Growth and carbon balance are differently regulated by tree and shoot fruiting contexts: an integrative study on apple genotypes with contrasted bearing patterns

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In plants, carbon source–sink relationships are assumed to affect their reproductive effort. In fruit trees, carbon source–sink relationships are likely to be involved in their fruiting behavior. In apple, a large variability in fruiting behaviors exists, from regular to biennial, which has been related to the within-tree synchronization vs desynchronization of floral induction in buds. In this study, we analyzed if carbon assimilation, availability and fluxes as well as shoot growth differ in apple genotypes with contrasted behaviors. Another aim was to determine the scale of plant organization at which growth and carbon balance are regulated. The study was carried out on 16 genotypes belonging to three classes: (i) biennial, (ii) regular with a high production of floral buds every year and (iii) regular, displaying desynchronized bud fates in each year. Three shoot categories, vegetative and reproductive shoots with or without fruits, were included. This study shows that shoot growth and carbon balance are differentially regulated by tree and shoot fruiting contexts. Shoot growth was determined by the shoot fruiting context, or by the type of shoot itself, since vegetative shoots were always longer than reproductive shoots whatever the tree crop load. Leaf photosynthesis depended on the tree crop load only, irrespective of the shoot category or the genotypic class. Starch content was also strongly affected by the tree crop load with some adjustments of the carbon balance among shoots since starch content was lower, at least at some dates, in shoots with fruits compared with the shoots without fruits within the same trees. Finally, the genotypic differences in terms of shoot carbon balance partly matched with genotypic bearing patterns. Nevertheless, carbon content in buds and the role of gibberellins produced by seeds as well as the distances at which they could affect floral induction should be further analyzed.

Keywords: architecture, biennial bearing, crop load, Malus × domestica Borkh., non-structural carbohydrates, photosynthesis.

Introduction

Trees are composed of a population of meristems that can remain vegetative or be floral-induced depending on tree ontogenic characteristics (Costes et al. 2014), environmental factors (Wilkie et al. 2008) or agronomic practices (Samach and Smith 2013). The intensity of floral induction (FI) varies strongly among species and years, and different bearing patterns are observed in trees including continuous production in tropical trees (van Schaik et al. 1993), annual production for fruit trees (Koenig and Knops 2000) and production occurring during some specific years only in masting forest trees (Kelly 1994). For temperate trees, even if the production usually displays an annual rhythm, its intensity can dramatically change between years. In many fruit trees such as apple, pear, plum, prune, apricot, cranberry or blueberry (Monselise and Goldschmidt 1982), a biennial trend with years of high production (ON years) followed by years of low production (OFF years) is usually observed. This phenomenon results from the inhibition of FI during the years of high production, which causes a reduction in
the production in the following year. Hypotheses explaining this impact of fruit load on Fl are related to a high production of hormones such as gibberellins, which inhibit Fl in meristems, and/or a starvation of carbon during ON years (Lenahan et al. 2006, Hanke et al. 2007, Wilkie et al. 2008, Krasniqi et al. 2013). This was observed in experiments using defoliation, fruit removal or girdling that modify carbon source–sink ratios (Goldschmidt et al. 1985 on citrus; Palmer et al. 1991 on apple trees; Snelgar and Manson 1992 on kiwi fruit).

Former studies have also demonstrated the impact of crop load on many processes in fruit trees. Increasing the tree crop load induces a stimulation of the photosynthesis activity (Palmer et al. 1997, Wünsche et al. 2005), reduces shoot growth and plant leaf area (Wünsche et al. 2000) and decreases non-structural carbohydrate (NSC) concentration in annual and woody parts of the trees (Goldschmidt and Golomb 1982 on citrus; Spann et al. 2008 on pistachio; Naschitz et al. 2010 on apple). Nevertheless, some results appear contradictory such as the maintenance of the pool of carbohydrate reserve in olive trees in ON years (Bustan et al. 2011), suggesting that reserves act as an active sink at the expense of fruit growth for limiting reserve resource depletion in order to increase long-term survival (Sala et al. 2012).

In apple tree, a large variability in flowering patterns has been observed on commercial cultivars (Lauri and Lespinaesse 1993) and segregating populations (Guitton et al. 2012, Durand et al. 2013, 2017), with flowering patterns ranging from biennial to regular bearing. Genotypes with regular bearing patterns can display a high flowering rate over years (defined as ‘bourse over bourse’ genotypes by Lauri et al. 1997). In this case, their buds are highly synchronized in each year (Durand et al. 2013). Regular genotypes can also display desynchronized bud fates in each year, leading to alternating shoots with a production of almost 50% of vegetative shoots and 50% reproductive shoots at the plant scale each year (Durand et al. 2013). In apple, shoots arise from buds located terminally or in axillary positions that can be either reproductive or vegetative. Floral growth units (‘bourse’) consist of vegetative metamer with non-elongated internodes at the base and an inflorescence in terminal position (Abbott 1984). Inflorescences have five flowers and can bear from zero to five fruits. Floral growth units (also called ‘bourse’) may give rise to one or two sympodial shoots (hereafter called reproductive shoots) that develop immediately (Crabbé and Escobedo-Alvarez 1991). A large within-tree variability in length is observed for both vegetative and reproductive shoots. These shoots can be composed of preformed metamer with non-elongated metamer (called short shoots or spurs) or can display neoformation and elongated metamer (called medium/long or extension shoots, Costes et al. 2003, Selezyova et al. 2008). A close relationship between the type and the length of the shoot and the presence of floral buds in terminal position has been observed. Indeed, floral buds are more frequent in terminal position of medium and long shoots than short ones (Neilsen and Dennis 2000, Lauri and Trotter 2004). Moreover, Fl has been shown to be influenced by the presence of fruits on neighboring shoots and by the tree crop load (Haberman et al. 2016), suggesting that shoots are not autonomous entities for Fl.

