UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Jerneja JAKOPIČ

ANTOCIANI, KVERCETINI IN DRUGE FENOLNE SPOJINE PRI JABLANI (*Malus domestica* Borkh.) SORTE 'FUJI' MED DOZOREVANJEM

Doktorska disertacija

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DOKTORSKA DISERTACIJA

ANTHOCYANINS, QUERCETINS AND OTHER PHENOLIC COMPOUNDS IN APPLE (*Malus domestica* Borkh.) CV. 'FUJI' DURING RIPENING

DOCTORAL DISSERTATION

Ljubljana, 2011

Doktorska disertacija je zaključek podiplomskega študija bioloških in biotehniških znanosti in se nanaša na znanstveno področje agronomije. Praktičen del poskusa je bil opravljen v sadovnjaku Sadjarstva Mirosan, Kasaze pri Žalcu. Laboratorijski del je bil izveden na Katedri za sadjarstvo, vrtnarstvo in vinogradništvo, Oddelka za agronomijo, Biotehniške fakultete v Ljubljani.

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Naloga je rezultat lastnega raziskovalnega dela. Izjavljam, da so vsa vključena znanstvena dela identična objavljeni verziji.

Jerneja JAKOPIČ

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IJ	sl
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AL	V dvoletnem poskusu smo ugotavljali, kako se pri jablani sorte 'Fuji' zaradi uporabe mreže proti toči in reflektivne folije spremenijo razmere v osvetlitvi. Določili smo štiri obravnavanja, (1) mreža proti toči, (2) reflektivna folija, (3) mreža proti toči + reflektivna folija in (4) kontrola (brez mreže in folije). Ugotavljali smo, kako spremenjene svetlobne razmere vplivajo na izbrane primarne in sekundarne metabolite, ki smo jih analizirali s sistemom visoko ločljivostne tekočinske kromatografije v kombinaciji z masno spektrometrijo (HPLC-MS). Čeprav je bila osvetlitev v medvrstnem prostoru pod mrežo proti toči manjša kot pri kontroli, se izmerjena osvetljenost plodov pod in izven mreže ni statistično razlikovala. Prekrivanje tal z belo reflektivno folijo je pozitivno vplivalo na osvetlitev v nasadu, posebno pa na osvetljenost posameznih plodov v krošnji. Od intenzivnosti osvetlitve plodov je bil odvisen razvoj rdeče krovne barve jabolk. Prekrivanje tal s folijo pod mrežo proti toči je pospešilo razvoj rdeče barve plodov in ob obiranju doseglo vrednosti, kakršne so bile izmerjene pri kontrolnem obravnavanju. Pomanjkanje osvetlitve v notranjosti krošnje je povzročilo nižje vsebnosti kvercetin glikozidov in večine cianidin glikozidov, medtem ko se vrednosti izmerjene pri plodovih, ki so rasli na vrhu in robu krošnje niso statistično razlikovale. Plodovi v notranjosti krošnje pa so bili slabše obarvani kot plodovi, ki so rasli na robu ali vrhu krošnje. Pokazalo se je, da je povečana osvetlitev zaradi uporabe reflektivne folije vplivala na vsebnost cianidin glikozidov, medtem ko mreža proti toči ni imela vpliva nanje. Plodovi s kontrolnih dreves so vsebovali najnižje vrednoti skoraj vseh posameznih kvercetin glikozidov. Mreža proti toči in reflektivna folija sta vplivali na povečano vsebnost kvercetin glikozidov. V poskusu smo spremljali tudi spremembe katehina, epikatehina in klorogenske kisline v plodovih, pri katerih nismo opazili enotnega odziva na spremenjene svetlobne razmere.

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AB In a two year experimental study the effects of hail net and reflective foil on light conditions were monitored on apple cv. ‘Fuji’. Four treatments were defined (1) hail net, (2) reflective foil, (3) hail net + reflective foil and (4) control (without hail net and reflective foil). We determined the modified light conditions and the influences on individual primary and secondary metabolites in apple fruit, which were detected by high performance liquid chromatography coupled with mass-spectrometer (HPLC-MS). Although the measured lighting in the orchard under the hail net was lower than at the control treatment, the differences in the lighting of fruits under and outside the hail nets was not statistically significant. Covering the floor with reflective foil positively influenced lighting conditions in the orchard, especially lighting of individual fruit in the tree canopy. Development of red coloration is dependent on the intensity and the amount of light that reaches the fruit. Covering the floor with reflective foil thus induced red coloration and at harvest time reached values comparable to the control treatment. Low light intensity inside the tree canopy caused lower contents of quercetin glycosides and most cyanidin glycosides. However, there were no statistically significant differences in their contents between fruits located at the perimeter and the top of tree canopy. On the other hand, fruits inside the canopy were less red than fruits at the perimeter and the top of tree canopy. Improved lighting conditions caused by the use of reflective foil influenced an increase in the content of cyanidin glycosides. Hail nets did not show a similar effect. Fruits from control trees had the lowest contents of almost all individual quercetin glycosides. Hail net in combination with reflective caused higher content levels of quercetin glycosides. The changes in catechin, epicatechin and chlorogenic acid content levels were also monitored; however, the effect of lighting conditions on these compounds was not uniform.

KAZALO VSEBINE

KAZALO SLIK

1 UVOD

Sorto jablane 'Fuji' so v poznih 30-ih letih prejšnjega stoletja vzgojili na Japonskem s križanjem sort 'Rdeči delišes' in stare sorte 'Virginia Ralls Genet'. Na trgu se je prvič pojavila leta 1962. Zaradi svoje sočnosti, čvrstosti mesa, sladkega okusa in rdeče barve kožice, je zanimanje zanjo hitro naraščalo med pridelovalci in potrošniki po vsem svetu. Da je interes zanjo tudi v Sloveniji velik, pričajo podatki o obnovi intenzivnih sadovnjakov v letih od 2003 do 2006 (Godec in sod., 2007), ko je bilo največ površin zasajenih prav s 'Fuji'-jem.

V pridelavi jabolk je na nekaterih pridelovalnih območjih velika nevarnost toče med rastno dobo. Toča lahko poškoduje liste in s tem zmanjša asimilacijsko površino ali povzroči direktne poškodbe plodov (Tartachnyk in Blanke, 2002; Štampar in sod., 2002). Poškodbe zaradi toče predstavljajo tudi vstopno mesto za patogene, kot je na primer bakterija *Erwinia amilovora* (hrušev ožig). Da bi zaščitili asimilacijsko (listno) površino in zagotovili visoko kakovost plodov, so proizvodni nasadi jablan vedno bolj pogosto prekriti z mrežo proti toči. Njena namestitev postaja običajen del investicije pri postavitvi sadovnjaka (Štampar in sod., 2002).

V evropskih nasadih jablan je večina mrež črne barve, nekaj belih, od leta 2007 pa so dostopne tudi barvne (rdeče in zelene) mreže (Blanke, 2007). Osvetlitev v sončnih dneh je pod mrežo slabša kot izven nje, najbolj kadar je mreža črne barve, manj pa pri rdeče-beli in zeleno-beli mreži (Solomakhin in Blanke, 2008).

Osvetlitev je poleg drugih okoljskih dejavnikov, kot so temperatura, vročinski stres, mraz, okužba s patogenom in mehanske poškodbe, ključno povezana z obarvanostjo kožice pri jabolkih (Ubi, 2004; Iglesias in sod., 2002). Pri rdeče obarvanih sortah je delež in odtenek krovne barve pomemben kriterij kakovosti. Vrednost slabo obarvanih plodov je na trgu manjša, odvisno od leta, za 10 do 15% (Guerrero in sod., 2002). 'Fuji', ki je pozno jesenska sorta, v Sloveniji zori v prvi dekadi oktobra, ko je osvetlitev zmanjšana že zaradi geografske lege.

Močno zmanjšanje razpoložljive sončne svetlobe so ugotovili zaradi črne mreže proti toči, kar negativno vpliva na razvoj rdeče barve plodov (Guerrero in sod., 2002; Štampar in sod., 2002). O slabši obarvanosti plodov pod mrežo proti toči poročajo tudi Guerrero in sod. (2002). Plodovi pridelani pod protitočno mrežo so slabše kakovosti, kar se kaže v slabši obarvanosti plodov, slabši čvrstosti mesa, manjši vsebnosti sladkorja in posledično slabšega okusa (Funke in Blanke, 2005). Bele in barvne mreže prepustijo več svetlobe kot črne, zato pri sorti 'Pinova' poročajo o slabšem razvoju krovne barve pod črno mrežo proti toči kot pri uporabi belih ali barvnih mrež (Blanke, 2007).

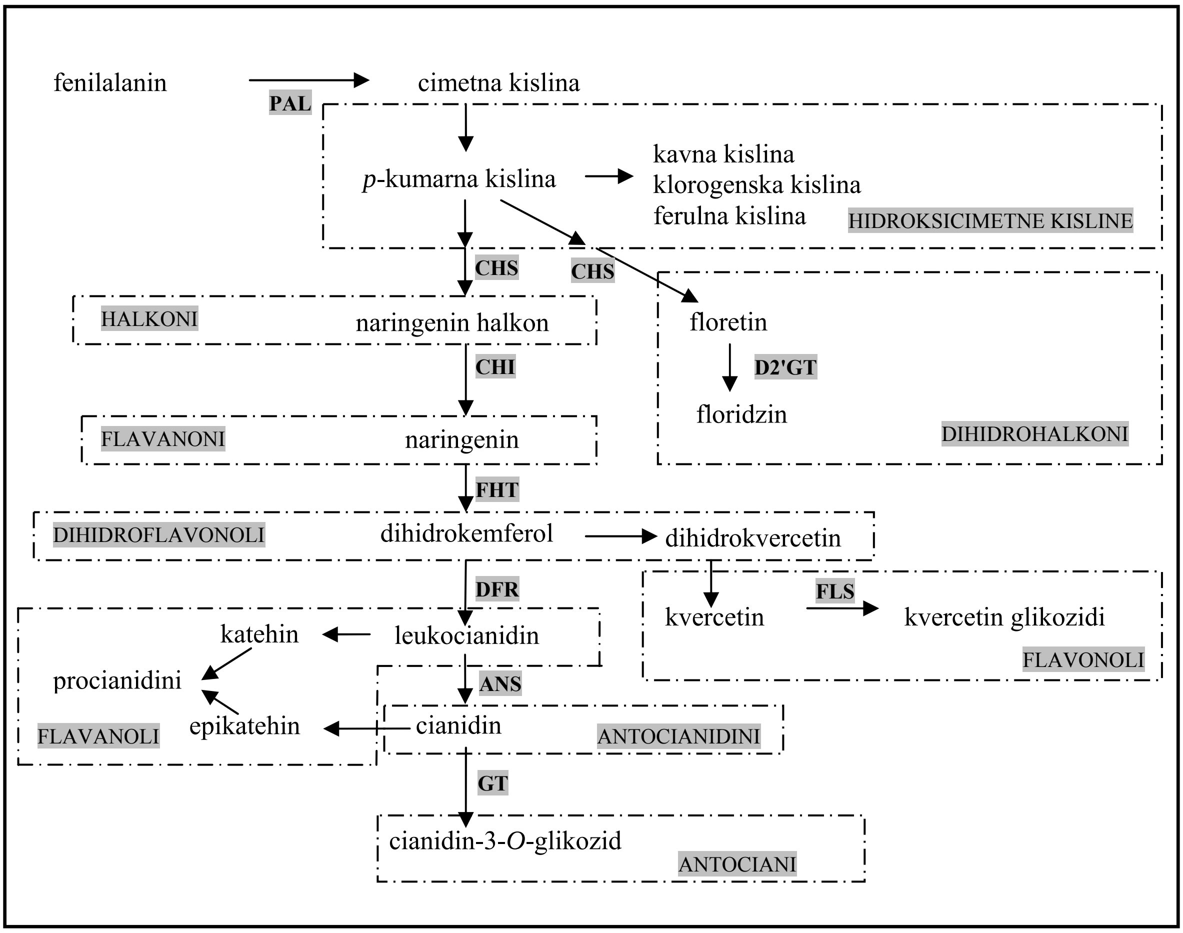
Z različnimi ukrepi se trudijo izboljšati kakovost in s tem povečati komercialno vrednost jabolk. Nekaj poskusov za izboljšanje obarvanosti plodov je že bilo narejenih, vendar so bili le redki uspešni. Prekrivanje plodov s pergamentnimi vrečkami se je izkazalo za učinkovito metodo za povečanje razvoja rdeče barve plodov, vendar ta metoda zahteva izjemno veliko dela in je ekonomsko nesprejemljiva (Ju in sod., 1999). Kemično tretiranje z etefonom, na primer, je učinkovalo na razvoj barve pri zgodnjih sortah, ne pa pri poznih sortah, kamor sodi tudi 'Fuji' (Curry, 1994). Poskušali so s hlajenjem z mirkorazpršilci (Iglesias in sod., 2002), itd.

Layne in sod. (2002) so odkrili, da prekrivanje tal v nasadu z reflektivno folijo spodbuja razvoj rdeče krovne barve pri sorti 'Gala' in ne vpliva na spremembo velikosti in mase plodov. Aluminizirana plastična folija je v glavnem povečala osvetlitev v krošnji in je vplivala na bolj intenzivno rdečo barvo (Gleen in Puterka, 2007).

Odtenek rdeče barve je pri jabolkih pogojen s sintezo antocianov (Li in sod., 2002). Različni avtorji (Lancaster, 1992; Saure, 1990) poročajo o povezavi med akumulacijo antocianov in intenzivnostjo osvetlitve. Osvetlitev vpliva na indukcijo ekspresije genov biosinteze antocianov v kožici jabolk (Kim in sod., 2003). Za začetek sinteze antocianov je potrebna svetloba z zadostno količino energije in primernim spektrom (Ubi, 2004). Raziskave so pokazale, kateri del spektra svetlobe je ključen za tvorbo antocianov v kožici jabolka. Sevanje z modro-vijolično (BV) in ultravijolično (UV) svetlobo se je pokazalo kot najbolj učinkovito, medtem ko je bilo dolgovalovno rdeče (FR) sevanje najmanj učinkovito ali je delovalo celo zaviralno. Osvetlitev plodov je odvisna od bujnosti podlage, gojitvene oblike, rezi, sistemov sajenja, orientiranosti vrst v nasadu, uporabe proti točnih mrež in tudi od pozicije plodov na drevesu (Awad in sod., 2001b). Plodovi, ki se nahajajo na robu krošnje in so torej izpostavljeni sevanju, kažejo mnogo večji potencial kopičenja antocianov kot tisti znotraj krošnje oz. senčeni (Reay in Lancaster, 2001). Plodovi znotraj krošnje, ki niso izpostavljeni svetlobi, namreč ne razvijejo rdeče krovne barve; so slabše obarvani ali ostanejo celo neobarvani (Ubi, 2004). Treutter (2001) navaja, da ima velik vpliv gojitvena oblika, saj mora biti za ustrezno obarvanost vsak plod posebej izpostavljen sončni svetlobi, da razvijejo zadostne količine antocianov.

