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Effects of Fruit Load and Reflective Mulch on Phenolic Compounds Accumulation in Nectarine Fruit

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Summary

The influence of fruit load per tree and reflective mulch on the content of phenol compounds was studied in two experiments conducted on nectarines (cultivar 'Stark Red Gold') from two orchards differing in tree density (1.660 and 830 trees ha^{-1}). Low crop load significantly increased fruit size and weight in both trials. Other parameters such as soluble solids, flesh firmness, acidity and skin color were affected only in nectarines from the low density orchard. At harvest, fruits from the low density orchard were more mature on low crop load trees compared to intermediate and high crop load trees. Crop load clearly affected the concentration of phenolic compounds: low-load fruits had a higher accumulation of phenolic compounds in skin and pulp tissues. Concentrations of cinnamic acids and flavan-3-ols consistently showed an inverse relationship to crop load.

Inter-row reflective mulching modified average maximum temperature and the intensity of reflected light in the canopy. The mulching also changed fruit quality at harvest by promoting the ripening and generally increasing the concentration of the main classes of phenolic compounds. Our data indicate that health promoting value of nectarine fruits can be enhanced by increasing phenolic content via crop load and reflective mulches.

Key words. *Prunus persica* – flavonoids – fruit thinning – crop load – light intensity – HPLC

Introduction

Phenolic compounds form an integral part of the human diet as they impart much of the color and flavour of fruits, vegetables, nuts and seeds (Parr and Bolwell 2000). These compounds and other secondary metabolites greatly influence the quality and health potential of foods (Stobiecki et al. 2002). Epidemiological data suggest that a high intake of fruit and vegetables can induce a number of beneficial effects on human health (Rissanen et al. 2003). Therefore, there is growing interest in the development of important food crops with optimised levels and composition of phenolic compounds.

While cultivar selection, traditional breeding programs and the metabolic engineering of the phenolic biosynthetic pathway (reviewed by Schijlen et al. 2004) are the most direct and powerful methods of improving crop nutritional potential, optimising field management practices and growing environments may also provide a rapid and efficient way to increase phenolic compound content (Schreiner 2005; Anttonen et al. 2006). Several studies have investigated the influence of management techniques and environmental conditions on phenolic compound accumulation in fruit species. Awad et al. (2001) showed little or no effect of crop load on flavonoids and chlorogenic acid concentration in 'Jonagold' apples, whereas the concentrations of anthocyanins, catechins and total flavonoids in 'Elstar' apples were generally decreased by increasing nitrogen supply (Awad and De Jager 2002). Experiments with 'bagged' fruit clearly showed the induction of anthocyanin biosynthesis by light in apple 'Cripps Red' (Takos et al. 2006). Moreover, postharvest irradiation with visible and UV light enhanced significantly the concentration of anthocyanins in both shade and sun-exposed apples (Hagen et al. 2007). Anthocyanin biosynthesis induction by light and UV radiation was also confirmed in peach fruit (cv. 'Hakuho') (Kataoka and Beppu 2004). The effect of temperature on anthocyanin biosynthesis was also studied on several apple cultivars. Post harvest treatments with temperature ranging from 10 to 30 °C, showed higher anthocyanin accumulation and related gene expression when low to intermediate temperature (around 17 °C) was combined with UV-light irradiation (Bakhshi and Arakawa 2006; Ubi et al. 2006; Ban et al. 2007). Different mulching techniques were also tested for their effect on phenolic compound accumulation in fruit. Foil and metallised films placed inter-row in the orchard were able to enhance colour and anthocyanin accumulation in 'Fuji' apples (Ju et al. 1999). Strawberries grown on white mulch were characterised by higher anthocyanin, ellagic acid and total phenolic compound levels in comparison to strawberries grown on brown mulch (Anttonen et al. 2006).

The composition of phenolic compounds in peach fruit has been correlated to the ripening stage in different cultivars (Senter and Callahan 1990; Tomas-Barberan et al. 2001; Andreotti et al. 2008). As for the cultivation methods, organically grown peaches ('Regina bianca')

showed a highly significant increase in polyphenols in comparison to conventionally grown peaches (CARBONARO and MATTERA 2001). The effects of rootstocks on 'Suncrest' peach fruit total phenolic compound levels were also demonstrated in a five-rootstock comparison trial performed by GIORGI et al. (2005). Regulated deficit irrigation technique was associated with higher total phenolic compounds in 'Flordastar' peaches when compared with fruits from fully irrigated trees (BUENDIA et al. 2008). The same authors also reported small effects of crop loads on the antioxidant compounds of the same peaches.

