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Fluctuations in sugar content are not determinant in explaining variations in vitamin C in tomato fruit

Capucine Massot¹, Michel Génard¹, Rebecca Stevens², Hélène Gautier^{1*}

¹ INRA UR 1115 Plantes et Systèmes de Culture Horticoles, Domaine St Paul, Site Agroparc, F-84914 Avignon, France

² INRA UR 1052 Génétique et Amélioration des Fruits et Légumes, Domaine St Maurice, F-84143 Montfavet, France

Hélène Gautier

Fax: +33 (0)4 32 72 22 80

E-mail: helene.gautier@avignon.inra.fr

* Corresponding author

Abstract

The present study aimed to clarify the relationship between sugars and vitamin C in fruit. The objective was to determine whether vitamin C content was regulated by sugar content due to the role of sugar as a precursor for vitamin C. During summer, maximal content in sugar and vitamin C were found in both genotypes tested *Solanum lycopersicum* ‘Cervil’ and ‘Levovil’. During autumn, fruit pruning increased fruit size and hexose content but fruit vitamin C content did not increase. Therefore sugar substrate was not limiting for vitamin C synthesis during autumn. We demonstrated for two cultivars, ‘Cervil’ and ‘Levovil’, with different sugar accumulation profiles during ripening, that sugar content was not determinant in the regulation of vitamin C content. The strong correlation observed between sugars and vitamin C in ‘Cervil’ was due to their concomitant increase during fruit ripening.

Keywords: fruit load, fruit quality, *Solanum lycopersicum*, sugars, tomato, vitamin C.

Abbreviations

D1: first period of fruit development from 0 to 29 Days after pollination for ‘Cervil’ and from 0 to 39 Days after pollination for ‘Levovil’

D2: second period of fruit development from 30 to 60 Days after pollination for ‘Cervil’ and from 40 to 67 Days after pollination for ‘Levovil’

DAP: days after pollination

FW: fresh weight

HL: high fruit load

LL: low fruit load

Introduction

Tomato fruit are produced year-round and their frequent and high levels of consumption mean they are an important source of antioxidant micronutrients and especially vitamin C for the human diet. Besides the well known role of vitamin C in preventing scurvy, recent studies have shown that vitamin C is involved in preventing various oxidative stress-related illnesses such as cancers, cardio-vascular diseases and aging [1-3]. However, vitamin C content in tomato fruit varies all year round [4] and depends on environmental factors, agricultural techniques and fruit ripening [5, 6]. Seasonal variations in vitamin C levels have been described in relation to light intensity variations and sugar content [4, 5, 7]. Murneek et al. [4] reported that fruit harvested during summer contained from 8 to 50% more vitamin C compared to fruit harvested during early spring. Moreover, Mc Collum et al. [7] showed that fruit exposed to light contained about 15% more reducing sugars and 35% more vitamin C compared to shaded fruit. Similarly, glasshouse-grown tomatoes are usually found to have lower levels of sugars and vitamin C than those grown outdoors, probably due to lower light intensities [6, 8]. Increased light can promote vitamin C content via increased sugar accumulation triggering increased vitamin C synthesis. Indeed, correlations between sugar and vitamin C content have been well described in fruit [7, 9, 10]. This correlation between vitamin C and sugar contents could be linked to the role of sugars as a substrate for vitamin C biosynthesis [11], but also to the well known role of sugars acting as a signal that promotes gene expression [12, 13]. In fact, increasing sucrose content has been shown to enhance expression of genes of the ascorbic acid biosynthesis pathway in broccoli [14]. Besides, Stevens *et al.* [9] showed that this relationship, between sugar and vitamin C contents, can be influenced by genetic factors, as for two populations out of three, low or no significant correlation was found ($r < 0.36$). Light can also have a direct effect on fruit vitamin C content

by promoting vitamin C biosynthesis through increased gene expression. Such an activation by light was reported in leaves of *Arabidopsis* by Tamaoki et al. [15] for the L-galactono- γ -lactone dehydrogenase gene which controls the final step of vitamin C biosynthesis from L-galactono-1,4-lactone. In the present work, we tested whether sugar content could regulate vitamin C content by studying the impact of increased sugar content on vitamin C content. In a first experiment, plants were grown outdoors during summer time in order to maximize fruit vitamin C content during fruit ripening. Then, in another experiment, plants were grown during autumn in a greenhouse to obtain fruit with low sugar and vitamin C content, and fruits from half of the plants were pruned in order to increase sugar content and follow the impact on vitamin C.

Material and Methods

The study was performed using *Solanum lycopersicum* cv. ‘Cervil’ a cherry tomato with high sugar and vitamin C contents and *Solanum lycopersicum* cv. ‘Levovil’ an intermediate sized fruit tomato with low sugar and vitamin C contents [10, 16].

Field experiment during summer

Plant growth. Plants were grown in a field in Avignon (Southern France, 44°N). On 21 March 2008, seeds were sown. On 22 April 2008 seedlings were planted in a field. Nutrient supply, chemical pest and disease control followed commercial practices. Plants were stopped above the second truss in ‘Levovil’ and the fourth truss in ‘Cervil’ to ensure good light interception for plant photosynthesis.

