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Two-Season Study of the Influence of Regulated Deficit Irrigation and Reflective Mulch on Individual and Total Phenolic Compounds of Nectarines at Harvest and during Storage

Eleni D. Pliakoni,[†] George D. Nanos,[†] and Maria I. Gil*[,‡]

[†]Laboratory of Pomology, School of Agricultural Science, University of Thessaly, Fitoko Street, 38446 Volos, Greece, and [‡]Research Group on Quality, Safety and Bioactivity of Plant Foods, CEBAS-CSIC, P.O. Box 164, Espinardo, Murcia E-30100, Spain

The influence of deficit irrigation (Deficit) and reflective mulch (Reflective) of Caldesi 2000 nectarines on the content of individual phenolic compounds was studied at harvest and during storage for 2, 4, and 6 weeks at 2 °C during two consecutive years (2007 and 2008). Individual phenolic groups in the edible fruit part consisted mainly of proanthocyanidins (200 mg/100 g fw), lower content of phenolic acids (17 mg/100 g fw), and minor content of flavonols (5 mg/100 g fw) and anthocyanins (1.2 mg/100 g fw). Deficit irrigation increased the content of total phenolics, including proanthocyanidins and phenolic acids, reaching similar amounts in both years. Sun-exposed fruit (upper part of canopy) showed higher content than shaded fruit (lower part of canopy). However, Reflective significantly increased the content of total phenolics, particularly phenolic acids and proanthocyanidins, of fruit located in the lower part of the canopy. During storage, Deficit and Reflective did not affect the content of phenolic acids, flavonols, and proanthocyanidins when compared to the content at harvest. Optimizing cultural practices can be a way to increase the phenolic content of nectarines.

KEYWORDS: ***Prunus persica*; cultural practices; anthocyanins; flavan-3-ols; proanthocyanidins; hydroxycinnamates; flavonols; HPLC-DAD; HPLC-MS**

INTRODUCTION

Peaches are widely cultivated in the Mediterranean basin but only when fertile soil and adequate amounts of water are available. Nowadays, water could be the limiting factor in various areas appropriate for peach cultivation. Irrigation in the Mediterranean basin is the biggest water consumer using 70−80% of the available irrigation water. It is clear that the efficient and sustainable use of irrigation water is a major priority. Deficit irrigation strategies can lead to irrigation water savings and high irrigation efficiencies (*1*).

The use of reflective mulch could be an innovative method for saving irrigation water. Lange and Geelen (*2*) reported for early season peaches that soil moisture levels were 10% higher under the Extenday reflective mulch compared to similar unmulched areas. The obvious explanation is that the mulch underneath the tree canopy and over the drippers will eliminate weed growth, water use by the weeds, and water evaporation. Apart from the expected water economy, reflective mulch is often used to improve light penetration inside the canopy and improve fruit quality and growth (*3*). Reflective mulch under the tree canopy increased light availability especially at the lower parts of the canopy (*4*). Light availability is a very important factor that can influence fruit productivity and quality. In Germany, the use of Extenday reflective mulch in apples increased red skin color, mainly in fruit from the lower part of the canopy (*5*). Also in New Zealand, the reflective mulch treatment improved peach quality, increasing soluble solids (SSC), dry matter content, flesh firmness, and taste panel scores (*3*). There are few reports on the positive influence of light availability on certain fruit constituents important to human health. Awad et al. (*6*) found that improved light conditions within the canopy, especially at the final stage of fruit development, improved both human health related constituents and skin color of apples.

Fruit quality may be influenced by deficit irrigation strategies. Apricots from deficit-irrigated trees had higher SSC, titratable acidity (TA), and hue angle than control fruit and similar fruit diameter, fresh mass, flesh firmness, and maturity index (*7*). Several authors reported that deficit irrigation improved peach fruit quality without affecting tree productivity (*8*, *9*). Deficit irrigation application in the second stage of fruit growth, in a mid- to late-maturing peach cultivar, resulted in higher SSC, lower TA, and higher SSC/TA ratio compared to those in control fruit (*10*). Regulated deficit irrigation may also affect fruit phenolic content. For example, in olive fruit, grown under different irrigation regimes, higher phenolic content was measured with decreased irrigation water volume (*11*). Also, in peaches, regulated deficit irrigation increased fruit phenolic content, especially anthocyanin and proanthocyanidin content (*12*).

The objectives of this study were to examine during two seasons (2007 and 2008) the effect of (1) regulated deficit irrigation during the third stage of fruit growth, (2) increased light

*To whom correspondence should be addressed. Tel: +34 968 396 315. Fax: +34 968 396 213. E-mail: migil@cebas.csic.es.

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availability inside the canopy using reflective mulch on the tree row, and (3) postharvest storage for up to 6 weeks on the individual and total phenolic compounds of nectarines. The main phenolic compounds were grouped as hydroxycinnamic acids (phenolic acids), flavan-3-ols (proanthocyanidins), flavonols, and anthocyanins.