Although phenolic compounds in fruits were the objects of different research studies, in our opinion further investigation is needed to gain knowledge on the optimisation of antioxidant compounds in ripened peach fruits. This study therefore investigate the effect of specific management practices, such as fruit load control and reflective mulching, on the phenolic compound profile and accumulation in nectarines as a way to increase the health promoting value of fruits.

Material and Methods

Experimental procedure

Fruit trials were run in 2005 and 2006 at Bologna University's Experimental Station located in Cadriano (Emilia-Romagna, Italy), using the yellow-flesh cultivar 'Stark Red Gold'. The 2005 experiment was conducted on eleven-year-old trees trained to transverse Y, grafted to *P. persica* and spaced 1.5 × 4 m for a density of approximately 1,660 trees ha^{-1} ('high density' orchard). Pest control and fertilization were those commonly used by growers. The alleyways were grassed and the irrigation was performed with a drip system settled to satisfy the whole crop evapotraspiration during the growing season. Nine trees were selected for their uniformity and on 31 May were hand-thinned to establish three different levels of final crop load per tree: low crop load at about 50 fruit tree^{-1} for approximately 10 kg tree^{-1} yield; intermediate crop load at about 80 fruit tree^{-1} for approximately 12.5 kg tree^{-1} yield; and high crop load at about 120 fruit tree^{-1} for approximately 18 kg tree^{-1} yield. The experimental design was a randomised block with three replicates of each tree. At harvest on 2 August (the second of three consecutive harvests performed at one week intervals) twenty fruits per tree were randomly collected from the outer part of the canopy at approximately 1 to 2 m above the ground, weighed and individually analysed for soluble solids (°Brix), flesh firmness (N) and skin colour (Hue-angle). Three bulk samples per replicate were later prepared for both fruit epicarp (skin) and mesocarp (pulp) and stored at −20 °C for later analysis.

The 2006 experiment was conducted on twelve-year-old-trees grafted to *P. persica*, trained to vase and spaced 3 × 4 m for a density of about 830 trees ha^{-1} ('low density' orchard). Pest control, fertilization and irrigation management were the same as reported above. Eighteen trees were chosen for their uniformity, and on 15 May reflective mulches (Extenday Europe Ltd, Egham, UK) were laid down on both sides of the grassed inter-row of half of them. Canopy temperature in mulch-treated and untreated trees was monitored during the experiment with a temperature data logger (Easylog USB-2 data logger, MicroDAQ.com, Ltd, USA). Reflective light intensity was evaluated using a lux meter (TES-1332, CL-Electronics GmbH, Buchs, Switzerland). Measurements were performed at noon on five different days (3rd – 7th – 10th – 14th – 17th of July 2006) and at four levels above the ground (0.5, 1.0, 1.5, and 2.0 m).

Mulch-treated and untreated trees were thinned to three loads: low with one fruit per branch, or about 150 fruit tree^{-1}, yielding approximately 30 kg tree^{-1}; intermediate with 2–3 fruit per branch, or about 250 fruit tree^{-1}, yielding approximately 45 kg tree^{-1}; and high with no fruit thinning resulting in about 500 fruit tree^{-1} and approximately 75 kg tree^{-1}. The experimental design was a split-plot with three replicates of each treatment: the first factor was the presence or absence of the reflective mulch (1 = control; 2 = reflective mulch) and the second factor the fruit load per tree (a = low; b = intermediate; c = high). During the main harvest on 5 August (second of three harvests), twenty fruits per tree were randomly collected from the outer part of the canopy at a height of approximately 1 to 2 metres above the ground. Fruit quality determination and sample preparation were performed as in 2005.

Fruit quality and analysis of phenol compounds

At harvest, fruit weight (g), total soluble solids content (°Brix measured with an Atago digital refractometer, Optolab, Modena, Italy), flesh firmness (N measured with an 8-mm probe EFFE.GI penetrometer, Ravenna, Italy), total acidity (titration in 20 mL of fresh juice using 0.25 N NaOH by a semiautomatic Compact-S Titrator, Crison, Modena, Italy) and skin hue colour (° measured with a Minolta Colorimeter Model 310 Tokyo, Japan) were determined. Phenolic compounds were analysed with a Gilson HPLC system at a 280 nm wavelength and a reverse-phase Supelcosil^{TM}LC-18 HPLC column (15 cm long, 4 mm ID, ODS particles of 5 μm diameter). 6-methoxy-flavone (0.025 mg ml^{-1} in methanol) was used as internal standard. The elution solvents were aqueous 0.01 M phosphoric acid (solvent A) and 100 % methanol for liquid chromatography (solvent B). The injection volume was 10 μl and the flow rate 1 ml min^{-1}.