Antociani ali antocianidini so glikozirani antocianidini, ki imajo na benzenov obroč vezan sladkor. Sladkorji so na aglikone antocianidinov navadno vezani na tretjem C-atomu benzenovega obroča, izjemoma lahko tudi na drugih. V kožici jabolk se glede na dosedanje raziskave vsi odkriti antociani nahajajo v obliki derivatov cianidina, med katerimi prevladuje cianidin 3-galaktozid (idaein). V manjši meri so pri nekaterih sortah prisotni cianidin 3-arabinozid, cianidin 7-arabinozid, cianidin 3-ksilozid in cianidin 3-glukozid (Lancaster, 1992). Odtenek rdeče barve je pogojen s koncentracijo antocianov v kožici ploda, vendar je barva kožice jabolk odvisna tudi od koncentracije drugih pigmentov, kot so flavonoli, klorofil in karotenoidi (Lancaster, 1992).

Jabolka poleg antocianov vsebujejo širok spekter drugih fenolnih snovi. Le-te so definiramo kot spojine, ki imajo na benzenov obroč vezano hidroksilno (-OH) skupino in so kemijsko zelo raznolika skupina. Njihova sinteza poteka po več poteh, med katerimi je najpomembnejša pot šikimske kisline, pri čemer nastaja aromatska aminokislina fenilalanin. Iz fenilalanina nastane *trans*-cimetna (*t*-cimetna) kislina. To reakcijo katalizira encim fenilalanin amonij liaza (PAL), ki je vezni člen med primarnim in sekundarnim metabolizmom (Taiz in Zeiger, 2002). V jabolkih je aktivnost PAL-a največja pri razvijajočih se plodičih in se med rastno dobo zmanjšuje. Encim halkon izomeraza sodeluje pri nastanku naringenina, ki je prvi v sintezi flavonoidov (Treutter, 2001). Njegova aktivnost se pri jabolkih do 139 dni po polnem cvetenju zmanjšuje podobno kot pri PAL-u, potem pa se do zrelosti spet poveča. UDP-flavonoid-3-*O*-glikoziltransferaza je zadnji encim v biosintezi antocianov in katalizira glikozilacijo antocianidinov v antociane. Njegova aktivnost se med dozorevanjem povečuje (Li in sod., 2002). Isti avtorji ugotavljajo, da je sinteza antocianov povezana z aktivnostjo omenjenih encimov, od katerih ima najpomembnejšo vlogo prav UDP-flavonoid-3-*O*-glikoziltransferaza. Sekundarni metabolizem do sinteze antocianov je prikazan na sliki 1.



Slika 1: Biosintetska pot fenolnih snovi. PAL, fenilalanin amonij liaza; CHS, halkon sintaza; CHI, halkon izomeraza; FHT, flavanon 3-hidroksilaza; DFR, dihidroflavonol 4-reduktaza; FLS, flavonol sintaza; D2'GT, dihidrohalkon 2'-*O*-glukoziltransferaza; ANS, ???? GT, UDPG-flavonoid-3-*O*-glikoziltransferaza.
Figure 1: Biosynthetic pathways of phenolic compounds in apple. PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; FHT, flavanone 3-hydroxylase; DFR, dihydroflavonol 4-reductase; FLS, flavonol synthase; D2'GT, dihydrochalcone 2'-*O*-glucosyltransferase; ANS, ???? GT, UDPG-flavonoid-3-*O*-glycosyltransferase.

Fenolne spojine v rastlinah povzročajo pigmentacijo cvetov in plodov, prispevajo k aromi in okusu plodov, predstavljajo obrambo pred škodljivci in patogeni mikroorganizmi. Mnoge med njimi se tvorijo kot odgovor rastline na kemične ali mehanske poškodbe, za molekule flavonoidov pa je znano, da ščitijo celice rastline pred absorpcijo potencialno nevarnega UV sevanja (Lister in sod., 1996), saj imajo tudi antioksidativno sposobnost.

Antioksidanti uničujejo in nevtralizirajo proste radikale, kar pomeni, da z vključevanjem antioksidantov v prehrano ljudi skrbimo za varstvo pred kardiovaskularnimi boleznimi in rakastimi obolenji (Biedrzycka in Amarowicz, 2008). Glavni antocian v jabolkih, cianidin 3-galaktozid, lahko uničuje kisikove radikale v in vitro sistemu (Yamasaki in sod., 1996).

Izmed fenolnih snovi so v kožici jabolk poleg antocianov prisotni kvercetin glikozidi, dihidrohalkon glikozidi, procianidin monomeri (npr. katehin) in klorogenska kislina (Lancaster, 1992). Vsebnost fenolnih snovi v jabolkih se spreminja med sortami, rastno dobo, dozorevanjem, lokacijo sadovnjaka, načinom pridelave, pogoji skladiščenja in okoljskimi razmerami. Eden od dejavnikov, ki vplivajo na koncentracijo fenolnih snovi v jabolkih je izpostavljenost sončnemu obsevanju (Awad in sod., 2000). Medtem ko je razvoj krovne barve pri rdečih sortah povezan s sintezo cianidin glikozidov, sorte brez rdeče krovne barve ('Granny Smith', 'Zlati delišes') kopičijo kvercetin glikozide in katehine (Lancaster, 1992). Kvercetin glikozide navajajo celo kot najbolj zastopane v kožici jabolk, saj so koncentracije okrog trikrat večje kot antocianov (Lister in sod., 1996). Na sintezo flavonoidov vplivajo tudi različni tehnološki ukrepi. Ugotovili so zelo majhne koncentracije flavonoidov, vključno s kvercetin glikozidi, v kožici plodov, ki so bili zaščiteni s pergamentno vrečko (Ju in sod., 1998).

Znani so torej tako pozitivni učinki reflektivne folije kot tudi negativni učinki mreže proti toči na osvetlitev. Je torej mogoče z uporabo reflektivne folije izničiti vpliv mreže proti toči? Cilj naše raziskave je bil odkriti kako uporaba mreže proti toči in prekrivanja tal s folijo vplivata na osvetlitev v nasadu in na osvetlitev posameznih plodov v krošnji. Nekateri avtorji so že raziskovali vpliv osvetlitve na razvoj skupnih antocianov, ni pa še znano kakšen je vpliv na posamezne antociane. Predmet naše raziskave je bil nadgraditi rezultate v kompleksnem poskusu, ki vključuje obarvanost plodov in vsebnost pigmentov, kot so cianidin glikozidi, klorofil in karotenoidi v plodovih jablane, sorte 'Fuji'. Proučevali smo tudi vpliv na nekatere primarne metabolite, kot so sladkorji in organske kisline, ter nekatere sekundarne metabolite, kot so kvercetin glikozidi, katehin, epikatehin in klorogenska kislina.

Poskus smo izvajali v intenzivnem nasadu jablan Sadjarstva Mirosan v Kasazah pri Žalcu. Izbrali smo leta 2003 sajene jablane, sorte 'Fuji', cepljene na podlago M9. V letih 2006 in 2007 smo med dozorevanjem, v zadnjem mesecu pred obiranjem, pogrnili belo reflektivno folijo po medvrstnem prostoru in pod drevesi pri polovici dreves, vključenih v poskus. Določili smo štiri obravnavanja:

(1) Drevesa pod mrežo proti toči;
(2) Drevesa izven mreže proti toči, brez reflektivne folije (kontrola);
(3) Drevesa pod mrežo, tla pogrnjena z belo reflektivno folijo;
(4) Drevesa izven mreže; tla pogrnjena z belo reflektivno folijo.

Vsakemu obravnavanju smo priredili 8 dreves, med katerimi smo izbrali 5 najbolj izenačenih in na vsakem označili 3 plodove (15 plodov v vsakem obravnavanju). Na njih smo spremljali barvo in osvetlitev med trajanjem poskusa. V nasadu smo tedensko merili osvetlitev v medvrstnem prostoru in na označenih plodovih ter vzeli po 10 plodov iz vsakega obravnavanja za kemijske analize, v zadnjem terminu pa obrali označene plodove.

Na plodovih smo merili barvo in izvedli kemične analize vsebnosti izbranih primarnih in sekundarnih metabolitov.

Postavili smo naslednje raziskovalne hipoteze:

- Uporaba mreže proti toči vpliva na zmanjšano osvetlitev v nasadu,
- Pozicija ploda v krošnji vpliva na dostopnost svetlobe za posamezen plod,
- Prekrivanje tal z reflektvino folijo v nasadu poveča osvetlitev v krošnji jablan,
- Večja osvetlitev vpliva na boljšo obarvanost plodov,
- Osvetlitev vpliva na sintezo posameznih antocinanov, kvercetin glikozidov in drugih fenolih snovi,
- Osvetlitev vpliva na vsebnost posameznih primarnih metabolitov.

Rezultati dvoletnih raziskav bodo pokazali, kako sprememba svetlobnih razmer v nasadu in v krošnji posameznega drevesa vpliva na posamezne produkte primarnega in sekundarnega metabolizma jablan. Zanimiva bo tudi ugotovitev, ali s polaganjem reflektivne folije v medvrstnem prostoru lahko nadomestimo zmanjšanje osvetlitve zaradi uporabe mreže proti toči.

2 ZNANSTVENI ČLANKI

2.1 VPLIV REFLEKTIVNE FOLIJE IN MREŽE PROTI TOČI NA OSVETLITEV, BARVO IN ANTOCIANE V JABOLKIH, SORTE 'FUJI'

JAKOPIČ Jerneja, VEBERIČ Robert in ŠTAMPAR Franci
The effect of reflective foil and hail nets on the lighting, color and anthocyanins of 'Fuji' apple.
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Medtem ko uporaba mreže proti toči zmanjša osvetlitev v nasadu, prekrivanje tal z reflektivno folijo povzroča odboj svetlobe in s tem izboljšuje osvetljenost krošenj. Od intenzivnosti osvetlitve plodov je odvisna sinteza antocianov in posledično krovna barva. V zadnjem mesecu pred obiranjem smo položili reflektivno folijo in določili štiri obravnavanja: (1) mreža proti toči, (2) mreža proti toči in reflektivna folija, (3) reflektivna folija in (4) kontrola (brez mreže proti toči in reflektivne folije).

Merili smo fotosintetsko aktivno sevanje (PAR) v nasadu in na označenih plodovih pri vseh štirih obravnavanjih. Pokazalo se je, da je PAR pod mrežo proti toči manjši v primerjavi s kontrolo za okrog 35% in da uporaba reflektivne folije poveča osvetlitev za 20% pod mrežo in 10% izven mreže v primerjavi z obravnavanji, kjer je tla v medvrstnem prostoru prekrivala trava. Med osvetlitvijo spodnjih delov plodov ni bilo razlik pod in izven mreže, medtem ko se je pokazal pozitiven učinek reflektivne folije. Kolorimetrično določena vrednost a* (-60 do 60 oz. zelena do rdeča), ki opredeljuje rdečo barvo, nakazuje, da so bila najmanj intenzivno rdeča jabolka pod mrežo in najbolj intenzivno rdeča izven mreže, kjer so bila tla pokrita s folijo. Vrednosti posameznih antocianov so bile določene s pomočjo tekočinske kromatografije visoke ločljivosti v kombinaciji z masno spektrometrijo (HPLC-MS). Spremljali smo spremembe petih cianidin glikozidov (cianidin 3-galaktozida, treh cianidin pentozidov in čistega cianidina) v zadnjem mesecu pred obiranjem. Koncentracija najbolj zastopanega, cianidin 3-galaktozida, se je med dozorevanjem povečevala pri vseh obravnavanjih. Ob obiranju pri nobenem od analiziranih cianidin glikozidov ni bilo razlike med obravnavanjem pod mrežo in kontrolnim obravnavanjem, pokazal pa se je pozitiven učinek reflektivne folije, kjer je bila vsebnost posameznih cianidin glikozidov in cianidina večja v primerjavi z obravnavanji, kjer tla niso bila pokrita.

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

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Abstract

We studied the influence of covering the orchard floor with reflective foil on photosynthetic active radiation (FAR) 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 FAR. 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 (FAR); 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).

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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

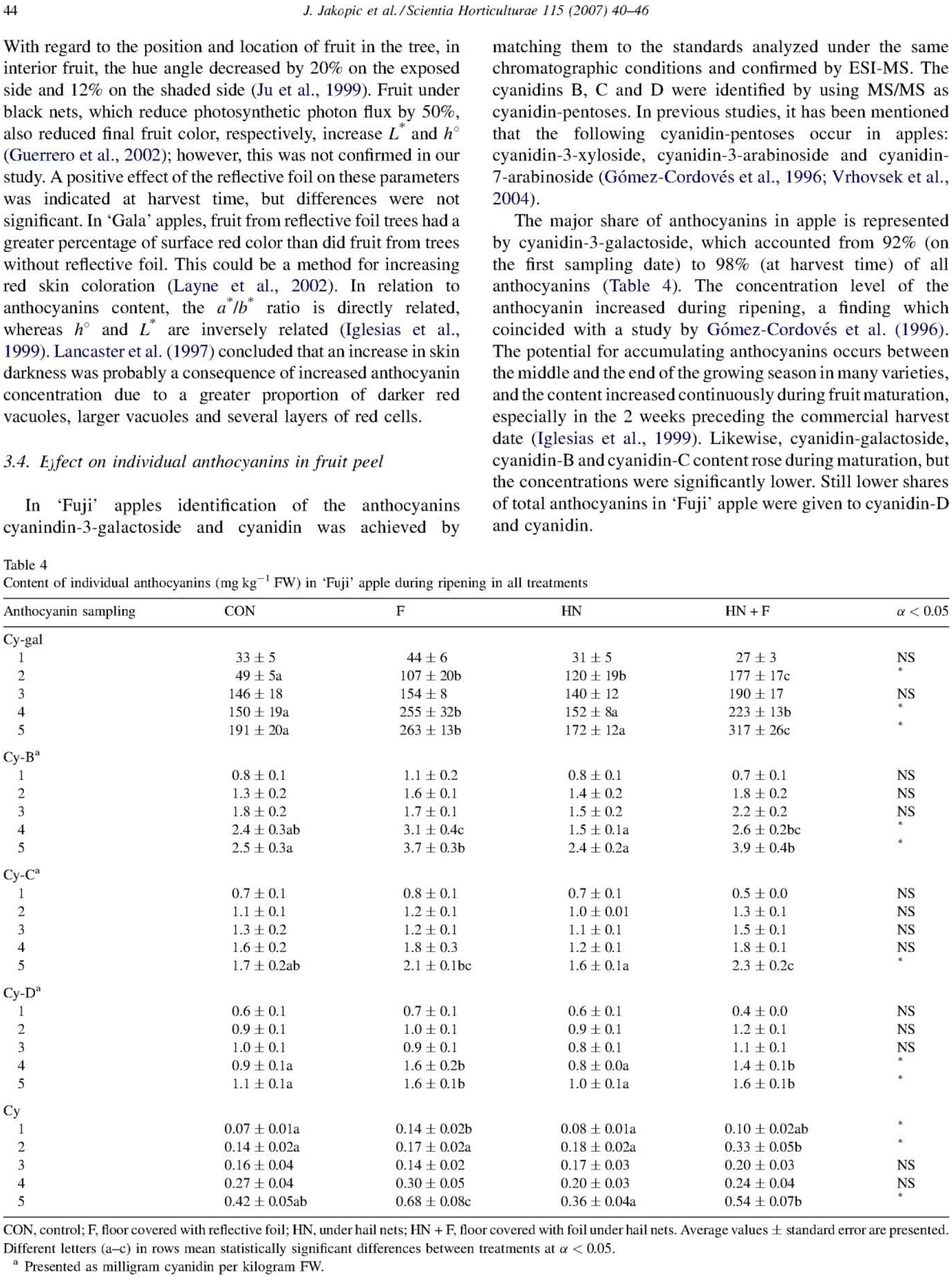
The intensity of fruit lighting influenced the development of red coloration. The CIE color coordinate a^*, a higher value of which indicates more red color, was on all sampling dates the lowest in the treatment under hail nets without reflective foil, but it was not always statistically significant (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.