MATERIALS AND METHODS

Plant Material and Experimental Conditions. Fruits were harvested from nine year old early to midseason (late June) ripening Caldesi 2000 nectarine trees, in an experimental field in the Velestino area in central Greece. Tree spacing was 5 × 5 m, and pruning was applied every year in February and summer (after harvest). Drip irrigation was applied using two 16 L/h drippers per tree situated around 0.5 m away on both sides of the trunk on the row. The farm was irrigated twice a week increasing the irrigation time during the last few weeks before harvest depending on weather and calculated evapotranspiration (ETc). The soil was heavy, and the irrigation water quality was good (conductivity < 700 μS/cm) but contained high nitrate content (25 mg/L nitrates), which was taken into consideration for fertilization. The experiments were conducted during the spring and summer of 2007 and 2008. Four trees were considered as experimental units or replications, and there were four treatments separated from each other with at least a row of nonexperimental trees. Four treatments were compared: (a) a control that consisted of irrigation with approximately 100% of ETc applied regularly twice a week; (b) regulated deficit irrigation (Deficit) using 75% of the water applied to control trees before harvest during the last part of the final fruit growth and 50% postharvest, with treatment initiation on 5/6/07 and 23/5/08; (c) reflective, irrigated as in the control treatment combined with reflective mulch (Extenday white reflective mulch) on the row, 1.6 m wide under the tree canopy, and above the drippers (applied almost one month before harvest on 6/6/07 and 15/5/08 and remaining in place until September); and (d) reflective plus regulated deficit irrigation (Reflective + Deficit), irrigated as in the deficit irrigation treatment, but combined with reflective mulch under the tree canopy. Total water amounts applied during June, July, and August were 2680 and 1576 m^3/ha in 2007 and 2590 and 1542 m^3/ha in 2008 for control and Deficit treatments, respectively.

Partially ripe nectarines were harvested (on 21/6/07 and 24/6/08) from the sun-exposed upper part of the canopy (up) and from the shaded lower part of the canopy (down). In particular, in the morning hours of sunny days (photosynthetically active radiation on the outer canopy was > 1200 mmol m^{-2} s^{-1}) fruits were harvested from all directions at 1.5−2 m high at the outer part of the canopy and at 0.5 m high in the lower part of the canopy. Fruits were harvested relatively ripe with a mean soluble solids content of 14.6% in 2007 and 13.1% in 2008. Flesh firmness ranged between 24 and 49 N depending on the year, treatment, and fruit position. The softer fruits were found in 2008 for the control fruit at the lower part of the canopy. Fruits were immediately transported to the laboratory, and after 4 h, those that constituted the sample at harvest were sectioned, kept in a freezer for a few days, freeze-dried, and stored bagged in a freezer until analysis.

Fruits were stored in a commercial cold room at 2 °C and 90−95% RH for 2 (6/7/07 and 8/7/08), 4 (18/7/07 and 21/7/08), and 6 (1/8/07 and 4/8/08) weeks. Fruits were extracted for phenolic compound analysis after each storage period plus one day of shelf life at 20 °C and 45−55% RH. Three replicates of six fruits each were used per treatment, time, and fruit position. Fruit wedges were frozen and lyophilized for phenolic compound extraction as indicated above.

Extraction and Analysis of Phenolic Compounds. The procedure was as described by Buendia et al. (*12*). Briefly, freeze-dried powder (2 g) was homogenized with 20 mL of extraction solution (acetone/water/acetic acid, 70/29.5/0.5, v/v/v) and then centrifuged (1765*g*) for 10 min. After the acetone was evaporated, the aqueous residue was flushed through an activated Sep-Pak C_{18} cartridge (Waters), previously activated with methanol, followed by water, and then eluted with methanol. The methanol was evaporated at 35 °C under vacuum, recovered in 1 mL of extraction solution, and then filtered through a 0.45 μm Nylon filter. Samples of 50 μL were analyzed by HPLC (Merck Hitachi, Tokyo, Japan) equipped with a model L-7100 pump, a model L-7455 photodiode array UV/vis detector, and a model L-7200 autosampler. The separation was achieved on a reversed phase LiChrocart C_{18} column (250 mm × 4 mm i.d., 5 μm particle size) (Merck, Darmstadt, Germany) with water/formic acid (95/5, v/v) (A) and methanol (B) as the mobile phases. The linear gradient started with 3% B, at 5 min 5% B, at 10 min 8% B, at 15 min 13% B, at 19 min 15% B, at 47 min 40% B, at 64 min 65% B, and at 66 min 98% B, and then it was maintained isocratic up to 70 min. The flow rate was 1 mL/min, and chromatograms were recorded at 280, 320, 360, and 510 nm. Anthocyanins were quantified by comparisons with an external standard of cyanidin 3-rutinoside at 510 nm; flavonols as quercetin 3-rutinoside at 360 nm; hydroxycinnamic acid derivatives as chlorogenic acid at 320 nm; and flavan-3-ols as catechin at 280 nm. The results were expressed as milligrams per 100 g fresh weight (fw).