The analytical HPLC gradient and the identification and quantification of phenolic compounds followed the information reported in ANDREOTTI et al. (2008). Concentrations of the main classes of phenolic compounds were calculated as follows: cinnamic acids (as sum of chlorogenic acid and neo-chlorogenic acid); flavan-3-ols (as sum of catechin, epicatechin and procyanidins); cyanidins (as cyanidin-3-glucoside, the major compound); flavonol glycosides (as sum of quercetin-3-rutunoside and other quercetin derivatives). Standard molecules (catechin, epicatechin, chlorogenic acid, quercetin-3-rutinoside, 6-methoxy-flavone) were acquired from Sigma-Aldrich (St. Louis, MO, USA) and Extrasynthese (Lyon, France; cyanidin-3-glucoside).

Statistics

All results were subjected to analysis of variance and Student-Newman-Keuls Multi-Range test for comparison of means (SAS 9.0 Software Package).

Results

Crop load effect at high density

As expected, fruit collected from severely thinned, low load trees were significantly larger than those from high load trees (188.6 and 151.9 g, respectively; Table 1). The other quality parameters were not significantly affected by fruit load per plant. The total amount of phenolic compounds was significantly higher in low fruit load trees (Table 2). While phenolic compound concentrations were highest in both analysed tissues of the fruits originating from low load trees, the differences were statistically significant for fruit mesocarp only: 1.9, 1.5 and 0.7 mg g^{-1} DW (dry weight) for low, intermediate and high load, respectively. Cinnamic acids and flavan-3-ols were the two main classes of phenolic compounds detected in ripened fruits and showed significant differences in concentration. Skin concentration of cinnamic acids ranged from 1.6 mg g^{-1} DW at high fruit load to 2.4 mg g^{-1} DW at low fruit load. The highest accumulation in pulp was 1.1 mg g^{-1} DW at low load. Flavan-3-ols showed highest concentrations at 2.3 and 0.6 mg g^{-1} DW in the skin and pulp of low-load fruits, respectively. Two classes of phenolic compounds (cyanidins, mainly cyanidin-3-glucoside, and flavonol glycosides) were detected and quantified in skin tissue only. Cyanidin accumulation was highest in intermediate load fruit (1.32 mg g^{-1} DW), but the differences among fruit load treatments were not significant. Similarly, flavonol glycosides presented higher concentrations in the intermediate load fruit (1.25 mg g^{-1} DW), with no statistically significant differences among treatments.

Fruit load and mulch effect at low density

Table 3 shows the main fruit quality traits per thinning treatment. As expected, severely thinned trees produced the largest fruit: 214, 188 and 143 g for low, intermediate and high loads, respectively. Fruit from low load trees were also the ripest, as indicated by the soluble solids content (15.0 and 12.4 °Brix for low and high load, respectively) and flesh firmness (53.0 and 55.9 N for low and high load). Low load fruit also showed the highest significant accumulation of organic acids (11.4 in comparison to 8.4 g L^{-1} in high load fruit). No significant differences were found for H-angle index.

The main environmental parameters affected by reflective mulching were canopy temperature and reflected-light intensity (Table 4). Maximal daily temperature was enhanced by reflective mulching and reflected light intensity was 6.5 to 7.5 fold higher in mulch-treated trees, depending on the height at which measurements were taken.

The main fruit quality parameters, specifically fruit weight, soluble solids and flesh firmness were clearly affected by fruit load levels also under mulching (Table 3). Fruit weight and total soluble solids content were higher in low and intermediate crop load trees as compared to high crop load, whereas differences in flesh firmness were significant between intermediate and high fruit load only, with 42.2 and 54.9 N respectively. No significant difference was detected for H angle and acidity among the three fruit load levels under mulching. To establish the effects of mulching alone on fruit quality, values for each parameter at different load levels were calculated as an average and compared to control trees. The results

Table 1. Fruit quality traits of 'Stark Red Gold' nectarine at harvest as affected by fruit load (season 2005).

Fruit load	Fruit weight (g)	Brix (%)	Flesh firmness (N)	H-angle
High	151.9 b	13.0	36.3	43.5
Intermediate	173.7 ab	12.9	40.2	39.1
Low	188.6 a	12.6	39.2	40.0
SNK-test	*	NS	NS	NS

NS: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

Table 2. Phenolic compound concentration (mg g^{-1} DW) of 'Stark Red Gold' nectarine at harvest as affected by fruit load (season 2005).