Load treatments. Trusses were not pruned leading to a high fruit load per plant.

Fruit sampling. Fruit were harvested in the morning between 9am and 11am on 26 June 2008 for 'Cervil' and 10 July 2008 for 'Levovil'. Three ripening stages were collected: mature green stage (corresponding to 34 days after pollination (DAP) for 'Cervil' and 40 DAP for 'Levovil'); orange stage (43 DAP for 'Cervil' and 42 DAP for 'Levovil') and red ripe stage (40 DAP for 'Cervil' and 51 DAP for 'Levovil') as it was reported that ripening stage greatly influence vitamin C and sugar content [8]. Following harvest, equatorial fruit diameter, fruit fresh weight and external fruit color near the pistil scar were measured using an electronic digital caliper (Codium Scientific), a balance and a Minolta chromameter (CR 300, Minolta, France SA) using the LAB colour space (Hunter colour coordinates L, a and b; L=lightness ranging from 0 for dark to 100 for light, a ranging from green to red, b ranging from blue to yellow), respectively. Fruit were partitioned into five replicates per treatment each replicate corresponds to a sample of three tomatoes for 'Levovil' and five for 'Cervil'. Fruits were then frozen in liquid nitrogen and store at -80°C before grinding to powder in liquid nitrogen.

Greenhouse experiment during autumn

Plant growth. Plants were grown in a glasshouse at Ctifl (Centre Technique Interprofessionnel des Fruits et Légumes) Bellegarde (southern France, 43.75°N, 4.5°E). On 30 July 2007, seeds were sown. Seedlings were transplanted into rock wool cubes. On 30 August 2007, they were planted on coco slabs (Grodan Master (dimension 100x15x25cm) Grodan BV, Netherlands) at a density of 2.5 plants m⁻² in two modules of 250m² of a multispan Venlo-type glasshouse orientated north-south. Nutrient supply, chemical pest and disease control followed commercial practices. Water was supplied to the plants according to the potential evapotranspiration in order to maintain 20–30% drainage. Flowers were open-pollinated by bumble bees and all side shoots were removed as they appeared. Every fortnight, old leaves were removed up to the youngest turning truss.

Load treatments. In the first module, plants were conducted in low fruit load (LL). So, the inflorescences were pruned, after the anthesis of each flower, to five flowers per truss in ‘Cervil’ and to two flowers per truss in ‘Levovil’. In the second module, plants were conducted in high fruit load (HL), twenty flowers per truss were kept for ‘Cervil’ and five for ‘Levovil’.

Fruit sampling. From 20 October to 19 December, once a week, fruits were harvested in the morning between 9am and 11am on truss n°7 for ‘Cervil’ and truss n°3 for ‘Levovil’ according to their stage of development expressed in days after pollination (DAP). Only the two first fruit of ‘Levovil’ were harvested (5 first fruit of ‘Cervil’) whatever the fruit load treatment. Consequently a total of 88 fruit for LL (130 for HL) were harvested and analysed in ‘Levovil’ (615 fruit for LL and 433 fruit for HL in ‘Cervil’). Following harvest, the same fruit characterization was carried out as previously described for field experiment. Fruits were then frozen in liquid nitrogen for ‘Cervil’ or cut into quarters and the two opposite quarters frozen in liquid nitrogen for ‘Levovil’ and stored at -80°C before grinding to powder in liquid nitrogen (Retsch mixer mills, MM301, Eragny sur Oise, France).

Fruit analyses.

Assays of total, reduced and oxidized vitamin C content were carried out as previously described by Stevens et al. [17] on ground powder conserved at -80°C. Briefly, 0.5 to 1g of powder was homogenized with 600µL of ice cold 6% trichloroacetic acid (TCA). Samples were centrifuged for 15 min at 16,000g at 4°C. 20µL of supernatant were used in each assay. Two assays were carried out on each sample, one to measure the total vitamin C (including addition of 5mM dithiothreitol (DTT)) and one to quantify the reduced vitamin C content (omission of DTT from the assay). So, 20µL of each sample (or standard) were distributed

into two wells for two repetitions) of a 96-well microplate and mixed with 20 μ L of 5 mM DTT (total ascorbic acid assay) or 0.4 M phosphate buffer (pH 7.4, reduced ascorbic acid assay). The plate was incubated at 37°C for 20 minutes. Then 10 μ L of N-ethyl maleimide (total ascorbic acid assay) or of 0.4 M phosphate buffer (pH 7.4, reduced ascorbic acid assay) were added and mixed followed by the addition of 80 μ L of color reagent [17]. After incubation at 37°C for 50 min, the absorbance was read at 550nm using the multiscan Ascent MP reader (Labsystems, Thermo Fisher Scientific, Courtaboeuf, France). The standard curve obtained from the standard solution values allowed calculation of the vitamin C concentration of the samples after correction for the quantity of water introduced by the tomato sample.