The first aim of this study was to evaluate if the large variability in bearing patterns observed in apple trees could be linked to the variability in the within-tree source–sink relationships. More precisely, we aimed at comparing biennial genotypes with regular genotypes displaying either high and constant rate of Fl in all shoots or medium rate of Fl at tree scale due to desynchronized shoots. For this, we compared 16 genotypes with contrasted bearing pattern for their leaf photosynthesis activity and shoot carbohydrate content in organs belonging to either reproductive or vegetative shoots. The second aim was to investigate to what extent Fl, shoot growth, photosynthesis and NSC concentration are affected by the source–sink status at the tree scale (tree crop load) or at shoot scale.

**Materials and methods**

**Plant material and growing conditions**

The experiment was carried out in 2014 and 2015 on genotypes belonging to an apple tree progeny composed of 261 genotypes that originated from a cross between the INRA hybrid X3263 and the cultivar ‘Belrène’. The population was planted in 2005 at the INRA experimental unit Diascope in Mauguio (France, 43°36’N, 3°58’E). The site displays a typical Mediterranean climate with hot and dry summers and mild winters. The parents of the progeny were chosen because they displayed contrasted bearing behaviors and architectures. ‘Belrène’ exhibits an errect architecture and was observed to be prone to biennial bearing. X3263 is considered to have an intermediate growth habit, to be insensitive to alternation and to exhibit self-thinning traits (Celton et al. 2013). Trees were grafted on Pajam 1 rootstock and were planted with an inter-row distance of 5.0 m, a within-row distance of 2.0 m and a northwest–southeast orientation. From planting until the end of the experiment, the field plot was irrigated (120 l tree⁻¹ week⁻¹) from mid May until October with a system of microsprayers located in the row. In both years, 200 kg ha⁻¹ of NPK (10–5–20%) fertilizer and 100 kg ha⁻¹ of nitrate fertilizer (33%) were added in the field in April and June, respectively. These practices are known to be adequate for avoiding any mineral or water deficiency at this location. Trees were neither pruned nor thinned to be able to observe an unmodified architecture and bearing habit.

**Determination of genotype bearing pattern based on indices**

The determination of genotype bearing patterns in the population was done using statistical indicators adapted from Durand et al. (2013). The first indicator (BBI_res_norm) gives quantitative information about the intensity of the variations in production between two consecutive years. This indicator is defined as the normalized biennial bearing index (BBI) (Wilcox 1944) and computed on the...
residuals between the values of the observed variable \( X_i \) (harvest flower or fruit number or yield) and its general trend over years that accounts for the changes in production during the first years after planting. It was computed as follows:

\[
BBI_{\text{res}}_{\text{norm}} = \frac{\sum_{i=1}^{y} y_{i} - \bar{y}_{i} - \bar{y} - 1}{\sum_{i=1}^{y} X_{i}/y},
\]

where \( y \) is the number of years and \( e_i \) are the residuals of the linear regression between \( X_i \) and years.

The second indicator (autocor) was the entropy, which represents the synchronization of flowering or fruiting fates among the buds of a tree for each year. This indicator was computed as follows:

\[
\text{entropy} = -\frac{1}{\sum_{y} n_{y}} \sum_{y} n_{y,fl} \log \left( \frac{n_{y,fl}}{n_{y}} \right) + n_{y,v} \log \left( \frac{n_{y,v}}{n_{y}} \right)
\]

with \( n_{y,fl} \) and \( n_{y,v} \) and \( n_{y} \) being the number of floral buds, the number of vegetative buds and the number of flowering and vegetative buds, respectively, in year \( y \). \( \log(n_{y,fl}/n_{y}) \) or \( \log(n_{y,v}/n_{y}) \) was set to zero if \( n_{y,fl} \) or \( n_{y,v} \) was equal to zero, respectively. Therefore, entropy ranges from 0 (perfect synchronicity among buds) to \( \log2 \) (perfect desynchronicity). We also computed a last indicator, the mean rate of flowering, as the ratio of the number of flowering to the total number of buds in each year.

Based on the first two indicators, Durand et al. (2013) defined three classes of genotypes: (i) regular genotypes with low values of \( BB1_{\text{res}}_{\text{norm}} \) and autocor values close to 0, (ii) biennial genotypes displaying high values of \( BB1_{\text{res}}_{\text{norm}} \) and autocor values close to −1 and (iii) irregular genotypes with intermediate values of both \( BB1_{\text{res}}_{\text{norm}} \) and autocor. In this study, two sub-classes of regular genotypes were also considered based on entropy values. The first sub-class, hereafter called regular synchronized, ‘RegS’, includes genotypes with entropy values close to 0, i.e., with high synchronicity among buds, and a high mean flowering rate. The second sub-class, hereafter called regular desynchronized, ‘RegD’, includes genotypes with entropy values close to \( \log2 \) i.e., with a perfect desynchronicity among buds and a medium flowering rate, meaning that each year half of the buds were either floral or vegetative.

