Available online at www.sciencedirect.com
ScienceDirect
Scientia Horticulturae 115 (2007) 40–46
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

The effect of reflective foil and hail nets on the lighting, color and anthocyanins of 'Fuji' apple

Jerneja Jakopic *, Robert Veberic, Franci Stampar

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

Received 25 May 2007; received in revised form 20 July 2007; accepted 24 July 2007

Abstract

We studied the influence of covering the orchard floor with reflective foil on photosynthetic active radiation (PAR) both under and outside hail nets, and the possibility that the reflective foil under the hail net compensates for light reduction in last month before harvest time. On the lower side of fruit in the canopy, the reflective foil increased PAR. The chromaticity value a^* showed a difference in the intensity of red coloration in the reflective foil and hail net treatments. Amounts of individual cyanidins were detected by using HPLC–MS. The accumulation of five individual anthocyanins (cyanidin-galactoside, three cyanidin-pentoses and cyanidin) was investigated during last month before harvest time. Concentrations of the main, cyanidin-galactoside in 'Fuji' apple increased before harvest time, and at harvest time the reflective foil caused an increase in all identified anthocyanins.

Keywords: Photosynthetic active radiation (PAR); Anthocyanin; Reflective foil; Hail net; Light

1. Introduction

For competitive markets and consumers, the attractive red skin color of apples is an important and expected quality attribute. The intense red coloration of apple skin is a result of varying amounts of anthocyanins and flavonols. Anthocyanidins may be glycosylated, and they are then referred to as anthocyanins. In apples, anthocyanins are all derivatives of cyanidin. The main cyanidin glycoside in apple skin is cyanidin-3-galactoside, while cyanidin-3-arabinoside, cyanidin-3-glucoside and cyanidin-3-xyloside are present in small amounts in some red apple cultivars (Lancaster, 1992).

The intensity and quality of red skin coloration is regulated by internal as well various environmental factors (Veberic et al., 2007; Lancaster, 1992), such as light, temperature, heat and cold stress, pathogen attacks and mechanical lacerations (Ubi, 2004; Iglesias et al., 2002). Apples have better red color development in climates with clear bright days and cool nights during the preharvest period (Iglesias et al., 2002). The anthocyanin level in apple skin is inversely related to field temperature, and high night temperatures reduced anthocyanin accumulation (Iglesias et al., 1999).

Light is a key regulatory factor affecting color development in apples (Lancaster, 1992); apples that are not exposed to light do not redden (Ubi, 2004). Light with sufficient amount of energy and appropriate spectral composition is necessary to initiate anthocyanin synthesis (Ubi, 2004). Reports of experiments (Lancaster, 1992; Saure, 1990) have shown a relationship between anthocyanin accumulation and light intensity. Studies have also been carried out to determine the most active part of the spectrum for anthocyanin formation in apple skin. Irradiation with blue-violet (BV) and ultraviolet (UV) light is shown to be most effective, while far-red (FR) is the least effective or even inhibitory. Response to radiation also depends on the position of the apple in the canopy. Fruit that face outward (and are thus exposed) show much greater potential to accumulate anthocyanins than did those facing in (shaded) (Reay and Lancaster, 2001). Therefore, apples in the shaded side of the canopy are often poor in red coloration. Sometimes climatic characteristics mandate the use of hail nets, because the hail injury on leaves decrease photosynthesis and also cause damage on fruits during the growing season (Tartachnyk and Blanke, 2002; Stampar et al., 1999). Most of the hail nets used are black, which greatly reduces incident solar light, and they may have a negative impact on the development of fruit and on its final color (Guerrero et al., 2002; Stampar et al., 2002).

* Corresponding author. Tel.: +386 1 423 11 61; fax: +386 1 423 10 88.
E-mail address: jerneja.jakopic@bf.uni-lj.si (J. Jakopic).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2007.07.014

Fuji has become an attractive, promising apple cultivar all over the world. One of its characteristics is its late harvest time—in autumn, when radiation is already reduced and poor coloration becomes a serious problem. Some experimental attempts are reported to have tried to improve fruit coloration, but only few have been successful. Although fruit bagging is an effective method for improving red skin color, this labor intensive method is not economically justifiable (Ju et al., 1999). Chemical treatment with ethephon, for example, is effective in early cultivars but not in the late ones, including 'Fuji' (Curry, 1994). Covering the orchard floor with reflective foil increases light intensity in the tree canopy and improves fruit coloration (Ju et al., 1999).

