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Water scarcity conditions affect peach fruit size and polyphenol contents more severely than other fruit quality traits

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Abstract

BACKGROUND: The literature abounds with the impacts of drought conditions on the concentration of non-structural compounds (NSC) in peach fruits without distinction as to the direct effect of drought on fruit metabolism and its indirect effect through dilution. Moreover, there is a need to investigate the sensitivity of the fruit composition to progressive water deficit in semi-arid conditions, as well as the origin of variations in fruit composition – not only in carbohydrates and organic acids, but also in secondary metabolites such as polyphenols.

RESULTS: The increase in stress intensity resulted in smaller fruits and a reduction in yield. Drought increased fruit dry matter content, structural dry matter (SDM) content and firmness due to lower water import to fruits, although drought reduced fruit surface conductance and its transpiration. Drought significantly affected the concentrations of each NSC either through the decrease in dilution and/or modifications of their metabolism. The increase in hexoses and sorbitol concentrations of fruits grown under drought conditions resulted in an increase in the sweetness index but not near harvest. Malic acid concentration and content:SDM ratio increased as drought intensified, whereas those of citric and quinic acids decreased. Polyphenol concentration and content increased under severe drought.

CONCLUSION: The increase in stress intensity strongly affected fruit mass. The concentration of total carbohydrates and organic acid at harvest increased mainly through a decrease in fruit dilution, whereas the concentrations of polyphenols were also strongly affected through an impact on their metabolism.

Keywords: *Prunus persica* L; drought; fruit surface conductance; carbohydrates; organic acid; structural material; sweetness index

INTRODUCTION

Fruit size is a major criterion of both final fruit quality and yield. Drought conditions affect the size of most fruits such as stone fruits,[1,2] pome fruits[3] and citrus[4,5] through a decrease in water import to fruit. Fruit dry matter content (DMC) and taste are also affected by drought.[6] Indeed, severe drought can increase citrus acidity more than sweetness, thus reducing fruit quality.[5] In the case of peach and grape, it has been shown that moderate stress improved fruit quality.[7,8] Drought mostly increases sugar and organic acid concentrations in the fruit through their higher import rates to the fruit, linked with possible osmotic adjustment or simply through the effect of concentration.[4,9,10] On the other hand, high levels of secondary metabolites such as polyphenolic compounds and anthocyanins could result from water stress.[11,12] These compounds improve fruit nutritional quality by increasing the content of antioxidants in the fruit, and are directly related to the organoleptic characteristics of the fruit, particularly to its color and flavor.[13]

Solute concentrations vary throughout fruit development according to the changes in fruit metabolism, phloem supply, cell wall material production and dilution caused by an increase in fruit volume.[14,15] It has been shown that sugar metabolism and transport which occur in relation to photosynthesis levels change under drought.[16] Drought can affect the uptake rates of water and solutes by the fruit due to an increase in fruit transpiration and a decrease in plant water potential.[4,17] For citrus fruit, water deficit resulted in the decomposition of cell wall polymers into soluble solutes, leading to a higher decrease in fruit osmotic potential.[4] The changes in fruit DMC could be linked to changes in its structural components, including cell walls, and its non-structural components, mainly soluble sugars, organic acids and polyphenols.

Peach (*Prunus persica* L.) is a fleshy fruit that contains about 87% water.[18] Although water has an important effect on fruit

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growth and quality traits, the literature abounds with reports of high concentrations of non-structural compounds in peach fruits under drought conditions without distinction as to the direct effect of drought on fruit metabolism and its indirect effect through dilution.[15,19,20] Climate change projections indicate dryer and warmer conditions – especially during the summer – and also higher risks of drought occurrence over the Mediterranean region. The future climate of the Mediterranean region could be close to the arid and semi-arid Iranian climate, with low rainfall, high temperature and vapor pressure deficit and a maximum daily potential evapotranspiration as high as 15 mm. We hypothesized that under drought conditions different fruit components (sugars, acids, polyphenols, structural dry matter and water) will not be affected to the same extent.

Given these factors, in this research we performed an experiment using field-grown late-mature peach cultivar to assess seasonal variations in the main fruit quality components when exposed to long-term drought in Iranian semi-arid climate conditions, with specific focus on (i) fruit growth and yield, (ii) DMC, (iii) structural DMC, (iv) non-structural composition (concentration of carbohydrates, organic acids and polyphenols) and (v) content:structural dry mass ratio.

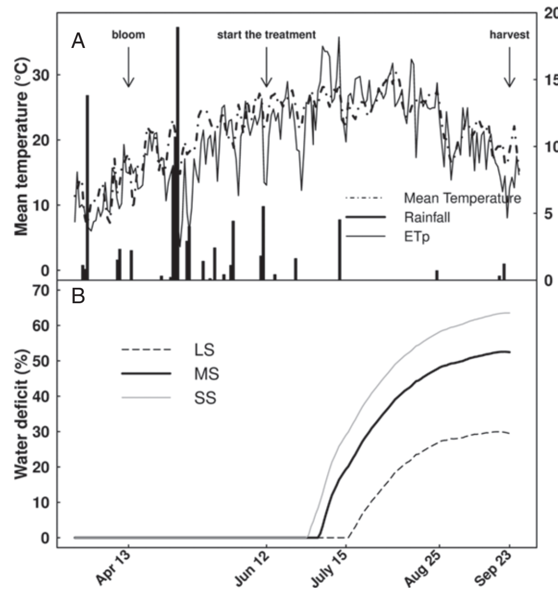

Figure 1. Daily air temperature, rainfall and reference evapotranspiration (ETp) at the Golmakan meteorological station, Iran, during the 2011 growing season (A); water deficit for different irrigation treatments (B). Abbreviations: LS, low stress; MS, moderate stress; SS, severe stress treatment.

MATERIALS AND METHODS

Study site and plant material

This study was conducted during spring and summer 2011, in Shahdiran commercial orchard, Golmakan, Iran (36° 29′ N, 59° 17′ E, around 1176 m above sea level). The orchard soil is sandy loam (64.0% sand, 30.0% silt) with pH 7.51 and 2.5 m depth. The average annual rainfall is about 212 mm. The maximum daily vapor pressure deficit was about 5.8 kPa and the temperature ranged between 23 and 38 °C, at solar noon over the study period. Rainfall and reference evapotranspiration (ETp) were monitored near the orchard at Golmakan's meteorological station (Fig. 1A). ET_c was calculated by multiplying ET_p by the crop coefficient (K_c), itself depending upon growth stage.[21]

A randomized complete-block design with four blocks, each made up of nine trees, was used for the experiment. Four rows of vigorously growing 8-year-old "Elberta" peach (*Prunus persica* L.) trees grafted on to GH Hale seedling rootstocks were selected. The trees were heavily cropped (∼270 fruits per tree), spaced 4 × 5 m apart and goblet trained. Each row was divided into three parts, which received three levels of irrigation imposed from the mid-pit hardening stage (12 June) until harvest (23 September). Trees were managed according to commercial practices for fertilization, pest and weed control, and hand thinning was done before the start of treatment. The measurements were made on four trees for each irrigation treatment.

Irrigation was carried out using a drip irrigation system with two lateral pipes per tree row and six emitters per plant. Irrigation was scheduled according to conventional irrigation applied in commercial orchards as follows: 5 days a week for 3 h each day, with each emitter delivering 8 L h^{-1} (low stress, LS), 4 L h^{-1} (moderate stress, MS) and 2 L h^{-1} (severe stress, SS) for the lowest to highest drought stress, respectively. Supplied water by soil, precipitation and irrigation under different irrigation treatments compared to cumulative ETc during the growing season led us to a water deficit estimation (Fig. 1B). The soil water balance was estimated at full bloom depending upon on the soil texture, soil water content and rooting depth.[21] According to this simplified soil–water balance, the trees grown under conventional irrigation (LS) were subjected to no stress until mid July and reached a water deficit of 30% at harvest (Fig. 1B and Table 1), meaning that water supply was 30% less than cumulative ETc. The water deficit rose to 53% and 64% at harvest for MS to SS modalities, respectively, and was observed 2–3 weeks earlier than that of LS modality.