Sugars were extracted as described in Gomez et al. [18]. Briefly, the soluble sugars were extracted at 4°C from 10 mg of freeze-dried fruit powder. Then 1mL of methanol/water solution (1:1, v/v) was added and 0.3mL of chloroform. Samples were shaken for 30 min at 4°C and centrifuged (5min at 16,000g at 4°C). A 0.8mL of methanol/water supernatant was recovered, evaporated under vacuum (Speed Vac). Then, for measurement of soluble sugars, samples were homogenized with 1.6mL of water at 4°C for 10 min before adding 10 mg of PVPP (polyvinylpolypyrrolidone). After 30 min at 4°C, PVPP and phenolics were removed by centrifugation (10 min at 16,000g at 4°C) and the supernatant was purified and filtered using a C18 cartridge and a 0.2 μ m filter. Finally, the soluble sugars (glucose, fructose and sucrose) were assayed by HPLC. For starch measurement, 1mL of methanol was added to the tube containing chloroform and fruit powder, and the tube was shaken for 20 min before centrifugation (5 min at 16,000g at 4°C). The supernatant was discarded and the pellet was used for starch assay. Starch was dispersed by autoclaving for 1h (120°C) and then hydrolyzed for 1.5h at 56°C by addition of amydoglucosidase solution (concentration 1g/L). The glucose released by starch hydrolysis was measured as described previously by Gomez et

al. [19] using 150 μ L of diluted extract, 100 μ L of a solution containing ATP, NAD and 20 μ L of solution containing glucose-6-phosphate dehydrogenase and hexokinase.

Data analyses.

Fruit traits measured during the greenhouse experiment in autumn were expressed versus time (in day after pollination, DAP). As fruit development was quicker in the field experiment during summer, field data were positioned on the same graph according to their external fruit coloration (a/b) in order to compare fruit traits at similar ripening stage (similar a/b ratio). Indeed, this ratio was previously reported to be linearly related to fruit stage of development [20]. Analyses of variance considering two factors 'fruit load' and fruit age expressed in 'days after pollination' and their interaction were performed on data from autumn experiment. For data collected in summer, only the factor 'fruit age' has been considered. Significant differences among treatments were assessed using a Tukey test at 5 %. A linear model was fitted to data describing fruit content in vitamin C =Y and hexose=X with fruit load treatment as a dummy variable (D) in autumn ($Y = b_0 + b_1 * X + b_2 D + b_3 * X * D + E$ where E is the error term) during ripening. The hypotheses of coincidence, parallelism and equality of intercepts for the linear regressions, obtained for the two load treatments, were tested using this model as described by Wonnacott and Wonnacott [21]. The same test was used with the dummy variable season, to compare linear regression for fruit harvested in high fruit load in autumn and summer during ripening. All statistical analyses were performed using R software (R development Core team, <http://www.R-project.org>).

Results

In summer, under HL, ‘Cervil’ fruit weight was about 5g and ‘Levovil’ about 120g, similar to those obtained in autumn under HL. Reducing fruit load increased fruit weight for both genotypes (*figure 1*). This was likely to be due to a limiting carbon and water supply under HL compared to LL. Moreover, in ‘Cervil’ and ‘Levovil’, reducing fruit load increased dry matter (data not shown). Fruit dry matter under high fruit load were similar in summer and autumn and were lower to the one obtained in autumn under low fruit load, indicating the major role of fruit load to modulate fruit dry matter accumulation within the fruit.

In ‘Cervil’, starch was very high in young fruit and decreased during ripening (*figure 2A*). In contrast, sucrose and hexoses strongly accumulated during fruit development, especially hexoses from 30 to 60 DAP (*figure 2B and C*). In ‘Levovil’, starch also decreased during fruit development. Other sugars showed different pattern compared to ‘Cervil’: sucrose decreased during development and hexoses only slightly accumulated mostly from 6 to 18 DAP (*figure 3*). Moreover starch, sucrose and hexose contents were two to three times lower in ‘Levovil’ than in ‘Cervil’.

Reducing fruit load in autumn increased starch content and soluble sugars in ‘Cervil’ (+40% for sucrose and +30% for hexoses, *figure 2*). In ‘Levovil’, reducing fruit load did not significantly modify starch or sucrose content, but hexose content increased (*figure 3*). In summer, ‘Cervil’ had lower starch content and slightly higher soluble sugar content compared to autumn in high fruit load. Furthermore, sucrose and hexose contents were also higher during summer in ‘Levovil’ (+40%) although starch content was not greatly modified. We have therefore illustrated that ‘Cervil’ and ‘Levovil’ have different sugar variations during fruit ripening and in response to fruit load or season. Moreover, as expected, reducing fruit load led to increased hexose content for both genotypes and increased sucrose content in

‘Cervil’ only. In ‘Cervil’, reducing fruit load led to similar or even higher soluble sugar contents compared to those in summer. In contrast, in ‘Levovil’, soluble sugars under low fruit load did not reach levels observed in summer.