Accordingly, the positive effects of reflective foil as well as the negative effects of hail nets on light intensity are known. Is it possible to compensate for the influence of hail nets with the effect of reflective foil? The aim of our study was to ascertain the effect of covering the orchard floor on lighting the fruit in the canopy. Therefore, the development of red skin coloration and of individual anthocyanins – the latter are responsible for the red coloration – were measured last month before harvest time. Some authors had already ascertained the total level of anthocyanins, but rarely has the influence on individual anthocyanin been studied. At harvest, the influence of hail nets and reflective foil on some primary metabolites was also detected.

2. Material and methods

2.1. Plant material

The measurements were carried out during last month before harvest time – from the middle of September to harvest time on 11th October 2006 – on 3-year-old 'Fuji' apple trees grafted on M9 rootstock, grown in the productive orchard of Sadjarstvo Mirosan in eastern Slovenia. The experiment included trees grown under hail nets 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 to improve fruit coloration. We had eight trees in each of four treatments:

- CON (control; without hail nets or reflective foil);
- F (floor covered with foil, without hail nets);
- HN (hail nets without reflective foil);
- HN + F (floor covered with foil under hail nets).

For each tree, all measurements and samplings were done on all sampling dates (1, 12th September; 2, 20th September; 3, 29th September; 4, 6th October and 5, 11th October). The crop load on individual trees was similar and did not significantly differ among treatments.

2.2. Light measurement

During the experiment, the photosynthetic active radiation (PAR) was measured using the LI-COR quantum sensor (μmol m^{-2} s^{-1}). On each tree, two fruits were marked and the PAR was measured on the upper (oriented upward) and the lower (oriented downward) part of the fruit every week during ripening. Control lighting was measured on the floor between rows of trees with one meter sensing length in all treatments. On the last sampling date (at harvest time), the measurements of radiation were not carried out because of cloudy weather.

2.3. Fruit color measurement

Apple color was measured using the Minolta CR-10 Chroma portable colorimeter (Minolta Co., Osaka, Japan) with C illuminant. Fruit chromaticity was recorded in Commission Internationale d'Eclairage (CIE) parameters L^*, a^* and b^* color space coordinates. The colorimeter was calibrated with a white standard calibration plate before use. In this system of color representation, the L^* value corresponds to a dark-bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). The a^* and b^* scales extend from −60 to 60, where a^* is negative for green and positive for red and b^* is negative for blue and positive for yellow. The hue angle (h°) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 360° = blue. For each sampling date, color was measured at the reddest point of the fruit equator.

2.4. Analysis of individual carbohydrates and organic acids

The apple fruit were analyzed for carbohydrates (sorbitol, sucrose, glucose and fructose) and organic acids (malic, citric, fumaric and shikimic acids) content levels. In the laboratory, stalk, sepal and core were removed from the fruit, and 10 g of the fresh mass was immersed in 50 mL of bidistilled water and homogenized with the T-25 Ultra-Turrax (Ika-Labortechnik).

The fruit samples were left for extraction for half an hour at room temperature, with frequent stirring. Then the samples were centrifuged for 7 min at 4200 rpm (Eppendorf Centrifuge 5810R). The supernatants were filtered through a 0.45 μm filter (Macherey-Nagel), poured into vials and analyzed according to the method described by Sturm et al. (2003) using high-performance liquid chromatography (HPLC; Thermo Scientific, Finnigan Spectra System, Waltham, MA, USA). For each analysis of sugars and organic acids, the amount of 20 μL of sample was used. Sugars were analyzed in the column Aminex-HPX 87C with a flow of 0.6 mL min^{-1} and at 85 °C. For the mobile phase, bidistilled water was used, and an RI detector for identification. Organic acids were analyzed in the Aminex-HPX 87H column with a flow of 0.6 mL min^{-1} and at 65 °C. For the mobile phase, 4 mM sulphuric acid (H_2SO_4) was used together with a 210 nm wavelength UV detector for identification. The concentrations of carbohydrates and organic acids were calculated with the help of corresponding external standards.

