Influence of Hail Net and Reflective Foil on Cyanidin Glycosides and Quercetin Glycosides in ‘Fuji’ Apple Skin

Jerneja Jakopič¹, Franci Štampar, and Robert Veberič

University of Ljubljana, Biotechnical Faculty, Agronomy Department, Chair for Fruit Growing, Viticulture and Vegetable Growing, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

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Abstract. The objective of this work was to compare the contents of cyanidin glycosides and quercetin glycosides in the skin of apples grown with or without hail nets and using reflective foil or not. Under hail nets, photosynthetically active radiation was 10% to 30% lower in comparison with the control treatment. Covering the orchard floor with reflective foil had a positive effect on lighting, particularly on the lower parts of the fruit. Fruit coloration depends on the contents of anthocyanins copigmented with flavonols, the synthesis of which is light-dependent. The content of the main cyanidin glycoside in ‘Fuji’ apple, cyanidin galactoside, was lowest in the control treatment as well as concentrations of cyanidin arabinoside and two other cyanidin pentosides. Reflective foil caused a higher cyanidin glycoside content. Among flavonols, quercetin galactoside, quercetin glucoside, quercetin pentoside, quercetin arabinofuranoside, quercetin xyloside, quercetin rutinoside, quercetin rhamnoside, and quercetin were detected. Hail net and reflective foil both affected the increasing quercetin–glycosides contents. The highest amounts were achieved in the treatment under the hail net, where the orchard floor was covered with reflective foil. We also analyzed catechin, epicatechin, and chlorogenic acid. The lowest amounts of these were measured in the skin of fruit grown on trees under hail nets. In the control treatment, contents of those phenolic compounds were equal or higher, whereas the highest concentrations were detected in the treatments using reflective foil, where lighting was also higher in comparison with the treatments without it.

In apple production, the incidence of hailstorms during the growing season is high in some regions. The damage to leaves caused by hail decreases photosynthesis, causes damage to the fruit during the growing season (Tartachnyk and Blanke, 2002), and is an infection entry point for diseases such as fireblight. To protect the assimilation area and ensure high fruit quality, apple trees are increasingly grown under hail nets. The nets used for protection against hail represent an additional investment (Štampar et al., 2002).

Several different types of nets are used to protect fruit crops against hail. In European fruit orchards, most of the hail nets used are black, some white, and since 2007 colored (red and green) (Blanke, 2007) hail nets have also become available. However, on a sunny summer day, light intensities under the hail nets are lower compared with the outside control (Solomakhin and Blanke, 2008). Black hail nets greatly reduce incident solar light, and they may have a negative impact on fruit development and on the final color of fruit (Guerrero et al., 2002; Štampar et al.,

2002). Fruit grown under these hail nets suffers from lower fruit quality, i.e., less (red) coloration, less firm fruit flesh, less sugar, and therefore less taste (Funke and Blanke, 2005). White and colored hail nets reduced light less than black hail nets and the black hail nets decreased fruit coloration in the poorly colored apple cultivar Pinova more than white and colored (Blanke, 2007).

Reflective white woven cloth placed in the grass alleys between the tree rows under the hail net can overcome these shortcomings (Funke and Blanke, 2005). Relative to the grassed control, two tested reflective cloths increased the percentage of Class I fruit with greater than 25% coloration by 12% (from 82% to 94%) without hail nets and by 23% (from 69% to 89%) under hail nets (Solomakhin and Blanke, 2007). Reflective foil appears to be a method for increasing red skin coloration in ‘Gala’ apples (Layne et al., 2002). Glenn and Puterka (2007) reported that the use of reflective, aluminized plastic film increased fruit red color and that the use of reflective, particle films increased average fruit weight. These mulches reflect solar radiation into the tree canopy and may increase canopy absorption of photosynthetic photon flux by up to 40% (Green et al., 1995). This additional light is useful for both photosynthesis and anthocyanin pigment production (Jakopic et al., 2007; Layne et al., 2002). Although the red color of apple fruit is determined by the concentration of anthocyanin in the fruit peel, it is also

affected by concentrations of other pigments like flavonoids, chlorophyll, and carotenoids (Lancaster, 1992). A variety of red colors are produced by cyanidin glycosides copigmented with flavonols and other compounds (Lancaster, 1992).