The analytical conditions for the HPLC-MS-MS analyses were the same as those described above for the HPLC-DAD analysis but using water with 1% formic acid as solvent A for the mobile phase. The HPLC system equipped with a UV−vis DAD and an MS detector in series consisted of a G1322A binary pump, a G1313A autosampler, a G1322 degasser, a G1315 B photodiode array detector, and an ion trap mass spectrometer equipped with electrospray ionization (ESI) and operated in the negative ion mode controlled by software (v.4.0.025) from Agilent Technologies (Waldbronn, Germany). The capillary was maintained at 350 °C and at a voltage of 4 kV. Mass scan (MS) and daughter (MS-MS) spectra were measured from m/z 100−1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative mode for all phenolic compounds except anthocyanins that were in the positive one. The phenolic characterization was carried out by means of their UV spectra, molecular weight, and their MS-MS fragments and, whenever possible, by chromatographic comparisons with authentic standards.

Proanthocyanidin Analysis. To quantitatively evaluate the proanthocyanidins present in the fruit without a previous extraction, the acid catalysis of proanthocyanidins in the presence of excess phloroglucinol was carried out. Freeze-dried powdered nectarine (0.5 g) was vortexed with 5 mL of a 0.1 N HCl solution in MeOH containing 5 g/L phloroglucinol and 10 g/L ascorbic acid for 3 min and kept at 50 °C for 10 min, and then combined with 1.2 mL of aqueous sodium acetate to stop the reaction (*13*). Phloroglucinol adducts were analyzed by reversed-phase HPLC. The column used was a Develosil diol column (250 × 4.6 mm i.d. 5 μm) (Phenomenex, Torrance, CA). The method utilized a binary gradient with the mobile phase containing 1% v/v aqueous acetic acid (mobile phase A) and MeOH (mobile phase B). Eluting peaks were monitored at 280 nm. The elution was performed with 5% B isocratic for 10 min, and a linear gradient was then installed to reach 20% B at 20 min and 40% B at 25 min. The flow rate was 1.0 mL/min, and the injection volume was 20 μL. The column was washed with 90% B for 10 min and then re-equilibrated with 5% B for 5 min before the next injection.

Chemicals. Cyanidin 3-rutinoside, quercetin 3-rutinoside, chlorogenic acid, and catechin were purchased from Sigma (St. Louis, MO). Formic acid and acetic acid came from Merk (Darmstadt, Germany) and phloroglucinol from Sigma (St. Louis, MO). All chromatographic solvents were of HPLC grade.

Statistical Analysis. An analysis of variance (ANOVA) at $P \leq 0.05$ was performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL). LSD was calculated when significant differences were observed. Significant differences in ANOVA tables were as follows: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$.

RESULTS AND DISCUSSION

Characterization of Phenolic Compounds. The HPLC-DAD analysis allowed the characterization of 6 phenolic compounds classified into 3 groups at different specific wavelengths: hydroxycinnamic acids (320 nm), flavonols (360 nm), and anthocyanins (510 nm). The chromatograms recorded at 320 nm showed two main peaks with UV spectra characteristic of caffeic acid derivatives and with ESIMS spectra as caffeoyl-quinic derivatives ($M - H^-$, m/z 353). The main compound coincided with an authentic marker of chlorogenic acid (5-*O*-caffeoyl-quinic acid), and the first-eluting compound was identified as neochlorogenic acid (3-*O*-caffeoyl-quinic acid). In the same chromatogram, two different flavonol

Table 1. ANOVA Table for Individual Phenolic Groups and Total Phenolics of Caldesi 2000 Nectarines at Harvest[a]

		phenolic acids		flavonols		anthocyanins		proanthocyanidins		total phenolics	
	Df ($n-1$)	*F*	sig	*F*	sig	*F*	sig	*F*	sig	*F*	sig
year	24	49.3	***	4.9	***	56.4	***	51.8	***	49.0	***
treatment (Trt)	12	0.5	ns	2.2	ns	1.7	ns	5.1	**	4.7	**
position	24	19.0	***	0.0	ns	0.3	ns	16.2	***	16.8	***
year * Trt	6	8.6	***	0.6	ns	0.7	ns	11.8	***	11.6	***
year * position	12	0.4	ns	0.6	ns	0.4	ns	0.2	ns	0.2	ns
Trt * position	6	3.9	*	0.4	ns	0.6	ns	7.7	***	7.3	**
year * Trt * position	3	2.0	ns	1.1	ns	1.6	ns	0.2	ns	0.3	ns