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2.2 VPLIV IZPOSTAVLJENOSTI SVETLOBI NA VSEBNOST FENOLOV V JABOLKIH SORTE 'FUJI'

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The influence of exposure to light on the phenolic content of 'Fuji' apple.
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Na osvetljenost plodov poleg zunanjih dejavnikov (npr. uporaba mreže proti toči) vpliva tudi položaj plodov v krošnji, zato smo v tem poskusu spremljali spremembo osvetlitve plodov na robu krošnje, v notranjosti in v zgornjem delu (na vrhu) krošnje v zadnjem mesecu pred obiranjem pod in izven protitočne mreže. Proučevali smo vpliv uporabe protitočne mreže na osvetlitev, barvo, vsebnost pigmentov in drugih fenolnih snovi v kožici jabolk, sorte 'Fuji', ter vrednotili, kako je ta vpliv povezan s položajem plodov v krošnji Vsebnost osmih kvercetin glikozidov, petih antocianov, dveh flavan-3-olov in ene hidroksicimetne kisline smo analizirali s pomočjo tekočinske kromatografije visoke ločljivosti v kombinaciji z masno sperktrometrijo (HPLC-MS), skupni fenoli, klorofil in karotenoidi pa so bili določeni spektrofotometrično. Najmanjše fotosintetsko aktivno sevanje (PAR) je bilo izmerjeno na plodovih znotraj krošnje, večje na zunanjih plodovih in največje na plodovih, ki so se nahajali na vrhu krošnje. Mreža proti toči ni vplivala na zmanjšanje osvetlitve v primerjavi s kontrolnim obravnavanjem. Svetlobne razmere v krošnji so vplivale na nižji nivo kvercetin glikozidov in večine cianidin glikozidov v plodovih znotraj krošnje, medtem ko so bile v plodovih z vrha krošnje izmerjene največje vsebnosti kvertecin glikozidov in cianidin glikozidov. Koncentracija katehina, epikatehina in klorogenske kisline v kožici jabolk ni bila odvisna od položaja plodov v krošnji ali uporabe mreže proti toči, razen za klorogensko kislino, kjer je bila koncentracija večja, ko je bil nasad prekrit z mrežo proti toči. Plodovi z vrha in zunanjega roba krošnje so razvili temnejšo, bolj intenzivno rdečo barvo kot plodovi iz notranjega dela krošnje, medtem ko pozicija plodov ni vplivala na vsebnost klorofila a, klorofila b ali na vsebnost karotenoidov. Sklepamo lahko, da so kvercetin glikozidi in cianidin glikozidi povezani z razvojem rdeče barve, saj je bila ugotovljena intenzivnejša obarvanost plodov z roba in vrha krošnje. Intenzivnejša osvetlitev stimulira višje vsebnosti flavonoidov in posledično boljšo obarvanost, ki je pomemben dejavnik pri kakovosti jabolk.

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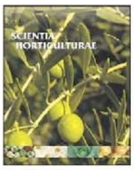

The influence of exposure to light on the phenolic content of 'Fuji' apple

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ABSTRACT

In this study, the influence of changes in fruit lighting on individual and total phenols in 'Fuji' apple, as well as color development was studied. Content levels of eight quercetin glycosides, five anthocyanins, two catechins and a hydroxycinnamic acid in the skin of apples were analyzed, using high-performance liquid chromatography, tandem mass spectrometry (HPLC-MS). Total phenol, chlorophyll and carotenoid contents were determined by spectrometry. The purpose of this study was to compare content levels of those compounds in apple skin of fruit grown in different parts of the tree canopy, under and outside of the hail net. Lighting of fruit was measured during the last month before harvest. The lowest values were measured in the inner fruit and higher ones in the outer parts of the canopy, while the highest values were measured in fruit growing at the top of the tree. The hail net had no influence on the decrease of lighting in comparison to the control. Light conditions in the tree canopy influenced lower content levels of quercetin glycosides and most anthocyanins in the fruit skin in the inner part of the tree canopy, whereas fruit from the canopy top contained the highest levels of quercetin glycosides and cyanidin glycosides. Catechin, epicatechin and chlorogenic acid content levels in apple skin were independent of fruit position in the canopy or hail net usage, except for chlorogenic acid, where the content level was higher in cases when the orchard was covered with a hail net. Fruit from the top and outer parts of the canopy had a darker and redder coloration than inner fruit, while no influence of canopy position on chlorophyll and carotenoids was detected. Since quercetin glycosides and cyanidin glycosides are influential in red skin color development, better coloration of fruit from the outer and top canopy was observed. More intensive lighting stimulated a higher content level of flavonoids and, consequently, better coloration, which is an important factor in fruit quality.

1. Introduction

Apples are a rich source of various phytochemicals, such as carotenoids, flavonoids, isoflavonoids, and phenolic acids (Veberic et al., 2007; Wojdylo et al., 2008). Boyer and Liu (2004) suggested that apples and apple products possess a wide range of biological activities, which may have beneficial health effects on cardiovascular disease, asthma and pulmonary dysfunction, diabetes, obesity, and cancer. The main structural classes of apple constituents include hydroxycinnamic acids, di-hydrochalcones, flavonols, catechins and oligomeric procyanidins, as well as anthocyanin in red apples (Jakopic et al., 2007; Veberic et al., 2007). Individual flavonoid and phenolic acid levels are not equally distributed within the fruit. Awad et al. (2000) reported that quercetin 3-glcyosides and anthocyanin were found almost exclusively in the skin, catechins mostly in the skin and occasionally in the flesh, and chlorogenic acid was mainly present in the seeds, with an intermediate level present in the flesh and a low level in the skin.

Phenolic compound levels in apple vary greatly among cultivars, seasons, orchards, organic or non-organic production and environmental conditions (Awad et al., 2000; Veberic et al., 2005; Lata, 2007; Solomakhin and Blanke, 2007). Among environmental factors, light is especially important. The effect of light has also been reported on the coordinate induction of the expression of the anthocyanin biosynthetic genes in apple fruit skin (Kim et al., 2003). Lighting of fruit depends on dwarf rootstock, training system, row orientation, hail net usage, and also on the position of the fruit in the tree canopy (Awad et al., 2001b; Jakopic et al., 2007). Sometimes climatic characteristics due to frequent hailstorms mandate the use of hail net, but because of its shading effect, incident solar light could be reduced and have a negative impact on fruit development as well as on its final color (Guerrero et al., 2002; Stampar et al., 2002). Several experiments have explored the possibility of increasing lighting in the tree canopy. Certain authors (Jakopic et al., 2007; Blanke, 2008) experimented with covering the orchard floor with reflective foil, while Gómez-Cordovés et al. (1996) tested the treatment with ethephon and seniphos, but these arrangements are labour intensive, expensive or low in effectiveness.

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Proper fruit coloration is very important topic in all apple growing regions, because it can be influenced by many technological measures applied (hail net, training system, etc.). Therefore it is a subject of continual research in connection with the influence of light reduction under hail nets or exposure of fruit to different light intensities. The objective of our study was to upgrade these results in a complex experiment with precise studies which also involve fruit metabolites. We tried to determine the changes in fruit lighting for different positions in the tree canopy under and outside the hail net, and the influence of position in the tree canopy and consequently differences in lighting on the content of phenolic compounds (phenolic acids and different groups of flavonoids), fruit coloration and content of pigments, such as individual cyanidin 3-glycosides, chlorophyll and carotenoids in apple skin, cv. 'Fuji'.

2. Material and methods

2.1. Plant material

The measurements were carried out during the last month before harvest time—from mid-September to harvest on 5 October 2007—on 4-year-old 'Fuji' apple trees grafted on M9 rootstock grown in the commercial orchard of Sadjarstvo Mirosan (46°15′N and 15°10′E) in eastern Slovenia. The experiment included fifteen trees grown under hail nets (double black longitudinal and double (black and green) transverse fibres with mesh size of 6 × 7 mm) as well as fifteen trees grown in the open. At the beginning of September, we marked the inner, outer and top fruit on each tree and determined following six treatments:

- CONTROL-OUTER (fruit outside the canopy, without hail net);
- CONTROL-INNER (fruit inside the canopy, without hail net);
- CONTROL-TOP (fruit from the canopy top, without hail net);
- HAILNET-OUTER (fruit outside the canopy, under hail net);
- HAILNET-INNER (fruit inside the canopy, under hail net); and
- HAILNET-TOP (fruit from the canopy top, under hail net);

There were no significant differences in crop load among individual trees or treatments. At harvest time, thirty marked fruit were picked up and used for chemical analyses.

2.2. Light measurement

During the experiment, the photosynthetic active radiation (PAR) was measured on a sunny day (μmol m^{-2} s^{-1}) using an LI-190 quantum sensor (LI-COR, Nebraska, USA). On each tree, PAR was measured on the lower (downward oriented) portion of the marked fruit every week during ripening, what means fifteen times per each treatment. On the last sampling date (at harvest), these radiation measurements were not carried out because of cloudy weather.

2.3. Fruit color measurement

Apple color was measured on red side of each of the thirty fruit using the Minolta CR-10 Chroma portable colorimeter (Minolta Co., Osaka, Japan). Fruit chromaticity was recorded in CIE (Commission Internationale d'Eclairage) parameters L^*, a^*, b^* and hue angle ($h°$) color space coordinates.

2.4. Spectrophotometric determination of chlorophylls and carotenoids in fruit skin

Two discs from the non-exposed side of each fruit (4 mm in diameter) were taken. For the extraction of chlorophyll and carotenoids, dimethyl sulfoxide (DMSO) was used. 0.5 ml DMSO was poured into microcentrifuge tubes, and one disc was added together with crystals of magnesium hydroxide carbonate to assure a sufficient concentration of Mg^{2+} ions in the solution. The disc was macerated for better extraction, and another 0.5 ml DMSO was added. Samples were left in a water bath (65 °C) in the dark for 2 h. After extraction, the samples were cooled to room temperature and decanted into cuvettes. Immediately afterwards, the absorptions were measured by spectrophotometer (Lambda Bio 20, PerkinElmer) at a wavelength of 480 nm (carotenoids), 649 nm (chlorophyll b) and 665 nm (chlorophyll a). Concentrations of chlorophylls a and b and carotenoids in the extract were counted up by using Wellburn (1994) equations.

2.5. Analysis of individual phenols

For the analysis of individual phenols, fresh apple peel was ground into fine powder in a mortal chilled with liquid nitrogen. 1 g of the sample was extracted with 5 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 HPLC (high-performance liquid chromatography) system.

Samples were analyzed using a Thermo Finningan Surveyor HPLC system (Thermo Scientific, San Jose, USA) with a diode array detector at 280 (catechins and chlorogenic acid), 350 (quercetin glycosides) and 530 nm (anthocyanins). 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 1% formic acid (A) and pure acetonitrile (B). The samples were eluted according to the gradient described by Marks et al. (2007).

Phenols 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. Quercetins and anthocyanins were confirmed using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray interface (ESI) operating in negative (quercetins) or positive (anthocyanins) ion mode. Analysis was carried out using MS^2 scanning from m/z 115 to 800.

2.6. Analysis of total phenols

The extraction of apple skin samples for the identification of total phenols was carried out by the same protocol as used for individual phenols, the difference being that no BHT was added. The total phenolic content of the extracts was assessed using the Folin–Ciocalteau phenol reagent method (Singleton and Rossi, 1965). 6 ml of bi-distilled water and 500 μl of Folin–Ciocalteau reagent were added to 100 μl of the sample extracts (diluted 1:5 (v/v) with MeOH). After a wait of between 8 s and 8 min at room temperature, 1.5 ml of sodium carbonate (20%, w/v) was added. The extracts were mixed and allowed to stand for 30 min at 40 °C before the absorbance was measured by 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 (GAE) in milligrams per kilogram fresh weight of apple skin. Absorptions were measured in three replications.

2.7. Chemicals

The following standards were used to determine chemical compounds: cyanidin 3-*O*-galactoside chloride, quercetin

Table 1
Lighting of fruit in the canopy (μmol $m^{-2}s^{-1}$) during ripening in year 2007 in all treatments and interaction between fruit position in the canopy and hail net.

Weeks before harvest	Control			Hail net			*p*-Value		
	Outer	Inner	Top	Outer	Inner	Top	Use of hail net	Position	Interaction
4	56 ± 7	17 ± 2	70 ± 16	69 ± 8	36 ± 5	77 ± 8	n.s.	0.0000	n.s.
3	93 ± 14	29 ± 5	84 ± 14	86 ± 15	34 ± 4	81 ± 9	n.s.	0.0000	n.s.
2	57 ± 6	18 ± 2	56 ± 7	57 ± 9	34 ± 6	62 ± 5	n.s.	0.0000	n.s.

Average values ± standard error are presented. n.s.—non-significant at $p < 0.05$.

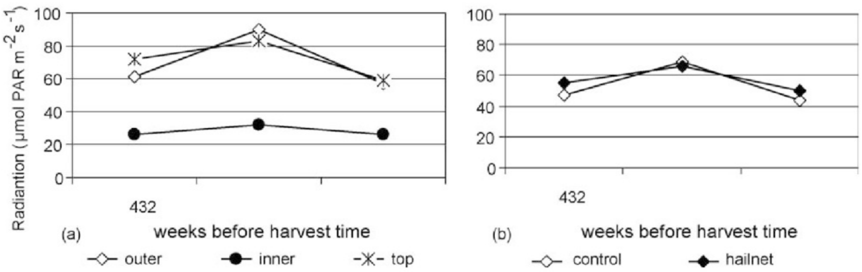

Fig. 1. The average photosynthetic radiation (PAR) of apple fruit in the canopy (μmol m^{-2} s^{-1}) during ripening in year 2007 for different fruit positions in the tree (a) and under or outside the hail net (b).

3-D-galactoside, quercetin 3-β-D-glucoside, quercetin-3-rhamnoside and cyanidin chloride from Fluka Chemie GmbH (Buchs, Switzerland); cyanidin 3-arabinoside from Polyphenols Laboratories (Sandnes, Norway); chlorogenic (5-caffeoylquinic) acid, quercetin, quercetin 3-rutinoside (rutin) and (−)-epicatechin from Sigma–Aldrich Chemie GmbH (Steinheim, Germany); (+)-catechin from Roth (Karlsruhe, Germany), and quercetin 3-*O*-xyloside and quercetin 3-arabinofuranoside from Apin Chemicals (Abingdon, UK).

Chemicals for the mobile phases were acetonitrile and formic acid from Fluka Chemie GmbH (Buchs, Switzerland).

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

2.8. Statistical evaluation

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

3. Results

3.1. Changes in photosynthetic active radiation (PAR) during ripening

Table 1 shows the photosynthetic active radiation (PAR) during ripening in different parts of the tree canopy for trees grown under and outside the hail net. Interaction between the latter two factors was not observed. Fruit lighting was independent of hail net coverage in the orchard, but it depended on fruit position in the canopy (Fig. 1). The lowest values were measured on fruit grown inside the canopy, where PAR was around 30 μmol m^{-2} s^{-1} (Fig. 1a). Lighting was higher on outer fruit, while the highest values were achieved for fruit from the top of the tree (Fig. 1a). In this year, the hail net did not result in a decrease of lighting in comparison to the control (Fig. 1b). Differences in PAR under the hail net and the control treatment were not statistically significant during the last month before harvest.