Fruit load	Cinnamic acids		Flavan-3-ols		Cyanidins		Flavonol gly.		Total phenol	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
High	1.62b	0.57a	1.28b	0.15	0.73	–	1.07	–	4.98	0.77b
Intermediate	1.93b	0.89ab	1.54b	0.43	1.32	–	1.25	–	6.20	1.54ab
Low	2.49a	1.15a	2.32a	0.60	1.23	–	0.81	–	7.04	1.93a
SNK-test	*	*	**	NS	NS	–	NS	–	NS	*

–: not detected
NS: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

Table 3. Fruit quality characteristics of 'Stark Red Gold' nectarine at harvest as affected by fruit load and reflective mulching (season 2006).

	Fruit weight (g)	Brix (%)	Flesh firmness (N)	H-angle	Acidity (g L^{-1})
Fruit load					
High	143.1 c	12.4 c	55.9	60.2	8.4 b
Intermediate	188.2 b	13.7 b	52.0	56.2	10.2 a
Low	214.9 a	15.0 a	53.0	62.0	11.4 a
SNK-test	**	**	NS	NS	**
Reflective mulching at different fruit load					
High	152.6 b	13.3 b	54.9 a	49.9	9.3
Intermediate	252.2 a	14.7 a	42.2 b	48.2	9.6
Low	233.9 a	14.7 a	47.1 ab	50.1	10.2
SNK-test	**	**	**	NS	NS
Treatment					
Control	178.7 b	13.6 b	54.0 a	59.3 a	10.0
Reflective mulching	209.1 a	14.2 a	48.1 b	49.4 b	9.7
SNK-test	**	**	**	**	NS

NS: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

Table 4. Influence of reflective mulching on temperature (average values of the first 20 days of July 2006) and light intensity (average of five detections).

	Temperature (°C)		Reflected light intensity (Lux)			
	Min	Max	0.5 m	1 m	1.5 m	2 m
Control	17.7	34.9	7600	5600	4500	4300
Reflective mulching	17.2	39.3	51800	42300	32600	28300
SNK-test	NS	**				

NS: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

clearly indicate an effect of mulching on the enhancement of fruit growth (209 compared to 178 g in control fruit), ripening (°14.2 compared to 13.6 ° Brix in control fruit), and lower flesh firmness (48.1 compared to 54.0 N). Although no significant difference was found in acidity, the H-angle value was higher for control fruit (59.3 compared to 49.4).

Total phenolic compound levels were influenced by fruit load. Their accumulation was highest in the skin and pulp of low load fruit. Concentration in the skin ranged from 6.9 for low load to 5.0 mg g^{-1} DW for high load and in the pulp the highest levels were detected in intermediate and low load fruit at 1.8 mg g^{-1} DW for both (Table 5). The main classes of phenolic compounds showed the highest concentration in low load fruit. Flavan-3-ols (catechin, epicatechin and oligomeric procyanidins) differed significantly in the skin at the three loads: high, intermediate and low respectively logged 1.2, 1.7 and 2.7 mg g^{-1} DW. Pulp flavanols (0.1 mg g^{-1} DW) and cinnamic acids concentrations in both skin (1.8 mg g^{-1} DW) and pulp (0.4 mg g^{-1} DW) of high load fruits consistently differed significantly from those of thinned trees. Only skin cyanidins and pulp flavonols recorded the highest concentrations in high load fruit.

Differences in the cinnamic and flavanol classes of compounds remained significant when high load fruit are compared against the other loads also after mulching application. The same trend for cyanidins and flavonols was also found for mulch-treated fruit. Skin concentrations from fruit of non-thinned trees were significantly higher than in low load fruit: 0.8 and 1.4 mg g^{-1} DW, respectively, compared to 0.2 and 0.7 mg g^{-1} DW.

To establish the effects of mulching alone on fruit phenolic compounds, values for each class of compounds at different load levels were calculated as an average and compared to control treated fruit. Mulching significantly enhanced the accumulation of cinnamic acids and flavan-3-ols. As a consequence, total phenolic compound

Table 5. Concentration of phenolic compounds (mg g^{-1} DW) 'Stark Red Gold' nectarine at harvest as affected by fruit load and reflective mulching (season 2006).