2.5. Analysis of individual anthocyanins

For the analyses of individual anthocyanins, fresh apple peel was ground into fine powder. Five grams of sample was

extracted with 25 mL methanol containing 1% (v/v) HCl and 1% (w/v) 2,6-di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for half an hour. After extraction, the treated samples were centrifuged for 7 min at 10,000 rpm. The supernatant was filtered through the Chromafil AO-45/25 polyamide filter produced by Macherey-Nagel (Düren, Germany) and transferred into a vial prior to injection to the high-performance liquid chromatography (HPLC) system.

The samples were analyzed using a Thermo Finningan Surveyor HPLC system (Thermo Scientific, San Jose, USA) with a diode array detector at 530 nm. A Phenomenex HPLC column S18 (150 mm × 4.6 mm, Gemini 3u) protected with a Phenomenex Security guard column, operated at 25 °C, was used. The injection volume was 20 μL, and the flow rate was 1 mL min^{-1}. The elution solvents were aqueous 0.01 M phosphoric acid (A) and pure methanol (B). The samples were eluted according to the linear gradient described by Escarpa and Gonzalez (1998).

The anthocyanins were identified by comparing their UV–vis 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 (ESI) operating in positive ion mode. Analysis of anthocyanins was carried out using MS^2 scanning from *m/z* 115 to 800.

2.6. Chemicals

The following standards were used to determinate the chemical compounds: sucrose, fructose, glucose, sorbitol; malic, citric, fumaric and shikimic acid, cyanidin 3-*O*-galactoside chloride and cyanidin chloride from Fluka Chemie GmbH (Buchs, Switzerland).

The chemicals for mobile phases were methanol from Sigma–Aldrich Chemie GmbH (Steinheim, Germany), and phosphoric acid from Fluka Chemie GmbH (Buchs, Switzerland).

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

2.7. Statistical evaluation

The results were statistically analyzed with the Statgraphics Plus program for Windows 4.0, using the one-way analysis of variance (ANOVA). The differences in the content levels were estimated with Ducan's test. *P*-Values of less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Effect on lighting

In the areas where hail damage could be a problem, hail nets are obligatory to protect the yield. Hail nets are often dark and reduce lighting and consequently red fruit coloration to a certain degree. We have measured the decrease of photosynthetic active radiation (PAR; 400–700 nm) from 1120 μmol m^{-2} s^{-1} in control to 700 μmol m^{-2} s^{-1} under hail nets. Guerrero et al. (2002) report a reduction of photosynthetic photon flux under black hail nets by more than half in comparison to the control. One of the ways to counter the above mentioned reduction could be to cover the orchard floor with reflective foil. The PAR, measured between rows at 1-m sensing distance, increased from 10% in treatments without hail nets up to 20% under the hail nets. Green et al. (1995) report that ground cover with reflective foil increased the total absorption of PAR radiation in the canopy by almost 40%, leaf photosynthesis by about 32% and leaf transpiration by about 26%.

Fruit at different locations in the canopy received different amounts of reflected light (Ju et al., 1999). In the tree canopy, fruit were more intensively lighted where the orchard floor was covered with reflective foil. The differences in PAR radiation between reflective foil and sod on the floor were especially high in the lower parts of the canopy when the sensor was oriented downward. The highest lighting level for fruit was achieved in those treatments where the orchard floor was covered with reflective foil both outside the hail net cover and under the hail net (Table 1). Under the hail net, the photosynthetic active radiation was four to five times higher where the floor was covered with reflective foil. The lowest values were measured in the control and hail net treatment accompanied by a sod orchard floor. Likewise, as on the upper part of the shaded fruits, PAR was higher where the orchard floor was covered with reflective foil under hail nets. Green et al. (1995), too, mention that the PAR reflected by the foil caused a significant increase in PAR radiation entering into 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.

