI_res_norm and $autocor$ and E, F, G: flowering rate recorded on successions of floral and vegetative growth units along axes and used for computing $entropy$. On A, B, C, red lines represent the linear regression between year and harvested fruit number used to estimate the values of residuals for computing BBI_res_norm .

Plant measurements

In 2014 and 2015, shoots either vegetative or reproductive were selected depending on their genotype fruiting context. Five fruiting context - shoot type combinations were considered: bourse shoots on 'bourse over bourse' genotypes (called afterwards 'bourse over bourse_F' shoots), on 'biennial' genotypes in ON years ('ON_F'), and on 'desynchronized' genotypes ('desynchro_F'); vegetative shoots on 'biennial' genotypes in OFF years ('OFF_F') and on desynchronized genotypes ('desynchro_V'). No measurement was performed on vegetative shoots on 'ON' or 'bourse over bourse' genotypes and on

bourse shoots on biennial genotypes in 'OFF' years because these types of shoots were not present in these trees. Indeed, growth units in 'ON' and 'bourse over bourse' genotypes were almost 100 % reproductive, and those on biennial genotypes in 'OFF' years were almost 100% vegetative.

Photosynthesis measurements were performed twice during each year (mid-April and early-July in 2014 and mid-June and mid-August in 2015) on the four selected genotypes of each bearing type in 2014 and on only three genotypes per bearing type in 2015. Measurements were performed on a fully expanded, mature and well-sun exposed leaves of 4 to 6 shoots per tree with a leaf gas exchange analyzer (LI-6400, LICOR, Lincoln, Nebraska, USA). Photosynthesis measurements were carried under controlled conditions in the leaf chamber known to be non-limiting for photosynthesis (Massonnet et al., 2008) (PPFD = 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, VPD= 1kPa, T°= 25°C, CO₂= 400ppm). We also counted the number of leaves on the shoots on which photosynthesis was performed. At harvest, the number of harvested fruits was evaluated on each tree. At this time, the trunk cross sectional was estimated based on the measurement of the trunk circumference at the base of the tree and assuming a cylinder shape for the trunk.

Statistical analyses.

Statistical analyses were performed with R software (R Development Core Team (2008)). The effect of each fruiting context - shoot type combination on shoots characteristics and leaf photosynthesis was estimated with a one-way ANOVA considering each sampling date separately (between 16 and 25 replicates were used for each combination). If statistical differences at $P < 0.05$ were observed, this analysis was followed by a pairwise t-test. For each date, differences between vegetative and bourse shoots within 'desynchronized' genotypes were tested with a two-way ANOVA with shoot (V vs F) and tree effects.

RESULTS

Crop load between genotypes

The crop load (number of harvested fruits per trunk cross sectional unit) strongly differed between treatments. In both 2014 and 2015, biennial genotypes in 'ON' years had the highest crop load (8.29 and 5.90 fruits cm^{-2} in 2014 and 2015, respectively) whereas biennial genotypes in OFF years had crop load values close to 0. Trees were correctly chosen since biennial genotypes that were in 'ON' (OFF) years in 2014 were then in 'OFF' (ON) years in 2015 (Table 1). 'Desynchronized' genotypes displayed intermediate crop load (3.31 and 3.98 fruits cm^{-2} in 2014 and 2015) with low variations between years except for genotype 246. Even if 'bourse over bourse' genotypes had a flowering rate close to 1 they reached lower crop loads than biennial genotypes in 'ON' years (2.09 and 1.67 cm^{-2} in 2014 and 2015) mainly because these 'bourse over bourse' genotypes had a low fruit set in both years (data not shown).

Impact of fruiting behavior and shoot type on shoot growth

A significant impact of the shoot type on leaf number per shoot was observed in both years (Figure 2). Indeed, the number of leaves was significantly higher ($P < 0.001$) in vegetative shoots than in bourse shoots whatever the year and the tree fruiting behavior ('biennial', 'bourse over bourse' or 'desynchronized'). Conversely, for either the bourse or vegetative shoots, the fruiting behavior did not have any significant effect on the leaf number (around 17 and 9 for vegetative and bourse shoots, respectively). The two-ANOVA performed on desynchronized trees revealed a significant shoot type ($P < 0.001$ in 2014 and 2015) and a non-significant tree ($P = 0.12$ and 0.41 in 2014 and 2015, respectively) effect.

For all the genotypes, a small year effect characterized by a tendency to get longer vegetative shoot in 2015 than in 2014 was also observed. This is probably due to differences in the timing of measurements between 2014 and 2015. Indeed, we can assume that some of the shoots did not finish growing in early July 2014 whereas it is likely that vegetative growth ended for all the shoots in mid August 2015.