Fruit load	Cinnamic acids		Flavan-3-ols		Cyanidins		Flavonol gly.		Total phenol	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
Fruit load										
High	1.79 b	0.39 b	1.28 c	0.14 b	0.91	–	0.86	0.17 a	5.00 b	0.83 b
Intermediate	1.73 b	0.91 a	1.78 b	0.75 a	0.69	–	0.91	0.08 b	5.19 b	1.84 a
Low	2.46 a	0.89 a	2.72 a	0.74 a	0.77	–	0.84	0.08 b	6.94 a	1.80 a
SNK-test	*	**	**	**	NS	–	NS	**	**	**
Reflective mulch per fruit load										
High	2.04 b	1.18	2.10 b	1.03 b	0.88 a	–	1.42 a	0.11 a	6.53 b	2.37 b
Intermediate	2.48 a	1.31	3.44 a	1.59 a	0.63 a	–	1.23 a	0.08 ab	7.96 a	3.07 a
Low	2.53 a	1.42	3.11 a	1.12 b	0.22 b	–	0.70 b	0.07 b	6.70 b	2.69 ab
SNK-test	**	NS	**	**	**	–	**	*	*	*
Treatment										
Control	1.99 b	0.73 b	1.93 b	0.54 b	0.79	–	0.87 b	0.11	5.71 b	1.49 b
Reflective mulching	2.35 a	1.30 a	2.88 a	1.24 a	0.58	–	1.12 a	0.09	7.03 a	2.71 a
SNK-test	**	**	**	**	NS	–	*	NS	**	**

–: not detected
NS: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

accumulation was higher in mulch-treated fruit skin (7.0 mg g^{-1} DW) and pulp (2.7 mg g^{-1} DW) than in control (5.7 and 1.4 mg g^{-1} DW, respectively). Mulching did not increase cyanidin accumulation in skin despite the enhancement of reflected light and the reddish external colour visualised by the Hue angle (Table 3). Flavonol glycosides were higher in mulch-treated fruit skin (1.1 mg g^{-1} DW) but no significant differences were detected in comparison to pulp.

Discussion

Our results indicate that crop load affects phenolic compound accumulation in fruit at harvest and that reflective mulching is a technique able to enhance fruit yield and quality.

Low load fruit had the highest content of phenolic compounds (carbon based secondary metabolites) and compounds from primary metabolism, as shown by the higher levels of sugars and organic acids. This finding suggests that the greater availability of assimilates in fruit lowered the internal trade-off between primary and secondary metabolism, as previously reported by Poll et al. (1996). *In vitro* studies conducted in apple have shown how the content of total phenolic compounds was increased by increasing the amount of sucrose in agar medium and that changes also occurred in the qualitative composition of phenolic compounds (Lux-Endrich et al. 2000). We found that cinnamic acids and flavan-3-ols consistently enhanced concentrations under both high and low density orchard conditions (2005 and 2006 experiments respectively) and in both the mesocarp and the epicarp.

Reflective mulching increased fruit size and accelerate fruit ripening, as shown by °Brix, flesh firmness, acidity and skin-colour values at harvest. Similar results are reported for pear in Denmark (Bertelsen 2005), where the use of reflective mulches resulted convenient for cultivars such as 'Clara Frijs', characterised by high economic returns. Light reflection at canopy level was increased by several folds, confirming the results also reported in Solomakhin and Blanke (2007). The observed general increment of the phenolic compounds concentration can be seen as a consequence of the changes induced on canopy light micro-climate by mulching. These findings were in part expected since phenolic compounds are UV-absorbing pigments in all plant organs and, therefore, are known to be sensitive to environmental conditions like light intensity (Cockell et al. 2000; Burchard et al. 2000; Kolb et al. 2003; Andreotti et al. 2006). Our results also agree with findings reported for strawberry by Anttonen et al. (2006), who showed that white reflecting mulch increased total phenolic compound content and antioxidant activity in comparison to brown mulch. They also found that total cyanidin content was higher in fruit grown on brown mulch, which agrees with our finding that cyanidins were the only class of compounds not enhanced by reflective mulching. This is surprising since, as shown in the apple cv. 'Jonathan' by Bakhshi and Arakawa (2006), the metabolism of cyanidins is light-dependent, with biosynthesis being enhanced by irradiation. This suggests that the higher temperature induced by the mulching in the canopy may have stimulated cyanidin

catabolism. Temperature is one of the major factors affecting anthocyanin synthesis in fruits. For instance, it is known that cool temperatures at ripening lead to more intensive red color apples than warmer conditions (Ubi et al. 2006; Ban et al. 2007). A positive co-relation was also detected in blood orange, linking low temperature, storage conditions and cyanidin-3-O-glucoside synthesis (Lo Piero et al. 2005). Alternatively, it is possible that, given the enhanced vegetative growth of the mulch-treated canopy, fruits may have been partially shaded by the higher number and larger size of adult leaves. Fruit shading and bagging are known to reduce apple peel concentration of compounds such as anthocyanins and flavonols (Takos et al. 2006). This may have occurred in our severely thinned trees with low fruit load and high vegetative vigour.