3.2. Effect on maturity

At harvest time, flesh firmness and soluble solids concentration (SSC) were measured (Table 2). The flesh

Table 1
The lighting of lower and upper parts of fruit in the canopy (μmol m^{-2} s^{-1}) during ripening in all treatments

Sampling	CON	F	HN	HN + F	$\alpha < 0.05$
Lower					
1	46 ± 4a	215 ± 12c	30 ± 2a	146 ± 12b	*
2	29 ± 3a	193 ± 24c	29 ± 3a	146 ± 17b	*
3	26 ± 3a	190 ± 19c	21 ± 2a	134 ± 14b	*
4	24 ± 2a	166 ± 15c	20 ± 1a	110 ± 16b	*
Above					
1	116 ± 9bc	139 ± 15c	74 ± 6a	103 ± 10b	*
2	67 ± 9a	80 ± 5ab	59 ± 5a	90 ± 9b	*
3	84 ± 8ab	110 ± 8c	66 ± 6a	93 ± 10b	*
4	51 ± 6	61 ± 4	50 ± 4	56 ± 5	NS

CON, control; F, floor covered with reflective foil; HN, under hail nets; HN + F, floor covered with foil under hail nets. Average values ± standard error are presented. Different letters (a–c) in row mean statistically significant differences between treatments at $\alpha < 0.05$.

Table 2
Firmness (kg cm^{-2}), soluble solids concentrations (%) and content of individual carbohydrates (mg g^{-1} FW) and organic acids (mg kg^{-1} FW) at harvest time in all treatments

	CON	F	HN	HN + F	$\alpha<0.05$
Soluble solids	15.6 ± 0.2b	15.8 ± 0.2b	15.8 ± 0.2b	15.0 ± 0.2a	*
Firmness	6.6 ± 0.1a	6.7 ± 0.1a	7.2 ± 0.2b	7.4 ± 0.2b	*
Sucrose	46.7 ± 0.9c	42.4 ± 1.1b	39.3 ± 2.0ab	36.9 ± 1.0a	*
Fructose	85.0 ± 2.0c	78.9 ± 1.4b	77.9 ± 1.5b	71.7 ± 1.1a	*
Glucose	31.1 ± 0.9	30.4 ± 1.3	31.9 ± 1.1	28.8 ± 1.2	NS
Sorbitol	10.1 ± 0.7b	10.0 ± 0.8b	8.3 ± 0.7ab	7.7 ± 0.3a	*
Sum sugars	172.9 ± 4.5c	161.7 ± 4.6b	157.4 ± 5.3b	145.1 ± 3.6a	*
Malic acid	3302 ± 108c	2501 ± 106ab	2585 ± 95b	2264 ± 73a	*
Citric acid	24.9 ± 2.7c	19.2 ± 1.5b	17.3 ± 1.6b	9.8 ± 1.5a	*
Fumaric acid	0.37 ± 0.03	0.32 ± 0.04	0.32 ± 0.05	0.43 ± 0.03	NS
Shikimic acid	73.9 ± 2.7b	55.5 ± 2.1a	72.5 ± 4.0b	66.0 ± 1.8b	*
Sum acids	3401 ± 113c	2576 ± 110ab	2675 ± 101b	2340 ± 76a	*

CON, control; F, floor covered with reflective foil; HN, under hail nets; HN + F, floor covered with foil under hail nets. Average values ± standard error are presented. Different letters (a–c) in rows mean statistically significant differences between treatments at $\alpha < 0.05$.

firmness was higher in both treatments under hail nets, which indicates later ripening and consequently a delay in picking. The soluble solids concentration was lower in the treatment with reflective foil under hail nets in comparison to all other treatments. The percentage of full sunlight incident on each test limb was correlated negatively with fruit firmness and positively with average fruit soluble solids, starch and total solids content (Robinson et al., 1983).