Apple fruit is known to be rich in flavonoid compounds such as anthocyanins, dihydrochalcones, quercetin 3-glycosides, catechin, and epicatechin and its polymers, which are mainly located in the skin (Awad et al., 2001; Lata et al., 2009). Polyphenols are a major antioxidant in apples. Antioxidants scavenge and neutralize free radicals, which in turn play a role in the onset of cardiovascular disease and cancer (Biedrzycka and Amarowicz, 2008). The main anthocyanin pigment is cyanidin 3-galactoside, which can scavenge superoxide radicals in an in vitro system (Yamasaki et al., 1996).

One factor among those that may affect the concentration of phenolic components in apples is light exposure (Awad et al., 2000). Flavonoid and chlorogenic acid contents in fruit vary greatly among cultivars, orchards, positions within the tree, and even within individual fruit (Awad et al., 2000).

Jakopic et al. (2007) demonstrated that light use of ‘Fuji’ apple trees grown under hail nets could be improved using reflective groundcover, resulting in better fruit coloration. The objective of the present work was to evaluate the effect of netting and reflective foil on the contents of individual phenolics compounds. We measured changes in the concentrations of four cyanidin glycosides, seven quercetin glycosides and aglycone quercetin as well as catechin, epicatechin, and chlorogenic acid as a result of the lighting changes created by hail nets and reflective foil.

Material and Methods

Plant material. The measurements were carried out during the last month before harvest, from the beginning of September to harvest time at the beginning of October in 2006 (12, 20, 29 Sept., 6 Oct.) and 2007 (3, 12, 21 Sept., 1 Oct.). The experiment was started on 3-year-old ‘Fuji’ apple trees grafted on M9 rootstock grown in the commercial orchard of Sadjarstvo Mirošan in eastern Slovenia. The experiment included trees grown under hail nets [double black longitudinal and double (black and green) transverse fibers with a mesh size of 6 × 7 mm] as well as trees in the open. At the beginning of September, the orchard floor under half the trees included in the experiment was covered with reflective foil (white plastic foil) to improve radiation. We had eight trees in each treatment, and five trees in the middle were more closely followed. The treatments were as follows: CON (control; without hail nets or reflective foil); RF (floor covered with foil, without hail nets); HN (hail nets without reflective foil); and HN + RF (floor covered with foil under hail nets).

Between different treatments, several trees were left for isolation. On each tree, three fruits were marked, a total of 15 fruits per treatment. On these fruit, lighting during the experiment

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¹To whom reprint requests should be addressed; e-mail jerneja.jakopic@bf.uni-lj.si.

Fig. 1. The lighting in the orchard ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) during ripening in all treatments in 2006 and 2007. CON = control; RF = orchard floor covered with reflective foil; HN = under hail nets; HN + RF = floor covered with foil under hail nets.

was closely monitored, and the fruits were picked for analysis of the phenolic content at harvest time. Each fruit was separately analyzed by high-performance liquid chromatography (HPLC) ($n = 15$).

Light measurement. During ripening, radiation was measured once a week on a sunny day at 1200 HR in five replications per treatment. The lighting was measured on the ground between rows of trees with a 1-m long sensor (LI-COR; $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for all treatments every week during ripening. On each tree, three fruit samples were marked, and the photosynthetically active radiation (PAR) was measured using the LI-COR quantum sensor on the upper (oriented upward) and the lower (oriented downward) parts of the fruit every week during ripening. On the first sampling date (4 weeks before harvest) in 2007, the radiation measurements were not taken because of cloudy weather. PAR values are presented as relative values compared with the control treatment. The measured values of each treatment were divided by the PAR value of the control treatment at the same measuring date.

Extraction of apple skin. Methods for extraction were performed as described by Jakopic et al. (2007). Fresh apple peel was ground into a fine powder using liquid nitrogen and extracted with methanol containing 1% (v/v) HCl and 1% (w/v) 2,6-di-*tert*-butyl-4-methylphenol in an ultrasonic bath. After extraction, the treated samples were centrifuged for 7 min at 10,000 rpm. The supernatant was filtered through a polyamide filter and transferred into a vial before injection into the HPLC system.

High-performance liquid chromatography/tandem mass spectroscopy analysis of individual phenolic compounds. The extracts were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 (catechin, epicatechin, chlorogenic acid), 350 (quercetin glycosides), and 530 nm (cyanidin glycosides). A Phenomenex HPLC column S18 (Phenomenex, Torrance, CA) (150 $\times$ 4.6 mm; Gemini 3u) protected with a Phenomenex Security guard column and operated at 25 $^{\circ}\text{C}$ was used. The injection volume was

20 μL , and the flow rate was 1 $\text{mL}\cdot\text{min}^{-1}$. The elution solvents were aqueous 1% (v/v) formic acid (A) and pure acetonitrile (B). The samples were eluted according to the gradient described by Marks et al. (2007).