Vitamin C content varied during fruit development but was different between ‘Levovil’ and ‘Cervil’. ‘Cervil’ fruit contained high vitamin C at the beginning of fruit development which slightly decreased first (from 6 to 40 DAP) and strongly increased during ripening to reach around 500 mg/kg FW at the end of maturation (from 42 to 60 DAP, *figure 4 A*). ‘Levovil’ fruit contained the highest vitamin C at the beginning of fruit development that decreased and stabilized during fruit ripening at around 200mg/kg FW (*figure 4 B*). Thus, despite the fact that ‘Levovil’ fruit had higher vitamin C content compared to ‘Cervil’ in younger fruit, at harvest, fruit vitamin C content in ‘Cervil’ was more than twice the one in ‘Levovil’. Both genotypes showed no significant difference in vitamin C between HL and LL (*figure 4*). In summer, fruit vitamin C contents were about 20% higher than those in autumn for both genotypes whatever the fruit load applied.

The relationship between vitamin C and the different sugars (sucrose, hexoses and starch) expressed per fresh weight in ‘Cervil’ and ‘Levovil’ during autumn and summer experiments are presented in table I. In ‘Cervil’, whatever the season and the fruit load, a similar trend was found: vitamin C content was always positively correlated to hexose and total soluble sugar content and negatively to starch content. No clear correlation was found between vitamin C and sucrose content. In ‘Levovil’, opposite correlations were found between vitamin C and hexoses or total soluble sugars depending on the season. Both starch and sucrose were positively correlated to vitamin C in autumn but no significant correlation could be found in summer.

The linear relationships between hexose and vitamin C contents in summer and in autumn was due to the concomitant increase in vitamin C and hexose contents during the

second period of fruit development (D2 from 30 to 60 DAA), which corresponds to the end of fruit growth and fruit ripening (*figure 5 A*). That is the reason why we further considered the linear regression on data collected during the second period of fruit development (D2). As ‘Levovil’ did not accumulate vitamin C and hexoses during fruit ripening, no positive relation was found (*figure 5 B*). Despite close or even higher soluble sugar contents in autumn under low fruit load compared to summer, fruit vitamin C content remained lower during autumn in both genotypes. The regression slopes between sugars and vitamin C were equal (between seasons) but the intercept significantly increased during summer ($p=0.002$) due to higher vitamin C levels in summer for the same sugar content. Moreover the regression slopes were significantly different for the two fruit load treatments in autumn ($P=0.003$), indicating that this correlation between hexoses and vitamin C contents was fruit load dependent. So, the positive linear correlation between vitamin C and sugar contents, in ‘Cervil’, was due to their concomitant increase during fruit maturation rather than to substrate dependence.

Discussion

We shown that ‘Levovil’ and ‘Cervil’ differ in vitamin C and hexose accumulation. Cervil’s fruit accumulated vitamin C and hexoses mostly during their second period of development. In contrast Levovil’s fruit contained high vitamin C at an early stage of fruit development which rapidly decreased during fruit growth and slightly increased during ripening. Moreover, hexoses mostly accumulated at the beginning of fruit development from 6 to 18 DAP in ‘Levovil’ and remained stable thereafter. This resulted in large differences in vitamin C and hexose contents in red-ripe fruit between ‘Cervil’ and ‘Levovil’. Cherry tomato ‘Cervil’ contained more sugars and vitamin C expressed per fresh weight than the medium

sized fruit tomato 'Levovil'. When expressed by dry weight, the differences in sugar and vitamin C content between genotypes remained the same so that they were unlikely to be due to an increase in water influx during the second period of fruit development in the bigger fruit (data not shown). This negative relationship between fruit size and sugar and vitamin C content was previously reported by Navez et al. when comparing 16 cultivated genotypes harvested at red-ripe stage [22]. Moreover, Zanor et al. [23] also found that Levovil's fruit contained lower hexoses compared to Cervil's. These observations are in agreement with the fact that large-fruited lines are generally considered to have inferior taste due to their lower sugar content [10, 16].

For both genotypes, increasing hexose content in autumn by reducing fruit load did not lead to increased vitamin C content. Vitamin C biosynthesis starts from hexoses, so we could have expected a substrate-dependent synthesis of vitamin C. Indeed, vitamin C and sugar contents in fruit or leaves often follow similar variations [7, 24]. Mc Collum (1946) showed that unshaded fruit had higher soluble sugars and ascorbic acid contents than shaded fruit [7]. Positive correlations between these two components have previously been described in both organs [9, 24]. In addition to being a substrate for vitamin C synthesis, sugars, especially sucrose, can also play an important signaling role, regulating gene expression of enzymes related to vitamin C metabolism [12, 14]. Indeed, Nishikawa et al. [14] observed an up-regulation of genes related to vitamin C biosynthesis and metabolism in chloroplasts by sucrose feeding of broccoli plants. However, we know that sugar content is not the only factor that might regulate vitamin C in tomato fruit. A major factor influencing vitamin C content in fruit and leaves is light [4, 5, 25, 26]. Many studies have reported a positive relationship between light, sugar and vitamin C contents [7, 14]. Gautier et al. [27] supposed that light affects vitamin C content in fruit either indirectly via sugar synthesis or directly. Nevertheless, the role of light received directly by fruit seemed to be predominant in the