3.2. Colorimetrically defined fruit color

Fruit color was determined colorimetrically, with parameters L^*, a^*, b^* and h° values. There was no interaction between fruit position in the canopy and covering the orchard with hail net in terms of fruit coloration. The only statistically significant differences were noticed between the inner fruit and fruit from other parts of the canopy; no differences were observed between outer fruit and fruit from the top of the trees (Table 2). Fruit from the outer parts and the top of the canopy had darker (lower L^* value), less yellow (lower b^*) and redder (higher a^* value) coloration than fruit from inner parts of the tree canopy.

3.3. Chlorophyll and carotenoid content

Content levels of chlorophyll a, chlorophyll b and carotenoids in apple skin are presented in Table 3. No influence of fruit position in the tree canopy or hail net coverage on the concentrations of these substances was observed, except for the content level of chlorophyll a, which was higher under hail net than in the open.

3.4. Content of phenolic compounds

Concentrations of different classes of flavonoids, catechins, chlorogenic acid and total phenols are shown in Table 4 for outer, inner and top fruit of trees grown under the hail net and in the open. There were no interactions between fruit position in the tree canopy and covering the orchard with hail net, except for quercetin 3-glucoside.

Table 2
Chromaticity values of red skin fruit color at different positions in 'Fuji' apple tree at harvest time in year 2007.

	Outer	Inner	Top	*p*-Value
L	39.8 ± 1.0 a	52.4 ± 1.1 b	38.5 ± 1.5 a	0.0000
a	32.3 ± 1.9 b	15.1 ± 2.2 a	33.3 ± 3.0 b	0.0000
b	19.2 ± 1.1 a	27.5 ± 1.1 b	17.8 ± 1.3 a	0.0000
h	31.4 ± 2.6 a	61.5 ± 4.3 b	29.7 ± 4.0 a	0.0000

Average values ± standard error are presented. Different letters in a row mean statistically significant differences between treatments at $p < 0.05$.

Table 3

Concentrations of chlorophyll a, chlorophyll b and carotenoids ($\mu g\,mm^{-2}$) in apple skin of fruit from different positions in 'Fuji' apple tree grown under hail net or in the open, and interaction between the treatments at harvest time in year 2007.

	Control			Hail net			*p*-Value		
	Outer	Inner	Top	Outer	Inner	Top	Use of hail net	Position	Interaction
Chlorophyll a	3.5 ± 0.7	3.3 ± 0.4	2.7 ± 0.1	3.8 ± 0.4	4.0 ± 0.4	4.0 ± 0.2	0.0245	n.s.	n.s.
Chlorophyll b	5.1 ± 1.2	4.8 ± 0.7	3.8 ± 0.3	5.1 ± 0.4	5.1 ± 0.4	4.5 ± 0.3	n.s.	n.s.	n.s.
Carotenoids	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.2	1.6 ± 0.4	1.1 ± 0.1	1.2 ± 0.1	n.s.	n.s.	n.s.

Average values ± standard error are presented. n.s.—non-significant at $p < 0.05$.

Table 4

Content levels of quercetin glycosides (Q-rut, quercetin 3-rutinoside; Q-gal, quercetin 3-galactoside; Q-glu, quercetin 3-glucoside; Q-xyl, quercetin 3-xyloside; Q-pent, quercetin pentoside; Q-araf, quercetin 3-arabinofuranoside; Q-rham, quercetin 3-rhamnoside and Q, quercetin), anthocyanins (Cy-gal, cyanidin 3-galactoside; Cy-glu, cyanidin 3-glucoside; Cy-ara, cyanidin 3-arabinoside; and Cy-pent1 and Cy-pent2, cyanidin pentosides), catechin, epicatechin, chlorogenic acid, and total phenols ($mg\,kg^{-1}$ FW) in 'Fuji' apple skin from different positions in the tree grown under hail net or in the open, and interaction between the treatments at harvest time in year 2007.

	Control			Hail net			*p*-Value		
	Outer	Inner	Top	Outer	Inner	Top	Use of hail net	Position	Interaction
Q-rut	86 ± 34	12 ± 5	117 ± 40	193 ± 32	28 ± 8	283 ± 51	0.0015	0.0000	n.s.
Q-gal	331 ± 102	84 ± 31	475 ± 62	540 ± 45	134 ± 30	603 ± 32	0.0101	0.0000	n.s.
Q-glu	85 ± 28 ab	20 ± 6 a	103 ± 20 b	234 ± 35 c	54 ± 11 ab	288 ± 26 c	0.0000	0.0000	0.0089
Q-xyl	189 ± 56	69 ± 18	260 ± 24	309 ± 28	110 ± 17	318 ± 6	0.0056	0.0000	n.s.
Q-pent[a]	19 ± 6	5 ± 2	28 ± 4	34 ± 4	8 ± 2	38 ± 2	0.0039	0.0000	n.s.
Q-araf	230 ± 56	86 ± 20	320 ± 19	341 ± 29	125 ± 18	331 ± 11	0.0364	0.0000	n.s.
Q-rham	179 ± 38	84 ± 16	250 ± 15	287 ± 30	143 ± 22	301 ± 18	0.0014	0.0000	n.s.
Q	58 ± 15	27 ± 11	90 ± 27	161 ± 11	67 ± 18	179 ± 13	0.0000	0.0001	n.s.
Cy-gal	140 ± 12	96 ± 19	216 ± 6	225 ± 43	157 ± 39	200 ± 34	n.s.	0.0301	n.s.
Cy-glu	4.7 ± 0.3	2.7 ± 0.5	8.3 ± 0.9	8.7 ± 2.7	5.7 ± 0.9	11.4 ± 2.4	0.0147	0.0060	n.s.
Cy-ara	13 ± 1	9 ± 2	20 ± 0.8	23 ± 4	16 ± 4	24 ± 2	0.0041	0.0078	n.s.
Cy-pent1[b]	2.0 ± 0.2	1.5 ± 0.3	2.7 ± 0.2	2.6 ± 0.7	2.3 ± 0.5	2.7 ± 0.3	n.s.	n.s.	n.s.
Cy-pent2[b]	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.2	1.6 ± 0.4	1.1 ± 0.1	1.2 ± 0.1	n.s.	n.s.	n.s.
Catechin	40 ± 5	30 ± 5	45 ± 2	54 ± 6	42 ± 4	46 ± 4	0.0189	n.s.	n.s.
Epicatechin	320 ± 19	368 ± 44	365 ± 22	369 ± 29	362 ± 22	304 ± 34	n.s.	n.s.	n.s.
Chlorogenic acid	59 ± 9	47 ± 8	68 ± 8	43 ± 3	40 ± 7	35 ± 5	0.0020	n.s.	n.s.
Total phenols[c]	2264 ± 270	1685 ± 109	2767 ± 282	3248 ± 333	1794 ± 148	3253 ± 219	0.0129	0.0000	n.s.

Average values ± standard error are presented. n.s.—non-significant at $p < 0.05$.

[a] Q-pent in equivalents of quercetin is present.

[b] Cyanidin pentosides in equivalents of cyanidin are present.

[c] Total phenols in equivalents of gallic acid are present.

Content levels of total phenols in fruit skin varied from 1.68 $g\,kg^{-1}$ FW for fruit inside the canopy, uncovered, up to 3.25 $g\,kg^{-1}$ FW for fruit on the tree top under hail net. A clear influence of fruit position in the tree canopy on the total phenol concentration was noticed. These values were lower for inner fruit than for outer and top fruit and unrelated to hail net coverage of the orchard.

For the determination of quercetin glycosides, HPLC-MS was used, and the following compounds were defined: quercetin 3-rutinoside, quercetin 3-galactoside, quercetin 3-xyloside, quercetin pentoside, quercetin 3-arabinofuranoside and pure quercetin. On HPLC chromatogram (Fig. 2), the peak between quercetin 3-xyloside and quercetin 3-arabinofuranoside was identified with MS as quercetin pentoside, using the fragmentation pattern of a negatively charged molecular ion ($[M-H]^-$) at *m*/*z* 443, the MS^2 fragmentation at *m*/*z* 301. Among quercetin glycosides, quercetin 3-galactoside was the principal glycoside. Except for quercetin 3-arabinofuranoside, no interaction between fruit position in the tree and hail net coverage was observed for quercetin glycosides content levels, but the influence of fruit position in the tree proved to be statistically significant. The absolute amount of quercetin glycosides was higher in outer than in inner fruit and highest in top fruit, but differences between outer and top fruit were not statistically significant. The same response was observed for all individual quercetin glycosides. Hail net affected the increasing quercetin glycoside levels.

Among anthocyanins, content levels of the main one, cyanidin 3-galactoside, as well as cyanidin 3-glucoside and cyanidin 3-arabinoside were lowest for inner fruit, they were followed by outer fruit, while top fruit achieved the highest values. Concentrations of other cyanidin pentosides were not dependent on fruit position in the tree canopy. Therefore, in our study no decrease in cyanidin glycosides content levels due to hail net was observed.

The content levels of catechin, epicatechin and chlorogenic acid in apple skin were not dependent on fruit position in the tree

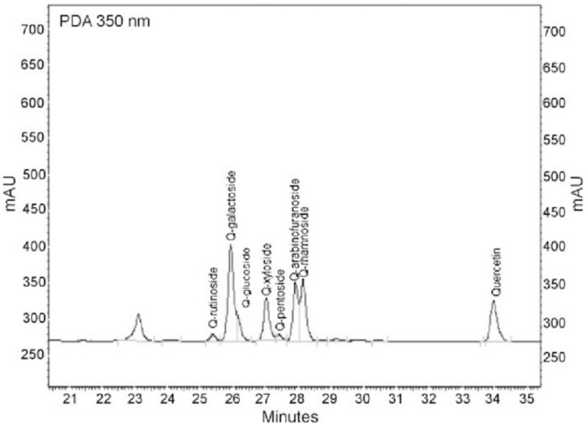

Fig. 2. HPLC chromatogram at 350 nm of 'Fuji' apple skin at harvest time in year 2007.

canopy or hail net coverage, except for chlorogenic acid, which had higher content levels when the orchard was covered with hail net than when it was uncovered.

4. Discussion

Lighting of the fruit depended on their position in the tree canopy (Fig. 1a). Inner fruit received less light, irrespective of the use of hail net. Lighting on outer and top fruit was higher under and outside the hail net, and PAR was comparable between the treatments under the hail net and in the open (Fig. 1b). Romo-Chacon et al. (2007) also reported that position in the canopy showed a highly significant interaction for PAR distribution. The inner canopy of trees under black hail net received the least PAR, and PAR measured in the upper parts of the canopy was significantly higher than in its lower parts.

Coloration of apples is a light-dependent process. Apples kept in the dark or in low light do not redden (Lancaster, 1992). Low values of colorimetrically measured parameter a^* mean that the surface shows more green coloration and, correspondingly, less red. The lowest amounts were measured for inner fruit, while outer and top fruit developed a redder blush (Table 2). Therefore, the inner fruit were darker (higher L^*), with higher hue angle (h°) than outer fruit. These measurements confirmed the fact that low light could be a limiting factor for fruit coloration, and that fruit in the lower canopy require improved light conditions (Barrit et al., 1991). To improve lighting in the tree canopy, various provisions were tested, for example, covering the orchard floor with reflective foil (Jakopic et al., 2007; Solomakhin and Blanke, 2007), treatment with ethephon and seniphos (Gómez-Cordovés et al., 1996), etc.

The chlorophyll in the fruit peel is responsible for the ground color of apples. In our experiment, content levels of chlorophyll a, chlorophyll b and carotenoids showed no statistically significant differences with regards to position of the fruit in the tree (Table 3). Hail net only influenced chlorophyll a concentrations but not chlorophyll b or carotenoids. Solomakhin and Blanke (2007) measured the highest chlorophyll content for fruit in the lower canopy from trees with grass alleys without hail net. They also reported that, unexpectedly, more chlorophyll occurred in the red, outer, sun-exposed fruit side of 'Elstar' apples cv. relative to the green, shaded side. The increased chlorophyll content in the red side of the apple fruit is also in line with chlorophyll and anthocyanin measurements on a bi-colored 'Gala' apple cv. in New Zealand (Reay et al., 1998).

Decreasing lighting inside the tree canopy caused lower content levels of quercetin glycosides and most anthocyanins (Fig. 3a) in the fruit skin from the inner part of the tree canopy. Fruit from the top of the canopy contained the highest levels of quercetin glycosides and cyanidin 3-galactoside, they were followed by the fruit from the outside of the canopy, whereas the lowest levels were found in fruit from the inner tree. Our data confirm those of Awad et al. (2000), who found that the levels of cyanidin 3-galactoside, quercetin 3-glycosides and total flavonoids were significantly higher in fruit from the top of the tree than in fruit from other positions, as well as in fruit from the outer position compared to fruit from the inner position. Awad et al. (2001a) reported that quercetin glycosides were the most abundant flavonoids in the skin of 'Elstar' and 'Jonagold' cultivars, and their accumulation showed a strong dependency on fruit position on the tree. In contrast, Ju et al. (1996) found for 'Delicious' and 'Ralls' a similar level of flavonoids (flavonols and procyanidins) in both sides, although in the sun-exposed peel twice as much anthocyanin was found than in the shaded peel. Reay and Lancaster (2001) compared the response to irradiation between the outer facing (exposed) and inner facing (shaded) sides of 'Royal Gala' and 'Gala' fruit when on the tree. The fruit from tree-shaded side showed a much greater potential to accumulate anthocyanins and quercetin glycosides than the fruit from tree-exposed side. The inner canopy portion of plants covered with black hail protection net received lower levels of ultraviolet, blue, green, red, and far red radiation, and light with a lower red/far red ratio, in comparison to uncovered plants (Amarante et al., 2007). These light changes could influence the synthesis of anthocyanin and other flavonoids for different fruit positions in the tree (Lancaster, 1992; Guo et al., 2008).

Lighting measured on fruit regardless of the position in the canopy was not significantly different under and outside hail net (Fig. 1b). This could be a consequence of diffused light under the hail net, mainly in the top and outer parts of the canopy. In our previous study (Jakopic et al., 2007), fruit lighting was in general slightly higher in the control treatment than under hail net, but the differences were not statistically significant. With regard to position in the canopy, we confirmed previous research studies (Awad et al., 2000, 2001b) that anthocyanin and quercetin glycoside synthesis is a light-dependent process. It has been demonstrated that antioxidants in plants which include also phenolics are part of a complex defence mechanism against a wide range of stress and thus accumulate in response to these stresses (Oh et al., 2009). A significant accumulation of quercetin-3-*O*-glucoside was noticed in lettuce after exposure to high light treatments. The authors conclude that certain flavonoid derivates can be induced by environmental stress, especially high light (Oh et al., 2009). Similar results were achieved also in our study. The fruits from the outer part and the top of the tree canopy had significantly higher amounts of different quercetins than the fruits in the inner part of the canopy which were less exposed to the light. Possible explanation could be in the protection function of the flavonols against UV-A radiation which causes the generation of reactive oxygen species. The flavonols have the maximum absorbance around 350 nm what corresponds to the wavelength range of UV-A radiation (315–400 nm) (Solovchenko and Merzlyak, 2007). The hail net had little influence on the content of quercetin glycosides and no on the anthocyanin concentration in the apple skin (Fig. 3b).