Table 1. Monthly water balance components: crop evapotranspiration (ETc) and sum of rainfall, irrigation and soil supply for each irrigation treatment in the 2011 growing season

		Sum of rainfall, irrigation and soil supply (mm)		
Month	ETc (mm)	Low stress	Moderate stress	Severe stress
April	6	94	94	94
May	80	306	306	306
June	254	469	422	397
July	604	632	507	442
August	950	790	588	482
September	1154	909	649	514

Midday leaf water potential

Midday leaf water potential measurements were made using a pressure chamber (ELE, UK) eight times throughout the fruit growing season on three fully expanded mature leaves – exposed to direct solar radiation – per tree (i.e. 12 per treatment).

Fruit growth and composition measurements

A random sample of 10 fruit-bearing shoots on a scaffold located in the same spatial position on all trees was tagged. On each fruit-bearing shoot, the cheek diameter and height of fruits were recorded every week from 6 weeks after full bloom (13 June) until harvest (23 September). Mean diameter was calculated in all treatments using both cheek diameter and fruit height. Peach fruit volume was calculated assuming that the fruit was a sphere. During the growing season up to harvest, five fruits per treatment were sampled every 1–2 weeks (six times) in order to determine fruit and stone fresh mass, dry mass (oven dried at 70 °C) and DMC. The yield was calculated for each tree by multiplying fruit numbers by fruit mass.

Two opposite parts of each fruit were placed in liquid nitrogen, stored at −20 °C, freeze-dried and ground in liquid nitrogen. Soluble carbohydrates and organic acids were determined after extraction with a water–methanol–chloroform mixture as described by Gomez *et al.*[22] Soluble carbohydrates were measured using a high-performance liquid chromatography (HPLC) system under the following specific conditions: one guard column (sugar pack, Waters, Milford, MA, USA); one column (sugar pack I, Waters) at 85 °C; eluent, 50 mg L^{-1} EDTA-CaNa_2 aqueous solution; flow rate 0.6 mL min^{-1}; detection: refractometer (model 410, Waters). Organic acids were measured using an HPLC system under the following specific conditions: one guard column (Shodex RSpak KC-G, New York, NY, USA); one column (Shodex RSpak KC-811) at 80 °C; eluent, 1 mL L^{-1} aqueous solution of phosphoric acid; flow rate 0.7 mL min^{-1}; detection: UV spectrophotometer (model SPD-10A VP, Shimadzu, Kyoto, Japan) at 210 nm. Starch was measured using an enzymatic micro-plate assay as described by Gomez *et al.*[23]

Phenolic compounds were analyzed at harvest. An aliquot of the lyophilized fruit powder (50 mg) was weighed in a 2 mL centrifuge tube, shaken (1 min) with 1.5 mL ethanol 70% and centrifuged (5000 × *g*, 10 min, 5 °C). The supernatant was transferred into a centrifuge tube and this procedure was repeated once on the pellet. The combined supernatants were evaporated to dryness with a centrifuge vacuum evaporator (speed Vac) for 15 h without heating. The residue was redissolved by the addition of 300 μL ultra-pure water and 700 μL HPLC-grade methanol, shaken and then filtered on to a 0.22 μm membrane disk prior to injection. The analysis of phenolic compounds was performed by reverse-phase HPLC. Separation was achieved on a Uptispher HDO C18 column (15 cm × 3.0 mm, particle size 3.0 μm) heated at 30 °C (Interchim, Montluçon, France) with water–phosphoric acid (adjusted to pH 2.6) (A) and methanol (B) as the mobile phases. A diode array detector (DAD) and also a fluorescence detector (model 2475, Waters) were used. UV chromatograms were recorded at 280, 330 (detection of chlorogenic and neochlorogenic acids) and 515 nm (detection of anthocyans). The fluorescence detector was set to 275 nm (excitation) and 322 nm (emission) for detection of catechin and epicatechin.

In order to assess the dilution effect on fruit concentration and the possible effect of drought on flesh structural dry mass proportion, the concentration for each soluble compound (g kg^{-1} fresh mass) was expressed according to Léchaudel *et al.*[15] as

$$[X] = \frac{X}{\mathrm{SDM}} \cdot \frac{\mathrm{SDM}}{\mathrm{DM}} \cdot \frac{\mathrm{DM}}{\mathrm{FM}} \qquad (1)$$

with *X*, the amount (g) of soluble compounds *X*, SDM the flesh structural dry mass (g), DM the flesh dry mass (g), FM the flesh fresh mass (g). DM/FM represents the dry matter content (g/g) which is sensitive to dilution by water (i.e. an increase in FM). SDM/DM represents the ratio of the structural dry mass to the total dry mass (g/g). The flesh structural dry mass, SDM, was calculated as the difference between the flesh dry mass and the sum of non-structural compounds (carbohydrates, organic acids and polyphenols).

A sweetness index (g equivalent sucrose per 100 g flesh fresh mass) was computed as a linear combination of sugar concentrations, using the sweetness ratings of each sugar as coefficients:[24]

$$\text{Sweetness index} = 0.77 \times [\text{Glucose}] + 1.75 \times [\text{Fructose}] + 1 \times [\text{sucrose}] + 0.06 \times [\text{sorbitol}] \qquad (2)$$

Fruit firmness (N) was determined with a hand-held penetrometer (Effegi, Italy) with an 8 mm plunger in two opposite positions of five fruits per treatment. These fruits were those which were used to chemical analysis.

Calculation of fruit surface conductance

On three occasions over the 2 weeks before harvest, 20 fruits per treatment were harvested. After the peduncle surface was sealed by glycerin, the fruits were weighed and placed in front of a ventilator. Each fruit was weighed hourly for about 11 h. The temperature and relative humidity of the atmosphere were continuously measured.

Hourly fruit surface conductance, *g* (cm h^{-1}) was calculated according to Gibert *et al.*[1] as follows:

$$g = \frac{T_f}{\left(M_w/_{(R \times \mathrm{Temp})}\right) \times (P^*) \times \left(H_f - H_a\right) \times S_f} \qquad (3)$$

where T_f is the fruit transpiration per unit time (g h^{-1}), which is equal to the weight loss rate measured on each fruit the day of the harvest over approximately 8 h, M_w is the molecular mass of water (18 g mol^{-1}), *R* is the gas constant (83 cm^3 bar mol^{-1} K^{-1}), Temp is the temperature (K), *P** is the saturation vapor pressure (bar) (depending on temperature according to the equation of Fishman and Génard:[6] $P^* = 0008048 \times \exp(00547 - (\mathrm{Temp} - 273.15)]$, H_f is the relative humidity within the fruit (assumed to be equal to 100%), H_a is the relative humidity of the atmosphere and S_f is the fruit surface area. Fruit surface was calculated using fruit mean diameter.