absence of substrate limitation [27]. This was confirmed by the present experiment as the increase in hexose and sucrose contents did not trigger any increase in fruit vitamin C content in autumn. As previously shown in other non photosynthetic tissues, fruit vitamin C regulation did not seem to be tightly linked to primary metabolism and sugar content [28-30]. Moreover, maximal vitamin C contents were obtained during summer in field experiments. We showed that, outdoors in summer, tomato fruit could contain about 20% more vitamin C than in autumn under glasshouses. However, such vitamin C contents were not reached in autumn even when sugar content was increased to the same level as the one in summer especially for ‘Cervil’. These results underlined that substrate was not the limiting factor for vitamin C accumulation in fruit. The difference in light intensity between outside in summer and under glasshouses in autumn may explain differences in vitamin C content [7, 26, 31]. Indeed, increasing light was reported to increase vitamin C biosynthesis in leaves and fruit via increased gene expression or enzyme activities of the vitamin C biosynthetic pathway [15, 27, 32]. We confirmed in this study that environmental conditions are important factors regulating fruit vitamin C content [27].

Finally, the strong positive linear correlation described here for ‘Cervil’ between total hexoses and vitamin C was due to their concomitant increase during fruit maturation. Indeed, we found no positive relationship for the medium sized tomato ‘Levovil’ because contrary to ‘Cervil’, hexoses and vitamin C did not accumulate during maturation. Differences between genotypes for sugar and vitamin C correlations were also found by Stevens et al. [9]: only the population with high vitamin C content showed correlation with sugars.

In conclusion, we have showed that reducing fruit load in autumn increased fruit size and improved fruit quality due to increased dry matter and sugar contents in fruit. As fruit quality varies during the year, this cultural practice can help producers to improve it in winter when quality deteriorates because of a large accumulation of water in fruit and low assimilate

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supply in this period. However this improvement in gustative quality is not linked with an improvement of nutritional quality, as measured by ascorbic acid content, despite the decrease of crop yield. This might be explained by the important role of light on vitamin C regulation in the absence of substrate limitation.

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Figure captions

Figure 1: Mean fruit weight variations during fruit development in summer under high fruit load (-▲-) or in autumn under high fruit load (-●- HL) and low fruit load (···○··· LL) for fruit of 'Cervil'(A) and 'Levovil'(B). Data are means of 2 or 3 samples, a sample corresponding to 2 fruit for 'Levovil' and 5 for 'Cervil'. P-values of the anova considering the factors fruit age (P_{age}), fruit load (P_{load}) and their interaction (P_{A*L}) were given in the tables.

Figure 2: Sucrose (A), hexose (B) and starch (C) content during fruit development of 'Cervil' fruit in summer under high fruit load (-▲-) or in autumn under high fruit load (-●- HL) and low fruit load (···○···LL). Legend similar to Figure 1.

Figure 3: Sucrose (A), hexose (B) and starch (C) content during fruit development of 'Levovil' fruit in summer under high fruit load (-▲-) or in autumn under high fruit load (-●- HL) and low fruit load (···○···LL). Legend similar to Figure 1.

Figure 4: Total vitamin C in 'Cervil'(A) and 'Levovil' (B) content during fruit development in summer under high fruit load (-▲-) or in autumn under high fruit load (-●- HL) and low fruit load (···○···LL). Legend similar to Figure 1.

Figure 5: Relationship between vitamin C and hexose contents during fruit development in summer and autumn for 'Cervil' (A) or 'Levovil' (B). Fruit cultivated in autumn were separated in D1 LL (○): first period of fruit development (0 to 29 days after pollination for 'Cervil') under low fruit load; D2LL (□): second period of development (30 to 60 days after pollination for 'Cervil') under low fruit load; D1HL (●): first period of development under high fruit load; D2HL (■): second period of development under high fruit load. In summer

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fruit was harvested during the second period of development (D2SUM *). The linear regressions were calculated on data obtained during the second period of development (D2). The equation of linear regression for 'Cervil', in summer, is $Y=6.8X+308$ ($R^2=0.59$). In autumn, in high fruit load (D1HL+D2HL) the equation of the linear regression is $Y=5.3X+187$ ($R^2=0.8$) and in low fruit load (D1LL+D2LL), $Y=8.5X+152$ ($R^2=0.93$).

Table

Table I: Correlations between total vitamin C and sugars (sucrose, hexoses, starch or total soluble sugars), expressed per fresh weight during fruit development. Data are presented for autumn experiment in high and low fruit load in ‘Cervil’ and ‘Levovil’ and for summer experiment in high fruit load. Significant correlations are in bold.

		CERVIL				LEVOVIL				
		Sucrose	Hexoses	Starch	Total soluble sugars	Sucrose	Hexoses	Starch	Total soluble sugars	
Autumn	High load	Vitamin C	0.09	0.66	-0.41	0.67	0.76	-0.35	0.69	-0.30
	Low load	Vitamin C	0.20	0.66	-0.52	0.64	0.55	-0.50	0.50	-0.48
Summer	High load	Vitamin C	0.33	0.77	-0.91	0.76	0.39	0.51	-0.01	0.54