All the trees of the population were harvested at maturity and the number of harvested fruits was recorded from 2008 (first year of production) until 2013 (see Figure S1 available as Supplementary Data at Tree Physiology Online). The total number of harvested fruits was then used to estimate the \( BB1_{\text{res}}_{\text{norm}} \) and autocor. The entropy and the mean rate of flowering were estimated on a subset of 50 genotypes that displayed contrasted values of \( BB1_{\text{res}}_{\text{norm}} \) and autocor. On these trees, the successions of floral and vegetative growth units along axes were recorded retrospectively in spring 2014. These data were collected on 16 axes arising from the trunk and the first-order branches in each genotype and allowed us to estimate the proportion of floral and vegetative buds in each year from 2006 to 2014 (see Figure S2 available as Supplementary Data at Tree Physiology Online). The mean rate of flowering and entropy were calculated from 2010 until 2014 and from 2009 until 2014, respectively, for removing the first years of production when the flowering rate remained low. Based on the indicator values, four RegS genotypes and four RegD with one tree per replicate were chosen. The mean \( BB1_{\text{res}}_{\text{norm}} \), autocor, entropy and flowering rate for these two classes of genotypes were equal to [0.41, 0.17, −0.09, 0.81] and to [0.41, 0.17, 0.40, 0.51], respectively (see Table S1 available as Supplementary Data at Tree Physiology Online). For the biennial genotypes, the trees that were either in ON or OFF years in 2014 were distinguished. For each case, four genotypes with one tree per genotype were chosen. These genotypes displayed similar mean values of \( BB1_{\text{res}}_{\text{norm}} \) and autocor, equal to [1.91, −0.88] and [1.93, −0.88] for the genotypes in ON and OFF years in 2014, respectively. In the present study, we did not consider irregular genotypes that display intermediate production patterns (Durand et al. 2013).

### Shoot categories

In 2014 and 2015, vegetative and reproductive shoots were selected in each tree (Table 1). Six shoot categories were considered: (i) reproductive shoots with fruits on the inflorescence of regular synchronized genotypes (‘RegS_F’), (ii) reproductive shoots with fruits on the inflorescence of biennial genotypes in ON years (‘ON_F’), (iii) reproductive shoots without fruit on the inflorescence of biennial genotypes in ON years (‘ON_abort’), (iv) reproductive shoots with fruits on regular desynchronized genotypes (‘RegD_F’). (v) vegetative shoots on regular desynchronized genotypes (‘RegD_V’) and (vi) vegetative shoots on biennial genotypes in OFF years (‘OFF_V’).

### Shoot growth, photosynthesis and non-structural carbohydrates measurements

All the measurements were performed on the four selected genotypes per genotypic class in 2014. In 2015, measurements

<table>
<thead>
<tr>
<th>Genotype class</th>
<th>Shoot type</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biennial in ON years</td>
<td>Reproductive shoots with fruits</td>
<td>ON_F</td>
</tr>
<tr>
<td></td>
<td>Reproductive shoots without fruits</td>
<td>ON_abort</td>
</tr>
<tr>
<td>Biennial in OFF years</td>
<td>Vegetative shoots</td>
<td>OFF_V</td>
</tr>
<tr>
<td>Regular – Desynchronized</td>
<td>Reproductive shoots with fruits</td>
<td>RegD_F</td>
</tr>
<tr>
<td>Regular – Synchronized</td>
<td>Vegetative shoots</td>
<td>RegD_V</td>
</tr>
<tr>
<td>Genotypes</td>
<td>Reproductive shoots with fruits</td>
<td>RegS_F</td>
</tr>
</tbody>
</table>
were performed on three genotypes per class, only. For each genotype and at each measurement date, three to six extension shoots (Seleznyova et al. 2008) were randomly chosen per category (Table 1), in terminal position of a 1-year-old stem with elongated internodes, with an eastern exposure and in outer part of the canopy. These shoots were all analyzed for non-structural carbohydrates (NSC) content, photosynthesis, and growth.

For NSC content, sampling was performed four times in 2014, early May (about 4 weeks after full bloom), early July, late August and late October after harvest, and twice in 2015 in mid-June and mid-August (see Table S2 available as Supplementary Data at Tree Physiology Online for the description of environmental conditions) during the measurement periods. All the shoot categories were sampled at all dates, except ON_abort shoots that were sampled in late August 2014 and mid-August 2015, only. Sampling was performed between 8 and 10 a.m., on three organ types: the leaf displaying the maximal area on each shoot, the entire annual stem (current year shoot) and a section of around 5 cm of the 1-year-old stem. The samples were placed immediately in liquid nitrogen and stored at −20°C for <1 week. Samples were then freeze-dried and ground using a ball grinder. Soluble sugars (i.e., glucose, fructose, sucrose and sorbitol) were extracted on 30 mg dry mass of sample with 80% EtOH. Erythritol was added as internal standard in the extract. After evaporation of EtOH, soluble sugars were dissolved in H2O and quantified by high-performance ion chromatography with a Supelcogel Ca column at 54°C and a refractometer detector. Unfortunately, soluble sugar contents on the first sampling date in 2014 were not analyzable due to storage troubles. The insoluble residue of the extract containing starch was solubilized with 0.02 N sodium hydroxide at 100°C for 1 h and hydrolyzed with α-amyloglucosidase. Glucose was quantified spectrophotometrically with hexokinase, glucose-6-phosphate-dehydrogenase and NADP. Sugars concentration was quantified as milligram per gram of dry matter.

Photosynthesis measurements were done on the leaf with maximal area on the shoot in early May and early July 2014 and in mid-June and mid-August 2015. Measurements were performed with a leaf gas analyzer (LI-6400, LICOR, Lincoln, NE, USA) under controlled conditions in the leaf chamber known to be non-limiting for photosynthesis in apple tree (Massonnet et al. 2007), photosynthetic photon flux density = 1800 μmol m−2 s−1, vapor pressure deficit = 1 kPa, T = 25°C, CO2 = 400 ppm). On the same leaf, chlorophyll concentration (SPAD value) was measured with a SPAD-502 (Konica Minolta Sensing, Sakai, Osaka, Japan) at all dates except early May 2014.

We also counted the number of leaves on each annual shoot selected. In autumn 2015, the trunk sectional area was estimated based on measurements of the trunk circumference at the base of each tree and assuming a cylinder shape for the trunk. For each year, the crop load (e.g., Wünsche et al. 2000) was computed as the ratio of the number of harvested fruits to the trunk sectional area measured in 2015.