3.3. Effect on fruit coloration

The intensity of fruit lighting influenced the development of red coloration. The CIE color coordinate a^*, a higher value of which indicates more red color, was on all sampling dates the lowest in the treatment under hail nets without reflective foil, but it was not always statistically significant (Table 3). The highest values were mainly achieved by fruit growing on trees where the orchard floor was covered by reflective foil, except on the third sampling date. This means that the reflective foil induced more intensive red coloration in comparison to hail nets. Covering the orchard floor with foil under the hail nets increased the intensity of red coloration and at harvest time almost achieved values similar to those of the control. Differences in the photosynthetic photon flux values received by the fruit on the trees had an effect on the final fruit color (Guerrero et al., 2002).

At harvest time, the effect of reflective foil was shown on the CIE factor L^*, which indicates lightness, as well as on factor b^* (blue to yellow) and hue angle. The values were lower when the reflective foil was used under the hail nets as well as in treatments without hail nets (Table 3). Differences were not always statistically significant. Lower L^* and hue angle (h°) values translate into a darker, redder fruit color. The reflective foil thus affected lighter, less yellow and redder fruit coloration.

Table 3
Chromaticity values of red skin color during ripening in all treatments

Sampling	CON	F	HN	HN + F	$\alpha < 0.05$
L					
3	43. 6 ± 0.9	43.8 ± 0.6	44.8 ± 0.9	44.8 ± 0.9	NS
4	48.4 ± 2.2	42.9 ± 0.9	45.5 ± 1.1	45.0 ± 1.6	NS
5	44.1 ± 1.2b	41.4 ± 1.0ab	43.5 ± 0.7b	39.8 ± 1.0a	*
a					
3	28.2 ± 0.9b	27.2 ± 1.3b	23.6 ± 1.5a	25.9 ± 1.0ab	*
4	23.2 ± 1.5a	33.0 ± 1.7b	22.9 ± 1.3a	26.0 ± 1.6a	*
5	30.7 ± 1.6ab	33.5 ± 1.0b	28.0 ± 1.1a	30.4 ± 0.9ab	*
b					
3	10.7 ± 0.8a	13.5 ± 0.7b	16.3 ± 0.6c	14.2 ± 0.8bc	*
4	16.1 ± 1.4	16.0 ± 1.1	15.1 ± 0.6	13.9 ± 0.7	NS
5	14.1 ± 1.1	12.0 ± 0.5	12.7 ± 0.7	11.3 ± 0.9	NS
h					
3	21.0 ± 1.7a	26.7 ± 1.8ab	35.2 ± 2.1c	29.3 ± 2.7bc	*
4	35.1 ± 3.6b	26.0 ± 1.7a	34.0 ± 2.3b	28.4 ± 2.1ab	*
5	25.4 ± 2.6	19.8 ± 0.9	24.7 ± 1.7	22.2 ± 2.3	NS

CON, control; F, floor covered with reflective foil; HN, under hail nets; HN + F, floor covered with foil under hail nets. Average values ± standard error are presented. Different letters (a–c) in row mean statistically significant differences between treatments at $\alpha < 0.05$.

With regard to the position and location of fruit in the tree, in interior fruit, the hue angle decreased by 20% on the exposed side and 12% on the shaded side (Ju et al., 1999). Fruit under black nets, which reduce photosynthetic photon flux by 50%, also reduced final fruit color, respectively, increase L^* and h° (Guerrero et al., 2002); however, this was not confirmed in our study. A positive effect of the reflective foil on these parameters was indicated at harvest time, but differences were not significant. In 'Gala' apples, fruit from reflective foil trees had a greater percentage of surface red color than did fruit from trees without reflective foil. This could be a method for increasing red skin coloration (Layne et al., 2002). In relation to anthocyanins content, the a^*/b^* ratio is directly related, whereas h° and L^* are inversely related (Iglesias et al., 1999). Lancaster et al. (1997) concluded that an increase in skin darkness was probably a consequence of increased anthocyanin concentration due to a greater proportion of darker red vacuoles, larger vacuoles and several layers of red cells.