Reflective mulches could also have reduced soil water evaporation therefore determining differences in the trees water status. According to Buendia et al. (2008) water stress implies an higher biosynthesis and accumulation of phenolic compounds, especially at fruit skin level. Although direct investigation of plant water level was not performed, we can exclude such situation giving the fact that trees did not present any water stress symptoms (both at leaf and fruit level) and that the final nectarines water content was similar in control and mulch-treated fruits (measured average fruit dry weight of 12 %). It is our opinion that the differences observed in the final phenolic accumulation in fruits have to be therefore ascribed to the already reported changes induced by reflective mulches in the micro-climate conditions at tree canopy level.

Both, the enhancement of nutritional value and the development of management techniques for the optimisation of flavonoid levels in fresh fruit are important goals of modern horticulture (Stobiecki et al. 2002; Schijlen et al. 2004). Our data show that optimal fruit load (that means an average of 2 nectarine fruits per productive branches 50–60 cm long) and the improvement of canopy light environment can directly affect final quality parameters and specific phytochemical concentrations. As reported in Fig. 1, if we consider a peach

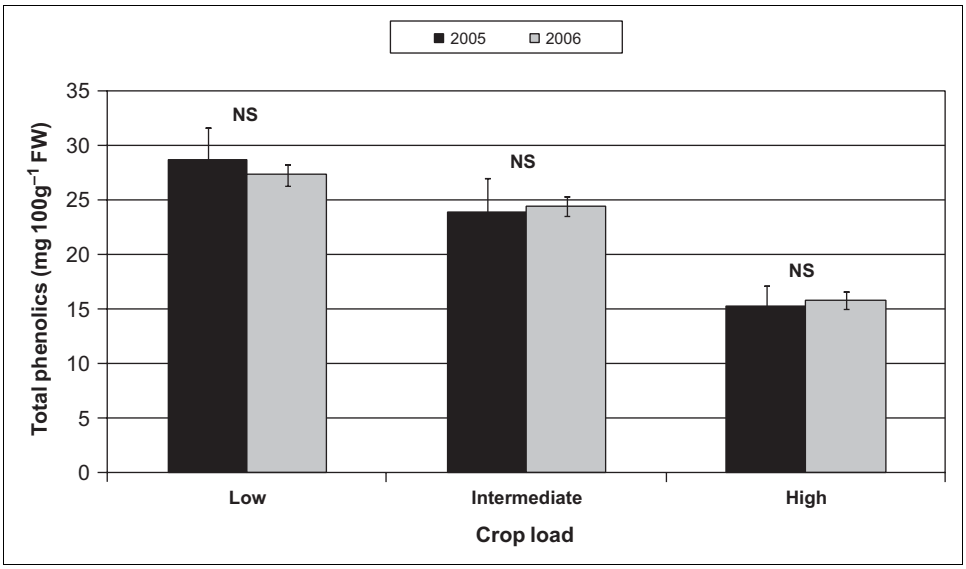


Fig. 1. Total phenol concentrations calculated in a 100-g portion of fresh nectarine fruit including both pulp (80 % of total weight) and skin (20 % of total weight) as affected by fruit load in two growing seasons (NS: not significant).