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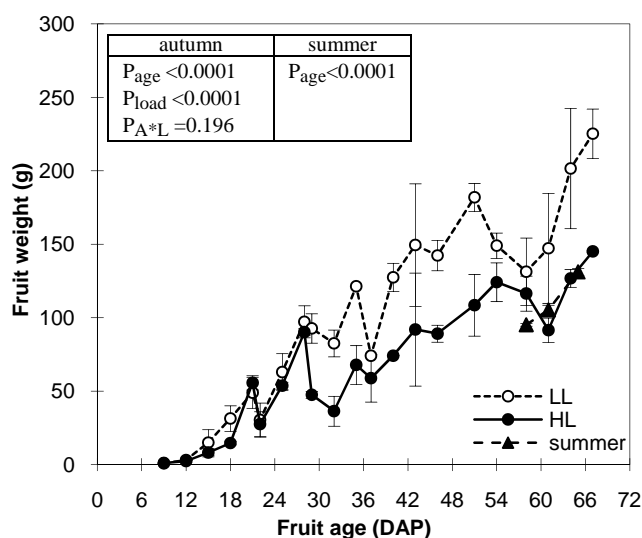
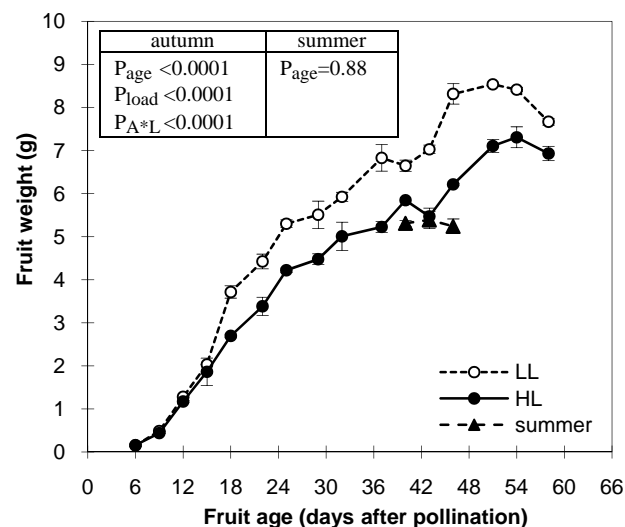


Figure 1: Mean fruit weight variations (\pm se) during fruit development in summer under high fruit load (\blacktriangle) or in autumn under high fruit load (\bullet HL) and low fruit load (\circ LL) for fruit of ‘Cervil’(A) and ‘Levovil’(B). Data are means of 2 or 3 samples, a sample corresponding to 2 fruit for ‘Levovil’ and 5 for ‘Cervil’. P-values of the anova considering the factors fruit age (P_{age}), fruit load (P_{load}) and their interaction (P_{A*L}) were given in the tables.