The phenolic compounds were identified by comparing their ultraviolet-visible spectra from 220 to 550 nm and retention times. Quantification was achieved according to concentrations of a corresponding external standard and was confirmed using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray interface operating in either negative (for quercetins) or positive ion mode (for anthocyanins) depending on the compound of interest. The analysis was carried out using full-scan data-dependent tandem mass spectroscopy (MS^2) scanning from m/z 115 to 1000. Two unknown cyanidin glycosides were identified as quercetin pentosides on the basis of a $[\text{M}-\text{H}]^+$ at m/z 419, a MS^2 fragment at m/z 287.

Analysis of total phenols. The total phenolic content of the extracts was assessed using the Folin-Ciocalteu phenol reagent method (Singleton and Rossi, 1965). Six milliliters of bidistilled water and 500 μL of Folin-Ciocalteu reagent were added to 100 μL of the sample extracts, and 1.5 mL of sodium carbonate (20%, w/v) was added after waiting between 8 s and 8 min at room temperature. The extracts were mixed and allowed to stand for 30 min at 40 $^{\circ}\text{C}$ before measuring absorbance on a spectrophotometer at 765 nm. A mixture of water and reagents was used as a blank. The total phenolic content was expressed as gallic acid equivalents in milligrams per kilogram fresh weight. Absorptions were measured in three replications.

Chemicals. The following standards were used to determine the chemical compounds: cyanidin 3-*O*-galactoside chloride, quercetin 3-*D*-galactoside, quercetin 3- β -*D*-glucoside, quercetin-3-rhamnoside, and cyanidin chloride from Fluka Chemie GmbH (Buchs, Switzerland); chlorogenic (5-caffeoylquinic) acid, quercetin, rutin, and (-)-epicatechin from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); (+)-catechin from Roth (Karlsruhe, Germany); and quercetin 3-*O*-xyloside and

Table 1. Relative comparison of photosynthetically active radiation on lower and upper parts of fruit in the canopy compared with control during ripening in all treatments in 2006 and 2007.

Weeks before harvest time	Yr	CON	RF	HN	HN + RF
Lower					
4	2006	1 a	4.7 c	0.7 a	3.2 b
3		1 a	6.7 c	1.0 a	5.1 b
2		1 a	7.3 c	0.8 a	5.1 b
1		1 a	6.9 c	0.8 a	4.6 b
3	2007	1 a	5.3 b	1.4 a	5.3 b
2		1 a	3.5 b	1.1 a	4.4 c
1		1 a	3.9 b	1.2 a	4.4 b
Upper					
4	2006	1 bc	1.2 c	0.6 a	0.9 b
3		1 a	1.2 ab	0.9 a	1.3 b
2		1 ab	1.3 c	0.8 a	1.1 b
1		1	1.2	1.0	1.1
3	2007	1	1.1	1.1	1.1
2		1	0.9	1.1	1.2
1		1	1.3	1.3	1.2

CON = control; RF = floor covered with reflective foil; HN = under hail nets; HN + RF = floor covered with foil under hail nets. The relative values compared with the control are presented. Different letters in a row mean statistically significant differences between treatments according to Duncan's multiple range test at $P < 0.05$. Where there are no letters in the row, differences between treatments were not statistically significant. On the first sampling date (4 weeks before harvest) in 2007, the radiation measurements were not taken because of cloudy weather.

quercetin 3-arabinofuranoside from Apin Chemicals (Abingdon, U.K.).

The chemicals for mobile phases were acetonitrile and formic acid from Fluka Chemie GmbH.

The water used in the sample preparation, solutions, and analyses was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

Statistical evaluation. Results were statistically analyzed with the Statgraphics Plus program for Windows 4.0 (Manugistics, Inc., Rockville, MD), using one-way analysis of variance. The differences between treatments were estimated with Duncan's multiple range test. P values < 0.05 were considered statistically significant.