[a] ns, *, **, *** = not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

peaks were detected. Their UV spectra suggested that they were quercetin derivatives glycosylated at the hydroxyl in the 3 position (*14*). The retention time, UV spectra, and HPLC-MS analysis confirmed that these compounds were quercetin-3-glucoside and quercetin-3-rutinoside. Two anthocyanidin pigments were identified as cyanidin-3-glucoside, the main pigment, and cyanidin-3-rutinoside the minor one. The HPLC-MS analysis confirmed these structures. As the HPLC-DAD analyses showed a very low response factor for the quantification of flavan-3-ols at 280 nm, the method used for the quantification of proanthocyanidins was the acid-catalyzed degradation in the presence of excess phloroglucinol (*13*). The analysis of proanthocyanidins after acid-catalyzed cleavage provides information on their subunit composition as well as the interflavonoid bond location. Under acidic conditions, proanthocyanidins were depolymerized, releasing terminal subunits as flavan-3-ol monomers and extension subunits as electrophilic flavan-3-ol intermediates. The electrophilic intermediates were trapped by a nucleophilic reagent to generate analyzable adducts. After the degradation, both the terminal units and the extension units corresponding to the phloroglucinol adduct were quantified by HPLC-DAD. The nature of both chromatographic peaks was determined by HPLC-MS analysis. The determination of the phloroglucinolysis degradation products gives information of the quantification of proanthocyanidins and the mean degree of polymerization (DPn) of the proanthocyanidins' oligomers (*13*).

In our study, the main groups of phenolic compounds identified and quantified in the edible part of nectarines (flesh plus peel) were hydroxycinnamic acids (phenolic acids), flavonols, anthocyanins, and flavan-3-ols (proanthocyanidins). The characterization of phenolic compounds agrees with a previous work by Tomas-Barberan et al. (*14*) in which they identified the phenolic compounds by HPLC-DAD and HPLC-ESIMS of different cultivars of stone fruits including nectarines. In that study, the peel and flesh of nectarines were examined separately, and higher concentrations of phenolic compounds were found in the peel than in the flesh. Our results agree with this previous study, although there are no previous studies on Caldesi 2000 nectarines. At harvest with both fruit positions pooled (up and down), total phenolic compounds consisted of 89.0−90.9% proanthocyanidins, 7.5−7.8% phenolic acids, 1.4−2.2% flavonols, and 0.2−1.0% anthocyanins. In our study, total phenolic compounds were almost double the content reported previously for other white flesh nectarine cultivars (*15*), i.e., in our study the total phenolic content for Caldesi 2000 nectarines ranged from 137 to 185 mg/100 g fw, while in a previous study, total phenolic compounds ranged from 13.6 to 102.4 mg/100 g of fruit (85% flesh and 15% peel) (*15*). This high phenolic content could be due to the cultivar Caldesi 2000, a white-fleshed nectarine particularly rich in proanthocyanidins. It could also be due to soil and climatic conditions of the experimental farm. This soil contains significant amounts of calcium, which is known to significantly affect fruit quality in various ways, but no work has correlated soil composition with fruit phenolic content. This cultivar ripens in late June, and preharvest temperatures are not high enough to positively affect total phenolic content, as a response to thermal stress (*16*). In addition in our study, Caldesi 2000 nectarines cultivated in 2007 showed significantly higher total phenolic content than those cultivated in 2008 (179 ± 33 and 139 ± 39 mg/100 g fw, respectively), even though data for air temperatures and solar radiation collected from a nearby meteorological station (100 m away) during the last weeks before harvest were similar in the two experimental years. From these data, it seems that factors other than preharvest air temperature affect the total phenolic content of Caldesi 2000 nectarines. It has been reported that environmental factors and agronomic practices affect phenolic compound synthesis through the regulation of PAL activity (*17*). In this context, water availability (basically through irrigation) and light intensity and quality are factors that affect phenolic accumulation of fruits and vegetables (*11*, *12*, *18*−*20*). In our experiment, the factors we examined that could affect the phenolic compound biosynthesis were water availability, sunlight, and the changes during postharvest storage. In order to interpret the experimental results in terms of whether one or more agronomical, environmental, and postharvest factors have a significant effect on the content of different classes of phenolic compounds, statistical evaluation of each factor and their interactions were established at harvest (**Table 1**) and during storage (**Table 2**).

Influence of Regulated Deficit Irrigation and Reflective Mulch on Individual and Total Phenolic Compounds at Harvest. Deficit irrigation and reflective mulch significantly affected the content of phenolic acids and proanthocyanidins of Caldesi 2000 nectarines at harvest. However, the influence varied depending on the year (**Table 1**). In 2007, the highest content of phenolic acids was observed in nectarines cultivated in reflective mulch, whereas in 2008, the highest level of these compounds was detected in nectarines cultivated in Deficit (**Figure 1A**). The content of phenolic acids of nectarines cultivated in reflective mulch in 2007 was twice that of 2008. When the content of proanthocyanidins was studied, behavior similar to that of phenolic acids was observed. In 2007, the highest content of proanthocyanidins was found in fruit grown in Reflective mulch without differences from those cultivated in reflective + deficit (**Figure 1B**). In 2008, a positive effect on the content of proanthocyanidins was observed in nectarines grown under deficit irrigation, significantly increasing the amount of these phenolic compounds when compared with that in the other treatments. In addition, the content of total phenolic compounds of nectarines at harvest was influenced by the treatments in a way similar to that observed for proanthocyanidins (data not shown). Deficit irrigation increased the content of total phenolics, including proanthocyanidins and phenolic acids, reaching a similar amount in both years. However, the content of flavonols and anthocyanins was not influenced by the treatments, and a similar behavior was observed in both years (**Table 1**).