Statistical analyses

Statistical analyses were performed with R software (R Development Core Team 2008). For all the variables, two consecutive two-way ANOVA were performed with shoot category and date effects and with shoot category and year effects, respectively.

The effect of each shoot category on the NSC concentration, photosynthesis and SPAD values was tested for each measurement date with a one-way ANOVA considering each sampling date separately. For leaf number and in both the year, the two-way ANOVA with date and shoot effects did not reveal any significant difference (P = 0.11 and 0.07 in 2014 and 2015, respectively) if the data collected in May 2014 were excluded, because at this date, shoot development was not finished. For subsequent statistical analyses and in both years, we thus gathered together the number of leaves collected at all sampling dates without considering data collected in May 2014. For all the variables (NSC concentrations, photosynthesis, SPAD and leaf number), if statistical differences were observed for shoot category effect, a Tukey’s HSD test for pairwise comparison was performed. The shoot category effect on proportions of the different types of soluble sugars was assessed with a Chi-square test.

In order to compare the shoots bearing fruits or not within the same trees belonging to regular desynchronized and ON genotypes (RegD_V vs RegD_F and ON_F vs ON_abort), a two-way ANOVA with shoot category and tree effects was performed on all the variables.

Sigmoidal functions were fitted on photosynthesis data for accounting for the crop load effect using the nls function of R software. The fitting quality was evaluated with the root mean square error (RMSE) and its normalized value (NRMSE). A linear model was used to fit the relationship between crop load and starch concentration and the significance of the crop load effect was assessed by a one-way ANOVA.

Results

Crop load and fruit production among genotype classes

The number of trees was too low to perform classical statistical analyses on crop load and fruit production differences among genotype classes. However, it can be observed that the crop load and the number of harvested fruits during the experimental period (2014–15) strongly differed among genotype classes (Table 2). In both 2014 and 2015, biennial genotypes displayed the highest mean harvested fruit number (593 and 437 fruits, respectively) and crop load (8.30 and 5.99 fruits cm−2, respectively) in ON years and the lowest values in OFF years with a fruit production close to zero. This supports the relevance of our selection based on production and shoot succession analyses done in years prior to the experiments, since these genotypes exhibited a typical biennial bearing pattern during the experimental period. Regular synchronized (RegS) or desynchronized (RegD) genotypes did
Crop load was computed as the ratio of the number of harvested fruits to the trunk cross sectional area (TCSA) estimated in autumn 2015.

<table>
<thead>
<tr>
<th>Genotype class</th>
<th>Genotype</th>
<th>Number of harvested fruits</th>
<th>Crop load (no. fruits/TCSA cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2014</td>
<td>2015</td>
</tr>
<tr>
<td>Biennial (OFF in 2014)</td>
<td>X0072$^1$</td>
<td>20</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>X0119</td>
<td>0</td>
<td>492</td>
</tr>
<tr>
<td></td>
<td>X0179</td>
<td>24</td>
<td>398</td>
</tr>
<tr>
<td></td>
<td>X0238</td>
<td>0</td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.10</td>
<td>437</td>
</tr>
<tr>
<td>Biennial (ON in 2014)</td>
<td>X0036</td>
<td>618</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>X0076$^1$</td>
<td>669</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X0222</td>
<td>415</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>X0303</td>
<td>671</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>593</td>
<td>38.0</td>
</tr>
<tr>
<td>Regular – Desynchronized (RegD)</td>
<td>X0042$^1$</td>
<td>362</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X0146</td>
<td>160</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>X0174</td>
<td>287</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td>X0246</td>
<td>152</td>
<td>326</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>240</td>
<td>275</td>
</tr>
<tr>
<td>Regular – Synchronized (RegS)</td>
<td>X0024</td>
<td>110</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>X0044$^1$</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X0067</td>
<td>148</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>X0183</td>
<td>181</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>122</td>
<td>106</td>
</tr>
</tbody>
</table>

Crop load was computed as the ratio of the number of harvested fruits to the trunk cross sectional area (TCSA) estimated in autumn 2015. 

$^1$Values are presented for the trees on which measurements were performed (four and three trees in 2014 and 2015, respectively).

Effect of shoot category and genotype class on leaf growth

A significant year effect on leaf number per shoot was observed (P < 0.001 for the two way ANOVA with shoot and year effect). This effect was associated with a higher leaf number in 2015 compared with 2014 (Figure 1). A significant effect of the shoot type (vegetative vs reproductive) on leaf number was observed in both years. Indeed, vegetative shoots (RegD_V, OFF_V) reached significant higher mean values (mean values = 12.5 and 16.3, in 2014 and 2015, respectively) than reproductive shoots (mean values = 5.4 and 7.7, in 2014 and 2015, respectively). This was also true when vegetative shoots were compared with reproductive shoots without fruits (ON_abort). This higher leaf number on vegetative shoots was also observed when reproductive and vegetative shoots were compared within the same trees, i.e. regular desynchronized (RegD) trees (two-way ANOVA with shoot category and tree effect). Moreover, the genotype bearing behavior had no direct effect on the leaf number per shoot since no significant difference was observed among the vegetative or reproductive shoots in the different genotype classes (Figure 1).

Effect of shoot category, genotype class and tree crop load on leaf photosynthesis

Regarding photosynthesis activity (Figure 2), a significant date effect (P < 0.001) was observed according to a two-way ANOVA with date and shoot category effects. The post hoc test showed the following ranking: early May 2014 < early July 2014 = mid-August 2014 < mid-June 2015.