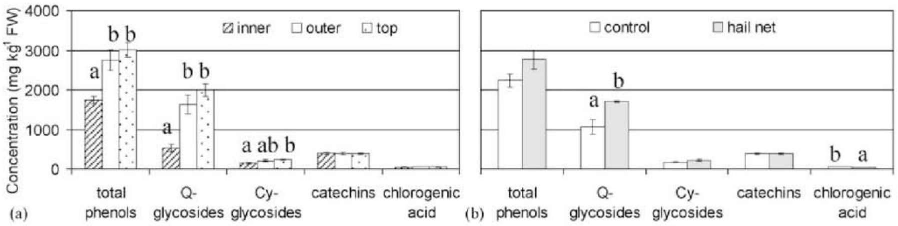

Fig. 3. The content levels of phenolic compounds (mg kg^{-1} FW) in apple skin of fruit at different positions in the tree (a) and under or outside the hail net (b) in year 2007. Average values ± standard error bars are presented. Different letters mean statistically significant differences between fruit positions in the tree canopy or hail net usage at $p < 0.05$. Total phenols are present as equivalents of gallic acid.

Beside flavonoids (quercetin glycosides and anthocyanins), catechin, epicatechin and chlorogenic acid were analyzed. There were no significant differences in their levels among fruit from different canopy positions. The amount of chlorogenic acid was slightly lower in the inner fruit than for top and outer fruit, but the difference was not statistically significant. There was also no significant difference in the content levels of catechins among fruit from different parts of the tree. Chlorogenic acid was not affected by the position of the fruit in the tree (Awad et al., 2000), and the amount of catechins was also independent of fruit position in the tree (Awad et al., 2001a). Flavan-3-ols and phenolic acids have absorbance maximums in lower wavelengths than the quercetin glycosides and therefore their function may be different that protection against UV-A radiation as already mentioned in the case of quercetin glycosides.

Hail net influenced a decrease in chlorogenic acid concentration, while catechin content levels showed no difference under the hail net and in the open. Awad et al. (2001a) also reported that the level of chlorogenic acid was slightly higher in outer than in inner fruit.

Awad et al. (2000) suggested that very low levels of anthocyanins, moderate levels of quercetin 3-glycosides and relatively high levels of phloridzin, catechins and chlorogenic acid, are found in the shaded skin of an individual fruit and also in the skin of a fruit borne inside the canopy, indicating that anthocyanin synthesis is a light-dependent process, while the synthesis of other phenolic metabolites is only slightly, if at all, light-dependent. Ju (1998) found that the genes controlling the synthesis of different phenolic compounds might have a different sensitivity to light. Since accumulation of quercetin glycosides and cyanidin 3-galactoside has no influence on accumulation of any of the other flavonoid classes, their biosynthesis seems to be regulated independently of the other classes, although they have the same biosynthetic pathway (Awad et al., 2001a).

Covering the orchard with hail net had no influence on lower lighting in comparison to the uncovered control. Hail net increased the content of total quercetin glycosides, as well as chlorophyll a, and decreased content levels of chlorogenic acid, while content levels of cyanidin glycosides, chlorophyll b, carotenoids, and coloration were independent of hail net usage in our experiment. On the other hand, fruit position in the tree canopy influenced fruit lighting, as well as the content of many phenolic compounds. Fruit from inner parts of the canopy contained lower amounts of total phenols, as well as a lower sum of quercetin glycosides and cyanidin glycosides than fruit from outer parts and the top of the canopy. Content levels of catechins and chlorogenic acid were independent of fruit position in the canopy. Considering that quercetin glycosides and cyanidin glycosides are responsible for red skin color development, better coloration of the fruit from the outer and top canopy was expected. We could conclude that black hail net in central Europe conditions had no influence on lower fruit quality and that higher lighting, at different positions in the canopy, stimulated higher content levels of flavonoids and better coloration, which are important factors in fruit quality.

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2.3 VPLIV MREŽE PROTI TOČI IN REFLEKTIVNE FOLIJE NA CIANIDIN GLIKOZIDE IN KVERCETIN GLIKOZIDE V KOŽICI JABOLK SORTE 'FUJI'

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Influence of hail net and reflective foil on cyanidin glycosides and quercetin glycosides in 'Fuji' apple skin.
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Trgovci in potrošniki so vedno bolj osveščeni in zahtevni glede kakovosti sadja. Pri jabolkih sta poleg notranje kakovosti, pomembna videz in obarvanost kožice ploda. Razvoj rdeče krovne barve je odvisen od vsebnosti antoicanov v povezavi s flavonoli, katerih sinteza je odvisna tudi od svetlobe. V tej študiji smo se osredotočili na količino kvercetin glikozidov in cianidin glikozidov v kožici jabolk, ki so rasla v sadovnjaku pod in izven mreže proti toči z ali brez uporabe reflektivne folije. Vsebnost cianidin 3-galaktozida, glavnega cianidin glikozida v jabolkih sorte 'Fuji', je bila najmanjša v kontrolnem obravnavanju, kot tudi koncentracije cianidin 3-arabinozida in drugih dveh cianidin pentoz. Uporaba reflektivne folije je vplivala na povečanje količin cianidin glikozidov. Izmed flavonolov smo določili kvercetin 3-galaktozid, kvercetin 3-glukozid, kvercetin pentozid, kvercetin 3-arabinofuranozid, kvercetin 3-ksilozid, kvercetin 3-rutinozid, kvercetin 3-ramnozid in kvercetin. Tako mreža proti toči kot tudi reflektivna folija sta vplivali na povečanje vsebnosti kvercetin glikozidov. Največje vrednosti smo izmerili na plodovih pod mrežo, kjer so bila tla prekrita z reflektivno folijo. Analizirali smo tudi katehin, epikatehin in klorogensko kislino, katerih največja koncentracija je bila izmerjena v kožici plodov, ki so rasli na drevesih pod mrežo proti toči. V kontrolnem obravnavanju je bila njihova vsebnost enaka ali večja, medtem ko so se največje vrednosti pojavljale v obravnavanjih pod mrežo proti toči, kjer so bila tla prekrita s folijo.

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

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Additional index words. phenolics, lighting, ripening, color, quality, *PAR*

***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 (Stampar 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; Stampar 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, quarcetin 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 Mirosan 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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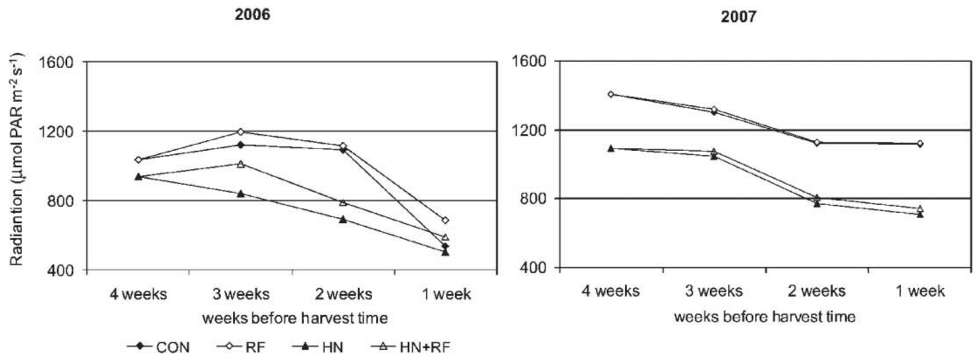

Fig. 1. The lighting in the orchard (μmol·m^{-2}·s^{-1}) during ripening in all treatments in 2006 and 2007. CON = control; RF = orchard floor covered with reflective foil; HN = under hail nets; HN + RF = floor covered with foil under hail nets.

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; μmol·m^{-2}·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 Finningan 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 × 4.6 mm; Gemini 3u) protected with a Phenomenex Security guard column and 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 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 [M-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-Ciocalteau phenol reagent method (Singleton and Rossi, 1965). Six milliliters of bidistilled water and 500 μL of Folin-Ciocalteau 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 °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 killigram 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 quercetin 3-arabinofuranoside from Apin Chemicals (Abingdon, U.K.).

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.

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 μmol·m^{-2}·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) μmol·m^{-2}·s^{-1} at the beginning of September to 1110 μmol·m^{-2}·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 *FAR* 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 *FAR* 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 μmol·m^{-2}·s^{-1} lower and under red–black and green–black 250 to 340 μmol·m^{-2}·s^{-1} lower. The decrease in *FAR* 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 *FAR* 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 *FAR* 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 μmol·m^{-2}·s^{-1}, whereas on the upper part, it extended from 50 up to 100 μmol·m^{-2}·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.

		Mean maximum temp. (°C)		Mean minimum temp. (°C)		Precipitation (mm)	
	Decade	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

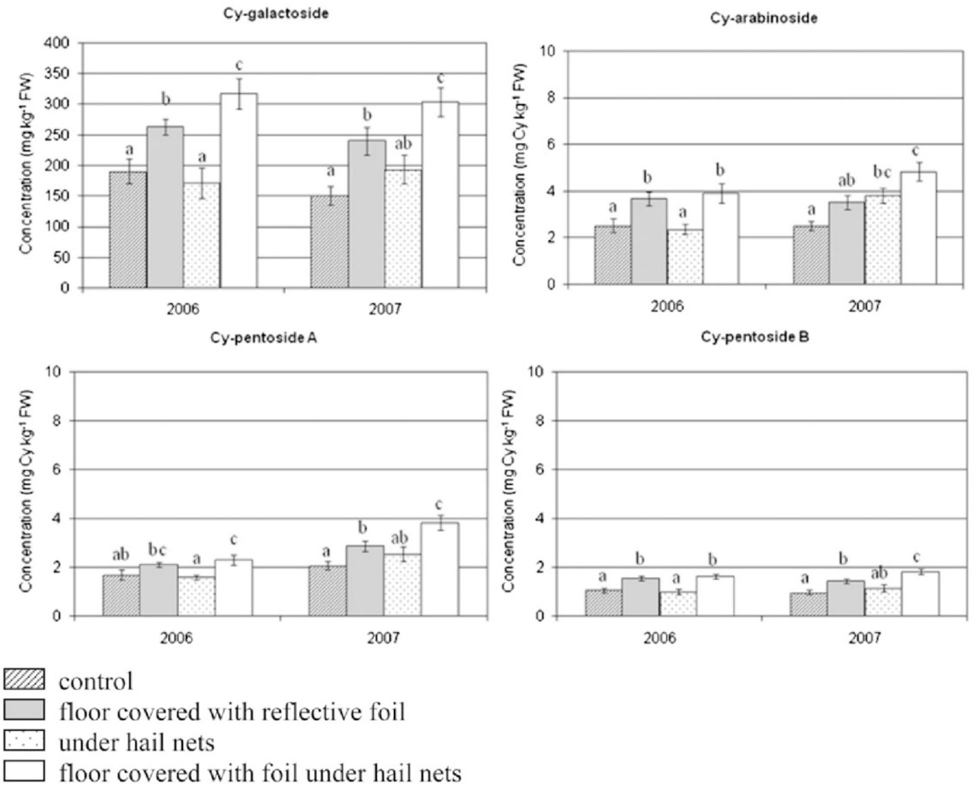

Fig. 2. Content of individual cyanidin glycosides in mg·kg^{-1} fresh weight for all treatments in 2 years. Concentrations of cyanidin pentoside are present in equivalents of cyanidin. Different letters mean statistically significant differences between treatments according to Duncan's multiple range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

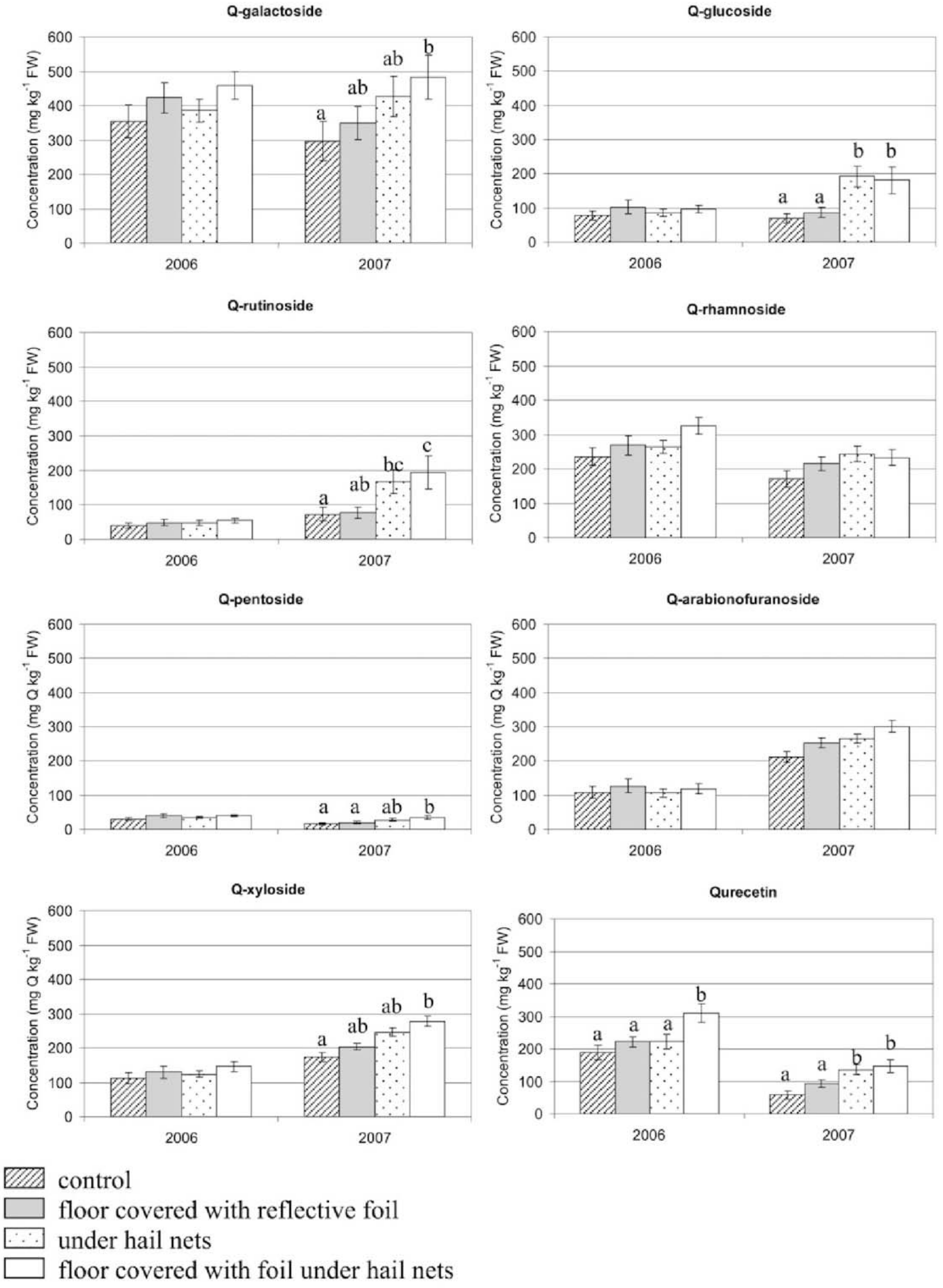

Fig. 3. Content of individual quercetin glycosides and quercetin in mg·kg^{-1} fresh weight for all treatments in 2 years. Concentrations of quercetin pentoside, quercetin arabinofuranoside, and quercetin xyloside are presented in equivalents of quercetin. The average values and SE bars are presented. 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 cyanindin 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 (UFGalT), 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 MS^2 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 MS^2 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 arabionofuranoside, 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 phoridzin 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

[z] Concentrations 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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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

V dvoletnem poskusu, ki smo ga izvajali v letih 2006 in 2007, na jablani sorte 'Fuji', smo ugotavljali, kako se zaradi uporabe mreže proti toči spremenijo razmere v osvetlitvi in kako to vpliva na sekundarne metabolite. Zaradi pričakovanega zmanjšanja osvetlitve smo v poskus vključili tudi prekrivanje tal z reflektivno folijo. Položili smo jo en mesec pred predvidenim obiranjem, da bi s tem povečali osvetljenost v krošnji. Določili smo štiri obravnavanja, (1) mreža proti toči, (2) reflektivna folija, (3) mreža proti toči + reflektivna folija in (4) kontrola. Poleg spremembe osvetlitve nas je zanimal predvsem odziv izbranih primarnih in sekundarnih metabolitov na te spremembe.