Results and Discussion

Compensation for lighting decrease under hail nets by reflective foil. During the last month before harvest in 2006, *PAR* decreased from 1030 (4 weeks before harvest, reflective foil) to 540 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (1 week before harvest, hail net). During the last month before harvest in 2007, *PAR* decreased from 1400 (4 weeks before harvest, reflective foil) $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the beginning of September to 1110 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (1 week before harvest, hail net) at the beginning of October (Fig. 1). In many European fruit orchards as well as in our experiment, hail nets are black. Hail nets decreased radiation from 10% to 30% in comparison with the control treatment. In contrast, the reflective foil treatment had *PAR* levels 2% to 10% higher than those in the control. Reflective foil under the hail nets also increased the radiation by 5% to 20% in comparison with hail nets only. The hail net treatment with reflective foil had *PAR* levels 10% to 20% lower than in the control

treatment. Solomakhin and Blanke (2008) reported that even under white or red-white net, *PAR* was 90 to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ lower and under red-black and green-black 250 to 340 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ lower. The decrease in *PAR* under hail nets and the increase in *PAR* where reflective foil was used are consistent with our earlier work (Jakopic et al., 2007). In that study, we compared the influence of hail nets and reflective foil on the lighting of fruit in the tree canopy. In the tree canopy, fruit were more intensively lighted where the orchard floor was covered with reflective foil, especially on the lower parts of the fruit. In this study, we confirmed that hail nets decreased lighting (Table 1). Covering the orchard floor with reflective foil had a positive effect on lighting in the tree canopy, which is especially desirable when the cultivar/rootstock combination is vigorous and trees are protected with hail nets. Lighting of lower parts of fruit was 3.5 to 7.3 times higher in both treatments with reflective foil in comparison with the control. Green et al. (1995) mention that the *PAR*

reflected by the foil caused a significant increase in *PAR* radiation entering the lower parts of the canopy and that little difference in the incoming *PAR* flux densities was measured in the top half of the canopy.

In the tree canopy, fruit were exposed to different lighting depending on the part of each fruit in the canopy. In the control treatment, *PAR* on the lower part of the fruit was from 25 to 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas on the upper part, it extended from 50 up to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (data not shown). *PAR* was higher in the treatment with reflective foil in comparison with those with sod on the ground (Table 1). The highest lighting level for fruit was achieved in those treatments in which the orchard floor was covered with reflective foil, both outside the hail net and under the hail net. In the previous study (Jakopic et al., 2007), we demonstrated that covering the orchard floor with foil under the hail nets increased the intensity of red coloration. It was expected that the reflective foil treatment would have the most extensive red color development because it had the greatest reflection of *PAR* compared with other treatments, especially compared with hail nets. Layne et al. (2002) reported that in 'Gala' apple trees with reflective foil, a greater percentage of the fruit surface had red coloration than in the fruit from trees without reflective foil. This is not yet an extended practice for increasing red skin coloration.

Table 2. Weather data for 2006 and 2007.

	Decade	Mean maximum temp. (°C)		Mean minimum temp. (°C)		Precipitation (mm)	
		2006	2007	2006	2007	2006	2007
September	First	26.0	19.6	11.3	9.3	0	4
	Second	21.8	21.7	11.4	9.6	62	108
	Third	23.3	19.4	8.6	6.7	0	49
October	First	20.8	19.5	8.1	7.4	29	39

Fig. 2. Content of individual cyanidin glycosides in $\text{mg}\cdot\text{kg}^{-1}$ fresh weight for all treatments in 2 years. Concentrations of cyanidin pentoside are present in equivalents of cyanidin. Different letters mean statistically significant differences between treatments according to Duncan's multiple range test at $P < 0.05$.

Phenolic compounds content. The intense red coloration of apple skin is a result of varying amounts of anthocyanins and flavonols. In general, low night temperatures and moderate daily temperatures promote red color development (Veberic et al., 2007) in 'Fuji' apples. In our research, no apparent differences in mean maximum and minimum decade temperatures were observed between

2006 and 2007 (Table 2) and therefore, the effect of the year on the total phenolic content should be negligible. In apples, anthocyanins are all derivatives of cyanidin. The main cyanidin glycoside in 'Fuji' apple, cyanidin galactoside, accounted for more than 97%. Its concentrations in fresh weight of apple skin varied from 150 mg·kg⁻¹ (control, 2007) to more than 300 (hail net + HN, 2007)

mg·kg⁻¹ (Fig. 2). The lowest values were established in the control treatment and under the hail nets. Reflective foil had a positive effect on cyanidin galactoside outside and especially under the hail nets, in which the highest levels were achieved. Similar results were shown for other cyanidin glycosides. Contents of cyanidin arabinoside and two other cyanidin pentosides were higher in