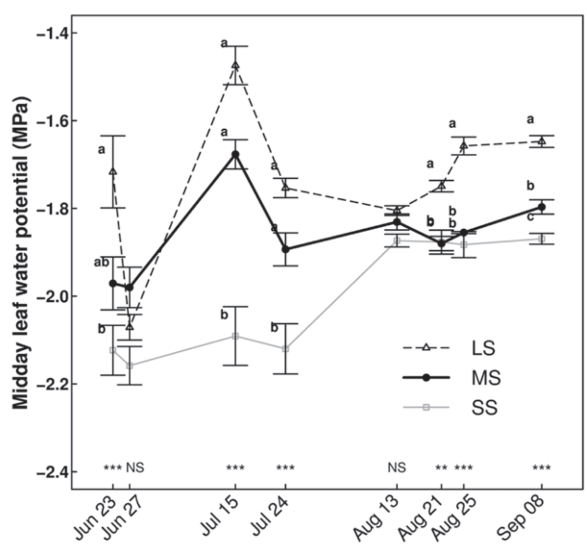

Figure 2. Evolution of midday leaf water potential under different irrigation treatments. Abbreviations: LS, low stress; MS, moderate stress; SS, severe stress treatment. Data are mean values of 12 replicates ± standard error. Differences between the irrigation treatments were either significant at $P < 0.10$*, $P < 0.05$** or $P < 0.01$*** or non-significant (NS) on each sampling date.

Data analysis

All statistical analyses were made using R 2.15.0 software (R Development Core Team, 2010). The comparison of mean values for the three irrigation levels on each of the sampling date was made by one-way ANOVA, followed by Fisher's least significant difference (LSD) test at the significance level of 0.05.

RESULTS

Midday leaf water potential

From 23 June onwards, i.e. less than 11 days after the treatment started, the SS midday leaf water potential was significantly lower than that of LS (Fig. 2). A significant difference between the LS and the MS treatment (Fig. 2) was observed 70 days after the treatment onset.

Fruit physical attributes

Both fruit volume and dry mass were strongly affected by the severity of water scarcity. At harvest, the fruit volume of MS and SS treatments was, respectively, only 66% and 44% of LS treatment (Fig. 3A). Fruit dry mass grown under MS and SS was, respectively, 85% and 73% of that grown under LS. Such a large decrease in fruit volume compared to fruit dry mass led to an increase in DMC at harvest for fruits grown under MS and SS, respectively, by 7% and 11%, compared to those grown under LS (Fig. 3B). The fruit DMC decreased throughout fruit growth from 19% to 15% for the LS treatment (Fig. 3B). Stone dry mass was not affected by irrigation levels, whereas stone fresh weight was affected (data not shown).

Both fruit flesh structural:total dry mass (SDM/DM) and structural DMC (SDM/FM) decreased up to harvest (Fig. 3C, D). The former was not affected by irrigation levels, except on 31 July, but the latter significantly increased as water scarcity intensified.

Fruit firmness progressively decreased with fruit development (Fig.4A). Fruits grown under SS presented higher firmness. However, MS treatment did not induce firmer fruits than LS treatment. Fruit firmness was positively related ($P \leq 0.001$) to structural DMC (Fig. 4B).

Later in fruit development, fruit surface conductance did not differ between measuring dates and ranged between 166 and 267 cm h^{-1} (Fig. 4C). The surface conductance of fruits grown under SS was 13–25% lower than that of those grown under LS at different measuring dates.

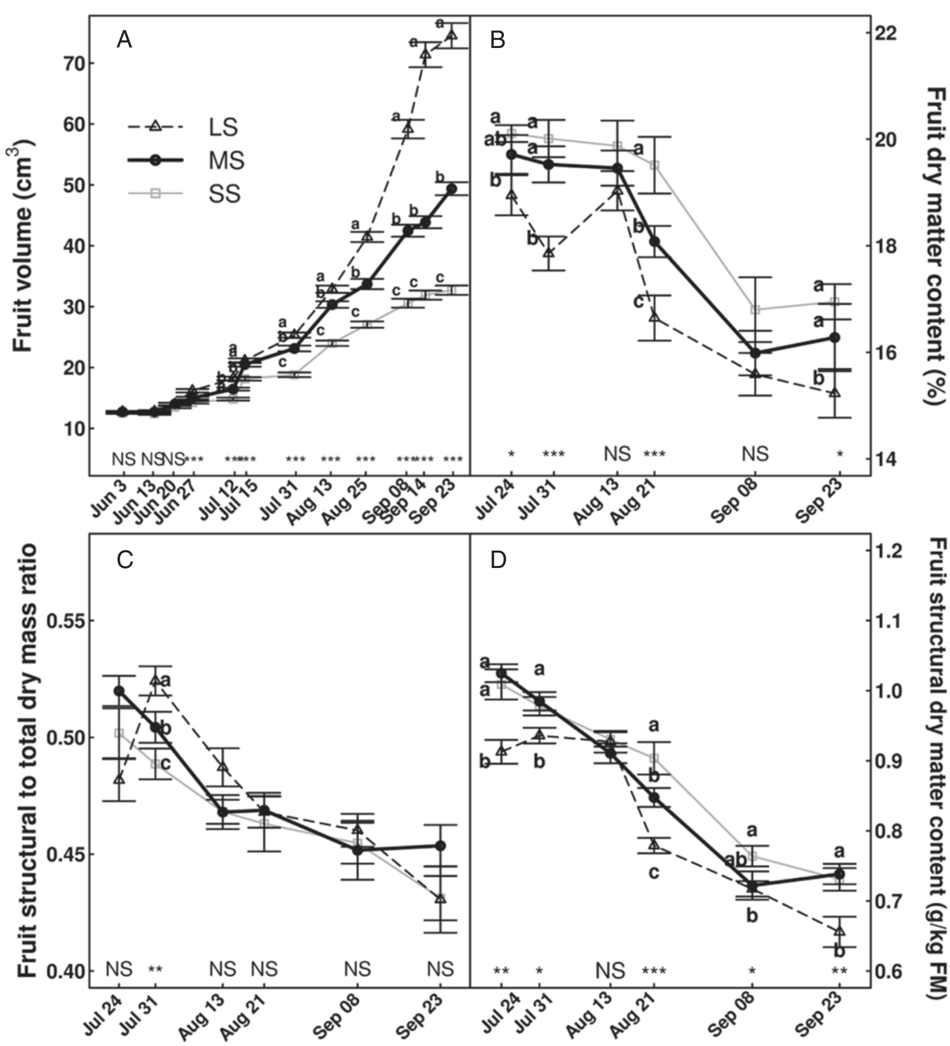

Figure 3. Evolution of the fruit volume (A), dry matter content (B), structural:total dry mass ratio (C) and structural dry matter content (D) under three different irrigation treatments during the 2011 growing season. Data are mean values of 10 replicates ± standard error. Labels and statistics are described in Fig. 2.