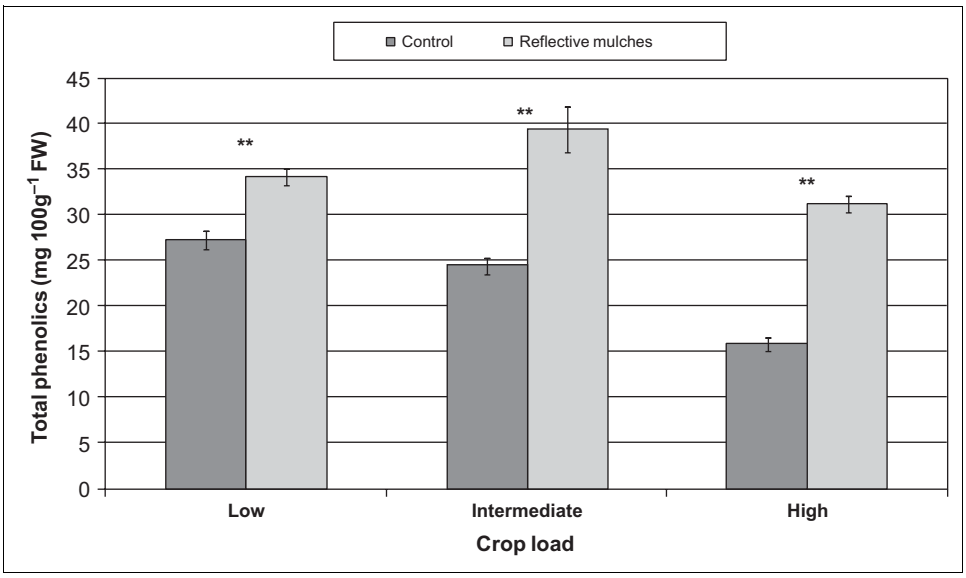


Fig. 2. Total phenol concentrations in 2006, calculated in a 100-g portion of fresh nectarine fruit including both pulp (80 % of total weight) and skin (20 % of total weight) at three fruit loads as affected by reflective mulching (** indicates significant differences at $P \leq 0.01$).

portion of 100 g FW composed of both edible pulp and skin, 80 and 20 % of total portion weight respectively, the total count of phenolic compounds was enhanced by lowering the crop load per tree in a similar way both in the high and in the low density orchard conditions. This shows the appreciable effect of tree fruit load on phenolic compound accumulation in fruit, regardless of tree training system and planting density. Moreover, application of reflective mulching increased final fruit phenolic compound content by 30–50 % (Fig. 2). This shows that also crop management tools, in addition to traditional plant breeding and genetic engineering (PARR and BOLWELL 2000), may also contribute to optimisation of the phenolic compound profile in nectarine fruits.

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References

ANDREOTTI, C., G. COSTA and D. TREUTTER 2006: Composition of phenolic compounds in pear leaves as affected by genetics, ontogenesis and the environment. Sci. Hortic. **109**, 130–137.

ANDREOTTI, C., D. RAVAGLIA, A. RAGAINI and G. COSTA 2008: Phenolic compounds in peach (*Prunus persica*) cultivars at harvest and during fruit maturation. Ann. Appl. Biol. **153**, 11–23.

ANTTONEN, M.J., K.I. HOPPULA, R. NESTBY, M.J. VERHEUL and R.O. KARJALAINEN 2006: Influence of fertilization, mulch color, early forcing, fruit order, planting date, shading, growing environment, and genotype on the contents of selected phenolics in strawberry (*Fragaria × ananassa* Duch.) fruits. J. Agric. Food Chem. **54**, 2614–2620.

AWAD, M.A., A. DE JAGER, M. DEKKER and W.M.F. JONGEN 2001: Formation of flavonoids and chlorogenic acid in apples as affected by crop load. Sci. Hortic. **91**, 227–237.

AWAD, M.A. and A. DE JAGER 2002: Relationships between fruits nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. Sci. Hortic. **92**, 265–276.

BAKHSHI, D. and O. ARAKAWA 2006: Effects of UV-B irradiation on phenolic compound accumulation and antioxidantactivity in 'Jonathan' apple influenced by bagging, temperature and maturation. J. Food, Agric. Environ. **4**, 75–79.

BAN, Y., C. HONDA, Y. HATSUYAMA, M. IGARASHI, H. BESSHO and T. MORIGUCHI 2007: Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. Plant Cell Physiol. **48**: 958–970.

BERTELSEN, M. 2005: Reflective mulch improves fruit size and flower bud formation of pear cv. 'Clara Frijs'. Acta Hort. **671**:87–95.

BUENDÍA, B., A. ALLENDE, E. NICOLS, J. ALARCN and M.I. GIL 2008: Effect of regulated deficit irrigation and crop load on the antioxidant compounds of peaches. J. Agric. Food Chem. **56**, 3601–3608.

BURCHARD, P., W. BILGER and G. WEISSENBOCK 2000: Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant, Cell Environ. **23**, 1373–1380.

CARBONARO, M. and M. MATTERA 2001: Polyphenoloxidase activity and polyphenol levels in organically and conventionally grown peach (*Prunus persica* L., cv. 'Regina bianca') and pear (*Pyrus communis* L., cv. 'Williams'). Food Chem. **72**, 419–424.