Table 2. ANOVA Table for Individual Phenolic Groups and Total Phenolics of Caldesi 2000 Nectarines during Storage[a]

		phenolic acids		flavonols		anthocyanins		proanthocyanidins		total phenolics	
	Df ($n-1$)	*F*	sig	*F*	sig	*F*	sig	*F*	sig	*F*	sig
year	96	71.4	***	44.9	***	231.6	***	120.3	***	113.8	***
treatment (Trt)	48	7.5	***	4.6	*	4.3	**	31.2	***	31.0	***
storage time (time)	24	4.8	**	1.1	ns	2.3	ns	7.6	***	8.1	***
year * Trt	24	2.2	ns	1.4	ns	2.0	ns	21.2	***	19.7	***
year * time	6	1.9	ns	1.3	ns	1.9	ns	5.2	**	5.2	**
Trt * time	12	0.9	ns	1.2	ns	2.8	**	1.2	ns	1.2	ns
year * Trt * time	6	2.5	*	1.9	*	3.8	***	1.6	ns	1.5	ns

[a] ns, *, **, *** = not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

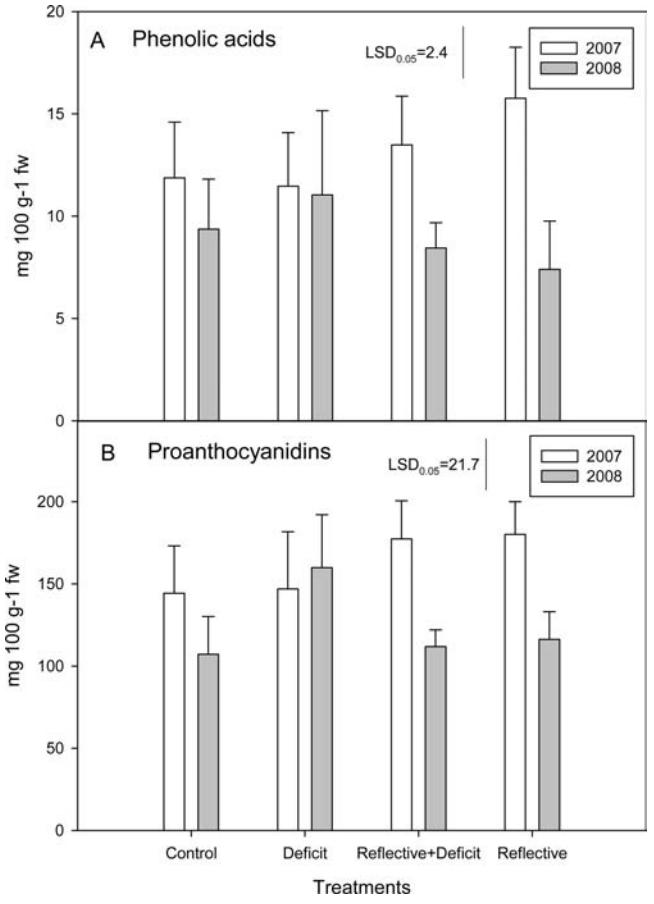

Figure 1. Influence of deficit irrigation, reflective mulch, and their combination on the content of phenolic acids (**A**) and proanthocyanidins (**B**) of Caldesi 2000 nectarines in two consecutive years at harvest. Each bar represents the mean of an irrigation treatment (n = 6) ± standard deviation.

Our results show that the content of total phenolics, mainly proanthocyanidins, increased when deficit irrigation was applied during the third fruit growth stage, considering samples at harvest and during storage (n = 48). This is in agreement with previous studies with peaches as well as with other fruit crops. For example, in a study with olives grown under different irrigation regimes, higher phenolic content was observed as irrigation water application decreased (*11*). Also, regulated deficit irrigation strategies in peaches increased fruit phenolic content, mainly anthocyanins and proanthocyanidins (*12*). It is interesting to mention that, in our study, nectarines from deficit irrigated trees had a higher content of proanthocyanidins in both experimental years than fruit from the other treatments studied, considering samples at harvest and during storage (n = 48). This may mean that whatever the cause of the differences between the two experimental years, the stress from deficit irrigation was the major cause of total phenolic accumulation in nectarines. As we reduced the amount of water available to the tree during the final fruit growth period early in the summer, this could stress the tree as, during that period, water is needed for shoot growth, carbohydrate production by the leaves, and fruit growth. According to Romero et al. (*21*) in olive trees and Roby et al. (*22*) in grapevines, the water stress implies an activation of the phenolic compound biosynthesis in the peel of fruits suffering from RDI. This fact was associated with an increase in the activity of L-phenylalanine ammonia lyase (PAL) as a response to water stress. Tovar et al. (*11*) associated a high PAL activity with the accumulation of anthocyanins and other phenolic compounds in olives.