Photosynthetic activity significantly differed between shoot categories whatever the year and the date of measurement. These significant differences result first from a significantly lower photosynthesis activity of the vegetative shoots on biennial genotypes in OFF years (OFF_V) compared with all other shoots in early July 2014, mid-June and mid-August 2015. In early May 2014, ON_F shoots displayed higher photosynthesis than all other shoots and only a small non-significant decrease was
observed for OFF_V if compared with other categories (RegD_V, RegD_F and RegS_F). No statistical difference was observed between shoots within the same trees differing by their local fruiting context: the vegetative and reproductive shoots belonging to RegD class (RegD_V vs RegD_F) and the reproductive shoots with or without fruit of ON trees (ON_F vs ON_abort) did not differ in their photosynthetic activity, according to a two-way ANOVA with shoot and tree effects. Significant differences were represented by the level of significance of the P-values above the corresponding box plots. For all the statistical tests: ***significant at P < 0.001. ON_F and ON_abort shoots refer to the reproductive shoots with and without fruit on biennial genotypes in ON years, respectively; OFF_V shoots are the vegetative shoots on biennial genotypes in OFF years; RegD_F and RegD_V shoots are the reproductive and vegetative shoots on regular desynchronized genotypes, respectively, and RegS_F shoots are the reproductive shoots on regular synchronized genotypes.

The effect of the tree crop load on photosynthesis could be modeled by a sigmoidal relationship (only shown in July 2014, Figure 3, and Figure S3 available as Supplementary Data at Tree Physiology Online for the other dates). In July 2014, this sigmoidal function showed a saturation of photosynthesis when the tree crop load was higher than 5 fruits cm$^{-2}$ with a maximum value of photosynthesis close to 15 μmol m$^{-2}$ s$^{-1}$ (Figure 3).

For all the dates and considering all the shoot categories together, this sigmoidal function well fitted the relationships between crop load and photosynthesis (NRMSE < 16%). This suggests a major impact of the tree crop load, irrespective of the shoot category or genotypic class.

The photosynthesis rate was strongly correlated with SPAD values with a mean increase in photosynthesis of about 0.25 μmol m$^{-2}$ s$^{-1}$.

![Figure 1](https://example.com/figure1.jpg)

**Figure 1.** Box plot representation of the number of leaves of the annual shoots in 2014 and 2015 depending on the shoot category. Leaf numbers in 2014 and 2015 were computed gathering the data collected in early July, late August and late October 2014 and the data collected in mid-June and mid-August 2015, respectively. Shoot category effect was estimated for each year with a one-way ANOVA considering all the treatments together. The analysis was followed by a Tukey’s HSD test for pairwise comparisons and different letters indicate statistically different values at P < 0.05. Statistical differences between RegD_V and RegD_F and between ON_F and ON_abort shoots were assessed by a two-way ANOVA with shoot category and tree effects. Significant differences were represented by the level of significance of the P-values above the corresponding box plots. For all the statistical tests: ***significant at P < 0.001. ON_F and ON_abort shoots refer to the reproductive shoots with and without fruit on biennial genotypes in ON years, respectively; OFF_V shoots are the vegetative shoots on biennial genotypes in OFF years; RegD_F and RegD_V shoots are the reproductive and vegetative shoots on regular desynchronized genotypes, respectively, and RegS_F shoots are the reproductive shoots on regular synchronized genotypes.

![Figure 2](https://example.com/figure2.jpg)

**Figure 2.** Box plot representation of leaf photosynthesis activity in early May and early July 2014 and in mid-June and mid-August 2015 for each shoot category (see Figure 1 legend and Table 1 for shoot category description). Shoot category effect was assessed for each date with a one-way ANOVA considering all the shoot categories together. The analysis was followed by a Tukey’s HSD test for pairwise comparisons. Different letters indicate statistically different values at P < 0.05. For all the statistical tests: ***significant at P < 0.001.

![Figure 3](https://example.com/figure3.jpg)

**Figure 3.** Relationship between photosynthesis (μmol m$^{-2}$ s$^{-1}$) and crop load (number of fruits per trunk cross sectional area) in early July 2014. Each point represents the value for one genotype and bars represent the standard deviation among measurements (from three to six measurements per genotype). See Figure 1 legend and Table 1 for shoot category description. Numbers close to the points refer to the name of the genotype. The continuous line is the sigmoidal function fitted on the whole dataset ($y = 15.90/(1 + \exp(-0.14(\frac{x-0.04}{1.25}))$).
per SPAD unit (Table 3). OFF_V shoots had the lowest SPAD values in all measurement dates and these differences were significant in early July 2014 and mid-August 2015. Here again, no statistical differences were observed between RegD_V and RegD_F shoots and between the ON_F and ON_abort shoots, probably showing a low influence of the local presence of fruits itself compared with the influence of the tree crop load.

**Effect of shoot category, genotype class and tree crop load on non-structural carbohydrate concentration**

Regarding soluble sugar concentration in leaves (Figure 4), no significant date effect \((P = 0.08, \text{ANOVA with shoot category and date effects})\) was observed in 2014 whereas a significant decrease \((P < 0.001)\) between mid-June and mid-August was observed in 2015. For the other organs, no noticeable variation with date was observed in leaves (Figure S4 available as Supplementary Data at Tree Physiology Online). A significant year effect was also observed in leaves (Figure 4; \(P < 0.001\) for the two-way ANOVA with shoot category and year effects), with a higher value in 2015 compared with 2014. No large significant differences among shoot categories were observed. Significant differences were mainly observed in leaves (Figure 4), at some dates with a general tendency to observe the highest soluble sugar concentration in OFF_V shoots and the lowest one in ON_F shoots. These differences were less visible in annual and 1-year-old stems (see Figures S4 and S5 available as Supplementary Data at Tree Physiology Online). OFF_V shoots could even reach significantly lower soluble sugar concentration than ON_F shoots in mid-August 2015 in 1-year-old stems \((66\, \text{mg}\, \text{g}^{-1} \text{FW} \text{and} 52\, \text{mg}\, \text{g}^{-1} \text{FW}, \text{respectively, see Figure S4 available as Supplementary Data at Tree Physiology Online}). Consistently, significant differences between the vegetative and reproductive with or without fruit within the same trees were rarely observed. However, sugar soluble concentrations were lower in leaves of ON_F than ON_abort shoots in mid-August 2015 (Figure 4) and were lower in 1-year-old stem of RegD_V than RegD_F shoots in early July 2014, only (see Figure S4 available as Supplementary Data at Tree Physiology Online).