COCKELL, C.S., A. SOUTHERN and A. HERRERA 2000: Lack of UV radiation in Biosphere 2 – practical and theoretical effects on plants. Ecol. Engineering **16**, 293–299.

GIORGI, M., F. CAPOCASA, J. SCALZO, G. MURRI, M. BATTINO and B. MEZZETTI 2005: The rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach (cv. 'Suncrest'). Sci. Hortic. **107**, 36–42.

HAGEN, S.F., G.I.A. BORGE, G.B. BENGTSSON, W. BILGER, A. BERGE, K. HAFFNER and K.A. SOLHAUG 2007: Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica* Borkh., cv. 'Aroma'): Effect of postharvest UV-B irradiation. Postharv. Biol. Technol, **45**, 1–10.

JU, Z., Y. DUAN and Z. JU 1999: Effects of covering the orchard floor with reflecting films on pigment accumulation and fruit coloration in 'Fuji' apples. Sci. Hort. **82**, 47–56.

KATAOKA, I. and K. BEPPU 2004: UV Irradiance Increases Development of Red Skin Color and Anthocyanins in 'Hakuho' Peach. Hort. Sci. **39**, 1234–1237.

KOLB, C.A., J. KOPECKY, M. RIEDERER and E.E. PFÜNDEL 2003: UV screening by phenolics in berries of grapevine (*Vitis vinifera*). Functional Plant Biol. **30**: 1177–1186.

LO PIERO, A.R., I. PUGLIESI, P. RAPISARDA and G. PETRONE 2005: Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. J. Agric. Food Chem. **53**, 9083–9088.

LUX-ENDRICH, A., D. TREUTTER and W. FEUCHT 2000: Influence of nutrients and carbohydrate supply on the phenol composition of apple shoot cultures. Plant Cell Tissue Organ Cult. **60**, 15–21.

PARR, A.J. and G.P. BOLWELL 2000: Phenols in plant and man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci. Food Agric. **80**, 985–1012.

POLL, L., A. RINDOM, T.B. TOLDAM-ANDERSEN and P. HANSEN 1996: Availability of assimilates and formation of aroma compounds in apples as affected by the fruit/leaf ratio. Physiol. Plant. **97**, 223–227.

RISSANEN, T.H., S. VOUTILAINEN, J.K. VIRTANEN, B. VENHO, M. VANHARANTA, J. MURSU and J.T. SALONEN 2003: Low intake fruits, berries and vegetables is associated with excess mortality in men: the Kuopio ischemic heart disease risk factor (KIHD) study. J. Nutr. **133**, 199–204.

SCHIJLEN, E.G.W.M., C.H.R. DE VOS, A.J. VAN TUNEN and A.G. BOVY 2004: Modification of flavonoid biosynthesis in crop plants. Phytochemistry **65**, 2631–2648.

SCHREINER, M. 2005: Vegetable crop management strategies to increase the quantity of phytochemicals. Eur. J. Nutr., **44**, 85–94.

SENTER, D.S. and A. CALLAHAN 1990: Variability in the quantities of condensed tannins and other major phenols in peach fruit during maturation. J Food Sci. **55**: 1585–1587.

SOLOMAKHIN, A. and M. BLANKE 2007: Overcoming adverse effects of hailnets on fruit quality and microclimate in an apple orchard. J. Sci. Food Agric. **87**, 2625–2637.

STOBIECKI, M., I. MATYSIAK-KATA, R. FRANSKI, J. SKALA and J. SZOPA 2002: Monitoring changes in anthocyanin and steroid alkaloid glycoside content in lines of transgenic potato plants using liquid chromatography/mass spectrometry. Phytochemistry **62**, 959–969.

TAKOS, A.M., S.P. ROBINSON and A.R. WALKER 2006: Transcriptional regulation of the flavonoid pathway in the skin of dark-grown 'Cripps' Red' apples in response to sunlight. J. Hortic. Sci. Biotech. **81**, 735–744.

TOMAS-BARBERAN, F.A., M.I. GIL, P. CREMIN, A.L. WATERHOUSE, B. HESS-PIERCE and A.A. KADER 2001: HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches and plums. J. of Agric. Food Chem. **49**: 4748–4760.

UBI, B.E., C. HONDA, H. BESSHO, S. KONDO, M. WADA, S. KOBAYASHI and T. MORIGUCHI 2006: Expression analysis of anthocyanin biosynthetic genes in apple skin: effect of UV-B and temperature. Plant Sci. **170**, 571–578.

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