Fig. 3. Content of individual quercetin glycosides and quercetin in mg·kg⁻¹ fresh weight for all treatments in 2 years. Concentrations of quercetin pentoside, quercetin arabionofuranoside, and quercetin xyloside are presented in equivalents of quercetin. The average values and SE bars are presented. Different letters mean statistically significant differences between treatments according to Duncan's multiple range test at $P < 0.05$.

treatments with reflective foil in comparison with treatments without foil, except for cyanidin arabinoside in 2007. In our case, the hail net had no effect on cyanidin glycoside concentrations, although in many cases a negative impact on the color development of fruit was observed under black hail nets (Guerrero et al., 2002; Solomakhin and Blanke 2007; Stampar et al., 2002). Also in the lighting of fruit in the canopy, there was no statistical difference between the treatment with hail nets and the control treatment. On the other hand, the positive influence of reflective foil on lighting and consequently on cyanidin glycosides content was clearly expressed. Their concentrations were significantly higher in both treatments with reflective foil in comparison with the treatments without it. Ju et al. (1999) also established that covering the orchard floor with foil stimulated anthocyanin accumulation in fruit peel. The light concentration was directly correlated with the concentrations of cyanidin 3-galactoside and with the percentage of blush in the fruit skin of the 'Jonagold' apple (Awad et al., 2001). Among anthocyanins, cyanidin glucoside was also detected in our study, but it was present only in trace amounts.

There are two different ways that light enhances anthocyanin synthesis: one is by increasing canopy photosynthesis and assimilating supply to the fruit and thus indirectly stimulating anthocyanin synthesis by providing a substrate. Another possibility is that the film treatments directly stimulate anthocyanin synthesis; the foil increases light intensity inside the canopy and increases the activity of UDP-Galactose: flavonoid-3-O-glycosyltransferase (UFGaT), an important enzyme in anthocyanin synthesis in apples (Ju et al., 1999).

Fruit coloration depends on the content of anthocyanins as well as flavonols (Lancaster, 1992), among which belong quercetin glycosides and quercetin. Seven quercetin glycosides and quercetin were detected and identified on the basis of cochromatography with a standard and mass spectral data. Quercetin galactoside, quercetin glucoside, quercetin rhamnoside, quercetin rutinoside, quercetin xyloside, quercetin arabinofuranoside, and quercetin were identified using an external standard and confirmed on the basis of an MS² fragment at *m/z* 301. Quantification was achieved according to the concentrations of a corresponding external standard. The other quercetin glycosides were identified as a quercetin pentoside on the basis of a [M-H]⁻ at *m/z* 433, a MS² fragment at *m/z* 301, and its concentration is presented as equivalents of quercetin. The contents of quercetin glycosides in apple skin varied from 20 (quercetin pentoside, reflective foil, 2007) to almost 500 (quercetin galactoside, hail net + reflective foil, 2007) mg·kg⁻¹ of fresh weight (Fig. 3). The highest share of quercetin glycosides in 'Fuji' apple was formed by quercetin galactoside, a finding also reported by Felicetti and Schrader (2009), but concentrations in our case were lower. Concentrations of quercetin galactoside were 297 (control, 2007) to 483 (hail net + HN, 2007) mg·kg⁻¹

fresh weight; this was followed by quercetin rhamnoside, quercetin arabinofuranoside, and others. Fruit from the control trees contained the lowest levels of almost all individual quercetin glycosides (quercetin rutinoside, quercetin glucoside, quercetin galactoside, quercetin xyloside, quercetin pentoside, quercetin rhamnoside, and quercetin). Hail nets as well as reflective foil increased quercetin glycosides. The highest concentrations of individual quercetin as well as the overall level of quercetin glycosides occurred under hail nets where the ground was covered with reflective foil. The differences were statistically significant in the case of quercetin galactoside, quercetin glucoside, quercetin rutinoside, quercetin pentoside, and quercetin xyloside in 2007 and quercetin in both years. Although hail nets decreased *PAR* and ultraviolet radiation (Blanke, 2007), in our case, contents of quercetin glycosides were unexpectedly higher in the treatment under hail nets. Also using the reflective foil additionally enhanced and achieved highest amounts mainly in treatments under the hail nets where the orchard floor was covered with reflective foil. Although lighting between rows under the hail nets was lower in comparison with the control, lighting in the canopy was not statistically different between treatments. In some cases, quercetin glycoside contents were higher in the treatment under hail nets than in the control treatment. On the other hand, increased lighting because of the reflective foil tended to have a positive effect on quercetin glycoside concentrations. We confirmed that light may have an enhancing effect not just on anthocyanin synthesis, but also on the accumulation of flavonoids in apple skin. Reay and Lancaster (2001) also reported that both quercetin glycosides and anthocyanins accumulated in the skin of 'Gala' and 'Royal Gala' after irradiation with white fluorescent and ultraviolet-B lamps.