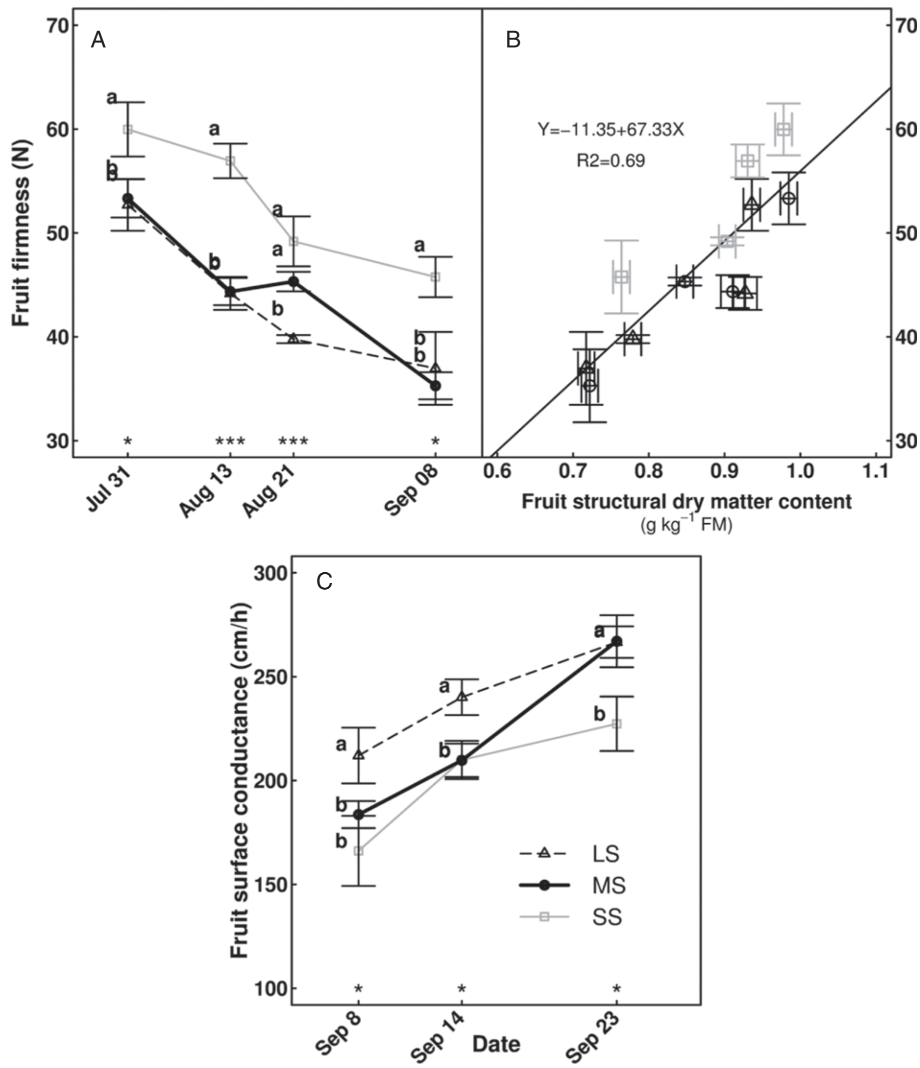

Figure 4. Evolution of the peach fruit firmness (A), relationship between fruit firmness and structural dry matter content (B) and fruit surface conductance (C) under different irrigation treatments. Data are mean values of five replicates ± standard error. Labels and statistics are described in Fig. 2.

Fruit-set and crop yield

A reduction of 36–43% in crop yield compared with LS trees was obtained on trees in MS and SS treatments respectively (Table 2). These reductions resulted from smaller fruit size on trees in MS and SS treatments. It can be noted that water deficit affected neither peach tree June drop nor fruit-set after the June physiological drop to maturity (Table 2).

Fruit carbohydrate and organic acid content:structural dry mass ratio

For LS treatment, the glucose and fructose content:structural dry mass ratios decreased during fruit development (Fig. 5A, B). For SS, this decrease was delayed by 1 month. Nevertheless, near harvest, the glucose and fructose content:structural dry mass ratios did not significantly differ among the treatments. The sucrose

Table 2. Peach yield and its parameters in the 2011 growing season responding to different irrigation treatments

	Yield parameters				
Treatment	June fruit drop[a] (%)	Fruit drop prior[b] to maturity (%)	Fruit number per tree	Fruit production (kg per tree)	Mean fruit weight (g)
Low stress	55.6 ± 3a	31.7 ± 7a	300 ± 28a	29.15 ± 2.08a	95.5 ± 8.76a
Moderate stress	54.5 ± 5a	22.2 ± 5a	268 ± 30a	18.58 ± 0.98b	71.5 ± 10.7b
Severe stress	52.7 ± 3a	28.5 ± 2a	259 ± 46a	16.56 ± 2.80b	63.3 ± 5.85c

Values followed by the same letter within a column are statistically different ($P < 0.05$).
[a] From full bloom to the end of physiological drop.
[b] After fruit physiological drop to harvest.

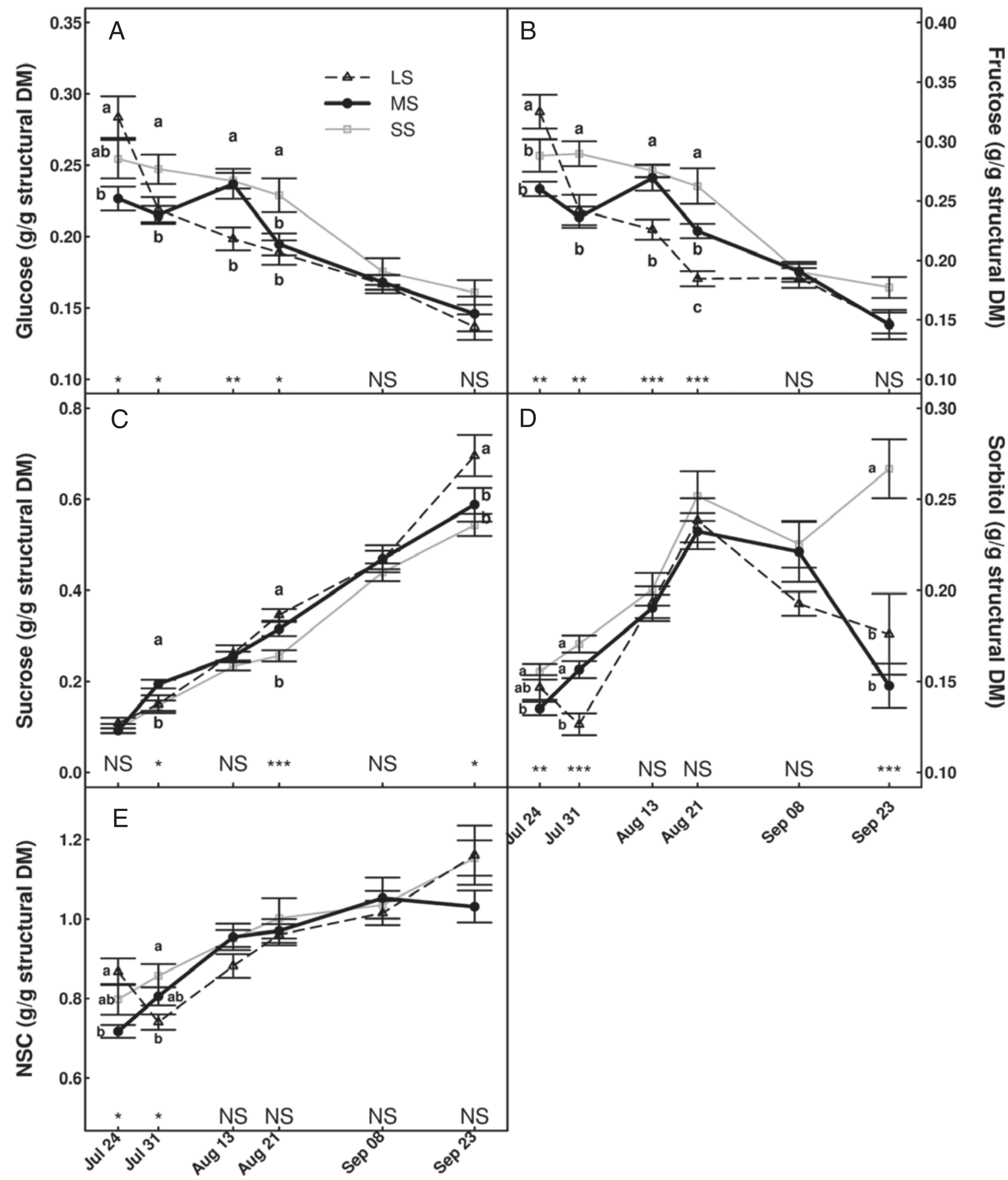

Figure 5. Seasonal variations in glucose (A), fructose (B), sucrose (C), sorbitol (D) and total non-structural carbohydrate (NSC) (E):structural dry mass ratios under three different irrigation treatments. Data are mean values of 10 replicates ± standard error. Labels and statistics are described in Fig. 2.

content:structural dry mass ratio increased during fruit development, irrespective of the treatments. At harvest, it reached values between 0.54 and 0.70 g g^{-1}, depending on the treatment (Fig. 5C). MS and SS treatments resulted in lower sucrose content than the LS treatment.