Sorbitol was the most important soluble sugar and represented between 62% and 78% of the total, sucrose between 8% and 20%, glucose between 4% and 20% and fructose between 2% and 14% in all the studied organs (Figure 5 and see Figure S6 available as Supplementary Data at Tree Physiology Online). The proportion of soluble sugars differed among shoots with a lower proportion of sorbitol and sucrose content in ON_F shoots compared with the other categories. This lower proportion was observable at almost all dates and in all organs and was significant in 1-year-old shoots in early July 2014 and mid-June 2015 \((P < 0.001\) for the Chi-square test on proportions in both years). The sorbitol proportion in ON_F shoots was equal to 61.6% and 64.7% in 2014 and 2015, respectively, whereas it was equal to 71.3% and 74.8% in the other shoot categories. Concomitantly, the proportion of glucose and fructose was higher in ON_F shoots. For instance in early July 2014 and in ON_F shoots, the proportions of glucose and fructose were equal to 13.3% and 16.2%, whereas their mean proportions were equal to 8.0% and 9.9% in the other shoot categories.

Starch concentration in the studied organs increased during the growing season in all the shoot categories (Figures 6 and 7) as revealed by the significant date effect according to a two-way ANOVA with shoot category and date effects for both years.

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**Table 3. SPAD values for the different shoot categories and related statistical analyses.**

<table>
<thead>
<tr>
<th>Measurement date</th>
<th>Shoot category</th>
<th>SPAD value</th>
<th>Shoot category effect</th>
<th>Correlation with photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early July 2014</td>
<td>OFF_V</td>
<td>37.0\textsuperscript{a}</td>
<td>(P = 0.004^\ast)</td>
<td>(P &lt; 0.001^{***} (+0.33))</td>
</tr>
<tr>
<td></td>
<td>RegD_V</td>
<td>44.3\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RegD_F</td>
<td>44.7\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RegS_F</td>
<td>42.9\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON_F</td>
<td>43.8\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-June 2015</td>
<td>OFF_V</td>
<td>39.7</td>
<td>(P = 0.13, \text{ns})</td>
<td>(P = 0.041^* (+0.21))</td>
</tr>
<tr>
<td></td>
<td>RegD_V</td>
<td>43.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RegD_F</td>
<td>42.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RegS_F</td>
<td>41.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON_F</td>
<td>42.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-August 2015</td>
<td>OFF_V</td>
<td>39.5\textsuperscript{a}</td>
<td>(P = 0.007^\ast)</td>
<td>(P &lt; 0.001^{***} (+0.24))</td>
</tr>
<tr>
<td></td>
<td>RegD_V</td>
<td>45.0\textsuperscript{b}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RegD_F</td>
<td>46.4\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RegS_F</td>
<td>42.3\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON_F</td>
<td>47.0\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON_abort</td>
<td>47.3\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A one-way ANOVA with shoot category effect was performed to estimate the significance of the shoot category effect. \(^{**, ***}\), significant at \(0.001 \leq P < 0.01\) and \(P < 0.001\), respectively; \(\text{ns}\), not significant. This analysis was followed by a Tukey’s HSD test for pairwise comparison. The significance of the correlation between SPAD and photosynthesis was tested with a Pearson test and the estimated value of the slope of the relationship is represented between brackets. Values followed by different letters were significantly different at \(P < 0.05\). See Figure 1 legend for shoot category description.
Furthermore, a higher starch concentration in annual and 1-year-old stem in 2015 compared with 2014 was observed if the data collected in early July 2014 and late August 2014 were compared with the data collected in mid-June 2015 and mid-August 2015, respectively. These differences were significant at 

$$P < 0.05$$

according to a two-way ANOVA with year and shoot category effects.

In leaves and during the spring–summer period in both years, OFF_V shoots had a significantly higher starch concentration (values close to 100 mg g\(^{-1}\) DM) than all the other shoot categories, which exhibited values lower than 20 mg g\(^{-1}\) DM. These differences disappeared in autumn 2014 when the only significant difference was between ON_F and RegD_F shoots. In annual and 1-year-old stem, the highest starch concentrations were observed in OFF_V shoots. RegD_V, RegD_F and RegS_F shoots had intermediate values and ON_F and ON_abort shoots displayed the lowest ones. These differences were significant at all dates except in mid-June 2015 for annual stems and in late October 2014 in 1-year-old stems.

Tree crop load significantly decreased starch concentration if all the shoot categories were considered together ($P < 0.001$, see Figure S7 available as Supplementary Data at Tree Physiology Online). Nevertheless, the local presence of fruits also tended to decrease starch concentration, at least at some dates if the shoots of different types, differing by their local fruiting context but belonging to the same tree, were compared. Starch content was significantly higher in annual stems of RegD_V shoots than Reg_F shoots in late August 2014, and in 1-year-old stems in early July 2014 and late August 2015.
A similar higher content was found in 1-year-old stems of ON_abort shoots compared with ON_F shoots in late August 2014 (Figure 6) and in annual and 1-year-old stems in mid-August 2015 (Figure 7).