Table 1. Crop load expressed in number of fruit per trunk cross sectional area (TCSA) in 2014 and 2015 for the 16 genotypes used in this study.

Genotype	Category	Crop load (Nb Fruits/TCSA cm ⁻²)	
		2014	2015
67	'Bourse over bourse'	1.48	1.58
183		2.84	0.94
24		2.8	2.5
44		1.24	¹
146	'Desynchronized'	1.36	1.47
246		2.72	5.83
174		4.11	4.66
42		5.05	¹
119	'Biennial'	0	4.51
238		0	5.25
179		0.39	7.94
72		0.37	¹
222	'Biennial'	6.99	0.56
36		7.44	0.06
303		8.77	0.99
76		9.99	¹

¹Crop load values are represented on the trees on which measurements were performed.

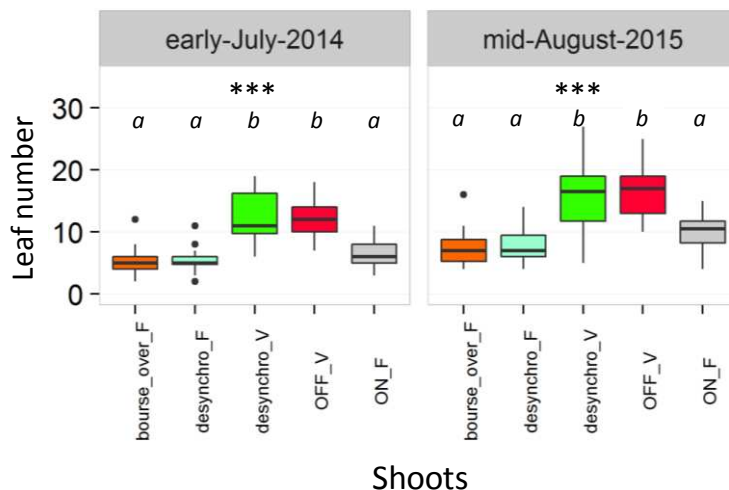


Figure 2. Number of leaves on shoots depending on the type of shoots (V/F) and the tree fruiting behaviour. ***significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$.

Impact of genotype fruiting behavior and shoot type on photosynthesis activity

Significant differences in photosynthesis depending on shoot type were observed for each year and date of measurement. The photosynthesis activity of the vegetative shoots of biennial genotypes in 'OFF' years was significantly lower ($P < 0.05$) than all other shoots, except in mid April 2014 (Figure 3). Furthermore, no significant difference in photosynthesis was observed among the other shoots (bourse_over_F, desynchro_F, desynchro_V and ON_F) whatever the year and date. The two-way ANOVA performed on desynchronized trees did not show any significant difference ($P = 0.78$; 0.57 ; 0.53 and 0.30 respectively in April 2014, July 2014, June 2015 and August 2015) in photosynthesis activity between vegetative and bourse shoots. No significant tree effect was found on photosynthesis of shoots in desynchronized genotypes for all the dates except in July 2014 ($P = 0.02$). Finally, some temporal variations in photosynthesis could be observed during the growth season, with an increase in photosynthesis between mid April and early July in 2014 for all the genotypes except the biennial genotypes in "ON" years. In 2015, photosynthesis tended to decrease at the end of the growth season (end of August).