The seasonal trend of the sorbitol content:structural dry mass ratio can be separated into two phases. During the first phase, the sorbitol increased to reach a maximum of 0.24–0.25 g g^{-1}; it then decreased to about 0.14 and 0.17 g g^{-1}, respectively, for MS and LS treatments at harvest, whereas it increased to 0.27 g g^{-1} for the SS treatment at harvest (Fig. 5D). The starch content in the flesh was negligible for all treatments (data not shown).

Total non-structural carbohydrates (NSC) to structural dry mass ratio increased throughout fruit development, irrespective of the treatments (Fig. 5E). It was only slightly affected by the irrigation treatment early in the fruit development period.

Organic acid content:structural dry mass ratio showed different seasonal trends depending on the organic acids considered. Malic acid content of fruits grown under LS and MS treatments significantly increased from 13 August onwards. This increase was delayed by 8 days under SS conditions (Fig. 6A). Such a delay led to a reduction in the final malic acid content under SS conditions.

The citric acid content:structural dry mass ratio steadily decreased during fruit development and ranged between 0.01–0.02 and 0.08–0.09 g g^{-1} throughout the season (Fig. 6B). The citric acid content was significantly higher under SS conditions near harvest. As for quinic acid, there was a general decreasing trend down to a plateau, reached about 2 weeks before harvest (Fig. 6C). For SS treatment, this decrease was smaller compared to LS treatment. Nevertheless, near harvest, the quinic acid content did not significantly differ among the treatments (Fig. 6C). The total organic acid:structural dry mass ratio sharply decreased early in the fruit development period and then remained constant up

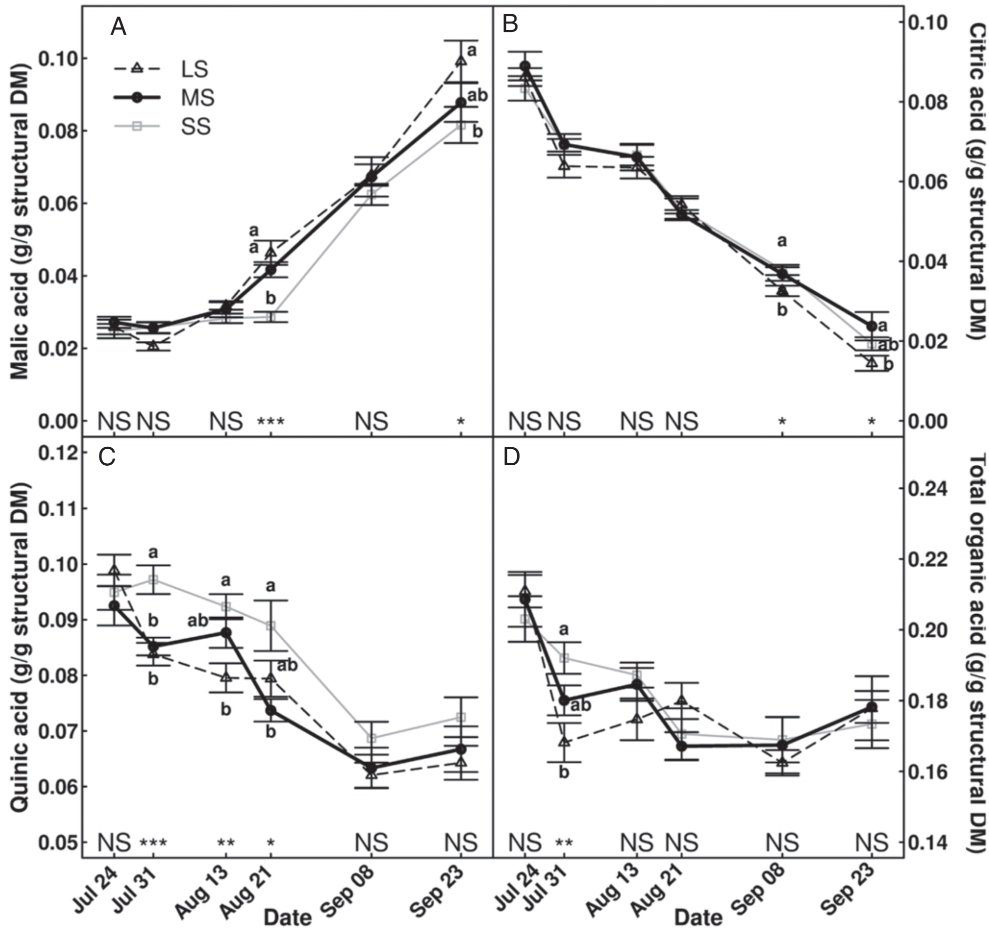

Figure 6. Seasonal variations in malic acid (A), citric acid (B), quinic acid (C) and total organic acid (D):structural dry mass ratios under three different irrigation treatments. Data are mean values of 10 replicates ± standard error. Labels and statistics are described in Fig. 2.

to harvest (Fig. 6D). The total organic acid:structural dry mass ratio did not significantly differ for the different irrigation levels at most dates.

Fruit carbohydrate and organic acid concentrations

The evolution of peach fruit composition is depicted in Figs 7 and 8 based on the changes in the carbohydrate and organic acid concentrations in fresh mass. SS treatment increased glucose, fructose and sorbitol concentrations in the flesh by about 12–70% during fruit development, whereas irrigation levels had no effect on sucrose concentration (Fig. 7A, B, C, D). The SS treatment increased total non-structural carbohydrate (NSC) concentration by about 8–21%, compared to LS treatment, during the growing season (Fig. 7E).

The sweetness index decreased with fruit development (Fig. 7F). Fruits grown under MS and SS conditions had higher sweetness index, respectively, by about 9–14% and 12–26% compared to LS conditions during fruit development. Near harvest, the fruit sweetness index of the different irrigation levels was not significantly different.

The SS treatment significantly decreased malic acid concentration compared to LS and MS treatments early in the fruit development (Fig. 8A). However, this decrease was only temporary and no significant differences appeared at harvest. The SS treatment led to significantly higher citric and quinic acid concentrations compared to those of LS and MS treatments at all dates (Fig. 8B, C). Total organic acid concentration increased by the same extent of total NSC concentration, as drought intensified (Fig. 8D).

Fruit polyphenol content:structural dry mass ratio and concentrations

Among the polyphenols in the flesh, anthocyanin (cyanidin-3-*O*-glucoside) and chlorogenic acid had the largest content, irrespective of the treatments (Table 3). The polyphenol concentration and content:structural dry mass ratio at harvest increased as drought intensified. SS treatment resulted in an increase of all polyphenol concentrations by about 62–85% compared to LS treatment (Table 3). Similarly, SS increased the content:structural dry mass ratio of all detected polyphenols, by about 46–67% compared to LS treatment (Table 3). At harvest, the total polyphenol concentration of fruits grown on trees in MS and SS treatments increased, respectively, by 35% and 70% compared to trees in LS treatment.