**Discussion**

**Seasonal variation of carbohydrate assimilation and metabolism**

Temporal variations in photosynthesis were observed in this study with an increase between May and June in 2014 (Figure 2). This increase could be due to the fact that the first sampling was performed early in the season (early May) when leaves may have not finished their expansion and did not reach their maximal photosynthesis potential (e.g., Lakso et al. 1999).

In 2015, leaf photosynthesis tended to be slightly lower in August, probably due to differences in environmental conditions. For instance the lower radiation in August 2014 compared with June 2015 (see Table S2 available as Supplementary Data at Tree Physiology Online) could have affected photosynthesis activity even if measurements were performed in controlled conditions in the leaf chamber of the leaf gaz analyzer. Starch concentration fluctuations (Figures 6 and 7) were similar to previous observations with an increase in starch concentration in annual and 1-year-old stems from the beginning of the growing season to reach maximal values after harvest (Jordan and Habib 1996 on peach; McQueen et al. 2004 and Naschitz et al. 2010 on apple).

**Impact of the tree crop load on carbon assimilation and balance**

This study was carried out on F1 hybrids issued from a segregating population where genotype classes were chosen for representing contrasted flowering patterns. Due to our experimental design, the effect of genotypic classes strongly overlapped with the effects of the tree crop load. Crop loads reached high values for biennial genotypes in ON years (between 5 and 10 fruits cm$^{-2}$ in our experiment), medium values (between 1 and 5 fruits cm$^{-2}$) for regular synchronized and desynchronized genotypes and values close to 0 for biennial genotypes in OFF years.
Even though the genotypes were grown in an experimental orchard without usual training and thinning, these values are consistent with those observed under commercial experimental conditions (e.g., Giuliani et al. 1997).

For almost all the dates when fruits were growing on trees, starch concentration in annual and 1-year-old stems was negatively correlated with the tree crop load whatever the genotype (see Figure S7 available as Supplementary Data at Tree Physiology Online), which clearly confirms that high fruit demand induces high utilization of carbohydrate at the shoot scale (Naschitz et al. 2010). Starch accumulation in leaves was observed only under low crop load conditions as observed in Wünsche et al. (2005). This suggests a sink saturation due to low demand at the tree scale which in turn leads to the down-regulation of leaf photosynthesis (Figures 2 and 3) as previously shown in other studies (Palmer 1992, Palmer et al. 1997, Wünsche et al. 2000). Nevertheless, during summer, photosynthesis was almost similar for medium and high crop load conditions (Figures 2 and 3). This could result from the maximal photosynthesis rate being reached when sink demand was high enough to allow sugar export out of the leaves (Goldschmidt and Huber 1992, Moore et al. 1999, Franck et al. 2006).

This hypothesis is consistent with the lack of starch accumulation in leaves under medium and high crop load conditions (Figures 6 and 7).

In this study, the effect of crop load on photosynthesis activity and starch accumulation in leaf was observed early in the season just after full bloom consistently with previous experiments (Fuji and Kennedy 1985). Even if the carbon demand for fruit growth at the early stage of fruit development are quite low compared with the values observed at the end of the growth season (Delong and Grossman 1995 on peach; Reyes et al. 2016 on apple), it has been shown that the competition for carbon is relatively high at the beginning of fruit growth (Lakso 2011, Reyes et al. 2016). Nevertheless, our result suggest that the level of competition for carbohydrates is lower during the early stages of plant development than during summer since no large up-regulation of photosynthesis was observed early in the season in trees with a medium crop load (RegD and RegS; Figure 2).

At all dates, photosynthesis activity was also strongly correlated with SPAD values, with the lowest values being observed for OFF trees (Table 3). This low chlorophyll content in trees with low crop loads has been hypothesized to be associated with a stronger partitioning of nitrogen to reserve organs when fruits are not developing (Choi et al. 2010). This can also be due to the enhanced vegetative growth of OFF trees that represents a huge sink of nitrogen (Neilsen et al. 2001).

Consistent with other studies (e.g., Naschitz et al. 2010), the impact of crop load on the total soluble sugar concentration was lower than on starch. Indeed, OFF trees did not display any increase in soluble sugar content in annual or 1-year-old stems (see Figures S4 and S5 available as Supplementary Data at Tree Physiology Online). A small increase in soluble sugar was observed in leaves of OFF trees at some dates, only (Figure 4). Nevertheless, significant changes in the proportion of soluble sugars were observed, with a higher proportion of hexoses compared with sorbitol and sucrose in trees with high crop load conditions (Figure 5 and see Figure S6 available as Supplementary Data at Tree Physiology Online). This increase in the proportion of hexoses under high crop load conditions could be associated with a greater metabolic activity on these trees to sustain fruit growth.

**Within-tree variation of on carbon assimilation, carbon balance and shoot growth**

The experimental design used allowed us to compare the variation in source–sink relationships and shoot growth among different shoot types belonging to the same trees. This was performed by comparing vegetative and reproductive shoots belonging to regular ‘desynchronized’ genotypes and reproductive shoots with or without fruit within ON trees. Interestingly, no
difference in leaf photosynthesis and leaf starch concentration was observed among these shoots even though vegetative shoots were located further from fruits than reproductive shoots and whatever the presence or not of fruits on the reproductive shoots (Figures 2 and 3). This suggests the existence of sugar export from leaves at long distances from vegetative and reproductive shoots without fruit to sustain the growth of the other sinks within the tree. Such long distance allocations of carbon from parts of the trees with a low fruit load to those with high fruit load have been described in trees during the fruit growth period (Palmer et al. 1991 on apple; Walcroft et al. 2004 on peach; Chaves et al. 2012 on coffee). This main impact of the tree crop load on carbon allocation compared with local conditions or shoot type was also confirmed by the high correlation between starch concentration and crop load in annual and 1-year-old stems whatever the shoot type (vegetative or reproductive; see Figure S7 available as Supplementary Data at Tree Physiology Online). The assumption of a mobile pool of carbohydrates within the tree to sustain the carbohydrate demand of the shoots with a low supply/demand ratio due to fruit growth has been already proposed in apple tree (Palmer et al. 1991). Nevertheless, it could be hypothesized that this mobile pool of carbon is not enough for supplying the potential demand of all the sinks and that a part of organ growth variation within the tree could be related to local source–sink imbalances. The existence of such a variation in organ growth associated with local variations in the source–sink balance has been suggested in modeling studies on fruit trees (Lescourret et al. 2011, Pallas et al. 2016b).