V osrednji Sloveniji zori sorta 'Fuji' v prvi dekadi oktobra. Jeseni se v naših klimatskih razmerah intenzivnost sončnega obsevanja zmanjšuje. Po podatkih agencije Republike Slovenije za okolje za leto 2006, se je dnevna vsota globalnega sončnega sevanja od začetka do konca septembra zmanjšala iz 6 na 3,75 kWh m^{-2} (Meteorološki letopis, 2006). V nasadu se je istega meseca fotosintetsko aktivno sevanje (PAR) zmanjšalo iz 1034 na 537 µmol m^{-2} s^{-1}, v letu 2007 pa iz 1410 na 1119 µmol m^{-2} s^{-1} (Jakopič in sod., 2010). Z uporabo mreže proti toči se je PAR še dodatno zmanjšal.

V evropskih nasadih je mreža proti toči običajno črne ali sive barve in tudi v našem poskusu smo uporabili črno protitočno mrežo. Pod njo smo izmerili 10 – 30% manjši PAR kot pri kontrolnem obravnavanju. Guerrero in sod. (2002) poročajo celo o več kot polovični redukciji fotosintetskega sevanja pod mrežo v primerjavi s kontrolo. Tudi uporaba barvnih mrež proti toči vpliva na PAR. Solomakhin in Blanke (2008) sta pod belo in belo-rdečo mrežo izmerila 6 do 15% manjši PAR, pod rdeče-črno pa 18 do 24% manjšega kot pri kontroli, kjer nista uporabila protitočne mreže.

V poskusu (2007) smo pri zadnjem vzorčenju, 1 teden pred obiranjem, izmerili zmanjšanje PAR iz 1119 µmol m^{-2} s^{-1} pri kontroli na 700 µmol m^{-2} s^{-1} pod mrežo proti toči. Eden od načinov zmanjšanja zgoraj omenjenega negativnega učinka mreže proti toči, je prekrivanje tal z reflektivno folijo. Green in sod. (1995) poročajo, da polaganje reflektivnih folij v nasadu poveča PAR v krošnji za skoraj 40%, fotosintezo za okoli 32% in listno transpiracijo za 26%.

Prekrivanje tal z belo reflektivno folijo je pozitivno vplivalo na osvetlitev v nasadu. PAR se je v medvrstnem prostoru povečal za 2 do 10% v primerjavi s kontrolnim obravnavanjem. Pod mrežo proti toči se je zaradi uporabe folije PAR povečal 5 do 20%. Če primerjamo obravnavanje folija + mreža s kontrolno, je bil PAR pri prvem še vedno 10 do 20% manjši kot pri kontrolnem obravnavanju, kar nakazuje na to, da reflektivna folija na tleh sicer poveča PAR v nasadu, vendar učinka zmanjšanja zaradi protitočne mreže v celoti ne izniči.

V obeh letih našega poskusa smo spremljali tudi spremembo osvetlitve v krošnji dreves pri uporabi mreže proti toči in reflektivne folijo v primerjavi s kontrolnim obravnavanjem. Prekrivanje tal z reflektivno folijo ima pozitiven učinek na osvetljenost krošnje, kar je še posebej pomembno kadar gre za kombinacijo bujne sorte in bujne podlage in je nasad zaščiten z mrežo proti toči. PAR je bil večji, kjer so bila tla pokrita z reflektivno folijo, kot tam, kjer je v medvrstnem prostoru rasla trava. Največje vrednosti so bile dosežene v tistih obravnavanjih, kjer so bila tla prekrita z reflektivno folijo, pod in izven mreže proti toči. Kadar smo uporabili reflektivno folijo, je bila osvetljenost spodnjih delov plodov statistično značilno večja. Tudi na zgornjem delu plodov se je nakazoval pozitiven vpliv folije, vendar povečanje ni bilo vedno statistično značilno.

Plodovi v krošnji so bili izpostavljeni močnejši osvetlitvi kadar so bila tla prekrita z reflektivno folijo, posebno spodnji deli plodov. Le-ti so bili 3,5 od 7,3-krat bolj osvetljeni v obravnavanjih z reflektivno folijo kot pri kontroli. Green in sod. (1995) omenjajo, da je od folije odbiti PAR signifikantno povečal sevanje v spodnjem delu krošnje, da pa so bile razlike v zgornji polovici krošnje manjše. Da bi lahko natančno proučili osvetlitev v krošnji in njene posledice na kakovost plodov, smo na drevesih označili po tri plodove: enega na zunanjem robu krošnje, enega v notranjosti krošnje in enega na vrhu krošnje, pod mrežo in izven nje. Na njih smo v zadnjem mesecu pred obiranjem merili PAR, spremljali razvoj rdeče barve in merili vsebnosti izbranih metabolitov. Interakcije med položajem plodov v krošnji in uporabo mreže proti toči nismo potrdili. Na osvetlitev plodov je odločilno vplival položaj v krošnji, medtem ko statističnih razlik zaradi prekrivanja nasada z mrežo proti toči nismo ugotovili. Najnižje vrednosti smo izmerili pri plodovih znotraj krošnje, kjer je bil PAR okrog 30 μmol m^{-2} s^{-1} (Jakopič in sod., 2009). Plodovi na robu in vrhu krošnje so bili bolj osvetljeni.

Ob obiranju smo merili tudi nekatere parametre zrelosti, suho snov in trdoto plodov. Trdota je bila večja v obeh obravnavanjih pod mrežo, kar bi lahko pomenilo počasnejše dozorevanje in posledično kasnejšo zrelost. Suha snov je bila nižja pri obravnavanju z reflektivno folijo pod mrežo v primerjavi z vsemi drugimi obravnavanji. O povezavi osvetljenosti s parametri zrelosti poročajo Robinson in sod. (1983). Navajajo, da je bil odstotek svetlobe, ki je dosegel veje v poskusu negativno povezan s trdoto plodov in v pozitivni povezavi s povprečno vsebnostjo suhe snovi in škroba. V našem poskusu nismo ugotovili enotnega vpliva osvetlitve na omenjene parametre.

Največji delež suhe snovi v plodovih jabolk predstavljajo sladkorji in organske kisline. Zato smo ugotavljali tudi vpliv mreže proti toči in reflektivne folije na vsebnost sladkorjev (glukoze, saharoze, fruktoze in sorbitola) in organskih kislin (jabolčne, citronske, šikimske in fumarne kisline) ob tehnološki zrelosti (Jakopič in sod., 2007). Skupna vsebnost tako sladkorjev kot organskih kislin je bila največja v kontrolnem obravnavanju. Prekrivanje tal v nasadu z reflektivno folijo je vplivalo na zmanjšanje skupnih sladkorjev in organskih kislin pod in izven mreže proti toči. Pri fruktozi, kot glavnem sladkorju v jabolkih 'Fuji', je bil učinek reflektivne folije podoben, prav tako pri jabolčni in citronski kislini, ne pa tudi pri drugih sladkorjih in organskih kislinah. Robinson in sod. (1983) poročajo, da je bila kislost plodov pri sorti 'Rdeči delišes' rahlo negativno povezana z odstotkom sončne osvetlitve.

Kot je znano, nizke temperature ponoči in visoke dnevne temperature ugodno vplivajo na razvoj rdeče krovne barve (Friedrich in Fischer, 2000). V našem poskusu ni bilo vidnih razlik v minimalnih in maksimalnih temperaturah med leti 2006 in 2007 (Jakopič in sod., 2010), tako da morebitnih razlik v barvi med letoma ne moremo pripisati različnim temperaturam med dozorevanjem.

Razlike v količini fotosintetskega sevanja, ki dosežejo plodove na drevesu, vplivajo na končno barvo plodov (Guerrero in sod., 2002). Pričakovali smo, da bodo plodovi pri obravnavanjih z reflektivno folijo bolj intenzivno rdeče obarvani, ker je bilo več odbitega PAR kot v drugih obravnavanjih, posebno pod mrežo proti toči. Layne in sod. (2002) poročajo, da je bil pri sorti 'Gala' na drevesih z reflektivno folijo večji delež plodov, ki so razvili rdečo barvo kot pri drevesih, kjer niso uporabili reflektivne folije. Barvo smo spremljali kolorimetrično s pomočjo CIE LAB parametrov. Barvna koordinata a*, katere višja vrednost pomeni bolj rdečo barvo, je bila v vseh terminih vzorčenja najmanjša pri obravnavanju z mrežo proti toči, kjer tla niso bila prekrita z reflektivno folijo, največja vrednosti pa večinoma na plodovih, ki so rasli na drevesih, izven mreže kjer so bila tla pokrita s folijo (Jakopič in sod., 2007). Med obravnavanjema, kjer sta bili hkrati uporabljeni mreža proti toči in reflektivna folija ter kontrolo ni bilo statističnih razlik. To pomeni, da je prekrivanje tal s folijo pod mrežo proti toči pospešilo razvoj rdeče barve plodov in ob obiranju doseglo vrednosti, kakršne so bile izmerjene pri kontrolnem obravnavanju.

Ob obiranju se je pokazal tudi vpliv folije na CIE faktor L*, ki predstavlja svetlost. Vrednosti so bile nižje, ko smo uporabili folijo, tako pod mrežo proti toči kot tudi izven nje. Reflektivna folija je torej povzročila temnejšo obarvanost plodov. Lancaster in sod. (1997) sklepajo, da je temnješa barva kožice lahko posledica večje koncentracije antocianov zaradi večjega deleža rdečih vakuol, večjih vakuol in posameznih slojev rdečih celic. Monoglikozidi so svetlejši, diglukozidi so temnejši in nastanejo na dobro osvetljenih delih krošnje. To lahko razloži dejstvo, zakaj so plodovi iz bolje osvetljenega dela krošnje močneje obarvani, kot plodovi iz notranjosti krošnje (Saure, 1990; Lancaster, 1992).

Podoben trend smo opazili pri faktorjih b*, ki določa odtenke od modre proti rumeni in h° (ki določa kot odtenka barve), vendar pri njima razlike niso bile statistično značilne. Plodovi pod črno mrežo proti toči, ki je reducirala fotosintetsko sevanje za 50%, je reducirala tudi končno barvo plodov, kar se je odražalo v povečanju L* in h° (Guerrero in sod., 2002); v našem posksu ni bilo razlik pri omenjenih dveh parametrih med kontrolo in mrežo proti toči. Z vsebnostjo antocianov je razmerje a*/b* direktno povezano, medtem ko sta h° in L* povezana obratno sorazmerno (Iglesias in sod., 1999). Omenjena korelacija se je potrdila tudi v naših poskusih. Pri jabolkih sorte 'Gala' so plodovi z dreves, kjer so bila tla pokrita s folijo, imeli večji delež krovne barve kot z dreves brez reflektivne folije (Layne in sod., 2002). Prekrivanje tal s folijo v nasadih še ni ustaljena praksa, vendar bi bila lahko učinkovita metoda za povečanje rdeče barve kožice jabolk.

Nižja vrednost kolorimetrično določenega parametra a* pomeni, da je površina bolj zeleno oz. manj rdeče obarvana. Najnižje vrednosti so bile izmerjene na notranjih plodovih, medtem ko so bili zunanji in vrhnji plodovi bolj rdeči. Razlike v barvi ni bilo med plodovi, ki so rasli na robu in tistimi z vrha krošnje (Jakopič in sod., 2009). Vrednost parametra h° je bila pri notranjih plodovih dvakrat večja kot pri zunanjih in vrhnjih plodovih. Ju in sod. (1999) poročajo, da se je notranjim plodovom h° zmanjšal za 20% na osvetljeni strani in 12% na senčni strani. Isti avtorji ugotavljajo, da se kombinacija povečanja antocianov in zmanjšanja klorofila odraža v manjšem h°. Jabolka, ki so v temi ali slabo osvetljena, ne pordečijo (Lancaster, 1992). Te meritve potrjujejo dejstvo, da je slaba osvetlitev lahko omejujoč dejavnik za razvoj krovne barve in da plodovi v spodnjem delu krošnje za zadovoljivo obarvanost potrebujejo izboljšanje razmer v osvetlitvi (Barrit in sod., 1991).

Osnovna zelena barva kožice jabolk izhaja iz pigmentov klorofila, od katerih je največ klorofila a in b, ki sta v razmerju 3:1. V dozorevajočih plodovih poteka razgradnja klorofila, pri čemer nastaja brezbarvni fitol, kar se kaže kot pojav rumene barve plodov (Hung in sod., 1997). V našem poskusu smo edino statistično značilno razliko opazili pri klorofilu a, katerega vsebnost je bila večja pod mrežo kot izven nje, pozicija plodov v krošnji pa nanj ni vplivala (Jakopič in sod., 2009). Na vsebnost klorofila b in karotenoidov v kožici jabolk ni vplivala niti pozicija v krošnji, niti uporaba mreže proti toči. Solomakhin in Blanke (2007) sta izmerila največje vsebnosti klorofila pri plodovih s spodnjega dela krošnje dreves, kjer je bila v medvrstnem prostoru trava brez mreže proti toči. Ista avtorja tudi poročata, da se je nepričakovano največ klorofila pojavljalo na zunanji, rdeči, soncu izpostavljeni strani plodov jabolk sorte 'Elstar' v primerjavi z zeleno, osenčeno stranjo. Povečano vsebnost klorofila na rdeči strani so izmerili tudi pri izvajanju meritev klorofila in antocianov na dvobarvni sorti 'Gala' v Novi Zelandiji (Reay in sod., 1998).

Intenzivnost rdeče barve plodov jabolk je rezultat različnih vsebnosti antocianov in flavonolov. V jabolkih so vsi antociani derivati cianidina. Pri sorti 'Fuji' smo določili cianidin 3-galakotozid, cianidin 3-glukozid in cianidin s pomočjo eksternih standardov na HPLC in jih potrdili z ESI-MS. Druge antociane smo identificirali z uporabo MS/MS kot cianidin pentozide. Glede na predhodne raziskave, bi bili to lahko cianidin 3-ksilozid, cianidin 3-arabinozid ali cianidin 7-arabinozid, saj o njihovi prisotnosti v jabolkih poročajo že Gómez-Cordovés in sod. (1996) ter Vrhovšek in sod. (2004).