When the influence of treatments was evaluated considering samples at harvest and during storage for 2, 4, and 6 weeks (n = 96), it was observed that the treatments significantly affected the content of proanthocyanidins and total phenolics depending on the year (**Table 2**). In 2007, Deficit, Reflective, and the combination of these two treatments significantly increased (and in a similar manner) the content of proanthocyanidins, when compared with that in the control treatment, whereas in 2008, only deficit irrigation significantly increased the content of proanthocyanidins (**Figure 2**). The same trend was observed for total phenolic content (data not shown). Romero et al. (*21*) observed that deficit irrigation influenced not only the amount of phenolic compounds in olive oil but also the phenolic profile.

In our study, anthocyanins and proanthocyanidins reacted differently to deficit irrigation treatment, although they share several early steps in the biosynthetic pathway. Contrary to our results, anthocyanin accumulation has been described to be modified by the water status of the plant (*23*). In particular, in red winegrapes, an early water stress is linked to an increase in anthocyanin biosynthesis. However, proanthocyanidins and other flavonoids were only slightly affected (*23*).

Influence of Fruit Position on Individual and Total Phenolic Compounds. Statistical evaluation on the influence of fruit position as a relevant factor to determine the impact of reflective mulch on the content of phenolic compounds is shown in **Table 1**. Treatments significantly affected the content of phenolic acids, proanthocyanidins, and total phenolics depending on the fruit position. During both experimental years, fruit from the upper part of the canopy had higher phenolic acids content than fruit from the lower part (**Figure 3**). However, when reflective mulch was used during fruit growth, the differences in the phenolic acid content diminished between the two positions as fruit from the lower part of the tree had phenolic acid content similar to that of the fruit from the upper canopy. In addition, the flavonol content of nectarines from the upper canopy was similar to that of the lower canopy without differences among treatments and years (**Table 1**). Surprisingly, no differences in the content of anthocyanins between fruit positions were observed among treatments and for both years. The content of anthocyanins was very low,

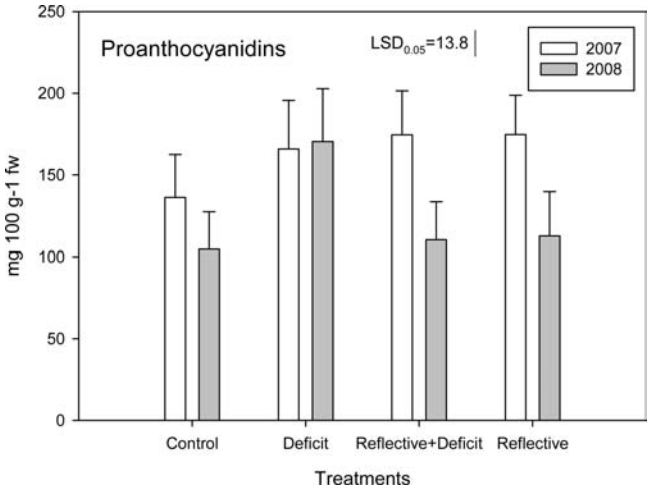

Figure 2. Influence of deficit irrigation, reflective mulch, and their combination on the content of proanthocyanidins of Caldesi 2000 nectarines in two consecutive years at harvest and during storage for 2, 4, and 6 weeks at 2 °C. Each bar represents the mean of an irrigation treatment ($n = 24$) ± standard deviation.