Some impact of the local presence of the fruit demand were observed on starch concentration in annual and 1-year-old stems (Figures 6 and 7), since differences between the shoots bearing fruits or not within the same tree (ON_abort vs ON_F and RegD_V vs RegD_F) were significant at some dates. This reveals that the common assimilate pool for carbon at the plant scale (Heuvelink 1995) is not fully relevant for describing the carbon fluxes within the tree. In this study, shoot growth depended on its type (vegetative or reproductive) since fruited or non-fruited reproductive shoots had a lower leaf number than vegetative shoots (Figure 1). This result was observed for reproductive and vegetative shoots belonging to trees with similar crop load (RegD_V vs RegD_F and ON_F vs ON_abort). A lower growth for reproductive shoots than for vegetative ones has been already observed in apple tree (Massonnet 2004, Willaume et al. 2004) and could be associated with the presence of fruits on reproductive shoots that likely reduces the amount of carbon available for growth. In our experiment, this lower growth for reproductive shoots was also observed on ON_abort shoots (reproductive shoots without fruit). This result remains consistent with the hypothesis of a local competition between fruits and shoots, since fruit drop in apple can occur during a long period ranging from 2 weeks after bloom to mid-June (Quinlan and Preston 1971) and reproductive shoots without harvested fruit could have grown during a long period in the presence of fruits. Moreover, the higher number of preformed leaves in vegetative buds compared with reproductive ones (Costes 2003, Lauri et al. 2008) could provide more leaf area and therefore carbohydrates for growth during the early stages of shoot development with possible consequences on the shoot neoformation processes. Another hypothesis for explaining this low growth of reproductive shoots could be related to the sympodial nature of reproductive shoots that could hamper carbon and water transport towards the apex.

**Relationships between genotypic bearing pattern, carbon balance and floral induction**

Starch concentration was strongly decreased in all the studied organs of biennial genotypes in OFF trees compared with biennial ones in ON years at the time of FL (early July 2014 or mid-June 2015, Foster et al. 2003). If we consider starch concentration as a good indicator of the organ carbohydrate availability, this was consistent with the previous results of Guitton et al. (2016), who showed a differential expression of genes related to carbon starvation between the meristems of OFF and ON trees. This starvation of carbon in meristems was hypothesized to be unfavorable for FL due to the strong carbohydrate requirement for cell differentiation during FL (Eveland and Jackson 2012). The analysis of variations in the carbon balance between vegetative and reproductive shoot was of particular interest within regular desynchronized genotypes the axes of which exhibit alternation in FL between consecutive years. Such a behavior suggests a local (at the shoot scale) determination of FL and is consistent with the higher starch concentration found at some dates in vegetative shoots (RegD_V) that are floral induced compared with reproductive ones (RegD_F), whose terminal meristem is likely to remain not induced.

Moreover, the genotypes with regular synchronized bearing patterns displayed lower crop loads than ON trees, probably due to a low fruit set. This could explain the high ability of these regular synchronized genotypes to initiate flowers each year. Nevertheless, the reproductive shoots of the regular synchronized genotypes (RegS_F) with a high FL rate displayed similar starch concentration to reproductive shoots of the regular desynchronized genotypes (RegD_F), which are not expected to be floral induced. This probably shows that carbon availability in the vegetative organs is not the only determinant of FL in the terminal meristem.

Other studies suggest that the presence of the fruit itself could have a role independently from the carbon source–sink relations. Indeed seeds secrete large amounts of hormones, and previous studies have shown that auxins and gibberellins may act together or independently to inhibit FL (Hanke et al. 2007). In particular, gibberellins are produced by seeds and could be transported to apical buds where FL occurs or could enhance polar auxin transport to
inhibit FI (Bangerth 2009). However, because fruits are simultaneously sinks for carbon and sources of hormones that are possible inhibiting signals, the analyses of their role remains complex and uncertain. Further investigations of carbohydrates and hormones availability and local specialization of the FI signal within the meristematic tissues themselves could provide further comprehension of FI. Indeed, the central zone where FI is triggered is tightly regulated and maintained in very isolated conditions (Lyndon and Battey 1985).

Conclusions
This study confirms that the tree crop load affects photosynthesis and NSC accumulation in vegetative organs. The within-tree variability of these processes depending on the presence of fruits is quite low and was observed for starch content, only. Conversely, shoot growth strongly depends on its vegetative or reproductive type. The differences of starch content in the vegetative organs partly matches with the genotypic bearing patterns. Nevertheless, the medium level of starch content in regular synchronized genotypes suggests that carbon availability in the shoots is not the only driver of FI and that hormonal signaling may have a role. For disentangling the intertwined relationships between hormone production and carbon source–sink relationships, functional structural modeling approaches could be a promising tool to test hypotheses by simulating the transport of both carbohydrates and hormones within a network of organs mixing sources and sinks (leaves and seeds producing carbohydrates and hormones, respectively) with target organs (terminal meristems) in which flower induction may occur (Pellerin et al. 2012, Pallas et al. 2016d).

Supplementary Data
Supplementary Data for this article are available at Tree Physiology Online.

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Conflict of interest
None declared.

References
Genotypic variability in apple source–sink relations