DISCUSSION

Fruit fresh and dry mass were very sensitive to drought and fresh mass was more affected than dry mass. Thus fruit dry matter content (DMC) increased as drought intensified, which implies that water inflow to fruit was more restricted than carbohydrate import for drought trees, in accordance with Génard *et al.*[14] and Lopez *et al.*[25,26] In the present study, under SS, despite a lower

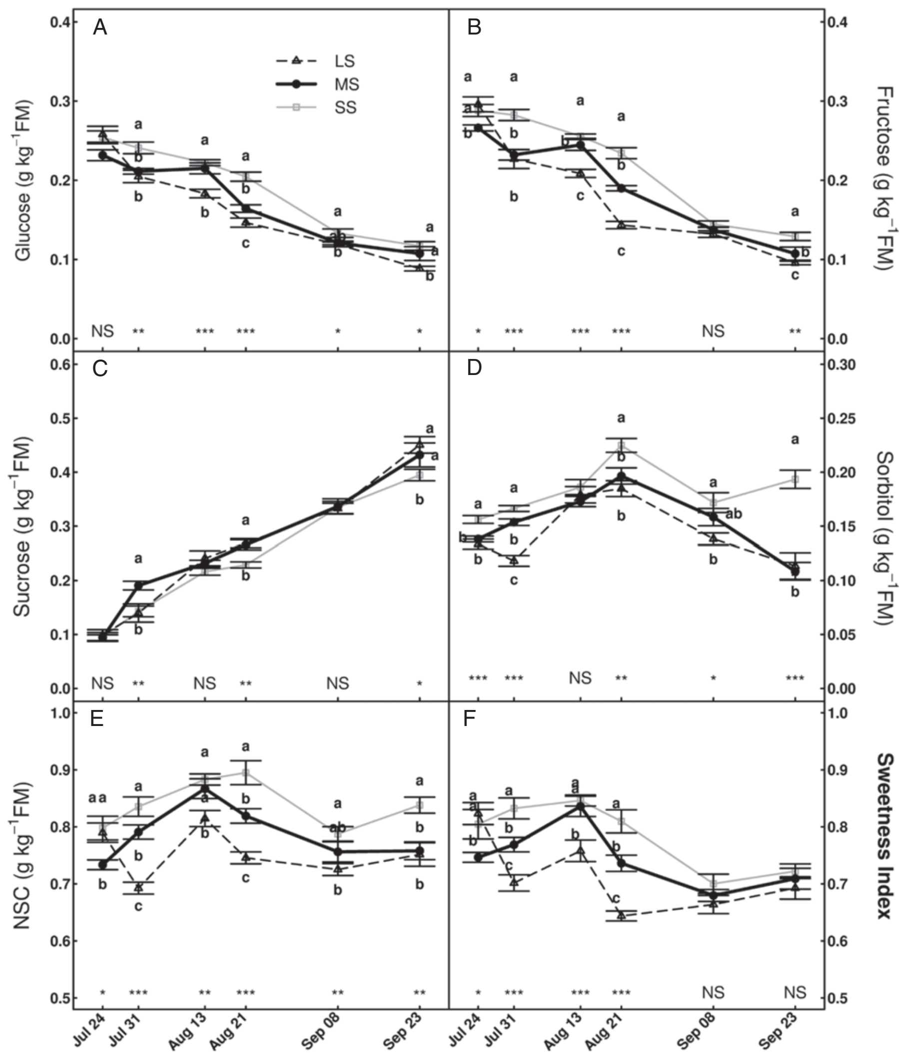

Figure 7. Seasonal variations of glucose (A), fructose (B), sucrose (C), sorbitol (D) and total non-structural carbohydrate (E) concentration as well as sweetness index (F) of peach under three different irrigation treatments. Data are mean values of 10 replicates ± standard error. Labels and statistics are described in Fig. 2.

fruit surface conductance, fruit weight decreased. This confirms that water inflow into the fruit under drought has been greatly reduced. Drought reduces fruit surface conductance through stomatal closure, development of thick cuticles and/or reduction in micro-crack occurrence and damage caused by fungal pathogens.[1] Decreased yield for MS and SS treatments was only due to a reduction in fruit size at harvest, with no effect on fruit numbers per tree, which is consistent with results previously reported by Lopez *et al.*[25]

SS resulted in higher peach flesh structural DMC, whereas flesh structural-to-total dry mass was similar in fruits from all treatments. in agreement with Léchaudel *et al.*[15] in mango fruit. The structural DMC mainly represents cell wall material concentration, while the structural:total dry mass ratio demonstrates the proportion of cell wall material produced. Such results suggest that the increase in cell wall material concentration is linked to the decrease in water import to the fruit. Peach fruit firmness increased due to severe drought, in agreement with the report by Lopez *et al.*[27] and Tavarini *et al.*[12] The positive correlation between peach fruit firmness and cell wall material concentration confirms the results obtained by Famiani *et al.*[28] in which kiwifruit structural DMC was related to fruit firmness after storage. Thus such an increase in fruit firmness by SS treatment could be related to an increase in cell wall material concentration.[29] Although the structural dry matter was lower for the SS treatment on 31 July, fruits were firmer. Such a discrepancy could be linked with a modification of the cell wall composition and possible variation in the cell wall remodeling (solubilization and depolymerization of pectic polymers) that could occur due to water deficit.[30]

Irrigation levels affected the concentration of each compound in peach fruits by changing either the fruit DMC or the compound content to flesh structural dry mass ratio, or both these two components at once. These results are in agreement with Léchaudel *et al.*[15] for mango fruit. As mentioned earlier, DMC increased as drought intensified. Thus the higher concentrations of glucose and fructose for MS and SS treatments during the third stage of fruit growth might be due to their higher levels:structural dry mass ratio, along with higher fruit DMC. The decrease in energy cost for fruit growth under drought conditions could result in a decline in the utilization of glucose and fructose in glycolysis, explaining the increase in their contents. However, the effect of irrigation decreased as fruit ripening proceeded, and at harvest

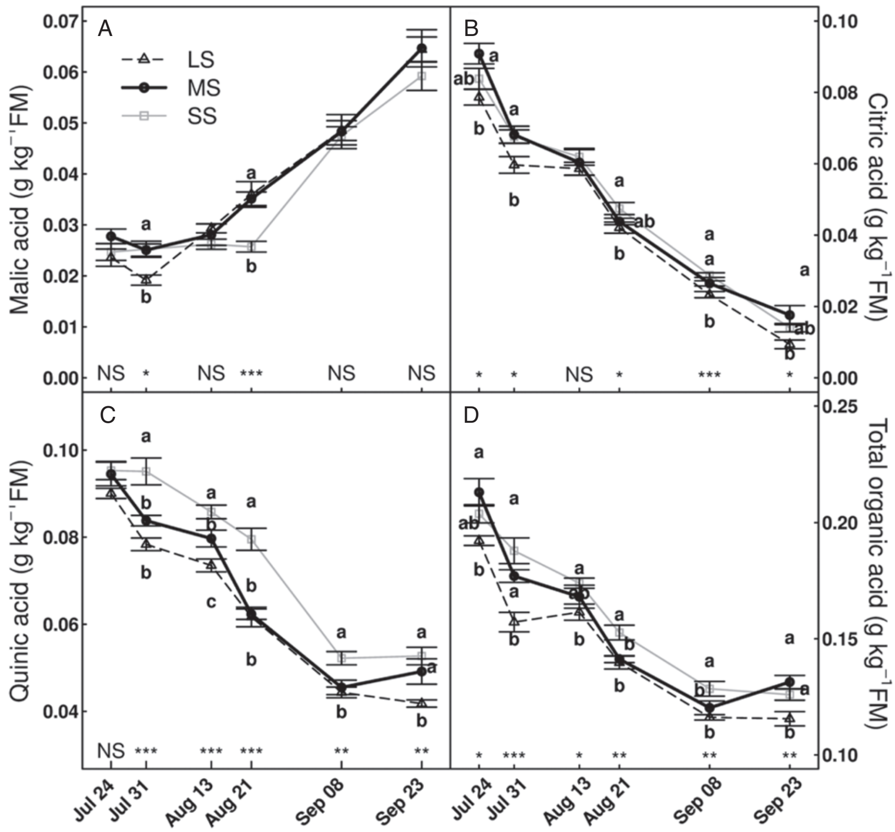

Figure 8. Seasonal variations in malic acid (A), citric acid (B), quinic acid (C) and total organic acid (D) concentration under three different irrigation treatments. Data are mean values of 10 replicates ± standard error. Labels and statistics are described in Fig. 2.

there were no significant differences in the glucose and fructose content:structural dry matter ratios across the different treatments, while their concentration remained higher under MS and SS conditions thanks to higher fruit DMC. This means that the decrease in dilution by water is a major consequence of drought treatment. Our data showed that drought induced an increase in glucose and fructose concentrations along with a decrease in fruit growth, which is in accordance with results of Génard *et al.*[31] Indeed, those authors reported a negative correlation between concentration of reducing sugars and fruit growth rate during the third stage of peach fruit development.