Glavni delež antocianov v jabolkih sorte 'Fuji' je cianidin 3-galaktozid, ki je predstavljal od 92 (pri prvem vzorčenju) do 98% (ob obiranju) vseh antocianov (Jakopič in sod., 2007). Koncentracija cianidin 3-galaktozida se je med dozorevanjem povečevala, kar so v svojih raziskavah potrdili tudi Gómez-Cordovés in sod. (1996). Tudi koncentracije cianidin 3-galaktozida, cianidin 3-arabinozida in cianidin pentozida A so se med dozorevanjem povečevale, vendar so bile njihove vsebnosti bistveno manjše. Še manjši delež sta med antociani predstavljala cianidin pentozid B in čisti cianidin. Sposobnost za kopičenje antocianov se pojavi med sredino in koncem rastne dobe pri mnogih sortah. Vsebnost med zorenjem narašča, posebno dva tedna pred tehnološko zrelostjo (Iglesias in sod., 1999).

Ob zrelosti se količina cianidin 3-galaktozida v sveži kožici giblje od 150 mg kg^{-1} do več kot 300 mg kg^{-1} (Jakopič in sod., 2010). Najmanjše vrednosti so bile določene v kontrolnem obravnavanju in pod mrežo proti toči. Reflektivna folija je imela pozitiven učinek na cianidin 3-galaktozid izven, še bolj pa pod mrežo proti toči, kjer so bile izmerjene največje vrednosti. Podobne rezultate smo dobili tudi za druge analizirane cianidin glikozide. Vsebnost cianidin 3-arabinozida in dveh drugih cianidin pentozidov so bile večje v obravnavanjih z reflektivno folijo, razen za cianidin 3-arabinozid v letu 2007. V našem poskusu mreža proti toči ni imela učinka na vsebnost cianidin glikozide, čeprav lahko v literaturi zasledimo poročila o negativnem vplivu črne mreže proti toči na razvoj rdeče barve (Guerrero in sod., 2002; Stampar in sod., 2002; Solomakhin in Blanke, 2007).

Po drugi strani je bil viden pozitiven učinek reflektivne folije na osvetlitev in posledično na vsebnost cianidin glikozidov. Njihove koncentracije so bile signifikantno večje v obeh obravnavanjih z reflektivno folijo kot v obravnavanjih brez folije. Tudi Ju in sod. (1999) so ugotovili, da je prekrivanje tal v nasadu stimuliralo kopičenje antocianov v kožici jabolk. Količina svetlobe je bila direktno povezana s koncentracijo cianidin 3-galaktozida in odstotkom krovne barve na plodovih sorte 'Jonagold' (Awad in sod., 2001c).

Barva plodov je odvisna od vsebnosti antocianov kot tudi od flavonolov (Lancater, 1992), kamor spadajo kvercetin glikozidi in kvercetin. Sedem kvercetin glikozidov in čisti kvercetin smo določili s pomočjo kromatografskih tehnik po metodi eksternega standarda in masnim spektrometrom (HPLC-MS). Kvercetin 3-galaktozid, kvercetin 3-glukozid, kvercetin 3-ramnozid, kvercetin 3-rutinozid, kvercetin 3-ksilozid, kvercetin 3-arabinofuranozid in kvercetin so bili določeni s pomočjo eksternih standardov in potrjeni na osnovi MS^2 fragmenta z *m/z* 301, kar je molska masa negativnega iona kvercetina. Drugi kvercetin glikozidi so bili določeni kot kvercetin pentozidi s pomočjo masnega spoktrometra na podlagi $[M\text{-}H]^-$ z *m/z* 433 in MS^2 fragmenta z *m/z* 301. Koncentracije smo preračunali glede na umeritveno krivuljo dostopnih standardov oz. na čisti kvercetin za katere standardi niso dostopni.

Vsebnost posameznih kvercetin glikozidov se je v kožici jabolk gibala od 20 do skoraj 500 mg kg^{-1} sveže mase. Kot najbolj zastopan kvercetin glikozid pri sorti 'Fuji' se je pokazal kvercetin galaktozid, kar poročata tudi Felicetti in Schrader (2009), čeprav so bile koncentracije v njunem primeru večje kot smo jih izmerili mi. Koncentracije kvercetin 3-galaktozida so bile od 297 do 483 mg kg^{-1} sveže mase; sledili so kvercetin 3-ramnozid, kvercetin 3-arabinofuranozid in drugi. Plodovi s kontrolnih dreves so vsebovali najnižje vrednoti skoraj vseh posameznih kvercetin glikozidov (kvercetin galaktozid, kvercetin glukozid, kvercetin ramnozid, kvercetin 3-rutinozid, kvercetin pentozid, kvercetin 3-ksilozid in kvercetin). Mreža proti toči in reflektivna folija sta obe vplivali na povečano vsebnost kvercetin glikozidov. Koncentracija posameznih kvercetin glikozidov kot tudi vsota kvercetinov je bila v večini primerov največja pod mrežo proti toči, kjer so bila pokrita z reflektivno folijo. Razlike so bile v letu 2007 statistično značilne pri kvercetin 3-galaktozidu, kvercetin 3-glukozidu, kvercetin 3-rutinozidu, kvercetin pentozidu in kvercetin 3-ksilozidu ter pri kvercetinu v obeh letih. Čeprav mreža proti toči zmanjšuje PAR in ultravijolično sevanje (Blanke, 2007; Jakopič in sod., 2007), je bila v našem poskusu vsebnost kvercetin glikozidov večja v obravnavanju pod protitočno mrežo kot pri kontroli. Pod drevesi položena reflektivna folija je to še dodatno povečala in tako so bile največje vrednosti dosežene v obravnavanju pod mrežo proti toči, kjer je bila uporabljena reflektivna folija, razen pri kvercetin 3-glukozidu in kvercetin 3-ramnozidu v drugem letu poskusa.

Osvetlitev v medvrstnem prostoru pod mrežo proti toči je bila manjša kot pri kontroli, vendar se osvetljenost plodov pod in izven mreže ni statistično razlikovala. Pri uporabi reflektivne folije je bila osvetlitev spodnjih delov plodov vsakokrat večja, zgornjih delov pa ne vedno. V večini primerov je bila vsebnost kvercetin glikozidov večja v obravnavanju pod mrežo kot pri kontroli. Na drugi strani je povečana osvetlitev zaradi reflektivne folije nakazovala pozitiven učinek na vsebnost kvercetin glikozidov. Potrdili smo dejstvo, da svetloba lahko vpliva ne le na sintezo antocianov, pač pa tudi na koncentracije kvercetin glikozidov. Reay in Lancaster (2001) namreč poročata, da se tako kvercetin glikozidi kot tudi antociani po obsevanju z belo fluorescentno in UV-B svetlobo kopičijo v kožici jabolk 'Gala' in 'Gala Royal'.

Razen za kvercetin 3-arabinofuranozid, se pri kvercetin glikozidih ni pokazala interakcija med uporabo protitočne mreže in položajem plodov na drevesu, ugotovili pa smo velik vpliv pozicije plodov v krošnji. Pomanjkanje osvetlitve v notranjosti krošnje je povzročilo nižje vsebnosti kvercetin glikozidov, cianidin 3-galaktozida, cianidin 3-glukozida in cianidin 3-arabinozida v kožici plodov, medtem ko statističnih razlik med zunanjimi in zgornjimi plodovi nismo opazili (Jakopič in sod., 2009). Koncentracije drugih cianidin pentozidov niso bile odvisne od pozicije plodov na drevesu. Zmanjšanja vsebnosti cianidin glikozidov zaradi uporabe mreže proti toči tudi v tem delu poskusa nismo zaznali, je pa mreža proti toči vplivala na povečanje koncentracij kvercetin glikozidov.

Tudi Awad in sod. (2000) poročajo, da je bil nivo cianidin 3-galaktozida, kvercetin glikozidov in skupnih flavonoidov signifikantno večji pri plodovih z vrha krošnje, kot iz drugih delov krošnje, kot tudi v plodovih z zunanjega dela v primerjavi z notranjimi plodovi. Awad in sod. (2001a) so ugotovili tudi, da so bili kvercetin glikozidi najbolj zastopani flavonoidi v kožici sort 'Elstar' in 'Jonagold' in da je njihovo nalaganje v veliki meri odvisno od pozicije plodov na drevesu. Nasprotno so Ju in sod. (1996) pri sortah 'Zlati delišes' in 'Ralls' odkrili podobne količine flavonoidov (flavonoli in procianidini) na obeh straneh plodov, čeprav je imela kožica na sončni strani dvakrat več antocianov kot na senčni strani. Reay in Lancaster (2001) sta primerjala odgovor na obsevanje med sončno in senčno stranjo plodov 'Gale' in 'Royal Gale', ko so bili še na drevesu. Plodovi s senčne strani so kazali večji potencial za akumulacijo antocianov in kvercetin glikozidov kot plodovih z sončne strani drevesa. Notranji deli krošnje pod mrežo proti toči dosežejo nižje vrednosti ultravijolične, modre, zelene, dolgovalovne rdeče (FR) svetlobe v primerjavi z nepokritimi rastlinami (Amarante in sod., 2007). Te spremenjene razmere v osvetlitvi lahko vplivajo na sintezo antocianov in drugih flavonoidov za različne pozicije plodov na drevesu (Lancaster, 1992; Guo in sod., 2008).

Glede na pozicijo plodov v krošnji smo potrdili prejšnje ugotovitve (Awad in sod., 2000; 2001b) da je sinteza antocianov in kvercetin glikozidov svetlobno odvisen proces. To nakazuje, da so antioksindanti, kamor spadajo tudi fenoli, v rastlini del kompleksnega obrambnega mehanizma pred različnimi oblikami stresa in se tako akumulirajo kot odgovor na stres (Oh in sod., 2009). Signifikantna akumulacija kvercetin 3-glukozida je bila opažena v solati po izpostavitvi močni svetlobi. Avtorji sklepajo da so posamezni derivati flavonoidov lahko odgovor na okoljski stres, posebno na močno osvetlitev (Oh in sod., 2009). Podobno smo ugotovili tudi v našem poskusu. Možna razlaga je v obrambni vlogi flavonolov pred UV-A sevanjem, ki povzroča reaktivne kisikove spojine. Flavonoli imajo absorpcijski maksimum okoli 350 nm, kar odgovarja valovni dolžini UV-A sevanja (315-400 nm) (Solovchenko in Merzlyak, 2007). Tudi drugi avtorji ugotavljajo, da svetloba spodbuja nastanek antocianov in drugih flavonoidov v povrhnjici ter drugih rastlinskih tkivih, ki veljajo za zaščitni mehanizem proti sončnemu sevanju pri višjih rastlinah (Merzlyak in Chivkunova, 2000). V našem poskus so spremenjene svetlobne razmere zaradi mreže proti toči imele majhen vpliv na vsebnost kvercetin glikozidov in nikakršnega na koncentracijo antocianov v kožici jabolk.

Možni sta dve razlagi, kako svetloba vpliva na sintezo antocainov. Prva je ta, da je za svetlobno povečanje sinteze antocianov in akumulacijo v jabolkih odgovorna povečana fotosinteza v krošnji in dotok asimilatov v plodove, kar posredno stimulirala sintezo antocianov s preskrbo (presežkom) substrata. Kawabata in sod. (1999) so zaznali pozitivno korelacijo med akumulacijo antocianov in vsebnostjo topnih sladkorjev, ne glede na svetlobne razmere. Obstoj tesne povezave med saharozo in svetlobno odvisno potjo sproži ekspresijo genov, ki sintetizirajo antocianidine pod svetlobnimi razmerami (Ubi, 2004). V našem poskusu nismo ugotovili povezave med vsebnostjo antocianov in ogljikovimi hidrati.

Druga možnost je, da obravnavanja s folijo direktno stimulirajo sintezo antocianov; folija poveča intenziteto svetlobe v krošnji, kar vpliva na večjo aktivnost flavonoid-3-*O*-glikoziltransferaze, pomembnega encima pri sintezi antocianov v jabolkih (Ju in sod., 1999). Nadaljnja razlaga, zakaj svetloba poveča sintezo antocaininov in kopičenje v jabolkih je, da svetloba direktno stimulira sintezo antocianov prek flavonoidnih encimov. V rdeče obarvani sorti jabolk 'Splendour' je bila aktivnost fenilalanin amonij liaze, halkon izomeraze in glikozil transferaze v pozitivni povezavi z nivojem antocianov med dozorevanjem (Lister in sod., 1996) in je njihova aktivnost odvisna od svetlobe (Treutter, 2001; Ju in sod., 1999; Dong in sod., 1995).

Poleg flavonoidov, so v jabolkih sorte 'Fuji' izmed fenolnih snovi odkrili tudi katehin, klorogensko kislino, epikatehin, kavno kislino, *p*-kumarno kislino in floridzin (Veberič in sod., 2007). V našem poskusu smo spremljali spremembe katehina, epikatehina in klorogenske kisline. Koncentracije katehina so se gibale od 29 do 75 mg kg^{-1} sveže mase kožice, epikatehina od 171 do 419 mg kg^{-1} in klorogenske kisline od 24 do 75 mg kg^{-1} sveže mase (Jakopič in sod., 2010). Vsebnosti katehina in klorogenske kisline so bile manjše, epikatehina pa večje v primerjavi s podatki Veberiča in sod. (2007), vendar še vedno v okviru vrednosti, ki jih za kožico jabolk navajajo Treutter (2001) ter Escarpa in Gonzalez (1998). Koncentracije analiziranih fenolov so bile najmanjše pri plodovih pod mrežo proti toči. Pri kontroli razlike niso bile statistično značilno različne v primerjavi z mrežo proti toči, z izjemo klorogenske kisline v drugem letu. Prekrivanje tal s folije se je na omenjenih fenolih odražalo v povečanju koncentracij, razen pri klorogenski kislini v letu 2006. Awad in de Jager (2002) sta proučevala vpliv svetlobe na vsebnost fenolov na sončni in senčni strani plodov. Ugotovila sta, da ima proti soncu obrnjena (sončna) stran ploda mnogo večjo vsebnost cianidin 3-galaktozida in kvercetin glikozidov kot senčna stran, medtem ko so bile količine floridzina, katehinov in klorogenske kisline enake na obeh straneh plodov.

Razlike v vsebnosti katehina, epikatehina in klorogenske kisline zaradi različnih pozicij plodov na drevesu nismo zaznali. Vsebnost klorogenske kisline je bila sicer nekoliko manjša v plodovih znotraj krošnje, vendar ne statistično značilno. Razlik v vsebnosti katehina in epikatehina zaradi različnega položaja plodov v krošnji nismo opazili. Klorogenska kislina ni odvisna od položaja plodov na drevesu (Awad in sod., 2000) in tudi vsebnost katehina v plodu se zaradi različne pozicija na drevesu ne spremeni (Awad in sod., 2001a). Flavan-3-oli in fenolne kisline imajo absorpcijski maksimum pri nižjih valovnih dolžinah kot kvercetin glikozidi in je zato njihova funkcija lahko drugačna kot zaščita pred UV-A sevanjem, kar smo omenili za kvercetin glikozide.