Besides flavonols, Veberic et al. (2007) detected catechin, chlorogenic acid, epicatechin, caffeic acid, *p*-coumaric acid, and phloridzin in 'Fuji' apple. In our study, catechin, epicatechin, and chlorogenic acid were analyzed. Concentrations of (+)-catechin were 29 (hail net, 2006) to 75 (hail net + reflective foil, 2007) mg·kg⁻¹ fresh weight skin, (-)-epicatechin 171 (hail net, 2006) to 419 (hail net + reflective foil, 2007) mg·kg⁻¹

fresh weight, and chlorogenic acid from 24 (hail net, 2006) to 75 (reflective foil, 2007) mg·kg⁻¹ fresh weight (Table 3). Contents of catechin and chlorogenic acid were lower and that of epicatechin were higher (Table 3) in comparison with Veberic et al. (2007) but still in the range reported by Treutter (2001) as well as Escarpa and Gonzalez (1998) for apple skin. Contents of these phenols were lowest in fruit grown under the hail nets. In the control treatment, values were not statistically different compared with the hail net treatment except for the content of chlorogenic acid in the second year. Covering the ground with reflective foil resulted in an increase of these phenolic compounds in both years, except for chlorogenic acid in 2006. Awad and de Jager (2002) studied the influence of light on phenols for the sun-exposed and the shaded parts of fruit. They established that the sun-exposed skin of individual fruit had much higher cyanidin 3-galactoside and quercetin 3-glycoside contents than the shaded skin, whereas phloridzin, catechins, and chlorogenic acid were similar in the skin of both sides.

Total phenols content varied from 1984 (control, 2007) to 2997 (hail net + reflective foil, 2006) mg·kg⁻¹. Although the hail net did not influence the total phenols content (Table 3), in the treatment with reflective foil under the hail net, contents were statistically higher compared with the control treatment.

The positive influence of reflective foil on lighting in the canopy, especially on the lower parts of the fruit, was demonstrable. Also, concentrations of cyanidin glycosides were higher in both treatments with reflective foil in comparison with the treatments without it. Contents of the quercetin glycosides were higher in the treatment under hail nets compared with the control. Using the reflective foil additionally enhanced their concentrations (except for quercetin glucoside and quercetin rhamnoside) and achieved the highest levels mainly in treatments under the hail nets where the orchard floor was covered with reflective foil. Contents of (+)-catechin, (-)-epicatechin, and total phenolics were lowest in the control treatment and did not differ in comparison with the treatment with hail net. Covering the ground with reflective foil resulted in an increase of (+)-catechin, (-)-epicatechin, and total phenolics. Results of our study indicate that

Table 3. Contents of (+)-catechin, chlorogenic acid, (-)-epicatechin, and total phenols in mg·kg⁻¹ fresh weight apple skin for all treatments in 2 years.^z

		CON	RF	HN	HN + RF
(+)-catechin	2006	32 ± 3 ab	39 ± 3 b	29 ± 2 a	40 ± 3 b
	2007	38 ± 3 a	67 ± 5 b	48 ± 3 a	75 ± 4 b
Chlorogenic acid	2006	25 ± 2	26 ± 3	24 ± 1	25 ± 2
	2007	58 ± 5 b	75 ± 5 c	39 ± 3 a	47 ± 4 ab
(-)-epicatechin	2006	186 ± 15 ab	220 ± 15 b	171 ± 10 a	225 ± 16 b
	2007	351 ± 17 a	417 ± 22 b	348 ± 17 a	419 ± 19 b
Total phenols	2006	2161 ± 117 a	2262 ± 171 a	2070 ± 95 a	2997 ± 113 b
	2007	1984 ± 133 a	2470 ± 125 bc	2189 ± 112 ab	2794 ± 126 c

^zConcentrations of total phenols are given in equivalents of gallic acid.

CON = control; RF = floor covered with reflective foil; HN = under hail nets; HN + RF = floor covered with foil under hail nets. The average means and SES are presented. Different letters in a row mean statistically significant differences between treatments according to Duncan's multiple range test at *P* < 0.05. Where there are no letters in the row, differences between treatments were not statistically significant.

reflective foil has a positive effect on content of mainly phenolic compounds and consequently on higher quality of apple crop, especially when apple trees growing under hail nets.

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