3.4. Effect on individual anthocyanins in fruit peel

In 'Fuji' apples identification of the anthocyanins cyanindin-3-galactoside and cyanidin was achieved by matching them to the standards analyzed under the same chromatographic conditions and confirmed by ESI-MS. The cyanidins B, C and D were identified by using MS/MS as cyanidin-pentoses. In previous studies, it has been mentioned that the following cyanidin-pentoses occur in apples: cyanidin-3-xyloside, cyanidin-3-arabinoside and cyanidin-7-arabinoside (Gómez-Cordovés et al., 1996; Vrhovsek et al., 2004).

The major share of anthocyanins in apple is represented by cyanidin-3-galactoside, which accounted from 92% (on the first sampling date) to 98% (at harvest time) of all anthocyanins (Table 4). The concentration level of the anthocyanin increased during ripening, a finding which coincided with a study by Gómez-Cordovés et al. (1996). The potential for accumulating anthocyanins occurs between the middle and the end of the growing season in many varieties, and the content increased continuously during fruit maturation, especially in the 2 weeks preceding the commercial harvest date (Iglesias et al., 1999). Likewise, cyanidin-galactoside, cyanidin-B and cyanidin-C content rose during maturation, but the concentrations were significantly lower. Still lower shares of total anthocyanins in 'Fuji' apple were given to cyanidin-D and cyanidin.

Table 4
Content of individual anthocyanins (mg kg^{-1} FW) in 'Fuji' apple during ripening in all treatments

Anthocyanin sampling	CON	F	HN	HN + F	$\alpha < 0.05$
Cy-gal					
1	33 ± 5	44 ± 6	31 ± 5	27 ± 3	NS
2	49 ± 5a	107 ± 20b	120 ± 19b	177 ± 17c	*
3	146 ± 18	154 ± 8	140 ± 12	190 ± 17	NS
4	150 ± 19a	255 ± 32b	152 ± 8a	223 ± 13b	*
5	191 ± 20a	263 ± 13b	172 ± 12a	317 ± 26c	*
Cy-B[a]					
1	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	NS
2	1.3 ± 0.2	1.6 ± 0.1	1.4 ± 0.2	1.8 ± 0.2	NS
3	1.8 ± 0.2	1.7 ± 0.1	1.5 ± 0.2	2.2 ± 0.2	NS
4	2.4 ± 0.3ab	3.1 ± 0.4c	1.5 ± 0.1a	2.6 ± 0.2bc	*
5	2.5 ± 0.3a	3.7 ± 0.3b	2.4 ± 0.2a	3.9 ± 0.4b	*
Cy-C[a]					
1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	NS
2	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.01	1.3 ± 0.1	NS
3	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	NS
4	1.6 ± 0.2	1.8 ± 0.3	1.2 ± 0.1	1.8 ± 0.1	NS
5	1.7 ± 0.2ab	2.1 ± 0.1bc	1.6 ± 0.1a	2.3 ± 0.2c	*
Cy-D[a]					
1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	NS
2	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	NS
3	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	NS
4	0.9 ± 0.1a	1.6 ± 0.2b	0.8 ± 0.0a	1.4 ± 0.1b	*
5	1.1 ± 0.1a	1.6 ± 0.1b	1.0 ± 0.1a	1.6 ± 0.1b	*
Cy					
1	0.07 ± 0.01a	0.14 ± 0.02b	0.08 ± 0.01a	0.10 ± 0.02ab	*
2	0.14 ± 0.02a	0.17 ± 0.02a	0.18 ± 0.02a	0.33 ± 0.05b	*
3	0.16 ± 0.04	0.14 ± 0.02	0.17 ± 0.03	0.20 ± 0.03	NS
4	0.27 ± 0.04	0.30 ± 0.05	0.20 ± 0.03	0.24 ± 0.04	NS
5	0.42 ± 0.05ab	0.68 ± 0.08c	0.36 ± 0.04a	0.54 ± 0.07b	*

CON, control; F, floor covered with reflective foil; HN, under hail nets; HN + F, floor covered with foil under hail nets. Average values ± standard error are presented. Different letters (a–c) in rows mean statistically significant differences between treatments at $\alpha < 0.05$.