Sorbitol and sucrose represent the main photosynthetic products and forms of translocated carbon in peach. It has been reported that drought enhances the production and accumulation of sorbitol relative to sucrose in peach leaves and phloem sap.[32] The increase in sorbitol content:structural dry mass ratio and its concentration under drought condition may be due to enhanced sorbitol unloading into fruit mesocarp. This increase could also be linked to the reduced sorbitol utilization and translocation in the fruit.[32]

Sucrose accumulation in the flesh during fruit development is linked with its translocation from leaves and its uptake from phloem by mass flow and active mechanism.[6] Sucrose concentration did not vary across all treatments. Since drought slightly decreased sucrose content:structural dry mass ratio, this reflects the fact that the increase in fruit DMC counteracted the decrease of sucrose content:structural dry mass ratio. According to Thakur and Singh,[33] the lower levels of sucrose in the fruit under drought suggest a greater hydrolysis of sucrose in response to water stress for osmotic adjustment; they may also be due

Table 3. Peach flesh individual phenolic compounds concentrations and content:structural dry mass ratios at harvest for different irrigation treatments

	Concentration (mg kg^{-1} FM)[a]			Content (mg g^{-1} structural DM)		
Phenolic compound	Low stress	Moderate stress	Severe stress	Low stress	Moderate stress	Severe stress
Cyanidin-3-*O*-glucoside	0.246 ± 0.04b	0.292 ± 0.04ab	0.407 ± 0.04a	0.37 ± 0.06b	0.40 ± 0.06ab	0.56 ± 0.06a
Chlorogenic acid	0.222 ± 0.01b	0.353 ± 0.04a	0.362 ± 0.03a	0.34 ± 0.02b	0.48 ± 0.06a	0.49 ± 0.04a
Neochlorogenic acid	0.155 ± 0.01b	0.186 ± 0.01b	0.286 ± 0.04a	0.24 ± 0.02b	0.25 ± 0.02b	0.39 ± 0.05a
Epicatechin	0.157 ± 0.02c	0.215 ± 0.02b	0.256 ± 0.02a	0.24 ± 0.04b	0.29 ± 0.02ab	0.35 ± 0.06a
Total phenolic compounds	0.776 ± 0.05c	1.046 ± 0.08b	1.320 ± 0.02a	1.18 ± 0.09b	1.42 ± 0.12b	1.81 ± 0.17a

[a] Values are mean ± standard error of 10 replications. Means followed by the same letter within a row are statistically different ($P < 0.05$).

to a reduced translocation from the leaf due to photosynthesis limitation, along with a declined leaf area.[31]

Our results indicate that the decrease in the concentration of total organic acid in the fruit over the third phase of peach development is classically related to the decreased concentrations of citric and quinic acids, while the malic acid concentration increased during fruit development.[34]

Regarding quinic and citric acids, their concentration increased under SS conditions, but the increase in their proportions from the structural dry mass was only observed during early fruit growth and near harvest, respectively. These are consistent with other reports indicating that water scarcity can affect organic acid concentration in fruits through a simple dehydration effect.[20,33] Since fruit growth was lower as drought intensified, the growth and maintenance respirations should be lower as well. Thus, according to the citrate model of Wu *et al.*,[20] since the respiration rate is reduced, TCA cycle activity should be lower and thus lead to a lower decline in citric acid. Malic acid concentration was relatively constant or slightly decreased as drought intensified. Since fruit DMC significantly increased under drought treatment, the decrease of malic acid concentration may arise from a high decline in malic acid to structural dry mass ratio. Low levels of malic acid found under severe drought conditions are in accordance with Thakur and Singh[33] for the nectarine.

High levels of anthocyanins such as cyanidin-3-O-glucoside and cinnamic acids – especially chlorogenic acid – found in our study are consistent with results previously reported,[12,35–37] for Elberta and other peach cultivars. The concentration and content of each polyphenol to structural dry mass increased as drought intensified; however, differences were only significant for SS treatment. The increase and accumulation of polyphenols in response to drought have been demonstrated for different crops, including peach[12,36] and grape.[7] In peaches, anthocyanins and phenolic compounds are mainly confined to peel tissues.[35] According to Romero *et al.*[38] and Roby *et al.*,[11] respectively, in olive trees and in grapevines, water stress implies an activation of phenolic compound biosynthesis in the peel of fruits suffering deficit irrigation. The increase in phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity – an enzyme involved in the biosynthesis of the polyphenol compounds – under drought was observed in olive flesh.[39] Assuming that polyphenol compounds are strictly concentrated in the peel, our results suggest that the increase in polyphenol content might be due to a higher ratio peel area/fruit fresh mass under drought due to the decrease in fruit size. Moreover, the increase in polyphenol concentration could also be related to an increase in accumulation of polyphenol compounds in the peel. It can be calculated that polyphenol content per peel area (mg cm^{-2}) showed a 51% increase under drought compared to LS treatment. The drought-related rises in polyphenol compounds in the peel could be attributed to the increase in incident radiation on the fruit.[7] Indeed, drought induces a large reduction in vegetative growth, even before any effect observed on photosynthesis,[40] as was the case in our study (data not shown).

Fruit size and polyphenol concentration were most sensitive to the severity of water scarcity. Total polyphenol concentration increased by 70% under SS treatment compared to LS treatment. Total NSC and total organic acid concentrations increased to the same extent under SS conditions (about 10% at harvest). This is in agreement with Lopez *et al.*[27] The sweetness index is only based on sugar concentrations. However, malic acid enhances sucrose perception when citric and quinic acids mask the perception of sugars.[26,41] Thus the decrease in malic acid concentration and the increase in citric and quinic acid concentrations under drought conditions may negatively affect fruit sweetness. Therefore, our results showing that drought significantly increased peach fruit sweetness index during fruit development but not near fruit harvest could be biased. Sensory evaluation of peach fruit quality grown under deficit irrigation indicated that drought increased fruit sourness, but it reduced sweetness and juiciness [25].

Fruit quality is a multi-criterion concept, so that each quality trait is the result of a complex chain of biological processes that are very much under the influence of environmental condition.[24] This work, inferring the sensitivity of peach fruit structural and non-structural dry mass to plant water availability, offers an opportunity to estimate the reduction in fruit growth in parallel with its quality changes under drought referring to semi-arid weather conditions.

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REFERENCES

1 Gibert C, Lescourret F, Génard M, Vercambre G and Pérez Pastor A, Modelling the effect of fruit growth on surface conductance to water vapour diffusion. *Ann Bot* **95**:673–683 (2005).

2 Girona J, Mata M, Goldhamer DA, Johnson RS and Dejong TM, Patterns of soil and tree water status and leaf functioning during regulated deficit irrigation scheduling in peach. *J Am Soc Hortic Sci* **118**:580–586 (1993).

3 Kilili AW, Behboudian MH and Mills TM, Postharvest performance of 'Braeburn' apples in relation to withholding of irrigation at different stages of the growing season. *J Hortic Sci* **71**:693–701 (1996).

4 Huang XM, Huang HB and Gao FF, The growth potential generated in citrus fruit under water stress and its relevant mechanisms. *Sci Hortic* **83**:227–240 (2000).