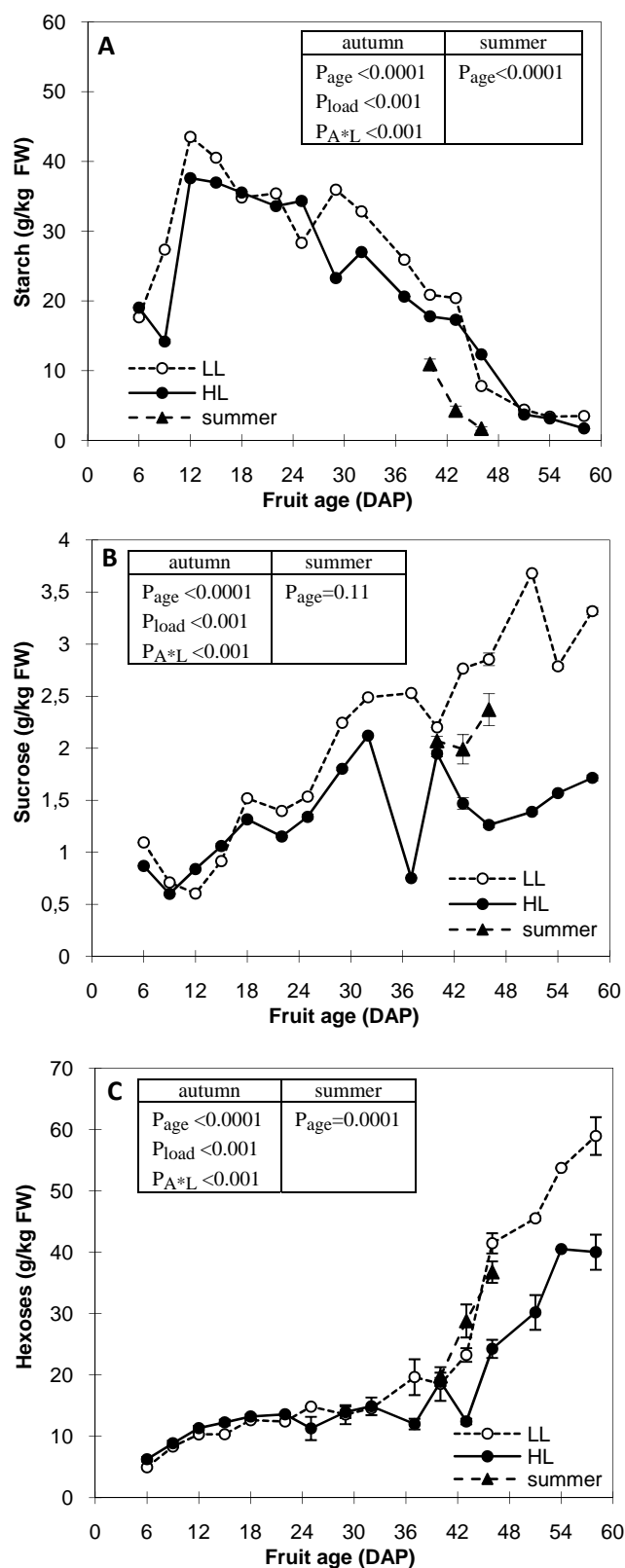


Figure 2: Sucrose (A), hexose (B) and starch (C) content (\pm se) during fruit development of ‘Cervil’ fruit in summer under high fruit load (--▲--) or in autumn under high fruit load (-●-) HL) and low fruit load (····LL). Legend similar to Figure 1.

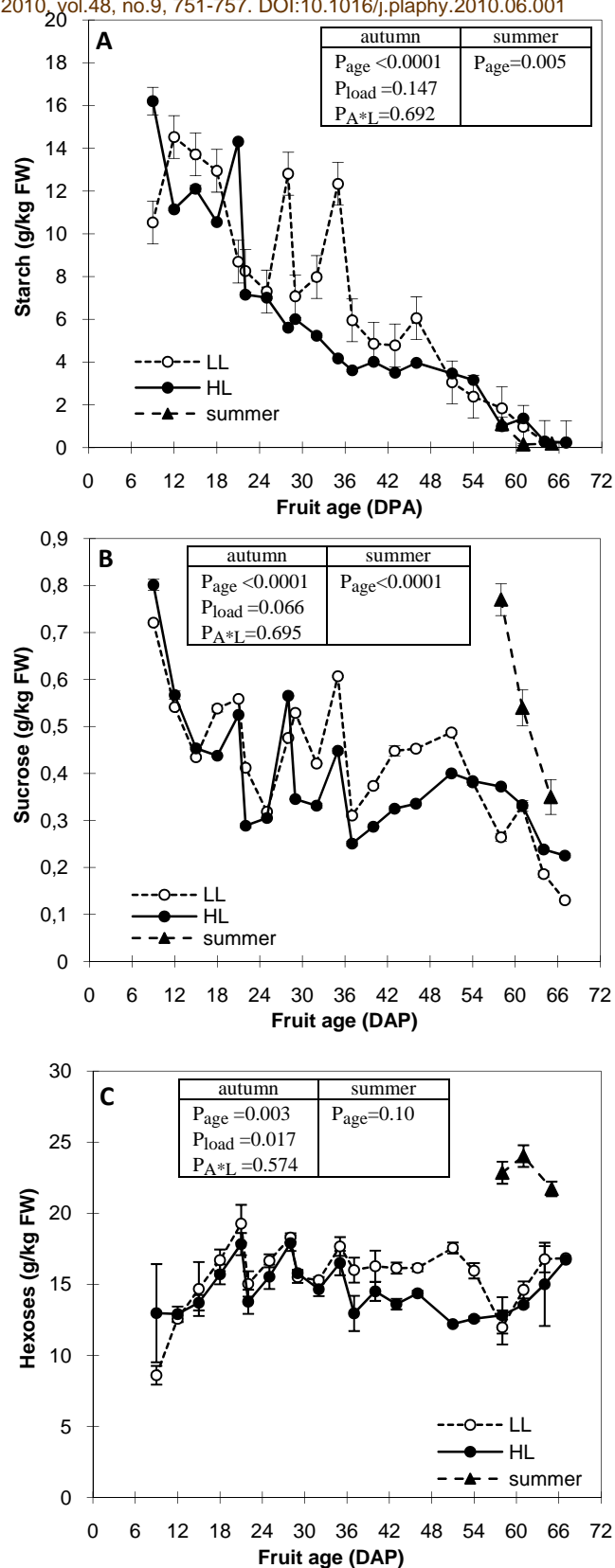


Figure 3: Sucrose (A), hexose (B) and starch (C) content (\pm se) during fruit development of 'Levovil' fruit in summer under high fruit load (-▲-) or in autumn under high fruit load (-●-HL) and low fruit load (-○-LL). Legend similar to Figure 1.