Awad in sod. (2000) sklepajo, da zelo majhna vsebnost antocianov, zmerna količina kvercetin glikozidov in relativno visoke količine floridzina, katehinov in klorogenske kisline, ki so bili odkriti v kožici na senčni strani posameznih plodov in tudi v plodovih, ki so rasli v notranjosti krošnje, nakazujejo, da je sinteza antocianov svetlobno odvisen proces, medtem ko je sinteza drugih fenolnih spojin samo delno, če sploh, svetlobno odvisna. Ju (1998) je odkril, da ima lahko genska kontrola sinteze za različne fenolne spojine različno občutljivost za svetlobo. Ker akumulacija kvercetin glikozidov in cianidin 3-galaktozida nima vpliva na akumulacijo nobene druge skupine flavonoidov, se zdi da je njihova biosinteza regulirana neodvisno od drugih skupin, čeprav imajo enako biosintetsko pot (Awad in sod., 2001a).

Vsebnost skupnih fenolov se je gibala od 1,68 g kg^{-1} pri plodovih znotraj krošnje do 3,25 g kg^{-1} pri plodovih z vrha krošnje. Tako kot pri posameznih fenolih se je tudi tukaj pokazalo, da na vsebnost skupnih fenolov močno vpliva razpoložljiva svetloba, ki je odvisna od položaja ploda v krošnji. Vrednosti so bile pri plodovih iz notranjosti krošnje manjše kot pri plodovih iz zunanjega dela in z vrha krošnje, ne glede na to, ali je bil sadovnjak prekrit z mrežo proti toči ali ne. Tudi v drugem delu poskusa, smo potrdili, da uporaba mreže proti toči na vsebnost skupnih fenolov ni vplivala in ugotovili večje vrednosti v obravnavanju z reflektivno folijo pod mrežo proti toči kot v kontrolnem obravnavanju.

3.2 SKLEPI

V ~~raziskvai~~raziskavi smo izvedli številne poskuse, s katerimi smo prišli do naslednjih ugotovitev:

- PAR se je v primerjavi s kontrolo pod mrežo zmanjšal za več kot 30%. Uporaba reflektivne folije je PAR povečala za 20% pod mrežo in 10% izven mreže. Kljub temu, da se je osvetlitev v nasadu zmanjšala zaradi uporabe mreže proti toči, le-ta ni vplivala na zmanjšano osvetljenost posameznih plodov v krošnji. Osvetlitev plodov v krošnji se je zaradi uporabe reflektivne folije povečala. Učinek je bil posebno velik na spodnjih delih plodov.

- Pri spremljanju razvoja rdeče barve smo ugotovili, da so se plodovi pod mrežo proti toči nekoliko slabše obarvali in vsebovali nekoliko manj cianidin 3-galaktozida. Povečana osvetlitev zaradi uporabe reflektivne folije je pozitivno vplivala na razvoj rdeče barve in vsebnost vseh analiziranih cianidin glikozidov. Ob obiranju so plodovi z dreves pod mrežo, kjer je bila položena folija, dosegli barvo, primerljivo s kontrolo.

- Med dozorevanjem se je v kožici jabolk kopičil cianidin 3-galaktozid, glavni antocian. Koncentracije so se povečevale. Prav tako se je povečevala vsebnost cianidin 3-arabinozida in enega cianidin pentozida, vendar so bile njihove koncentracije bistveno manjše kot cianidin 3-galaktozida. Še nižje vrednosti so bile izmerjene za drugi cianidin pentozid in čisti cianidin.

- Nakazovala se je povečana koncentracija večine kvercetin glikozidov pri uporabi mreže proti toči. Prekrivanje tal z reflektivno folijo je koncentracije še povečalo. Razlike so bile posebno izrazite v letu 2007 pri kvercetin 3-galaktozidu, kvercetin 3-rutinozidu, kvercetin pentozidu, kvercetin 3-ksilozidu in kvercetin 3-glukozidu ter v obeh letih pri kvercetinu.

- Položaj plodov v krošnji se je pokazal kot pomemben dejavnik osvetlitve. Plodovi z vrha in roba krošnje so bili enako osvetljeni, plodovi v notranjosti krošnje bistveno slabše. Plodovi iz notranjosti krošnje, ki so bili slabše osvetljeni, so vsebovali nižje vrednosti skupnih fenolov, kot tudi nižje vsote kvercetin glikozidov in cianidin glikozidov kot plodovi iz zunanjega roba ali z vrha krošnje.

- Vsebnosti katehinov in skupnih fenolov so bile najmanjše v kontrolnem obravnavanju in se niso statistično razlikovale od obravnavanj s protitočno mrežo. Prekrivaje tal z reflektivno folijo se je odrazilo v povečanju vsebnosti katehina, epikatehina in skupnih fenolov.

- Kadar so bila tla v nasadu prekrita z reflektivno folijo, je bila vsota analiziranih sladkorjev in organskih kislin manjša kot pri nepokritih tleh, ne glede na to, ali je bil nasad pokrit z mrežo proti toči ali ne. Fruktoza, kot glavni sladkor v plodovih jabolk 'Fuji', je pokazal isti odziv, prav tako jabolčna in citronska kislina, ne pa tudi drugi analizirani sladkorji in kisline.

- Pokazalo se je, da prekrivanje tal v nasadu z reflektivno folijo izboljšuje osvetlitev v krošnji in s tem vpliva na večjo vsebnost antocianov in boljšo obarvanost plodov. To je posebno pomembno pri plodovih v notranjosti krošnje. Rezultati našega poskusa kažejo tudi, da v naših razmerah v letih, ko je trajal poskus, mreža proti toči ni bistveno vplivala na slabšo obarvanost plodov ali manjšo vsebnost antocianov.

V raziskavi smo potrdili negativen vpliv mreže proti toči in pozitiven vpliv reflektivne folije na svetlobne razmere v nasadu jabolk. Bolje osvetljeni plodovi so razvili intenzivnejšo rdečo krovno barvo, vsebovali več cianidin glikozidov in več kvercetin glikozidov, medtem ko druge analizirane fenolne spojine niso enotno odzvale na spremenjene razmere v osvetlitvi.

Z našimi rezultati smo združili nekatera dognanja na področju osvetljenosti v nasadu in v krošnji. Z raziskavami smo nadgradili dosedanje ugotovitve o vplivu spremenjenih svetlobnih razmer na razvoj barve jabolk ter vsebnost posameznih cianidin glikozidov, kvercetin glikozidov, katehina, epikatehina in klorogenske kisline. Ugotovitve, da se njihove koncentracije pod vplivom spremenjenih svetlobnih razmer spremenijo, nakazujejo, da je njihova sinteza bolj ali manj odvisna od svetlobe. Študijo bi lahko nadgradili s še bolj natančnim spremljanjem mikroklime, ki bi vključevala stalno merjenje temperature, intenzivnosti in spektra sončnega sevanja ter določitev potencialnega vpliva indeksa listne površine. V nadaljnje analize bi bilo smiselno vključiti tudi spremljanje aktivnosti encimov, ki so odgovorni za sintezo posameznih fenolnih snovi, saj bi s tem dobili bolj celovito sliko sinteze sekundarnih metabo~~b~~litov.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

V dvoletnem poskusu smo ugotavljali, kako se pri jablani sorte 'Fuji' zaradi uporabe mreže proti toči spremenijo razmere v osvetlitvi. Zaradi predvidenega zmanjšanja osvetlitve, smo zadnji mesec pred obiranjem položili reflektivno folijo. Določili smo štiri obravnavanja: (1) mreža proti toči, (2) reflektivna folija, (3) mreža proti toči + reflektivna folija in (4) kontrola (brez mreže in brez folije). Zanimal nas je odziv izbranih primarnih in sekundarnih metabolitov na spremenjene svetlobne razmere.

Pod črno mrežo je bilo fotosintetsko aktivno sevanje (PAR) 10–30 % manjše kot pri kontrolnem obravnavanju. Čeprav je bila osvetlitev v medvrstnem prostoru pod mrežo proti toči manjša kot pri kontroli, se osvetljenost plodov pod in izven mreže ni statistično razlikovala. Prekrivanje tal z belo reflektivno folijo je pozitivno vplivalo na osvetlitev v nasadu, posebno pa na osvetljenost posameznih plodov v krošnji. Spodnji deli plodov so bili od 3,5 do 7,3-krat bolj osvetljeni v obravnavanjih z reflektivno folijo kot pri kontroli.

Od intenzivnosti osvetlitve plodov je odvisen razvoj rdeče krovne barve jabolk. Rdeča barva je bila v vseh terminih vzorčenja najmanj intenzivna pri obravnavanju pod mrežo proti toči, najbolj pa pri obravnavanju z reflektivno folijo. Prekrivanje tal s folijo pod mrežo proti toči je pospešilo razvoj rdeče barve plodov, ki so ob obiranju dosegla vrednosti, kakršne so bile izmerjene pri kontrolnem obravnavanju. Plodovi v notranjosti krošnje so bili slabše obarvani kot plodovi, ki so rasli na robu ali vrhu krošnje. Te meritve potrjujejo dejstvo, da je slaba osvetlitev lahko omejujoč dejavnik za razvoj krovne barve in da plodovi v spodnjem delu krošnje za zadovoljivo obarvanost potrebujejo izboljšanje razmer v osvetlitvi. Učinkovit način bi bilo prekrivanje tal z reflektivno folijo.

Glavni antocianin v jabolkih sorte 'Fuji' je cianidin 3-galaktozid, ki je predstavljal od 92 % (pri prvem vzorčenju) do 98 % (ob obiranju) vseh antocianinov. Koncentracija cianidin galaktozida se med dozorevanjem povečuje, kar velja tudi za cianidin 3-galaktozid, cianidin 3-arabinozid in cianidin pentozid, čeprav so njihove vsebnosti bistveno manjše. Še manjši delež med antocianini predstavljata drugi cianidin pentozid in čisti cianidin.

Pokazalo se je, da je povečana osvetlitev zaradi uporabe reflektivne folije vplivala na vsebnost cianidin glikozidov, medtem ko mreža proti toči ni imela vpliva nanje. Najmanj cianidin galaktozida smo določili v kontrolnem obravnavanju in pod mrežo proti toči, več v obravnavanju z reflektivno folijo in največ pod mrežo proti toči, kjer so bila tla prekrita s folijo. Podobne rezultate smo dobili za cianidin 3-arabinozid in dva druga cianidin pentozida. Koncentracije cianidin galaktozida kot tudi cianidin 3-glukozida in cianidin 3-arabinozida so bile najmanjše v kožici jabolk, ki so rasla v notranjosti krošnje, medtem ko so pri plodovih z vrha drevesa dosegali najvišje vrednosti. Pri drugih dveh cianidin pentozidih koncentracije niso bile odvisne od pozicije plodov na drevesu.

Plodovi s kontrolnih dreves so vsebovali najnižje vrednosti skoraj vseh posameznih kvercetin glikozidov (kvercetin 3-galaktozid, kvercetin 3-glukozid, kvercetin 3-ramnozid,

kvercetin 3-rutinozid, kvercetin pentozid, kvercetin 3-ksilozid in kvercetin). Mreža proti toči in reflektivna folija sta obe vplivali na povečano vsebnost kvercetin glikozidov. Koncentracija posameznih kvercetin glikozidov kot tudi vsota kvercetinov je bila torej v večini primerov najvišja pod mrežo proti toči, kjer so bila tla pokrita z reflektivno folijo. Pomanjkanje osvetlitve v notranjosti krošnje je povzročilo nižje vsebnosti kvercetin glikozidov, medtem ko se vrednosti izmerjene pri plodovih, ki so rasli na vrhu in robu krošnje niso statistično razlikovale.

V jabolkih sorte 'Fuji' smo spremljali tudi spremembe vsebnosti katehina, epikatehina in klorogenske kisline, pri katerih nismo opazili enotnega odziva na spremenjene svetlobne razmere.

Zelo nizek nivo antocianinov, zmerna količina kvercetin glikozidov in relativno visoki nivoji katehinov in klorogenske kisline, ki so bili določeni pri slabših svetlobnih razmerah, nakazujejo, da je sinteza antocianinov svetlobno odvisen proces, medtem ko je sinteza drugih fenolnih spojin samo delno, če sploh, svetlobno odvisna.

4.2 SUMMARY

In a two years experimental study the effects of hail net on light conditions were monitored on apple cv. ‘Fuji’. Due to the expected decrease in lighting, the orchard floor was covered with reflective foil in the last month before harvest. Four treatments were established, (1) hail net, (2) reflective foil, (3) hail net + reflective foil and (4) control (without hail net and reflective foil). The response of individual primary and secondary metabolites to the changed light conditions was the main aim of the study.

Under the black hail net, photosynthetic active radiation (PAR) was reduced 10 to 30 % compared to the control treatment. Although the measured lighting conditions in the orchard was lower under hail net than in the control, no statistically significant differences in lighting of fruits under and outside the hail net were detected. Covering the floor with white reflective foil positively influenced the amount of lighting in the orchard, especially the base of the tree canopy. Lower parts of fruits were 3.5 to 7.3 fold more lighted in the treatment with reflective foil than in the control treatment.

Development of red fruit coloration is strongly dependent on light intensity. At all sampling dates, red coloration was lowest under hail net and highest in the treatment with reflective foil. Covering the orchard floor with reflective foil improved red coloration in fruit under hail nets and at harvest time the apples reached values similar to the control treatment. Fruits inside the tree canopy were less red than fruits from the top and the perimeter of the tree canopy. These findings confirm the fact that lower lighting could reduce red coloration and that fruits from the base of the canopy need improved light conditions for suitable fruit coloration. Covering floor with reflective foil could present an effective method.

The main anthocyanin in ‘Fuji’ apple was cyanidin 3-galactoside, which accounted from 92% (at first sampling date) up to 98% (at harvest time) of all anthocyanins. Its

concentration rose during ripening. Similar results were obtained for cyanidin 3-galactoside, cyanidin 3-arabinoside and cyanidin pentoside, although their content levels were much lower. A second cyanidin pentoside and pure cyanidin were detected in even lower content in apple fruit.

Higher lighting caused by the use of reflective foil influenced an increase in cyanidin glycosides content. However, hail nets had no similar effect. The lowest content of cyanidin glycosides were measured in the control treatment and under hail net. Higher amounts were detected in fruit at the reflective foil treatment and the highest at hail net treatment in combination with reflective foil. Similar results were measured for cyanidin 3-arabinoside and two others cyanidin pentosides. Concentrations of cyanidin 3-galactoside, cyanidin 3-glucoside and cyanidin 3-arabinosides were the lowest in the skin of the apples grown inside the canopy, and highest in apples from the top of the canopy. The content of the other cyanidin pentosides were not dependent on fruit position in the canopy.

Fruits from the control treatment contained lower amounts of nearly all individual quercetin glycosides (quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, quercetin 3-rutinoside, quercetin pentoside, quercetin 3-xyloside and pure quercetin). Hail net as well as reflective foil both resulted in the increased content of quercetin glycosides. Therefore, the content of individual quercetin glycosides and the sum of quercetin glycosides were generally the highest in fruit under the hail net where the reflective foil was used. Lower lighting inside the canopy caused lower quercetin glycosides contents. Content levels of quercetin compounds measured in fruits from the top and from the perimeter of the tree canopy were not statistically different.

The changes in catechin, epicatechin and chlorogenic acid content levels were also monitored in ‘Fuji’; however, the effect of lighting conditions on these compounds was not uniform.

We can conclude that low anthocyanin levels, moderate contents of quercetin 3-glycosides and relatively high levels of catechins and chlorogenic acid which were measured at lower lighting show that the synthesis of antocyanins is a light dependent process and that synthesis of other phenolic compounds is only partly light dependent if dependent at all.

5 VIRI

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