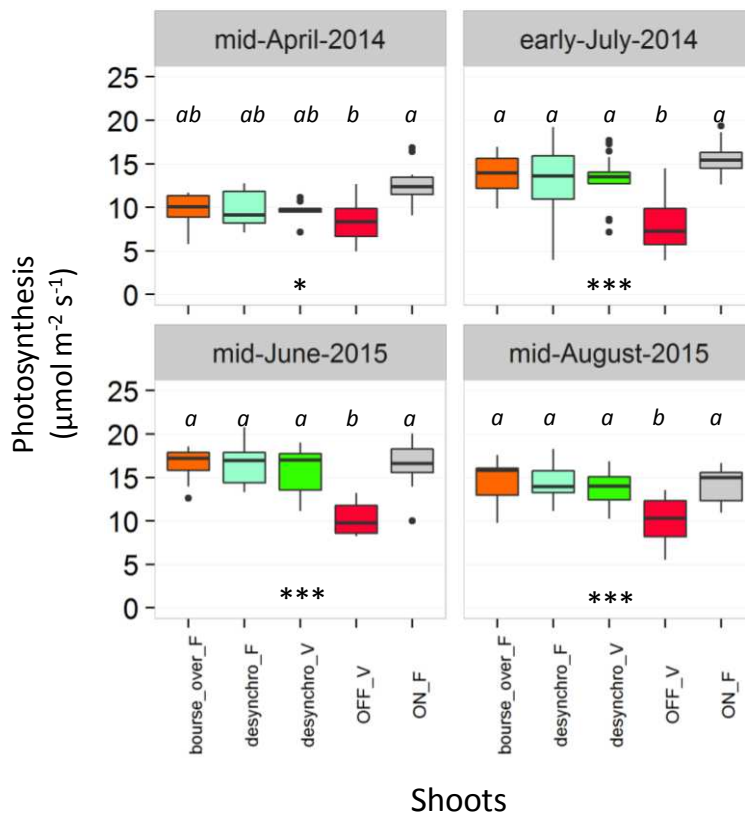


Figure 3. Leaf photosynthesis in 2014 and 2015 depending on the type of shoots. *, *** significant at $0.01 < P < 0.05$ and $P < 0.001$, respectively. Different letters indicate significant differences at $P < 0.05$.

DISCUSSION

This study was carried out in an experimental orchard on unpruned and unthinned trees in order to observe unmodified tree architectures and fruiting habits. Large variability in bearing patterns was observed in this F1 segregating population consistently with previous results obtained on another bi-parental cross (Starkimnson x Granny, Durand et

al., 2013). Crop load levels reached in this experiment were in accordance with observations collected on trees trained according to commercial practices (e.g. Giuliani et al. 1997) and correspond to commercial high crop load values for biennial genotypes in ON years (between 5 and 10 fruits.cm⁻² in our experiment) and medium crop load values (between 1 and 5 fruits.cm⁻²) for 'desynchronized' genotypes.

Based on the results, differences in bearing patterns cannot be explained by differences in photosynthesis between genotypes. Indeed, if the amount of carbon available affected floral induction, photosynthesis would be expected to be higher in 'bourse over bourse' genotypes compared to 'biennial' genotypes. Such a result was not observed in this study and the 'bourse over bourse' bearing pattern was more associated with a low fruit set (as observed with the low crop load values for these genotypes) than with higher leaf photosynthetic activity. This low fruit set for "bourse over bourse" genotypes was mainly observed on adult trees from the fifth year after planting (data not shown). Consistently, no difference in photosynthesis was observed between bourse shoots and vegetative shoots within desynchronized trees whereas the fate of the terminal meristems of these shoots differed (vegetative for bourse shoot and floral for vegetative shoots). This also supports the assumption of no direct impact of photosynthesis activity on floral induction.

This study also confirmed previously results such as the down-regulation of leaf photosynthesis under low crop load conditions (Palmer, 1992; Palmer et al., 1997; Wünsche et al., 2000), likely associated with a low export of sugars from leaves. This low export could result from the low demand of carbohydrate at the tree scale that led to increase reserve sugar content (soluble sugar or starch) in leaves (Goldschmidt and Huber, 1992; Moore et al., 1999; Frank et al., 2006). Interestingly, leaf photosynthesis of both vegetative and bourse shoots in desynchronized trees displayed the same rate even though vegetative shoots were located further from fruits than bourse shoots. This result suggests the existence of a regulation of photosynthesis at the tree scale and thus of long distance carbon fluxes that can allow exporting sugars from the leaves of non-fruited shoots.

Temporal variations in photosynthesis were also observed in this study with an increase in photosynthesis between April and June in 2014. This increase could be explained by the fact that the first harvest was performed early in the season (mid April) on leaves that were not mature and which did not reach their maximal photosynthesis potential. This low photosynthesis rate on immature leaves was observed in previous studies (e.g. Hieke et al., 2002 on litchi). In 2015, leaf photosynthesis tended to be slightly lower than in July, probably due to the beginning of leaf senescence at the end of August that can cause a drop in photosynthesis (Sinclair and Horie, 1989).

Conversely, shoot growth seemed determined by the local presence of fruits itself since bourse shoots (fruited shoots) reached a lower leaf number than vegetative shoots whatever the tree crop loads. This was observed even if bourse and vegetative shoots belonged to the same tree ("desynchro_V" vs "desynchro_F"). A lower growth of bourse shoots has been already observed in apple tree (Willaume et al. 2004) and has been associated with the presence of fruits on the bourses that likely reduce the amount of carbon available for growth.

CONCLUSIONS

Although this study did not show any impact of source activity on tree bearing pattern, it gives new insights into the variability of source-sink relationships in apple. Indeed, shoot growth seemed mainly determined by the local context of the shoot itself whereas leaf functioning was regulated at the plant scale by the tree crop load. These results will be complemented with non structural carbohydrate analyses which will allow us to further analyze the storage and transport of carbohydrates within the trees.

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