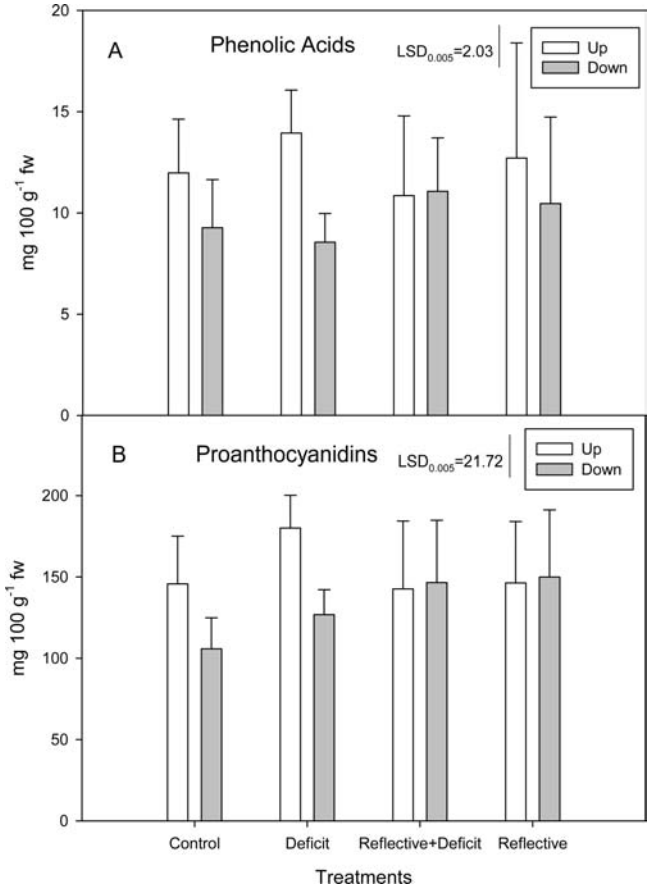

Figure 3. Influence of deficit irrigation, reflective mulch, and their combination on the content of phenolic acids (**A**) and proanthocyanidins (**B**) of Caldesi 2000 nectarines located in the upper and lower canopies at harvest. Each bar represents the mean of an irrigation treatment ($n = 3$) ± standard deviation.

and means for both positions and years were 0.78, 0.67, 0.84, and 0.63 mg/100 g fw in fruit from the control, Deficit, Reflective + Deficit, and Reflective treatments, respectively. Anthocyanins in Caldesi 2000 nectarines were localized mainly in the pericarp (peel) of the fruit, while negligible amounts were detected in the mesocarp (flesh). As the samples used for analysis were composed of 85% flesh + 15% peel and this nectarine cultivar accumulates anthocyanin pigments only in the light exposed side of the fruit, this could explain the large variability observed. Similar to the observations of phenolic acids, Deficit treatment significantly affected the proanthocyanidin content depending on the fruit position (**Figure 3**). Higher content of proanthocyanidins was found in fruit grown in deficit irrigation located in the upper canopy. In addition, total phenolic content, as the sum of individual phenolic groups, changed similarly to proanthocyanidins with fruit position and in both years (data not shown). However, reflective mulch significantly increased total phenolic content, particularly phenolic acids and proanthocyanidins, in fruits located in the lower part of the canopy (**Figure 3**). This fact could be explained by the increase in light reflection in the lower part of the canopy above the reflective mulch (data not shown). Our results agree with a previous study in which strawberries grown on plastic mulches had increased total phenolic contents when temperature increased (*24*).

In a previous study, reflective mulch under the tree canopy increased light availability in the lower part of the tree and improved red skin color in apples by increasing their anthocyanin content (*25*). In our results, reflective mulch treatment did not significantly affect most phenolic compounds measured in the sun-exposed fruit but often increased them in the fruit from the lower canopy compared to that in the control. We did not observe any statistical difference in anthocyanidin content between the control and reflective mulch treatments probably due to the evaluation of phenolic compounds in the whole fruit (flesh plus peel) and its possible dilution, in agreement with previous studies in which anthocyanins were only detected in the peel of peaches (*12*). Another factor affecting anthocyanin accumulation in fruits is temperature. It has been reported that genes involved in the anthocyanin biosynthetic pathway are considered as cold regulated genes (*26*). Reflective mulch in our work increased nectarine fruit temperature (data not shown) in agreement with Miller and Greene (*27*), who previously found that mulching increased apple fruit temperature. This increase in fruit temperature could lead to reduced anthocyanin synthesis. The explanation of the light effect on flavonoid biosynthesis is complicated as it is difficult to discriminate between the effect of the light and that of the temperature. Indeed, it has been proposed that accumulation of anthocyanins is more a function of temperature than of light. Temperature has a great influence on anthocyanin biosynthesis, this process being inhibited at low and high values, with the critical higher temperature identified around 30 °C (*28*). Interestingly, proanthocyanidin content increased in our mulched trees especially in 2007 and in fruit from the lower part of the canopy, pointing to changes in the phenylpropanoid biosynthetic pathway in favor of proanthocyanidins in the presence of excess light and, thus, increased fruit temperature. From this set of data, we can also conclude that the fruit from the trees receiving the combination treatment Reflective + Deficit behaved similarly to the fruit from the Reflective treatment, possibly showing less stress due to deficit irrigation. Finally, our results are in agreement with previous studies concluding that light induces the enzymatic activity of PAL and that this leads to increased total phenolic content in fruit (*16*).