[a] Presented as milligram cyanidin per kilogram FW.

In the orchard covered with reflective foil, there were higher amounts of cyanidin-3-galactoside in the fruit than in the control treatment. In the treatment under hail nets without reflective foil, concentrations were mainly lower than in the control, but the difference was statistically significant only on the second sampling date. On the second sampling date, i.e. 2 weeks after the floor covering, the effect of reflective foil was evident in the increase of cyanidin-galactoside and was present till harvest time. At harvest time, all of the anthocyanins were significantly higher in the treatment under hail nets when the trees were treated with reflective foil. Our findings are in agreement with those of Ju et al. (1999), who report that covering the orchard floor with foil and metallized foil stimulated anthocyanin accumulation. Reay and Lancaster (2001) compared the response to irradiation between the exposed and shaded sides of 'Royal Gala' and 'Gala' fruit when on the tree. The shaded tree side showed greater potential to accumulate anthocyanins than the exposed tree side.

One of the possible explanations of light-enhanced anthocyanin synthesis and accumulation in apple is the increase in canopy photosynthesis and assimilate supply to the fruit, thus indirectly stimulating anthocyanin synthesis by providing substrate. Kawabata et al. (1999) observed a positive correlation between anthocyanin accumulation and soluble sugar content levels, regardless of light conditions. The existence of a close interaction between sucrose and the light signaling pathway induced the expression of the anthocyanidin synthase gene under light conditions (Ubi, 2004). However, in our experiment, no correlation between anthocyanins and carbohydrates was confirmed.

An alternative route by which light enhances antocyanin synthesis and accumulation in apples is that lighting treatments directly stimulate anthocyanin synthesis by flavonoid enzymes. In the red-skinned apple cultivar 'Splendour', the activity of phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI) and glycosyltrasferase (UFGT) correlated with anthocyanin levels during reddening (Lister et al., 1996), and their activity is light-dependent (Treutter, 2001; Ju et al., 1999; Dong et al., 1995).

3.5. Effect on primary metabolites

The influence of reflective foil and hail nets on individual sugars (fructose, glucose, sucrose and sorbitol) and organic acids (malic, citric, shikimic and fumaric acid) at harvest time was studied (Table 2). The sum of individual sugars as well total organic acid content was higher in the control treatment, what could be connected with lower firmness in both treatments without hail net. Covering the orchard floor with the reflective foil influenced a decrease in total sugar and organic acid content in the treatments both under and outside of the hail nets. Fructose, as the main carbohydrate in 'Fuji' apple, showed the same effect, as well as malic and citric acid, but not other individual sugars and organic acids. Robinson et al. (1983) reported that fruit total acidity was slightly negatively correlated with the percentage of full sunlight.

Acknowledgment

This work is part of the program Horticulture P4-0013-0481 supported by the Slovenian Ministry of Higher Education, Science and Technology.

References

Curry, E.A., 1994. Preharvest applications of ethephon reduce superficial scald of 'Fuji' and 'Granny Smith' apples in storage. J. Hortic. Sci. 69, 1111–1116.

Dong, Y., Mitra, D., Kootstra, A., Lister, C., Lancaster, J., 1995. Postharvest stimulation of skin color in Royal Gala apple. J. Am. Soc. Hortic. Sci. 120, 95–100.

Escarpa, A., Gonzalez, M.C., 1998. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. J. Chromatogr. A 823, 331–337.

Gómez-Cordovés, C., Varela, F., Larrigaudiere, C., Vendrell, M., 1996. Effect of ethephon and seniphos treatments on the anthocyanin composition of starking apples. J. Agric. Food Chem. 44, 3449–3452.

Green, S.R., McNaughton, K.G., Greer, D.H., McLeod, D.J., 1995. Measurement of the increased PAR and net all-wave radiation absorption by an apple tree caused by applying a reflective ground covering. Agric. Forest Meteorol. 76, 163–183.

Guerrero, V.M., Orozco, J.A., Romo, A., Gardea, A.A., Molina, F.J., Sastré, B., Martinez, J.J., 2002. The effect of hail nets and ethephon on color development of 'Redchief Delicious' apple fruit in the highlands of Chihuahua. Mex. J. Am. Pomol. Soc. 56, 132–135.