5 Pérez-Pérez JG, Robles JM and Botía P, Influence of deficit irrigation in phase III of fruit growth on fruit quality in 'lane late' sweet orange. *Agric Water Manag* **96**:969–974 (2009).

6 Fishman S and Génard M, A biophysical model of fruit growth: simulation of seasonal and diurnal dynamics of mass. *Plant Cell Environ* **21**:739–752 (1998).

7 Chaves MM, Santos TP, Ortun MF, Souza CR, Rodrigues ML, Lopes CM *et al*, Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Ann Appl Biol* **150**:237–252 (2007).

8 Kobashi K, Gemma H and Iwahori S, Abscisic acid content and sugar metabolism of peaches grown under water stress. *J Am Soc Hortic Sci* **125**:425–428 (2000).

9 Etienne A, Génard M, Lobit P, Mbeguié-A-Mbéguié D and Bugaud C, What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *J Exp Bot* **64** 1451–1469 (2013).

10 Hummel I, Pantin F, Sulpice R, Piques M, Rolland G, Dauzat M *et al*, Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiol* **154**:357–372 (2010).

11 Roby G, Harbertson JF, Douglas AA and Matthews MA, Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. *Aust J Grape Wine Res* **10**:100–107 (2004).

12 Tavarini S, Gil MI, Tomas-Barberan FA, Buendia B, Remorini D, Massai R *et al*, Effects of water stress and rootstocks on fruit phenolic composition and physical/chemical quality in Suncrest peach. *Ann Appl Biol* **158**:226–233 (2011).

13 Benard C, Gautier H, Bourgaud F, Grasselly D, Navez B, Caris-Veyrat C *et al*, Effects of low nitrogen supply on tomato (*Solanum lycopersicum*) fruit yield and quality with special emphasis on sugars, acids, ascorbate, carotenoids, and phenolic compounds. *J Agric Food Chem* **57**:4112–4123 (2009).

14 Génard M, Lescourret F, Gomez L and Habib R, Changes in fruit sugar concentrations in response to assimilate supply, metabolism and dilution: a modeling approach applied to peach fruit (*Prunus persica*). *Tree Physiol* **23**:373–385 (2003).
15 Léchaudel M, Joas J, Caro Y, Génard M and Jannoyer M, Leaf:fruit ratio and irrigation supply affect seasonal changes in minerals, organic acids and sugars of mango fruit. *J Sci Food Agric* **85**:251–260 (2005).
16 Hayano-Kanashiro C, Calderón-Vázquez C, Ibarra-Laclette E, Herrera-Estrella L and Simpson J, Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. *Plos One* **4**:e7531 (2009).
17 Guichard S, Bertin N, Leonardi C and Gary C, Tomato fruit quality in relation to water and carbon fluxes. *Agronomie* **21**:385–392 (2001).
18 Crisosto CH and Valero D, Harvesting and post harvesting handling of peaches for the fresh market, in *The Peach: Botany, Production and Uses*, ed. by Layne DR and Bassi D, CABI, Cambridge, MA, pp. 575–596 (2008).
19 Génard M, Baldazzi V and Gibon Y, Metabolic studies in plant organs: don't forget dilution by growth. *Front Plant Sci* **5**:85 (2014).
20 Wu BH, Génard M, Lobit P, Longuenesse JJ, Lescourret F, Habib R and Li SH, Analysis of citrate accumulation during peach fruit development via a model approach. *J Exp Bot* **58**(10):2583–2594 (2007).
21 Allen R, Pereira L, Dirk R and Smith M, *Crop evapotranspiration. Guidelines for computing crop water requirements*. FAO Irrigation and drainage paper no. 56, Rome (1998).
22 Gomez LR, Rubio E and Auge M, A new procedure for extraction and measurement of soluble sugars in ligneous plants. *J Sci Food Agric* **82**:360–369 (2002).
23 Gomez LR, Rubio E and Lescourret F, Critical study of a procedure for the essay of starch in ligneous plants. *J Sci Food Agric* **83**:1114–1123 (2003).
24 Lescourret F and Génard M, A virtual peach fruit model simulating changes in fruit quality during the final stage of fruit growth. *Tree Physiol* **25**:1303–1315 (2005).
25 Lopez G, Behboudian MH, Echeverria G, Girona J and Marsal J, Instrumental and sensory evaluation of fruit quality for 'Ryan's Sun' peach grown under deficit irrigation. *HortTechnology* **21**:712–719 (2011).
26 Marsh KB, Friel EN, Gunson A, Lund C and MacRae E, Perception of flavour in standardised fruit pulps with additions of acids or sugars. *Food Qual Prefer* **17**:376–386 (2006).
27 Lopez G, Behboudian MH, Vallverdu X, Mata M, Girona J *et al*, Mitigation of severe water stress by fruit thinning in 'O'Henry' peach: implications for fruit quality. *Sci Hortic* **125**:294–300 (2010).
28 Famiani F, Baldicchi A, Farinelli D, Cruz-Castillo JG, Marocchi F *et al*, Yield affects qualitative kiwifruit characteristics and dry matter content may be an indicator of both quality and storability. *Sci Hortic* **146**:124–130 (2012).
29 Rato AE, Nunes C, Agulheiro-Santos AC, Barroso JM, Riquelme F *et al*, Influence of cell wall calcium content in fruit firmness during the ripening of plums (*Prunus domestica* L.), in *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010)*, ed. by Cantwell MI, Almeida DPF. ISHS Acta Horticulturae, Lisbon, pp. 1047–1054 (2012).
30 Gribaa A, Dardelle F, Lehner A, Rihouey C, Burel C *et al*, Effect of water deficit on the cell wall of the date palm (*Phoenix dactylifera* 'Deglet nour', Arecales) fruit during development. *Plant Cell Environ* **36**:1056–1070 (2013).
31 Génard M, Reich M, Lobit P and Besset J, Correlation between sugar and acid content and peach growth. *J Hort Sci Biotech* **74**:772–776 (1999).
32 Lo Bianco R, Rieger M and Sung SS, Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. *Physiol Plant* **108**:71–78 (2000).
33 Thakur A and Singh Z, Responses of 'Spring Bright' and 'Summer Bright' nectarines to deficit irrigation: fruit growth and concentration of sugars and organic acids. *Sci Hortic* **135**:112–119 (2012).
34 Wu BH, Génard M, Lescourret F, Gomez L and Li SH, Influence of assimilate and water supply on seasonal variation of acids in peach (cv Suncrest). *J Sci Food Agric* **82**:1829–1836 (2002).
35 Andreotti C, Ravaglia D, Ragaini A and Costa G, Phenolic compounds in peach (*Prunus persica*) cultivars at harvest and during fruit maturation. *Ann Appl Biol* **153**:11–23 (2008).
36 Buendia B, Allende A, Nicolas E, Alarcon JJ and Gil MI, Effect of regulated deficit irrigation and crop load on the antioxidant compounds of peaches. *J Agric Food Chem* **56**:3601–3608 (2008).
37 Tomas-Barberan FA, Cienfuegos-Jovellanos E, Marin A, Muguerza B, Gil-Izquierdo A, Cerda B *et al*, HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *J Agric Food Chem* **49**:4748–4760 (2001).
38 Romero MP, Tovar MJ, Girona J and Motilva MJ, Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. cv. Arberquina) grown under different deficit irrigation strategies. *J Sci Food Agric* **50**:5349–5354 (2002).
39 Tovar MJ, Romero MP, Girona J and Motilva MJ, L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L cv Arbequina) fruit grown under different irrigation regimes. *J Sci Food Agric* **82**:892–898 (2002).
40 McDowell NG, Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol* **155**:1051–1059 (2011).
41 Lobit P, Génard M, Wu BH, Soing P, Habib R, Modelling citrate metabolism in fruits: responses to growth and temperature. *J Exp Bot* **54**:1–13 (2003).