Influence of Regulated Deficit Irrigation and Reflective Mulch on Individual and Total Phenolic Compounds during Storage. During storage, deficit irrigation and reflective mulch did not affect the content of phenolic acids, flavonols, and proanthocyanidins of nectarines Caldesi 2000 (**Table 2**). The only group of phenolic compounds that was significantly affected by the storage time

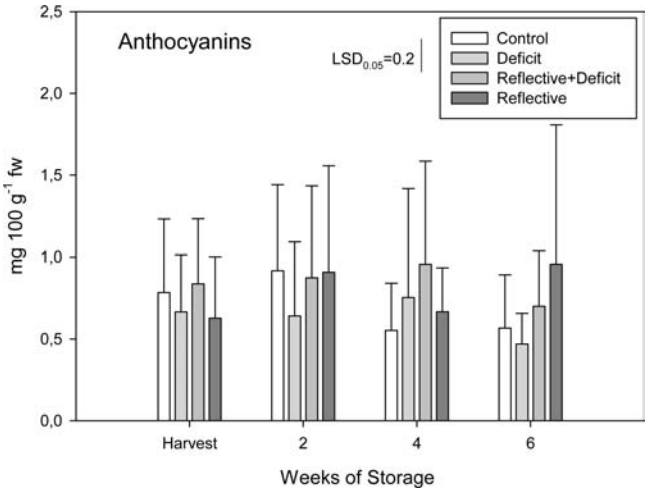

Figure 4. Influence of deficit irrigation, reflective mulch, and their combination on the content of anthocyanins of Caldesi 2000 nectarines during storage for up to 6 weeks at 2 °C. Each bar represents the mean of an irrigation treatment ($n = 6$) ± standard deviation.

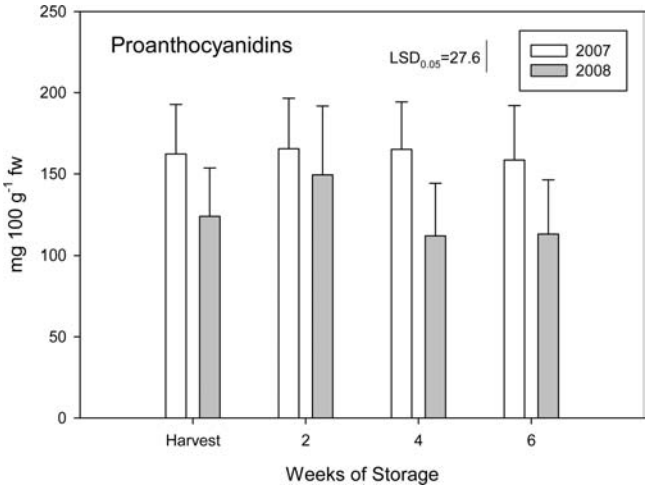

Figure 5. Influence of seasonal variation on the content of proanthocyanidins of Caldesi 2000 nectarines during storage for up to 6 weeks at 2 °C. Each bar represents the mean of different irrigation treatments ($n = 24$) ± standard deviation.

depending on the treatment was anthocyanins but without a clear tendency (**Figure 4**). As mentioned before, the content of anthocyanins was very low as peel and flesh were evaluated together and the fruit from the upper and lower canopy were pooled together. When the influence of seasons was studied, it was observed that postharvest storage at low temperatures did not affect the content of phenolic acids and flavonols for all treatments in both years (**Table 2**). The storage time influenced only the content of proanthocyanidins and total phenolic compounds depending on the year (**Table 2**). In 2007, the content of proanthocyanidins and total phenolic compounds was well preserved during storage, whereas in 2008 there was an increase after 2 weeks of storage followed by a drop, reaching the initial values, with extended storage. (**Figure 5**). We observed that in 2007, the phenolic content remained unchanged and close to the amounts found at harvest during storage in fruit with high phenolic content. This is in agreement with a previous study where the total phenolic content of peaches did not change during 2 weeks of storage at 4 °C (*29*). In our study, in 2008, in fruit with lower phenolic content than in 2007, the content of phenolic compounds increased after two weeks of storage, possibly due to cold stress. Theses observations were similar to a previous report by di Vaio et al. (*30*), who found that cold storage of peaches and nectarines cultivated in Italy enhanced total phenolic content by about 13%. During prolonged storage, phenolic content decreases with the appearance of chilling injury symptoms, even though it is expected that phenolic biosynthesis continues, due to the development of browning and the inability to evaluate these phenolic compounds with the methods used. Finally, during 2007, when the nectarines contained higher phenolic content, chilling injury was higher than in 2008 (data not shown).

This two-season study revealed that proanthocyanidins were the main phenolic compounds in Caldesi 2000 nectarines, when the edible part of the fruit was analyzed (composite samples of peel + flesh). Cold storage of up to 6 weeks did not modify individual and total phenolic compounds. Deficit irrigation increased phenolic content, in particular proanthocyanidins, mainly in sun-exposed fruit. Fruit from both positions in reflective mulched trees, even with reduced irrigation water, had similar phenolic content. This study shows that manipulation of horticultural practices including reduced use of irrigation water and increased light availability (through summer pruning, reflective mulch, etc.) may provide an efficient way to increase fruit phenolic content. This means that those horticultural practices, in particular, deficit irrigation, increasing the content of proanthocyanidins may enhance the health benefits of these fruit. However, although a high proanthocyanidin content is desirable, it may have a negative impact on the sensory characteristics of the fruit.

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