Iglesias, I., Graell, J., Echeverria, G., Vendrell, M., 1999. Differences in fruit color development, anthocyanin content, yield and quality of seven 'Delicious' apple strains. Fruit Varieties J. 53, 133–145.

Iglesias, I., Salvia, J., Torguet, L., Cabús, C., 2002. Orchard cooling with overtree microspinkler irrigation to improve fruit colour and quality of 'Topred Delicious' apples. Sci. Hortic. 93, 39–51.

Ju, Z., Duan, Y., Ju, Z., 1999. Effects of covering the orchard floor with reflecting films on pigment accumulation and fruit coloration in 'Fuji' apples. Sci. Hortic. 82, 47–56.

Kawabata, S., Kusuhara, Y., Li, Y., Sakiyama, R., 1999. The regulation of anthocyanin biosynthesis in *Eustoma grandiflorum* under low light conditions. J. Jpn. Soc. Hortic. Sci. 68, 519–526.

Lancaster, J.E., 1992. Regulation of skin color in apples. Crit. Rev. Plant Sci. 10, 487–502.

Lancaster, J.E., Lister, C.E., Reay, P.F., Triggs, C.M., 1997. Influence of pigment composition on skin color in a wide range of fruit and vegetables. J. Am. Soc. Hortic. Sci. 122, 594–598.

Layne, D.R., Jiang, Z., Rushing, J.W., 2002. The influence of reflective film and ReTain on red skin coloration and maturity of 'Gala' apples. HortTechnology 12, 640–645.

Lister, C.E., Lancaster, J.E., Walker, J.R.L., 1996. Developmental changes in enzymes of flavonoid biosynthesis in the skins of red and green apple cultivars. J. Sci. Food Agric. 71, 313–320.

Reay, P.F., Lancaster, J.E., 2001. Accumulation of anthocyanins and quercetin glycosides in 'Gala' and 'Royal Gala' apple fruit skin with UV-B-Visible irradiation: modifying effects of fruit maturity, fruit side, and temperature. Sci. Hortic. 90, 57–68.

Robinson, T.L., Seeley, E.J., Barritt, B.H., 1983. Effect of light environment and spur age on 'Delicious' apple fruit size and quality. J. Am. Soc. Hortic. Sci. 108, 855–861.

Saure, M.C., 1990. External control of anthocyanin formation in apple. Sci. Hortic. 42, 181–218.

Stampar, F., Hudina, M., Usenik, V., Sturm, K., Virscek Marn, M., Batic, F., 1999. Influence on leaf area on net photosynthesis, yield and flower bud formation in apple (*Malus domestica* Borkh.). Phyton Ann. Rei Bot. A 39, 101–106.

Stampar, F., Veberic, R., Zadravec, P., Hudina, M., Usenik, V., Solar, A., Osterc, G., 2002. Yield and fruit quality of apples cv. 'Jonagold' under hail protection nets. Gartenbauwissenschaf 67, 205–210.

Sturm, K., Koron, D., Stampar, F., 2003. The composition of fruit of different strawberry varieties depending on maturity stage. Food Chem. 83, 417–422.

Tartachnyk, I., Blanke, M.M., 2002. Effect of mechanically simulated hail on photosynthesis, dark respiration and transpiration of apple leaves. Environ. Exp. Bot. 48, 169–175.

Treutter, D., 2001. Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Regul. 34, 71–89.

Ubi, B.E., 2004. External stimulation of anthocyanin biosynthesis in apple fruit. Food Agric. Environ. 2, 65–70.

Veberic, R., Zadravec, P., Stampar, F., 2007. Fruit quality of 'Fuji' apple (*Malus domestica* Borkh.) strains. J. Sci. Food Agric. 87, 593–599.

Vrhovsek, U., Rigo, A., Tonon, D., Mattivi, F., 2004. Quantitation of polyphenols in different apple varieties. J. Agric. Food Chem. 52, 6532–6538.