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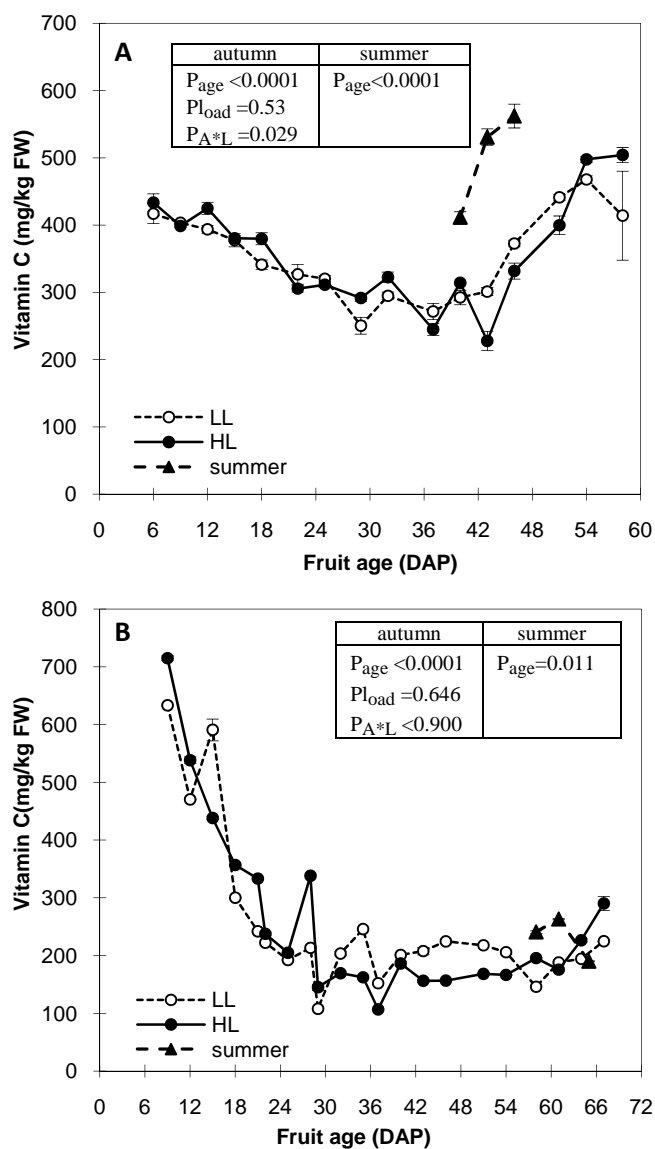


Figure 4: Total vitamin C in ‘Cervil’(A) and ‘Levovil’ (B) content (\pm se) during fruit development in summer under high fruit load (--▲--) or in autumn under high fruit load (-●- HL) and low fruit load (-○- LL). Legend similar to Figure 1.

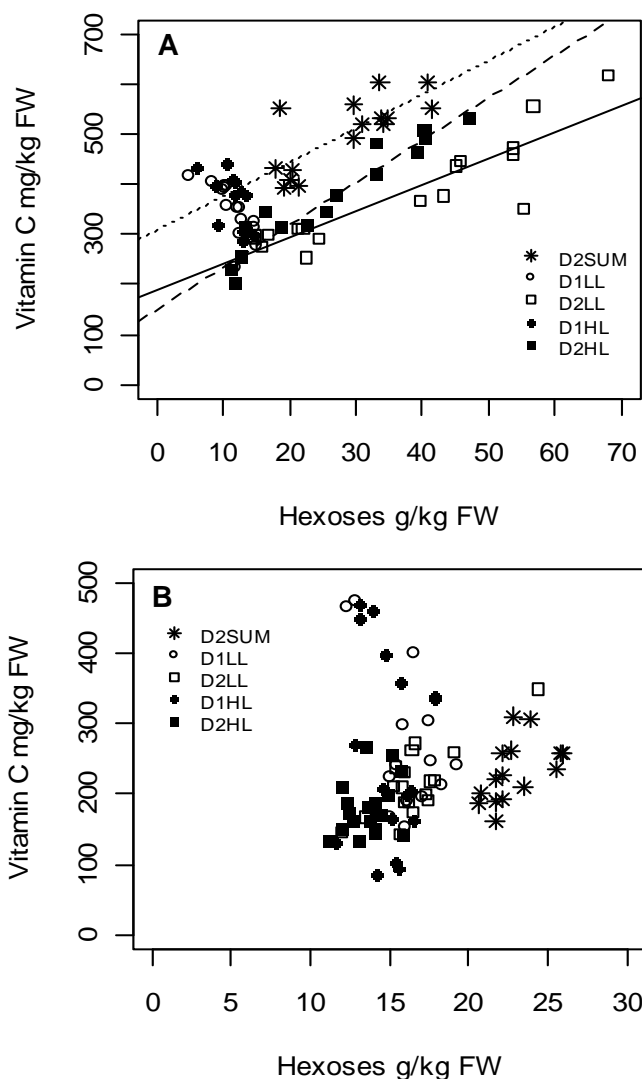


Figure 5: Relationship between total vitamin C and hexose contents during fruit development in summer and autumn for ‘Cervil’ (A) or ‘Levovil’ (B). Fruit cultivated in autumn were separated in D1 LL (○): first period of fruit development (0 to 29 days after pollination for ‘Cervil’) under low fruit load; D2LL (□): second period of development (30 to 60 days after pollination for ‘Cervil’) under low fruit load; D1HL (●): first period of development under high fruit load; D2HL (■): second period of development under high fruit load. In summer fruit was harvested during the second period of development (D2SUM *). The linear regressions were calculated on data obtained during the second period of development (D2). The equation of linear regression for ‘Cervil’, in summer, is $Y=6.8X+308$ ($R^2=0.59$). In autumn, in high fruit load (D1HL+D2HL) the equation of the linear regression is $Y=5.3X+187$ ($R^2=0.8$) and in low fruit load (D1LL+D2LL), $Y=8.5X+152$ ($R^2=0.93$).